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# Bio-stimulation Approach in Bioremediation of Crude Oil Contaminated Soil Using Fish Waste and Goat Manure

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author VGA designed the study, performed the statistical analysis. Author RRN wrote the protocol, wrote the first draft of the manuscript, managed the analyses of the study and literature searches under the strict supervision of author DNO. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** This study aimed to evaluate the ability of Fish waste and Goat manure to bio-stimulate the degradation process during bioremediation of crude oil-contaminated soil.
**Study Design:** Research was designed to evaluate and compare the strength of the organic nutrients (Goat manure and fish waste) to stimulate the biodegradation of crude oil contaminated soil within 56 days.
**Place and Duration of Study:** Study was carried out in Rivers State University Farm, Rivers state, Nigeria for 56 days from July to September 2018. Analyses were carried out weekly (per 7 days interval).
**Methodology:** Eight (8) experimental set-up were employed, each having 5kg farm soil, all were left fallow for 6 days before contamination with crude oil on the 7$^{th}$ day in the respective percentages. Four of the set-ups were contaminated with 5% Crude oil while the other four were contaminated with 10% Crude oil. The contaminated plots were further allowed for 21 days for proper contamination and

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exposure to natural environmental factors to mimic a crude oil spill site before the application of bio stimulating agents (fish waste and goat manure). The set-ups of 5% Crude Oil Contaminated Soil (5% COCS) and 10% Crude Oil Contaminated Soil (10% COCS) were then stimulated with nutrient organics; Goat Manure (GM) and Fish Waste (FW) except two setups (one 5% COCS and the other 10% COCS) which were used as controls. The treatments (setups) were as follows: 5% COCS (control 1), 5% COCS + GM, 5% COCS + FW, 5% COCS + GM + FW and 10% COCS (Control 2), 10% COCS +GM, 10% COCS + FW, 10% COCS + GM + FW. Physiochemical and microbiological status of the soil before and after contamination was evaluated while parameters including Nitrate, Sulphate, Phosphate and Total Petroleum Hydrocarbon (TPH), as well as Microbial analyses, were monitored throughout the experimental period. Bioremediation efficiency was estimated from percentage (%) reduction of Total Petroleum Hydrocarbon (TPH) from day 1 to the residual concentration at day 56 of bio-stimulation setups with the control. The bio-stimulating potentials of goat manure and fish waste were compared using statistical tools.

**Results:** The results revealed decrease in TPH with increasing time. The Amount (mg/kg) and Percentage (%) of Total Petroleum Hydrocarbon (TPH) remediated within the period of this study for 5% Crude Oil Contaminated Soil were as follows: 5% COCS-Ctrl 1 (563.52 mg/kg; 8.60%) < 5% COCS + GM (3608.84 mg/kg; 55.11%) < 5% COCS + FW (4156.49 mg/kg; 63.47%) < 5% COCS + GM + FW (4350.69 mg/kg; 66.44%) while 10% crude oil contaminated soil were: 10% COCS-Ctrl 2 (125.71 mg/kg; 1.21%) < 10% COCS + GM (4422.75 mg/kg; 42.82%) < 10%COCS + FW (5542.16 mg/kg; 53.66%) < 10% COCS + GM + FW (6168.66 mg/kg; 59.72%). This result shows that combination treatment with goat manure and fish wasteis more effective and has more bio-stimulating potentials than the single treatments. With respect to individual bio-stimulating agent, fish waste proves more effective and had a higher bioremediation efficiency than goat manure. The results of colonial counts obtained revealed that the total heterotrophic bacterial and total fungal counts generally increased during the study across the trend. The counts obtained from day 7 to 56 in the respective experimental set ups were as follows: Total Heterotrophic Bacteria counts increased from 6.32 to 8.20 $Log_{10}$CFU/g (Control) < 6.32 to 9.05 $Log_{10}$CFU/g (COCS+FW) < 6.41 to 9.13 $Log_{10}$CFU/g (COCS+GM) < 6.32 to 9.58 $Log_{10}$CFU/g (COCS+FW+GM). Similar progression was observed for total fungi, hydrocarbon utilizing bacteria and hydrocarbon utilizing fungi in all the experimental set ups although irregular differences were observed in the control set ups.

**Conclusion:** The combination of organic nutrient such as goat manure and fish waste as bio-stimulating agents have shown to have higher percentage (%) bioremediation efficiency than when applied singly. It was also observed that the microbial biomass increased with time; moreover the nutrient monitoring analysis revealed a continuous gradual increase of the soil nutrient as bioremediation increases with time. The nutrient inherent in the bio-stimulating agents' fish waste and goat manure resulted in increased soil nutrient (from day 7 to 56) as bioremediation period increase thereby enhancing soil nutrients at end of experiment. It is therefore recommended that bio-stimulating agents such as fish waste and goat manure should be employed in bioremediation of crude oil-contaminated soil especially due to its soil nutrient enhancement after bioremediation exercise. It's a very good nutrient amendment option.

*Keywords: Bio-stimulation; bioremediation; crude oil contamination; fish waste; goat manure.*

## 1. INTRODUCTION

Biostimulation involves the modification of the environment to stimulate existing bacteria capable of bioremediation. This can be done by addition of various forms of rate-limiting nutrients and electron acceptors, such as phosphorus, nitrogen, oxygen, or carbon (eg. In the form of goat manure and fish waste) according to Ogbonna [1]. Research had proven that the organic nutrient solution formulated from goat manure positively improved plant growth and yield performance of a tomato crop, and provided a technique feasible and alternative to conventional hydroponics.

The primary advantage of bio-stimulation is that bioremediation will be undertaken by already present native microorganisms that are well suited to the subsurface environment and are well distributed spatially within the subsurface [2]. The disadvantage is that the delivery of additives in a manner that allows the additives to be readily available to subsurface microorganisms is based on the local geology of the subsurface. Tight impermeable subsurface lithology (tight

clays or other fine-grained material) makes it difficult to spread additives throughout the affected area [3]. Fractures in the subsurface create preferential pathways in the subsurface which additives preferentially follow, preventing even distribution of additives.

Many substances known to have toxic properties are regularly introduced into the environment through human activity. These substances range in degree of toxicity and danger to human health. Many of these substances either immediately or ultimately come in contact with or are sequestered by soil. Conventional methods to remove, reduce, or mitigate toxic substances introduced into soil or groundwater via anthropogenic activities and processes include pump and treat systems, soil vapor extraction, incineration, and containment. Utility of each of these conventional methods of treatment of contaminated soil and/or water suffers from recognizable drawbacks and may involve some level of risk [4].

Bioremediation offers an alternative method to detoxify contaminants and is being used as an effective means of mitigating hydrocarbons, halogenated organic solvents and compounds, non-chlorinated pesticides and herbicides, nitrogen compounds, metals (lead, mercury, chromium) and radionuclides [5].

Bio-stimulation utilizes the indigenous microbial populations to remediate contaminated soils. The added nutrients and other substances in soil catalyze natural attenuation processes. Bio-augmentation involves introduction of exogenic microorganisms (Sourced from outside the soil environment) capable of detoxifying a particular contaminant, sometimes employing genetically altered microorganisms [6,7]. During bioremediation, microbes utilize chemical contaminants in the soil as an energy source and, through oxidation-reduction reactions, metabolize the target contaminant into useable energy for microbes. The by-products (metabolites) released back into the environment are typically in a less toxic form than the parent contaminants. For example, petroleum hydro-carbons can be degraded by microorganisms in the presence of oxygen through aerobic respiration. The hydrocarbon loses electrons and is oxidized while oxygen gains electrons and is reduced. The result is formation of carbon dioxide and water [8]. When oxygen is limited in supply or absent, as in saturated or anaerobic soils or lake sediment, anaerobic (Without oxygen) respiration prevails. Generally, inorganic compounds such as nitrate, sulfate, ferric iron, manganese, or carbon dioxide serve as terminal electron acceptors to facilitate biodegradation [9,10].

Primary ingredients for bioremediation includes; I) presence of a contaminant, ii) an electron acceptor, and iii) presence of microorganisms that are capable of degrading the specific contaminant. Generally, a contaminant is more easily and quickly degraded if it is a naturally occurring compound in the environment or chemically similar to a naturally occurring compound, because microorganisms capable of its biodegradation are more likely to have evolved [11]. Petroleum hydrocarbons are naturally occurring chemicals, therefore, microorganisms which are capable of attenuating or degrading hydrocarbons exist in the environment.

Crude oil is a naturally occurring complex mixture of hydrocarbon and non-hydrocarbon compounds such as Sulphur, Nitrogen and Oxygen; which at inappropriate concentrations possess toxicity towards living systems. The frequent oil spill incidents in Niger Delta area of Nigeria have become a problem to ecological protection efforts which usually employ conventional methods to remove, reduce, or mitigate toxic substances introduced into soil. Hence, due to recognizable drawbacks and the level of risk to biotic and abiotic component of the soil with the use of conventional remediation methods; this research was developed to assess the degree of bio-stimulating potential of goat manure and fish waste in crude oil contaminated soil.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out in the Rivers State University Farm, Port Harcourt Rivers State, Nigeria within the coordinates 7.30.08N and 8.13.46E.

### 2.2 Sample Collection

Topsoil samples were collected from the Agricultural Teaching Farm of Rivers State University, Port Harcourt, in accordance to the Food and Agricultural Organization guidelines [10] using a sterile manual soil auger to obtain a depth of 0-15 cm of topsoil as adopted by

Ogbonnaet al. [12]. The soil samples were transported in sterile black polythene bags perforated for aeration to the microbiology laboratory of the Rivers State University for analysis. While the crude oil used for the contamination of the soil sample was obtained from an oil company located at Nembe Creek, Bayelsa State. The crude oil was aseptically collected in large sterile plastic Jerry cans.

## 2.3 Application of Crude Oil

All setups were separately and deliberately contaminated with 5% and 10% of crude oil giving an initial Total Petroleum Hydrocarbon (TPH) value of 6548.06 mg/kg and 10328.03 mg/kg respectively. The setups were left for 21 days to ensure even distribution and soil-oil bonding.

## 2.4 Preparation of the Bio-stimulants (Goat manure and Fish waste)

The goat manure and fish waste used in this study were sun-dried for 2 weeks and then blended to fine particles before application (Plates 1 and 2).

## 2.5 Application of Nutrient Amendment for Bio-stimulation of the Contaminated Soil

Two hundred and fifty grams (250 g) of the organic nutrient (Goat manure and Fish wastes)were added to each setup except the controls, properly stirred with a sterile spatula to ensure the indigenous microorganisms thrive and have sufficient oxygen. Two (2) litres of water was added to each plot weekly, tilted slightly to enhance moisture content and microbial activity. Illustrative representations of the experimental setups are shown in Fig. 1.

## 2.6 Sampling Methods

Soil samples were collected from 5-10 random points from each setup, bulked to form acomposite sample after tilling using soil spatula according to the methods of Nrior and Echezolom [13]. Small portions measuring 5 g of the composite samples were collected into sterile bottles using a sterile spatula for physicochemical and microbiological analyses. Sampling was done for 56 days after contamination of the various setups at seven days interval (7, 14,21, 28, 35, 42, 49 and 56). Soil samples were stored at 14±2°C for further analysis.

## 2.7 Determination of Physico-chemical Properties of the Treated Samples

### 2.7.1 Determination of pH

The pH of soil sample was determined using a portable pH meter; code: HI9811-5 Hanna Instruments (Romania). The meter was switched on and allowed for some time. It was then calibrated with buffer solutions of higher pH range between 8 and 9 as well as a lower pH range between 1 and 6 by dipping the electrode into the buffer solutions. 10 g of soil was weighed into 100 ml beaker; 25 msl of distilled water was then added to allow immersion of the electrode**,** mixing was carried out by stirring frequently for a few minutes. Then beaker was allowed to stand for 15 minutes. The electrode was immersed in the slurry and the pH values for each sample were recorded accordingly.

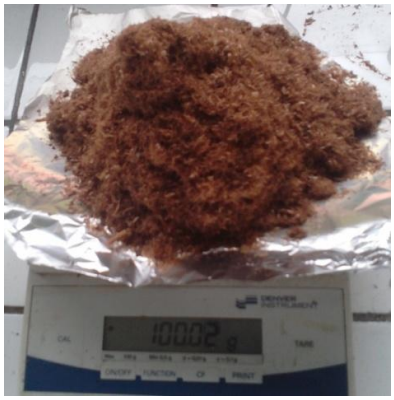


**Plate 1.Fish waste**


**Plate 2. Goat manure**


**Fig. 1. Illustrative representations of the experimental setup**

### 2.7.2 Determination of temperature

The Temperature for each sample was determined using a mercury-in-glass thermometer; code: G00127766-5 Hanna Instruments (Romania). The thermometer was immersed in the samples such that the mercury bulb was well covered by the samples. The final readings were considered the actual reading and were taken after it was allowed to stabilize.

### 2.7.3 Determination of nitrate

The nitrate levels for the samples were determined using an ultraviolet (UV) spectrophotometer method. Five gram (5 g) of each sample was weighed into a shaking bottle. 125 ml of distilled water was added and shaken for 10 minutes on a rotary shaker and then filtered to obtain the extract, 1 ml of the extract was transferred into 10 ml volumetric flask, 0.5 ml of Brucine reagent was added. Subsequently, 2 ml of concentrated sulphuric acid was rapidly added and mixed for about 30 seconds. The flasks were allowed to stand for 5 minutes. Two millilitres (2 mls) of distilled water was then added and mixed for about 30 seconds. Flasks were allowed to stand in cold water for about 15 minutes. The absorbance of the samples was measured using the spectrophotometer at the wavelength of 470 nm.

### 2.7.4 Determination of phosphate

The phosphate levels for the samples were determined using an ultraviolet (UV) spectrophotometer. The sulfuric acid - nitric acid Digestion method as described by APHA [14] was adopted. Twenty-five millilitres (25 mls) of 2.5% Acetic acid was added to 1 g of sample and shaken for 30minutes**.** The suspension was filtered through a filter paper, 10 ml of the extract was transferred into 50ml volumetric flask. The extract was diluted with distilled water until the flask was about two-thirds full. 2 ml of ammonium molybdate reagent was added and mixed with extract, 2 ml of stannous chloride was also added and mixed and the solution was diluted to 50 ml mark with distilled water. The flask was allowed to stand for 30minutes and the absorbance was measured at the wavelength of 690 nm.

### 2.7.5 Determination of sulphate

The sulphate levels for the samples were determined using the method as described by APHA [14]. Twenty-five millilitres (25 mls) of the extracting solution was added to 5 g of sample and shaken for 30minutes and the suspension was filtered through a filter paper. 5 ml of the extract was transferred into 50 ml volumetric flask. 5 ml of 50% acetic acid was added and 1 ml of $H_3PO_4$ was added and mixed. The solution was diluted with distilled water to about three-quarter (¾) full of the flask. One gram of Barium chloride was added and mixed. The solution was left to stand for 10 mins, then 1 ml of 0.5% gum acacia was added to the solution and made up to 50 ml with distilled water. The solution was allowed to stand for one hour, thereafter the absorbance was measured at 425 nm.

### 2.7.6 Determination of total petroleum hydrocarbon

Total Petroleum Hydrocarbon (TPH) analyses were carried out on all the eight setups using

Gas Chromatography (GC) for Day 7, 14, 21, 28, 35, 42, 49 and 56. Total Petroleum Hydrocarbon (TPH) in each of the set-ups was determined by a modified Environmental Protection Agency 8015 technique. The soil samples were extracted using a gas chromatograph, equipped with a flame ionization detector (FID). The residual Total Petroleum Hydrocarbon (TPH) in the different treatment set up was extracted with 40 ul of n-pentane (HPLC grade) by sonicating the sample 5min at each extraction for 3 times. The pentane extract was centrifuged at 3000 g for 5 min, the three organic phases were oven-dried over sodium sulphate ($Na_2SO_4$), pooled and adjusted to 150 ml after which 32 ul of cumene (isopropyl benzene) was added as internal standard analyses were carried out using a Varian 1440 GC-FID (Califoni, USA). The extractable TPH was identified and quantified by comparison using a sample chromatogram with standard calibration.

## 2.8 Microbiological Analysis

### 2.8.1 Total Heterotrophic bacterial

Total heterotrophic bacteria sample were enumerated using the spread plate technique as described by Prescott et al. [15]. An aliquot (0.1 ml) of the dilution $10^{-7}$ dilution was aseptically transferred unto properly dried nutrient agar plates in duplicate, spread evenly using a bent glass rod and incubated at 37ºC for 24 hours. After incubation, the bacterial colonies that grew on the plates were counted and sub-cultured unto fresh nutrient agar plate using the streak plate technique. Discrete colonies on the plates were aseptically transferred into 10% (v/v) glycerol suspension, well label and stored as stock cultures for preservation and identification [16]. Total Heterotrophic Bacteria Counts (THBC) was calculated from the mean value of colonies counted from the duplicate plates using the below formula:

THBC (CFU/g) = (Number of Colonies)/ Dilution ($10^{-7}$) x Volume plated (0.1 ml)

### 2.8.2 Total heterotrophic fungal

The total Heterotrophic fungi were enumerated using the spread plate method as described by Prescott et al.[15]. An aliquot (0.1 ml) of the dilution of $10^{-3}$ dilution was aseptically transferred unto properly dried Sabouraud Dextrose Agar plates containing antibiotic (tetracycline and penicillin) to inhibit bacterial growth in duplicate [17]. Plates were spread evenly using a bent glass rod and incubated at 35ºC for 3 days. After taking counts, the fungal colonies were sub-cultured onto Sabouraud Dextrose Agar slant in bijou bottle for preservation [18].

### 2.8.3 Hydrocarbon utilizing bacterial count

The population of hydrocarbon utilizing bacteria was determined by inoculating 0.1 ml aliquot of the serially diluted samples onto mineral salt agar media using vapour phase transfer method according to Okpokwasiliand Amanchukwu [18]. The mineral salt agar used for enumeration of hydrocarbon utilizing bacteria was amended with 2.5 ml of fungusol (Miconazole Nitrate B.P. 2%) [19], the plates were inverted and incubated at 28ºC for 5 days. The filter paper saturated with sterile crude oil served as the sole source of carbon in the mineral salt agar. Colonies formed in the duplicate plates were counted and the mean values were recorded and expressed as colony-forming unit per gram (CFU/g).

### 2.8.4 Hydrocarbon utilizing fungal count

The population of hydrocarbon utilizing fungi was determined by inoculating 0.1 ml aliquot of the serially diluted samples onto mineral salt agar media using vapour phase transfer method according to Nrior and Odokuma [7]. For hydrocarbon utilizing fungi, the mineral salt medium used was amended with 250mg of tetracycline to inhibit the growth of hydrocarbon utilizing bacteria. The plates were inverted and incubated at 28ºC for 5 days. The counts of fungi were expressed and recorded as spore-forming unit per gram (SFU/g) [19].

## 2.9 Determination of Amount and Percentage (%) Crude Oil Bioremediation

The method of Nrior and Echezolom [13] was used in calculating the percentage (%) bioremediation in the experiment on day 56. The process followed the steps stated below;

Step i: The amount of pollutant remediated equals to Initial Concentration of pollutant (Week 1) minus the Final concentration of a pollutant at the end of the experiment (Last day or Week 8).

Step ii: The percentage (%) Bioremediation equals Amount of pollutant divided by the initial concentration of pollutant (week 1), multiplied by 100.

Amount Remediated [$AR_{TPH}$]

$$\mathrm{AR} = Ic - \mathrm{Fc} \quad (1)$$

Percentage (%) Bioremediation [$\%B_{TPH}$]

$$\%B = \frac{AR}{Ic} x\ 100 \quad (2)$$

Where:

AR = Amount of pollutant remediated
Ic = Initial concentration of pollutant (week 1)
Fc = Final concentration of pollutant (week8)

## 2.10 Statistical Analysis

Results were subjected to statistical analysis using Analysis of Variance (Two way ANOVA) to test whether the different nutrient amendments given to the crude oil polluted plots were statistically significant. Regression analysis of Physiochemical parameters during bioremediation of crude oil-polluted soil showing regression equation of each parameter and their $R^2$ values was carried out.

## 3. RESULTS AND DISCUSSION

The physicochemical and microbiological analyses of the soil before and after crude oil contamination were carried out; the results are presented in Table 1. The following physicochemical parameters; sulphate, phosphate, phosphorus, temperature, electric conductivity, moisture content and, potassium, and sodium increased slightly after crude oil contamination while the pH value decreased from 6.8±0.31 to 5.95±0.26. The concentration of Total Hydrocarbon Content (THC) in the experimental soil before application of amendments was 2.023±0.02 mg/kg while after crude oil application, THC value increased to 6546±5.744 mg/kg. This value is above the intervention value of 5000 mg/kg according to Department of Petroleum Resources (DPR) standard for crude oil spill value (Above limit of 5000 mg/kg, the soil is considered polluted and needs intervention/ remediation) [20]. In the results of microbiological parameters; Total Heterotrophic Bacteria (THB), Total Heterotrophic Fungi (THF), Hydrocarbon Utilizing Bacteria (HUB) and Hydrocarbon Utilizing Fungi (HUF) there was an increase in microbial counts except the THB indicating that the crude oil used to inhibit the growth of some viable bacteria colonies that cannot utilized crude as sole source of carbon (Table 1).

The analysis carried out to evaluate bio stimulating potential of goat manure (GM) and fish waste (FW) on crude oil contaminated soil were studied which could serve as treatment options for crude oil-contaminated soil in case of an oil spill. The result obtained revealed that these bio stimulating agents helped the indigenous organisms as evident in increased bioremediation rate as well as reducing the contamination hazards caused by crude oil in the soil with time. The analyses carried out on weekly intervals; Day 1, 7, 14, 21, 28, 35, 42, 49 and 56 revealed the potentiality of how the stimulating agents were able to enhance by the indigenous organisms to degrade the petroleum in consonance with findings of Okerentugba and Ezeronye [20].

**Table 1.The physicochemical and microbiological analyses of the soil with crude oil before and after crude oil contamination**

| Parameter | Unit | Uncontaminated (Mean±SD) | Contaminated (Mean±SD) |
|---|---|---|---|
| Nitrate | mg/kg | 811.5±0.70 | 791.5±0.70 |
| Phosphate | mg/kg | 15782±8.09 | 15982±63.84 |
| Sulphate | mg/kg | 73413±20.10 | 73594±15.59 |
| pH | | 6.85±0.31 | 5.95±0.26 |
| Temperature | °C | 27.33±0.47 | 28.10±0.36 |
| Electrical Conductivity | µS | 0.073±0.02 | 0.083±0.01 |
| Moisture Content | (%) | 11.52±0.64 | 12.28±0.44 |
| Total Petroleum Hydrocarbon | mg/kg | 2.02±0.02 | 6546±5.74 |
| Calcium | mg/kg | 0.94±0.07 | 0.87±0.04 |
| Sodium | mg/kg | 1.97±0.04 | 1.98±0.08 |
| Magnesium | mg/kg | 3.38±0.07 | 3.18±0.07 |
| Potassium | mg/kg | 1.09±0.02 | 1.11±0.03 |
| Total Heterotrophic Bacteria | CFU/g | 2.58 x $10^8$±0.07 | 2.10 x $10^8$±0.50 |
| Total Heterotrophic Fungi | CFU/g | 1.6 x $10^5$±0.08 | 2.0 x $10^5$±0.050 |
| Hydrocarbon Utilizing Bacteria | CFU/g | 5 x $10^4$±0.50 | 8 x $10^5$±0.50 |
| Hydrocarbon Utilizing Fungi | CFU/g | 7 x $10^4$±0.50 | 9 x $10^4$±0.50 |

Total Petroleum Hydrocarbon (TPH) degradation was determined by the decrease in amount from initial contamination value of 6548.06 mg/kg (for 5% Crude oil contaminated soil) on day 7 to the last day (Day 56) on day 7 to treatment setup on the last day of the experiment were as follows; GM (55.11316 mg/kg) > FW (63.47669 mg/kg) GM+FW (66.44247 mg/kg) for 5% crude oil contaminated soil, while for 10% contaminated soil TPH value decreased in the following order from 10328.03 mg/kg on day 7 to GM (42.82275 mg/kg) > FW (53.66131 mg/kg) GM+FW (59.72732 mg/kg) on day 56 (Fig. 2).

Total Petroleum Hydrocarbon of the 5% and 10% crude oil contaminated soil with the various treatment of goat manure and fish waste as well as the control evaluated at a constant interval of one week within 56 days. Fig. 2, shows changes in the total hydrocarbon content within the period, the results revealed a decrease in TPH with increasing time. The decrease in THP varies with the various treatment as follows; GM+FW<GM <FW both in 5% and 10% crude oil-contaminated soil. This result shows that goat manure combined with fish waste is effective to have more bio stimulating potential than fish wastes. A similar observation had been made by other researchers [21,22].

Bioremediation evaluation from the initial TPH contamination value of 6548.06 mg/kg (5%) and 10328.03 mg/kg (10%)revealed the amount of remediated hydrocarbon and percentage (%) bioremediation efficiency at 56 days in the different treatment setup in decreasing order as shown in Figs. 3 and 4.

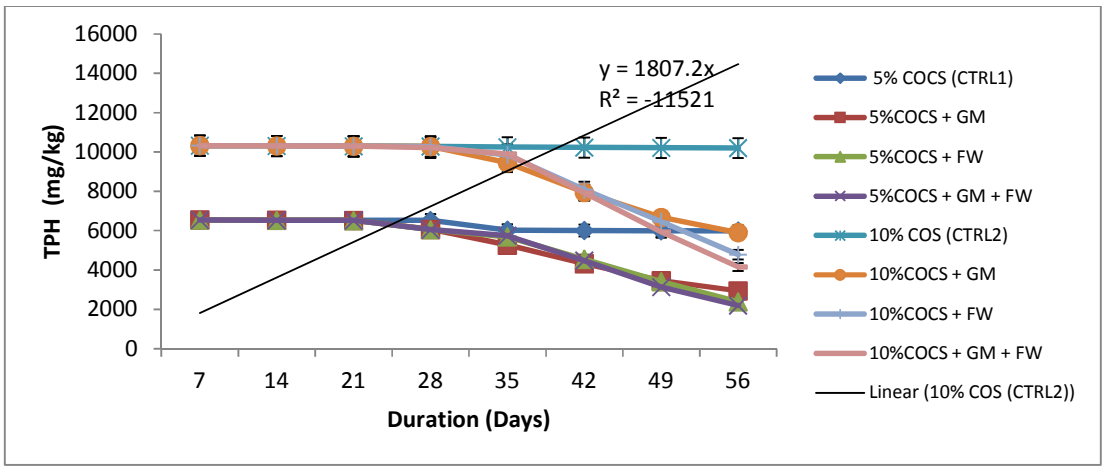


**Fig. 2. Changes in the Total Petroleum Hydrocarbon (TPH) contents during the Bioremediation Process**

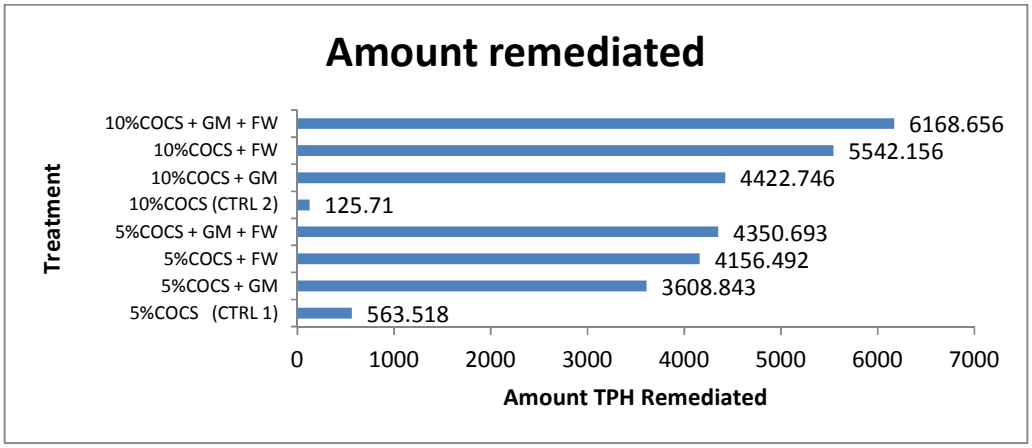


**Fig. 3. Amount of TPH remediation by the goat manure and fish waste in the 5%and 10% crude oil contaminated soil within 56 days**

The results of pH, Temperature, Nitrate, Magnesium, Potassium and Sulphateevaluated in this study within the period of bioremediation are presented in (Figs. 5-10).

The following Parameter; pH, Temperature, Magnesium, Potassium, Nitrate, and Sulphate were evaluated in the various set-ups in this study from day 7 to 56. The results show that pH obtained ranged from 6.3 to 7.6, this indicates that the soil used in this study is capable of supporting the growth of bacteria according to the report of Ogbonna [23] who stated clearly, that bacteria can proliferate in the soil that has a pH ranging from 5.0 to 8.5. Temperature value obtained in this result ranged from 27ºC to 28ºC.

The bacterial and fungal isolates from the experimental soil used in this study belong to the genera: *Proteus, Bacillus, Citrobacter, Pseudomonas, Micrococcus* and *Staphylococcus* species for bacteria while fungal genera were *Mucor*, *Rhizopussp* and *Penicillium*. This is in line with various researchers who reported similar bacterial and fungal isolates from crude oil contaminated soils [14,21]. The Results of colonial counts obtained revealed that the Total Heterotrophic Bacterial and Total Heterotrophic Fungal counts generally increased during the study as the treatment progressed resulting in corresponding bioremediation with time in the bio stimulated soil compared to the controls for both 5% and 10% crude oil contaminated soil (Figs. 11-14). The counts obtained from day 7 to 56 ranged in the respective experimental plots as follows; Total Heterotrophic Bacteria ranged from 6.32 to 8.20 $Log_{10}$CFU/g (control) < 6.32 to 9.05 $Log_{10}$CFU/g (CS+FW) < 6.41 to 9.13 $Log_{10}$CFU/g (CS+GM) < 6.32 to 9.58 $Log_{10}$CFU/g (CS+FW+GM).Similar progression was observed for total heterotrophic fungi, Hydrocarbon utilizing bacteria and Hydrocarbon utilizing fungi in all experimental setup except for the control setups that revealed irregular changes.

The Total Heterotrophic Fugal count (Fig. 12) was observed to show a similar pattern as THB on day 7 and day 56 with contaminated soil+ goat manure + fish waste revealing the highest value on day 56. Similar observations were observed in the Hydrocarbon utilizing bacterial and Hydrocarbon utilizing fungal counts in the various treatments setups (Figs.13-14). The result is consistent with the reports of Chikere et al.[17], Nrior and Mene, [21] and Ogbonna et al. [23] who observed that Total Heterotrophic Bacterial and Hydro-carbon Utilizing Bacterial counts increased over time in a nutrient amended crude oil contaminated soil undergoing bioremediation with time.

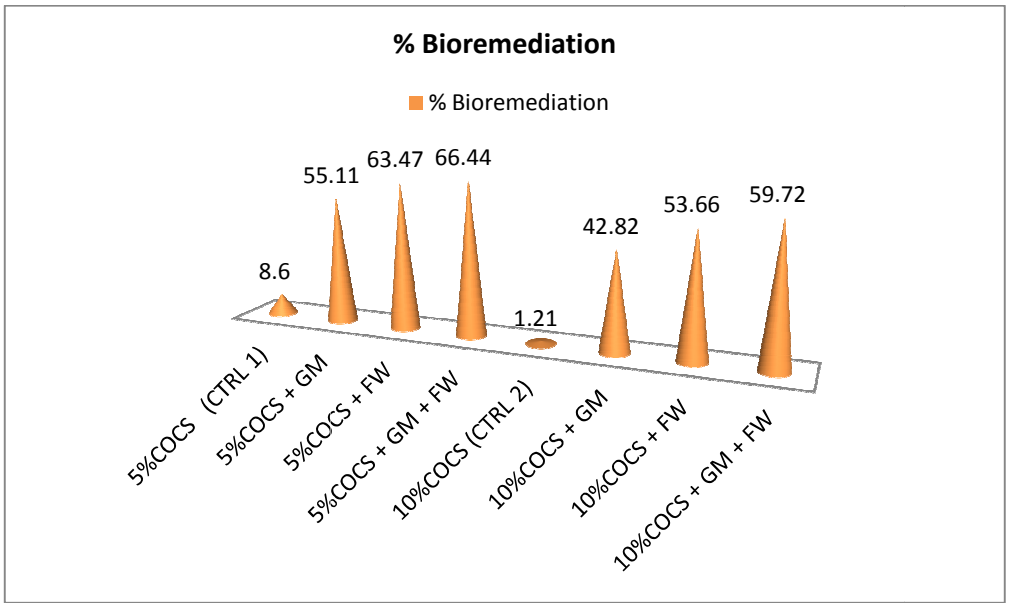


**Fig. 4. Percentage TPH bioremediation by the goat manure and fish waste in the 5%and 10% crude oil contaminated soil within 56 days**

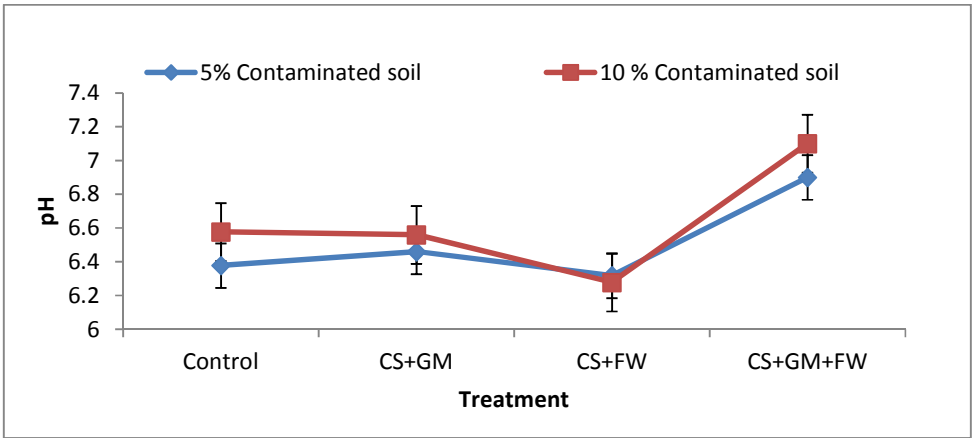


**Fig. 5. Changes in pH during bioremediation of crude oil contaminated soil**

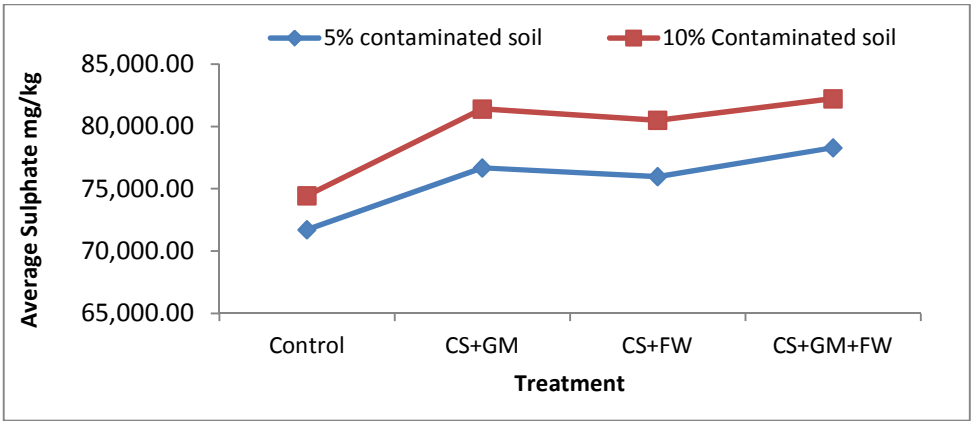


**Fig. 6. Changes in sulphate (mg/kg) during bioremediation of crude oil contaminated soil**

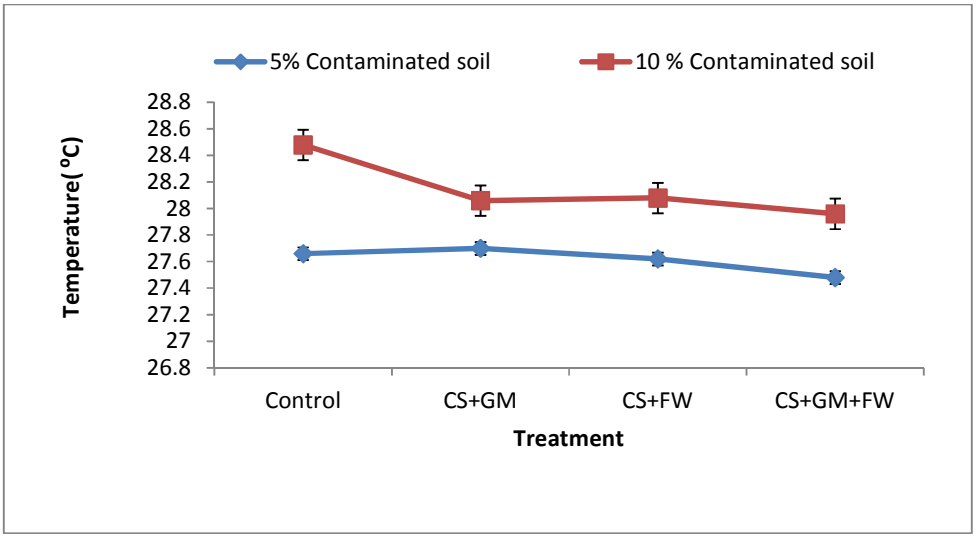


**Fig. 7. Changes in temperature (°C) during bioremediation of crude oil contaminated soil**

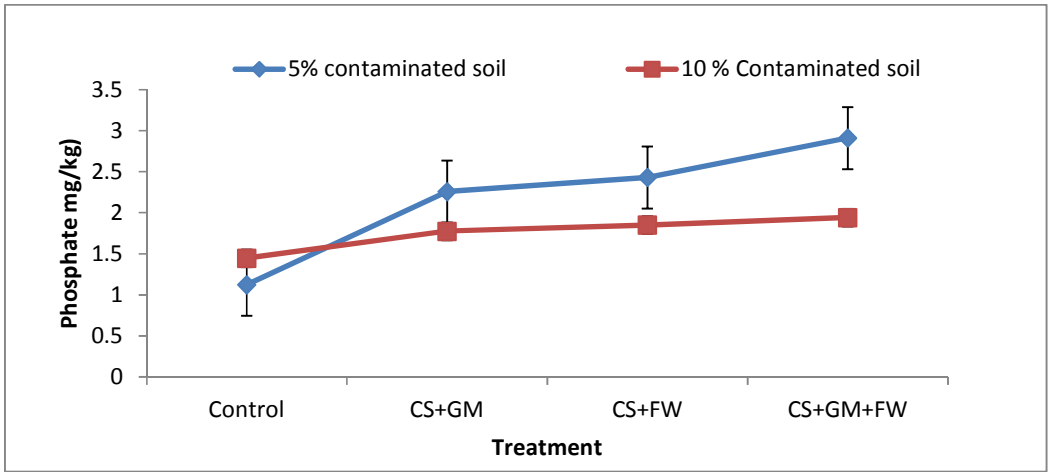


**Fig. 8. Changes in phosphate (mg/kg) during bioremediation of crude oil contaminated soil**

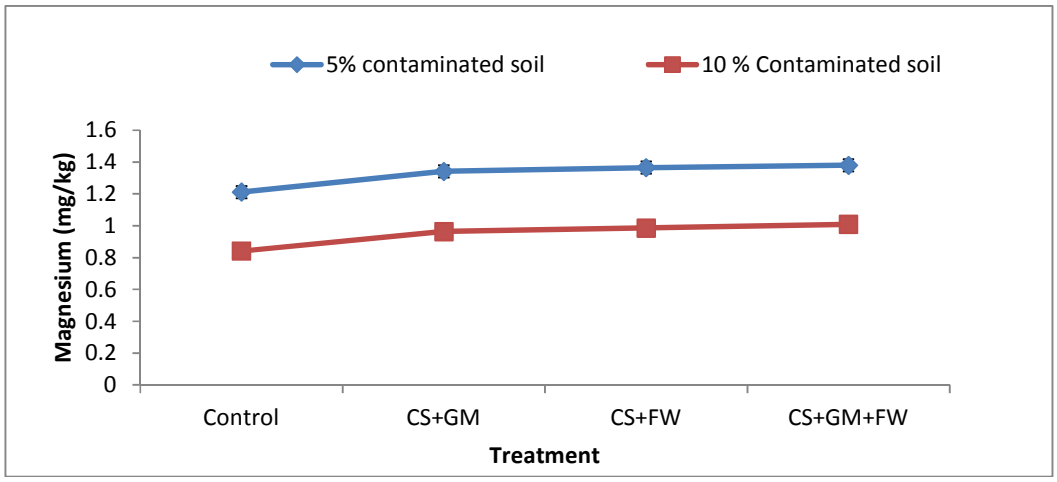


**Fig. 9. Changes in magnesium (mg/kg) during bioremediation of crude oil contaminated soil**

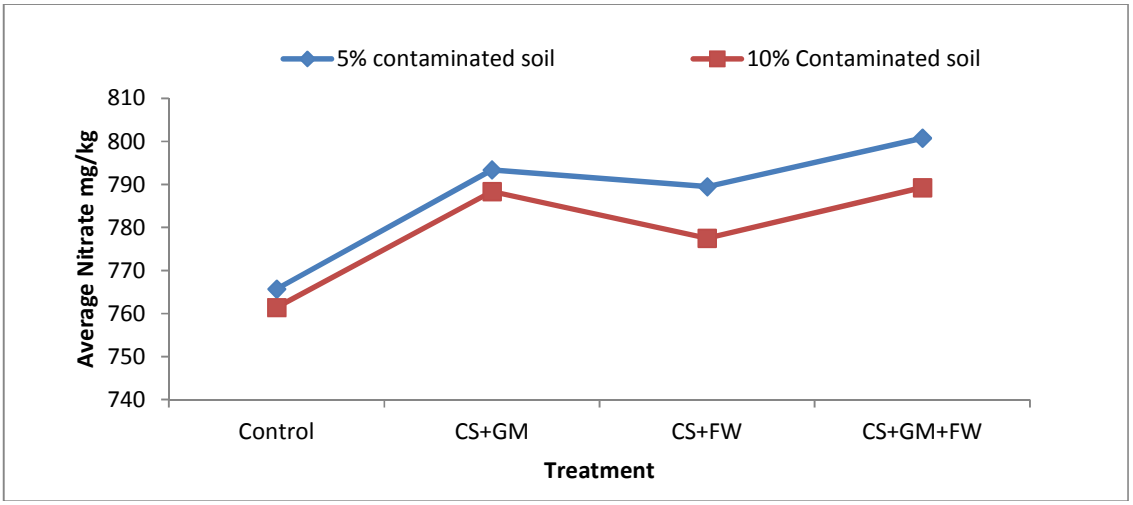


**Fig. 10. Changes in nitrate (mg/kg) during bioremediation of crude oil contaminated soil**

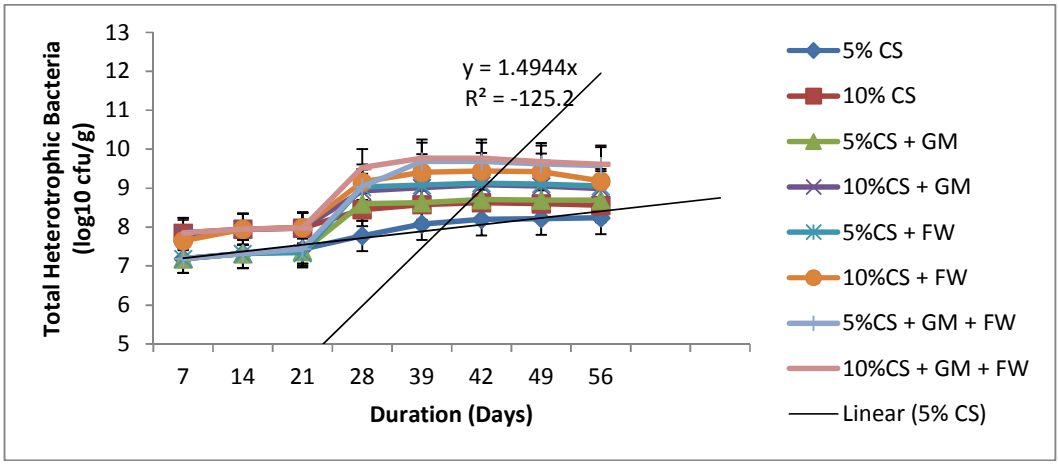


**Fig. 11. Total Heterotrophic Bacteria ($log_{10}$CFU/g) count during the 56 days of monitoring of the contaminated soil**

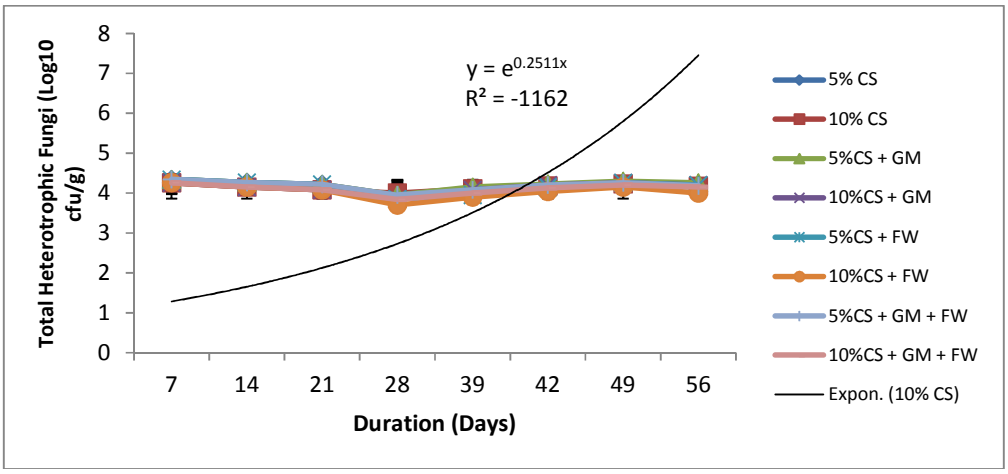


**Fig. 12. Changes in total heterotrophic fungi ($log_{10}$CFU/g) count during the 56 days of monitoring of the contaminated soil**

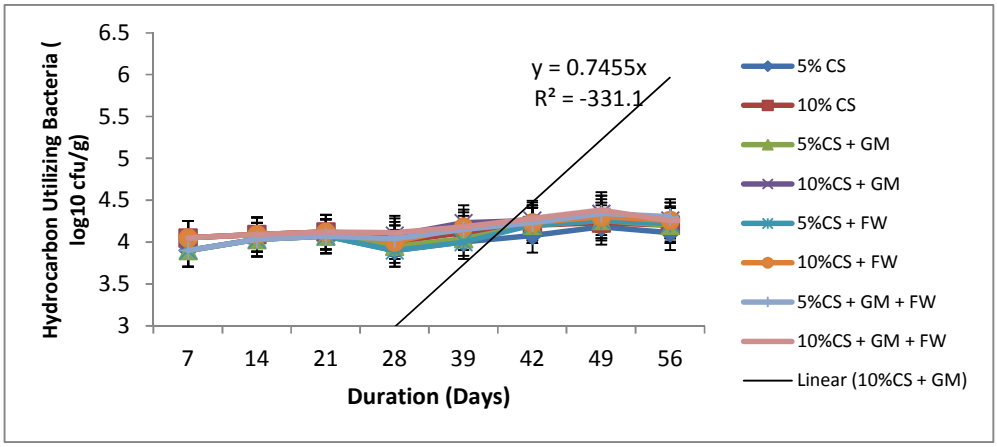


**Fig. 13. Changes in the hydrocarbon utilizing bacteria ($log_{10}$CFU/g) count during the 56 days of monitoring of the contaminated soil**

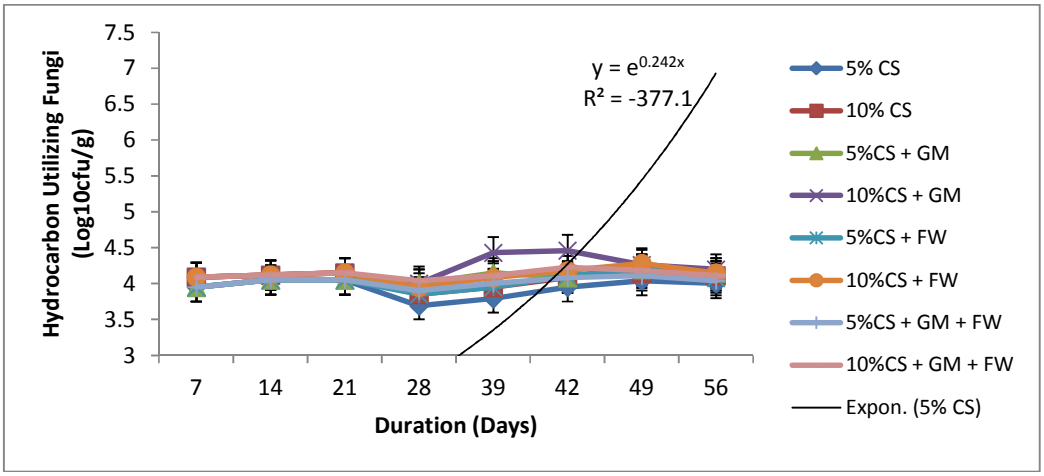


**Fig. 14. Changes in the hydrocarbon utilizing fungi ($Log_{10}$CFU/g) count during the 56 days of monitoring of the contaminated soil**

## 4. CONCLUSION AND RECOMMENDATION

The combination of organic nutrient such as goat manure and fish waste as bio-stimulating agents have shown to have higher percentage (%) bioremediation efficiency than when applied singly in both 5 or 10% crude oil-contamination. It was also observed that the microbial biomass increased steadily with time; moreover the nutrient monitoring analysis revealed a continuous gradual increase of the soil nutrient as bioremediation increases with time. This proffer a more efficient methods – as contaminant/pollutant (crude oil hydrocarbon) concentration is being remediated/ reduced the is increase in soil nutrient emanating from these tested bio-stimulating agents: Goat manure and fish waste.

It is therefore recommended that bio-stimulating agents such as fish waste and goat manure should be employed in bioremediation of crude oil-contaminated soil especially due to its soil nutrient enhancement after bioremediation exercise.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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