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# Bioremediation Efficiency of *Bacillus amyloliquefaciens* and *Pseudomonas aeruginosa* with the Nutrient Amendment on Crude Oil Polluted the Soil

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***Authors' contributions***

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

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## ABSTRACT

**Aim:** To assess the Bioremediation efficiency of *Bacillus amyloliquefaciens* and *Pseudomonas aeruginosa* strain CL 9 with nutrient amendment using bio-stimulating agents such as Fish waste and Goat manure on crude oil polluted soils in Rivers State, Nigeria.
**Study Design:** The study employs experimental design, statistical analysis of the data and interpretation.
**Place and Duration of Study:** A portion of Rivers State University demonstration farmland in Nkpolu-Oroworukwo, Mile 3 Diobu area of Port Harcourt, Rivers State was used for this study. The piece of land is situated at Longitude 4°48'18.50''N and Latitude 6°58'39.12''E measuring 5.4864 m x 5.1816 m with a total area of 28.4283 $m^2$. Bioremediation monitoring lasted for 56 days, analysis carried out weekly (per 7 days interval).
**Methodology:** Seven (7) experimental plots were employed using a Randomized Block Design each having dimensions of 100 x 50 x 20 cm (Length x Breadth x Height) were formed and mapped

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out on agricultural soil and left fallow for 6 days before contamination on the seventh day; after which it was allowed for 21 days for proper contamination and exposure to natural environmental factors to mimic crude oil spill site. Thereafter bio stimulating agents usually referred to as nutrient amendment organics in this study (fish waste and goat manure) and bio-augmenting microorganisms were applied. Soil profile before and after contamination was assayed while parameters like Nitrate, Sulphate, Phosphate, Total Organic Carbon (TOC) and Total Petroleum Hydrocarbon (TPH), were monitored throughout the experimental period. Microbial analyses such as Total Heterotrophic Bacteria (THB), Total Heterotrophic Fungi (THF), Hydrocarbon Utilizing Bacteria (HUB) and Hydrocarbon Utilizing Fungi (HUF) were recorded. Bioremediation efficiency was estimated from percentage (%) reduction of Total Petroleum Hydrocarbon (TPH) from day 1 to the residual hydrocarbon at day 56 of bio augmented/ biostimulation plots with the control.

**Results:** Results revealed amount of remediated hydrocarbon and % Bioremediation efficiency at 56 days in the different treatment plots (initial TPH contamination value of 9296.83 mg/kg) in a decreasing order as follows: PS+Bac+Pse+GF+FW (8032.825 mg/kg; 86.40%) >PS+GF+FW (6867.825 mg/kg; 73.87%) >PS+Bac+Pse (6587.825mg/kg; 70.86%) >PS+FW (6441.825mg/kg; 69.29%) >PS+GF (5909.825 mg/kg; 63.57%) >CTRL 2 (Polluted soil without amendment) (3604.825mg/kg; 38.78%). Microbiological results showed increased colonial values with increase time exposure. The results observed on day 56 indicate that Polluted soil + *Bacillus* + *Pseudomonas* (10.11 Log10 CFU/g) > Polluted soil but un-amended soil (8.76 Log10 CFU/g) > unpolluted soil (8.68 Log10 CFU/g). Comparatively, Polluted soil +*Bacillus* + *Pseudomonas* expressed higher heterotrophic bacteria of 9.77 and 9.67 Log10 CFU/g while fungal counts recorded 6.04 and 6.82 Log10 CFU/g.

**Conclusion:** Study showed that bioremediation of crude oil-polluted soils with bacteria singly is less effective but a combination with other organic nutrients is a better palliative measure. Therefore, amendment with organic nutrients like Goat manure and Fish wastes is recommended for crude oil polluted soils due to its high nutrient content as substrates for biostimulation of indigenous and augmenting biodegrading microbes. This process could be a source of enhanced natural attenuation of oil-contaminated environments in Nigeria.

*Keywords: Bioremediatio; bioaugmentation; biostimulation; goat manure; fish waste; petroleum hydrocarbon; Bacillus amyloliquefaciens; Pseudomonas aeruginosa; crude oil contamination.*

## 1. INTRODUCTION

Crude oil pollution is an environmental problem that has assumed a global dimension particularly in the Niger Delta region of Nigeria. Exploration, production, transportation and spillages of hydrocarbon and its products into the ecosystem resulting from constant accidental release, production failure, pipe ruptures, tanker accidents, sabotages, contributes to adverse impact on the environment. Tons of hydrocarbons are annually released into the environment prompting global concerns. The implication of these impacts on the environment includes groundwater contamination, reduction in the reproduction of plants and animals, de-vegetation, destruction of farmland, low agricultural production, mutation, reduction in the microbial diversity and abundance [1,2,3,4]. Physical and chemical methods used for the remediation of crude oil contaminated soils such as incineration, soil vapour extraction, containment, burial at landfills, evaporation, dispersion and washing are prohibitively expensive, these processes lead to incomplete decomposition of contaminants. Considerations required for bioremediation process include nature of the contaminant, an electron acceptor and the microorganisms. Microorganisms can degrade petroleum hydrocarbons by using them as a source of carbon and energy as a result of their genetic potentials and thereby exhibiting a wide range of degradation capabilities [4,5]. In this process, the hydrocarbon is oxidized by losing electrons while oxygen is reduced by gaining electron resulting in the formation of carbon dioxide, water, biomass and simple compounds which has no adverse impact on the environment [6,7]. This is achieved by the stimulation of natural activities and other environmental modifications using fertilizers or organic nutrients as substrates to increase rates of biodegradation or sometimes by the addition of exogenous microbes to enhance bioremediation. Therefore, bioaugmentation is the process of adding specific exogenous

microorganisms to the soil to enhance the biodegradation of the contaminants [8,9,10]. The success and efficiency of bioaugmentation depends on many factors, including ability of the inoculated microorganisms to survive and grow in the new environment, retention of its degradative potentials, contact and interaction with the contaminant, availability of electron donor/acceptors and sufficient amount of nutrient to remove the target contaminants [7,11]. Also, the survival ability and catabolic activity of exogenous microorganisms, as well as their resistance to other co-contaminants present in the soil and the bioavailability of the contaminants, is considered [8,10,12]. To overcome these factors, Beskoski et al. [12] suggested that the most practical approach is to use microorganisms isolated from the soil to be remediated.

The principles of biodegradation have been applied several times at pilot, field and laboratory scale levels with varying degrees of success [4,13,14,15,16,17,18]. Bacteria and fungi have been harvested and used for bioremediation for over thousands of years. The aim of this study, therefore, is to investigate bioaugmentation potentials of a combination of *Pseudomonas, Bacillus, Aspergillus, and Mucor* on bioremediation of crude oil-contaminated soils.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

A portion of the Rivers State University demonstration farmland in Nkpolu-Oroworukwo, Mile 3 Diobu area of Port Harcourt, Rivers State was used for this study. The piece of land is situated at Longitude 4°48'18.50''N and Latitude 6°58'39.12''E measuring 5.4864 m x 5.1816 m with a total area of 28.4283 $m^2$ was prepared for this study.

### 2.2 Experimental Plots Formation

Using the Randomized Complete Block Design (RCBD) the land was partitioned into seven (7) blocks of 100 cm x 50 cm x 20 cm giving 100,000 $cm^3$ each. Two of these plots were designated as pristine and crude oil polluted soil to serve as controls respectively.

### 2.3 Application of Crude Oil

Each of the experimental plots except the control was contaminated with 1,700 g of crude oil giving initial Total Petroleum Hydrocarbon (TPH) value of 9296.825 mg/kg. The plots were left for 21 days to ensure even distribution and soil-oil bonding.

All plots except Control 1 (plot 1) were separately and deliberately contaminated with 1,700 g of crude oil given Total Petroleum Hydrocarbon (TPH) value of 9296.825 mg/kg. The dimension of each plot had 100 x 50 x 20 (Length x Breadth x Height).

### 2.4 Sampling Methods

From each plot, 4-10 random points from 0-15 cm were bulked to form a composite sample after tilling using soil spatula according to the methods of Nrior and Echezolom [19]. Small portions measuring 5 g of the composite samples were collected into sterile bottles using a sterile spatula for microbiological and physic-chemical analysis. Sampling was done for 56 days after contamination of the various plots (7, 28, 35, 42, 49 and 56 respectively). Soil samples were stored at 14±2°C for further analysis.

### 2.5 Sources of Microbial Isolates

The microorganisms used were fungi specifically *Aspergillus nudilans* and *Mucor racemosus*. These organisms were isolated from the soil samples using Sabouroud Dextrose Agar as selective media for fungi. After which pure cultures obtained were inoculated onto Modified Sabouraud Dextrose broth in 500 ml Erlenmeyer flask loosely plugged with sterile cotton wool for the growth of the augmenting test organisms. Broth cultures with an optical density of 0.2 were used for augmentation.

### 2.6 Application of Bioaugmenting Microbes and Nutrient Amendment for Biostimulation on Experimental Plots

One hundred millilitres (100 ml) of the broth cultured bacterial isolates were added to each setup except the controls. These were properly stirred with a sterile spatula to ensure the microorganisms thrive and have sufficient oxygen. Four (4) litres of water was added to each plot weekly, tilted slightly to enhance moisture content and microbial activity. Illustrative representations of the experimental plots were shown in Table 1.

**Table 1. Illustrative representations of the experimental plots**

| S/N | Experimental plot on soil | Crude oil 1,700 | 300 g goat manure | 200 g dry fish waste | 150 ml Pseudomonas + 150 ml Bacillus broth |
|---|---|---|---|---|---|
| 1 | Control (CTRL)1 | - | - | - | - |
| 2 | CTRL 2 | + | - | - | - |
| 3 | PS + GM | + | + | - | - |
| 4 | PS + FW | + | - | + | - |
| 5 | PS + GM + FW | + | + | + | - |
| 6 | PS + Pse + Bac | + | - | - | + |
| 7 | PS + GM + FW + Pse + Bac | + | + | + | + |

*Key: PS = Polluted soil; Bac = Bacillus armyloliqquefaciens; Pse = Pseudomonas aeruginosa strain CL 9; GM = goat manure; FW = fish waste. NOTE: each experimental plot - length 100 cm x breath 50 cm x height 20 cm*

## 2.7 Total Petroleum Hydrocarbon (TPH) Analysis

Total Petroleum Hydrocarbon (TPH) analyses were carried out on all the seven setups using Gas Chromatography (GC) for Day 7, 14, 21, 28, 35, 42, 49 and 56.

## 2.8 Microbiological Evaluation

### 2.8.1 Isolation and enumeration of total heterotrophic bacteria

Total heterotrophic bacteria for each bioremediation set up were enumerated by spread plate method. 0.1ml aliquot of the $10^{-6}$ was transferred onto well-dried Nutrient agar plates and incubated at 37°C for 24 to 48 h after which the bacterial colonies that grew on the plates were counted and sub-cultured unto fresh Nutrient agar plates using the streaked plate technique. Discrete colonies on the plates were aseptically transferred into agar slants, properly labelled and stored as stock cultures for preservation and identification [20].

### 2.8.2 Isolation and enumeration of total fungal count

The total fungi population in the soil from experimental plots were enumerated and isolated by inoculating 0.1ml aliquot of the mixture onto well-dried Sabouraud Dextrose agar. Pure cultures of the fungi isolates were enumerated and transferred onto Sabouraud Dextrose agar slants as stock cultures for preservation and identification [21].

### 2.8.3 Isolation and enumeration of petroleum utilizing bacteria

Enumeration of Petroleum Utilizing Bacteria was performed by inoculating 0.1 ml aliquot of the dilutions unto Mineral Salt agar plates with 0.1% crude oil [20,21]. Colonies were counted after 48 to 72 h incubation at $37^0$C. The bacterial colonies on the plates after incubation were counted and sub-cultured onto freshly prepared Mineral Salt agar plates.

### 2.8.4 Isolation and enumeration of petroleum utilizing fungi

Vapour transfer phase method was adopted using Mineral Salt agar plates modulated with antibacterial agents (antibiotics: Tetracycline, Penicillin and Ampicillin) to inhibit bacterial growth. The presence or absence of septa in the mycelium, type of spore, presence of primary or secondary sterigmata, and other microscopic characteristics, as well as cultural characteristics, were used in the identification of the fungal isolates [20,22].

### 2.8.5 Preparation of stock culture

Discrete colonies of bacterial and fungal isolates were aseptically transferred onto agar slants and Sabouraud Dextrose agar slants respectively as stock cultures for preservation and identification [20,21]. Furthermore, pure cultures were inoculated into 10% glycerol solution (dispensed in McCartney bottles and autoclaved at 121°C for 15 minutes) to prevent contamination and for longer preservation.

## 2.9 Statistical Analysis

Two way ANOVA test was used to test whether the different nutrient amendments given to the crude oil contaminated plots were statistically significant while the percentage (%) of bioremediation was determined using the method of Nrior and Echezolom [19] based on the formula;

Amount of contaminant remediated (Bc):

$$Bc = Ic - Fc$$

$$\%\ Bioremediation = \frac{\text{Bc}}{\text{Ic}}\ \text{x}\ 100$$

Where:

Bc = Amount of contaminant remediated
Ic = Initial Concentration of contaminant (week 1)
Fc = Final Concentration of contaminant at end of the experiment (week 8)

## 3. RESULTS AND DISCUSSION

The physicochemical and microbiological analyses of the soil with crude oil before and after crude oil contamination are stated in Table 2. The concentration of Total Petroleum Hydrocarbon (TPH) in the experimental soil before application of amendments was 4.89 mg/kg while after crude oil application TPH value was 9296.85mg/kg. This value is above the intervention value of 5000mg/kg according to Department of Petroleum Resources (DPR) standard for crude oil spill value (Above limit of 5000mg/kg, the soil is considered polluted and needs intervention/ remediation) [23]. Nutrient parameters such as nitrate decreased from 801.00 to 686.25mg/kg, sulphate, phosphate, phosphorus and Total Organic Carbon (TOC) increased slightly after crude oil contamination. The pH value decreased from 7.0 to 5.0, Moisture content, Temperature and Electrical Conductivity increased slightly while particle size remains the same. In respect to 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 indicating that the agricultural soil used might have been previously exposed to crude oil contamination. (Table 2).

The analysis carried out to assess bioremediation efficiency of *Bacillus armyloliqquefaciens* (Bac) and *Pseudomonas aeruginosa strain CL 9* (Pse) with nutrient amendment using bio stimulating agent (Fish waste - FW and Goat manure - GM) on crude oil polluted soil were investigated which could serve as treatment options for crude oil-polluted soil in Nigeria, revealed that these organisms helped in bioremediation rate as well as reducing the contaminant 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 organisms were able to degrade the petroleum hydrocarbon in the pollution soil amended with nutrient organics. The Total Petroleum Hydrocarbon (TPH) degradation was determined by the decrease in amount from initial contamination value of 9296.85mg/kg day 7 to PS+Bac+ Pse+GF+FW (1264 mg/kg) >PS+GF+FW (2429 mg/kg) >PS+Bac+Pse (2709 mg/kg) >PS+FW (2855 mg/kg) >PS+GF (3387 mg/kg) >CTRL 2 (Polluted soil without amendment) (5692 mg/kg) on day 56 (Fig. 1).

**Table 2. The physicochemical and microbiological analysis of the soil with crude oil before and after crude oil contamination**

| Parameters | (Units) | Unpolluted soil | Polluted soil |
|---|---|---|---|
| Total petroleum hydrocarbon (TPH) | mg/kg | 4.89 | 9296.85 |
| Nitrate ($NO_3^{2-}$) | mg/kg | 801.00 | 686.25 |
| Sulphate ($SO_4^{2-}$) | mg/kg | 2,376.97 | 3,157.94 |
| Phosphate ($PO_4^{3-}$) | mg/kg | 0.28 | 5.78 |
| Phosphorus (P) | mg/kg | 3.52 | 3.92 |
| Total organic carbon (TOC) | % | 0.21 | 0.93 |
| Electrical conductivity | mg/kg | 100 | 103 |
| pH | None | 7.0 | 5.0 |
| Temperature | °C | 28 | 30 |
| Moisture content | mg/kg | 200 | 206 |
| Particulate size | mg/kg | 642 | 642 |
| Total heterotrophic bacteria (THB) | cfu/g | $6.0 \times 10^7$ | $2.2 \times 10^8$ |
| Total heterotrophic fungi (THF) | cfu/g | $4.0 \times 10^3$ | $7.0 \times 10^3$ |
| Hydrocarbon utilizing bacteria(HUB) | cfu/g | $1.0 \times 10^3$ | $1.5 \times 10^3$ |
| Hydrocarbon utilizing fungi (HUF) | cfu/g | $2.57 \times 10^2$ | $8.13 \times 10^3$ |

Bioremediation evaluation from the initial TPH contamination value of 9296.83 mg/kg revealed the amount of remediated hydrocarbon and % Bioremediation efficiency at 56 days in the different treatment plots in a decreasing order as follows: PS+Bac+Pse+GF+FW (8032.825 mg/kg; 86.40%)>PS+GF+FW (6867.825 mg/kg; 73.87%) >PS+Bac+Pse (6587.825 mg/kg; 70.86%) >PS+FW (6441.825 mg/kg; 69.29%) >PS+GF (5909.825 mg/kg; 63.57%)>CTRL 2 (Polluted soil without amendment) (3604.825 mg/kg; 38.78%). Study showed that bioremediation of crude oil polluted soils with bacteria singly is less effective but a combination with other organicnutrients is a better palliative measure (Figs. 2- 3).

The bacterial and fungal isolates from the experimental soil used in this study were characterized based on their microscopic, biochemical, morphological properties and they belong to the genera: *Pseudomonas, Nocadia, Micrococcus, Flavobacterium* and *Bacillus; Mucor, Aspergillus, Penicillum, Cladosporium,* and *Klebsiella* respectively. This is in line with various researchers who reported similar bacterial and fungal isolates from crude oil contaminated soils [4,16,19,24].

It was observed 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 augmented soil compared to the controls (Figs. 4-5). Also, there was a remarkable increase in the total heterotrophic bacterial count on day 35 compared to day 7 in the nutrient amended crude oil-contaminated soils with Polluted soil+ *Bacillus + Pseudomonas* to give 10.04 to 9.06 Log10 CFU/g; there was however a decrease in the uncontaminated soil with 9.90 to 8.66 Log10 CFU/g; while in contaminated soil but un-amended soil had 9.82 to 7.85 Log10 CFU/g. This, however, decreased on day 42 but subsequently increased on day 56.The results observed on day 56 indicated that Polluted soil + *Bacillus + Pseudomonas* (10.11 Log10 CFU/g) > Polluted soil but un-amended soil (8.76 Log10 CFU/g) > uncontaminated soil (8.68 Log10 CFU/g).Comparatively, contaminated soil +*Bacillus + Pseudomonas* expressed higher heterotrophic bacteria of 9.77 and 9.67 Log10 CFU/g while fungal counts recorded 6.04 and 6.82 Log10 CFU/g (Figs. 4-7).

The Total Heterotrophic Fugal count (Fig. 5) was observed to show a similar pattern as THB on day 7 and day 56 with Polluted soil +*Bacillus* + *Pseudomonas* showing the highest value on day 56. Similar observations were observed in the Hydrocarbon utilizing bacterial and fungal counts in the various treatments plot (Fig.6-7). The result is consistent with the reports of Chikere et al. [16] and Nrior and Mene [4] who observed that Total Heterotrophic Bacterial and Hydrocarbon Utilizing Bacterial counts increased over time in a nutrient amended crude oil contaminated soil undergoing bioremediation with time.

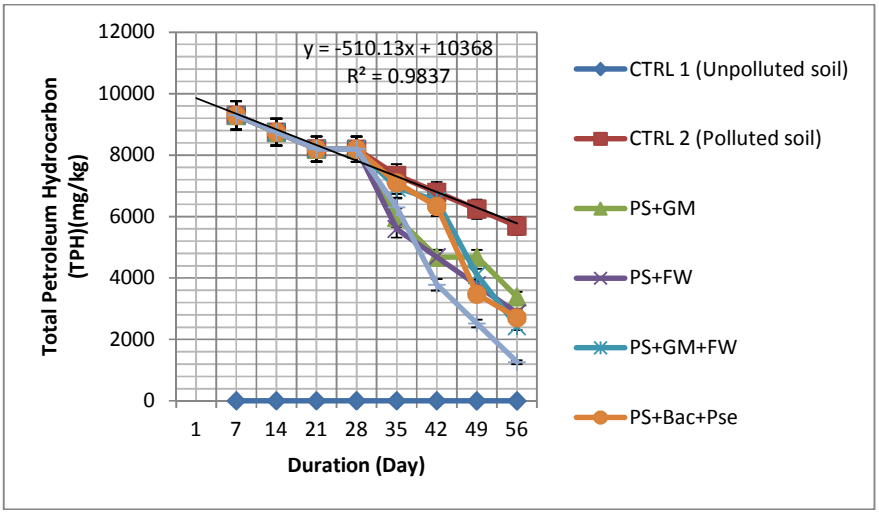


**Fig. 1. Variation in total petroleum hydrocarbon (TPH – mg/kg) during bioremediation crude oil polluted soil using *Bacillus amyloliquefaciens* FJAT-45825 and *Pseudomonas aeruginosa strain CL-9* with nutrient amendment organics goat manure and fish waste**

*(CTRL = Control, PS = Polluted soil with crude oil, GM = goat manure, FW = fish waste, Bac = Bacillus amyloliquefaciens FJAT-45825, Pse = Pseudomonas aeruginosa strain CL-9)*

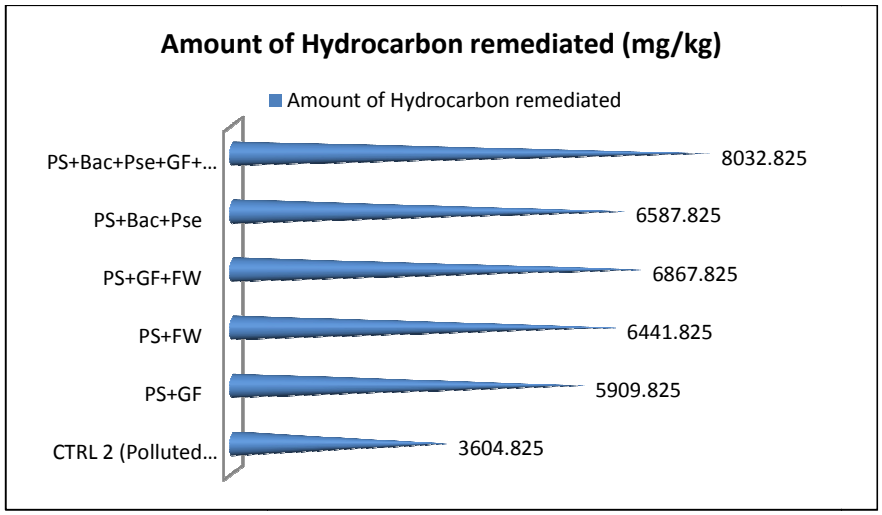


**Fig. 2. Amount of hydrocarbon remediated (mg/kg) during bioremediation crude oil polluted soil using *Bacillus amyloliquefaciens* FJAT-45825 and *Pseudomonas aeruginosa strain CL-9* with nutrient amendment organics goat manure and fish waste**

*(CTRL = Control, PS = Polluted soil with crude oil, GM = Goat manure, FW = Fish waste, Bac = Bacillus amyloliquefaciens FJAT-45825, Pse = Pseudomonas aeruginosa strain CL-9)*

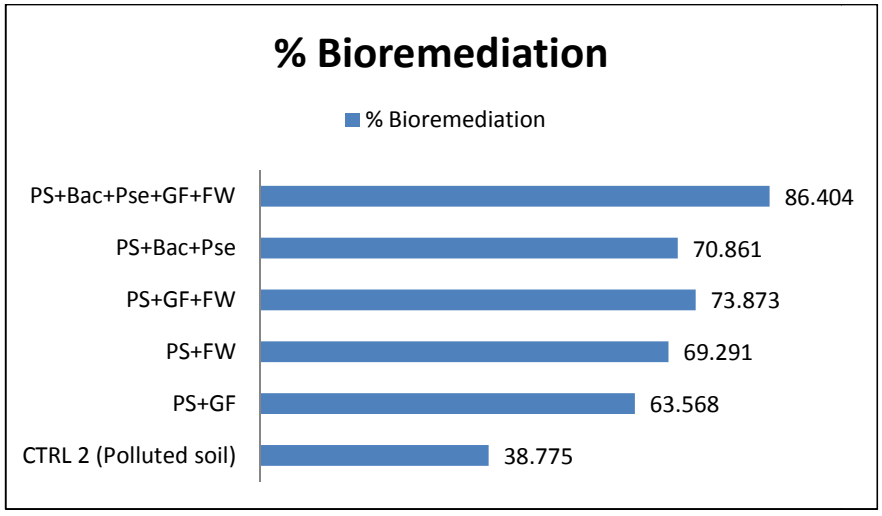


**Fig. 3. Percentage (%) bioremediation efficiency of total petroleum hydrocarbon (TPH – mg/kg) during bioremediation of crude oil-polluted soil using *Bacillus amyloliquefaciens* FJAT-45825 and *Pseudomonas aeruginosa strain CL-9* with nutrient amendment organics goat manure and fish waste**

*(CTRL = Control, PS = Polluted soil with crude oil, GM = Goat manure, FW = Fish waste, Bac = Bacillus amyloliquefaciens FJAT-45825, Pse = Pseudomonas aeruginosa strain CL-9)*

The Total heterotrophic bacterial and fungal counts; Hydrocarbon utilizing bacterial and fungal counts were comparatively observed to decrease with time (days) as shown in (Fig. 6-7). This can be attributed to the abundance of nutrients for the microorganisms to feed on during the first week, but started to deplete with acclimatization and competition for nutrients by the microorganisms. Shang-Hawan et al. [25] and Nrior and Echezolom [19] made similar observations and concluded that the microbial count of crude oil contaminated soils during bioremediation increases within the first 20 days.

Total Organic Carbon (TOC), Nitrate, Sulphate and Phosphate as soil nutrients evaluators were

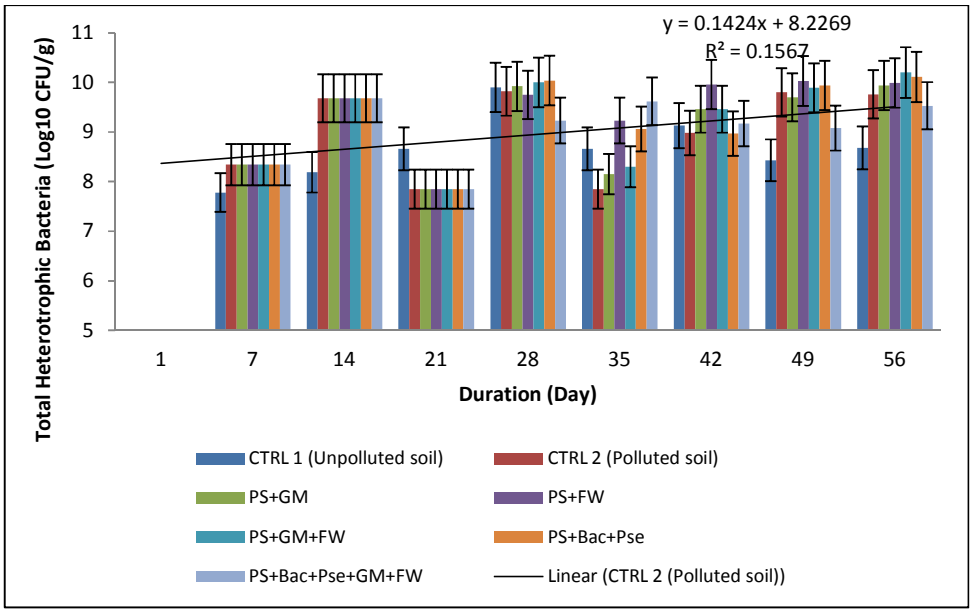


**Fig. 4. Variation in total heterotrophic bacteria (THB) (log10 cfu/g) count during bioremediation of crude oil polluted the soil**

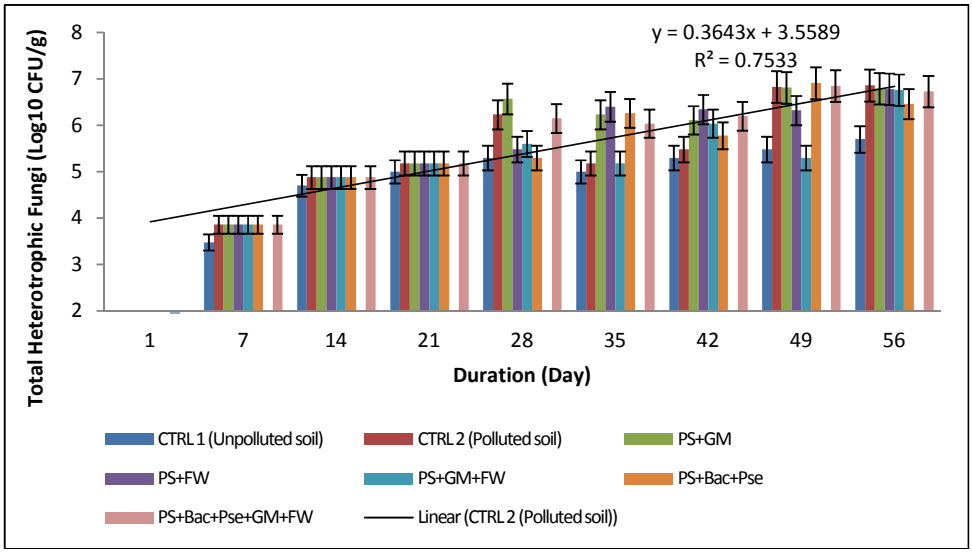


**Fig. 5. Variation in total heterotrophic fungi (THF) (log10 cfu/g) count during bioremediation of crude oil polluted the soil**

analysed throughout the experimental period of 56 days at weekly intervals. Results obtained as shown in Fig. 8-11 revealed a supportive role in nutrient amendment dynamics using organic substrates (goat manure and fish waste) which was particularly evident in soil Nitrate values with increase in time. These suggest the positive impact nutrient amendment with organic substrates had on the augmenting microbes (*Bacillus amyloliquefaciens* FJAT-45825, and *Pseudomonas aeruginosa strain CL-9*) thereby increasing the percentage (%) bioremediation; though fish wastes had a greater impact concerning goat manure or augmenting microbes without organic substrates. Several researchers have extensively examined and discussed the effect of using both organic and inorganic nutrients either singly or in combination for the bioremediation of crude oil-polluted soil such as cow dung [19], poultry droppings [15,18], goat

manure and fertilizer [14]. Goat manure contains a valuable source of nutrient and organic matter which enhances bioremediation [19]. This study observed that fish waste had a greater % bioremediation impact about the former nutrient application, thus could be preferred either singly or in combination with other organic substrates or as augmenting microbes' enhancer.

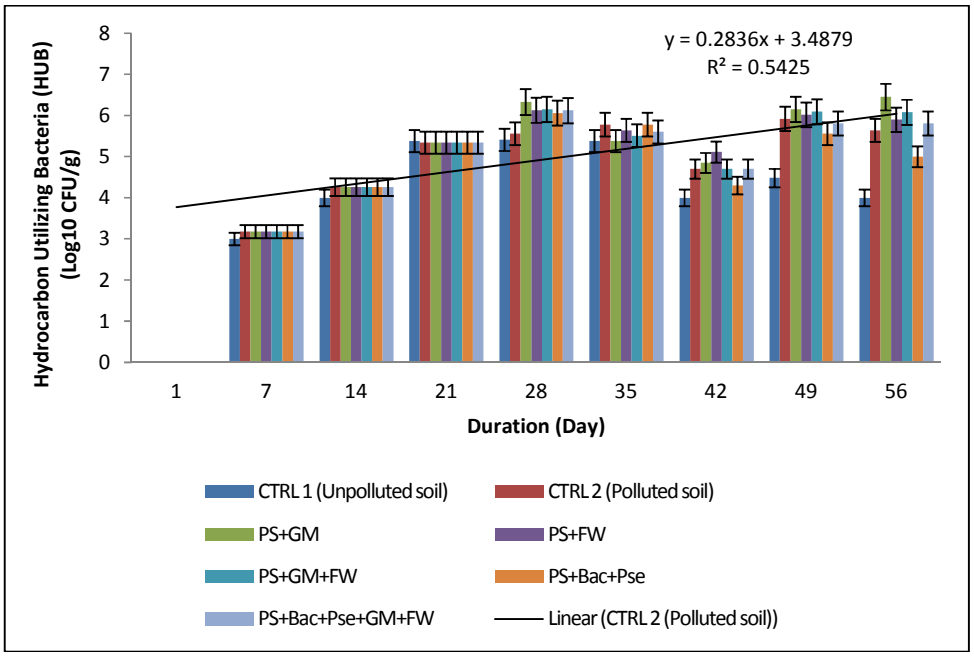


**Fig. 6. Variation in hydrocarbon utilizing bacteria (HUB) (log10 cfu/g) count during bioremediation of crude oil-polluted soil**

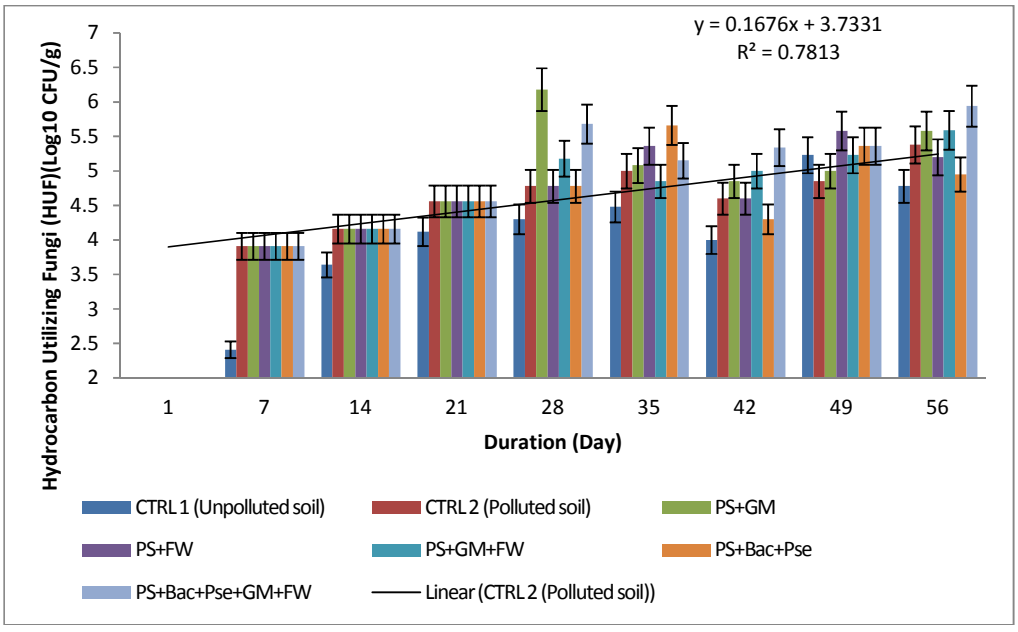


**Fig. 7. Variation in hydrocarbon utilizing bacteria (HUB) (log10 cfu/g) count during bioremediation of crude oil-polluted soil**

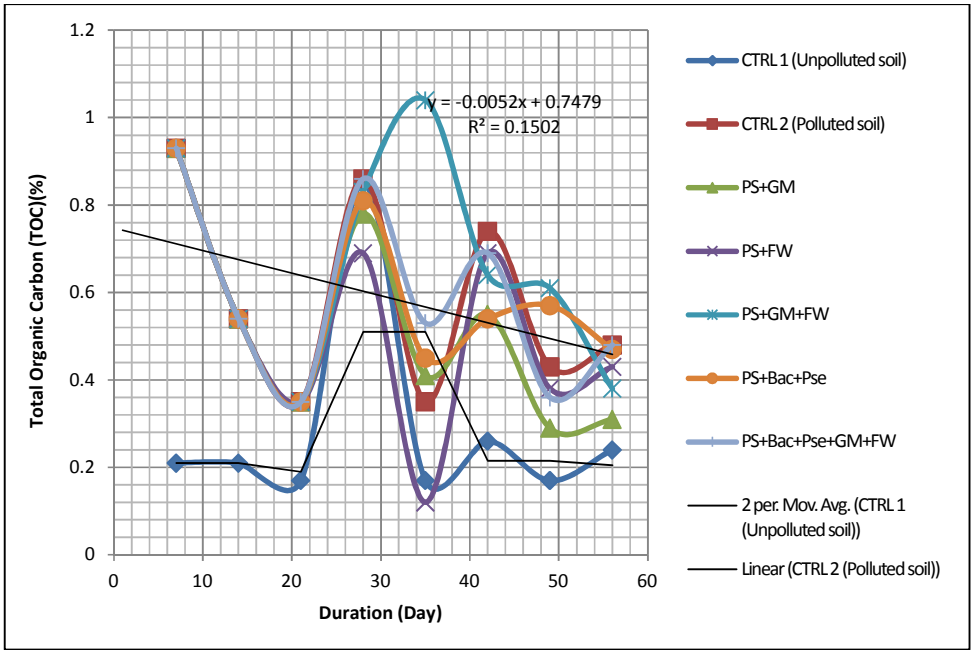


**Fig. 8. Total organic carbon (TOC)(%) during bioremediation crude oil polluted soil using *Bacillus amyloliquefaciens* FJAT-45825 and *Pseudomonas aeruginosa strain CL-9* with nutrient amendment organics goat manure and fish waste**

*(CTRL = Control, PS = Polluted soil with crude oil, GM = Goat manure, FW = Fish waste, Bac = Bacillus amyloliquefaciens FJAT-45825, Pse = Pseudomonas aeruginosa strain CL-9)*

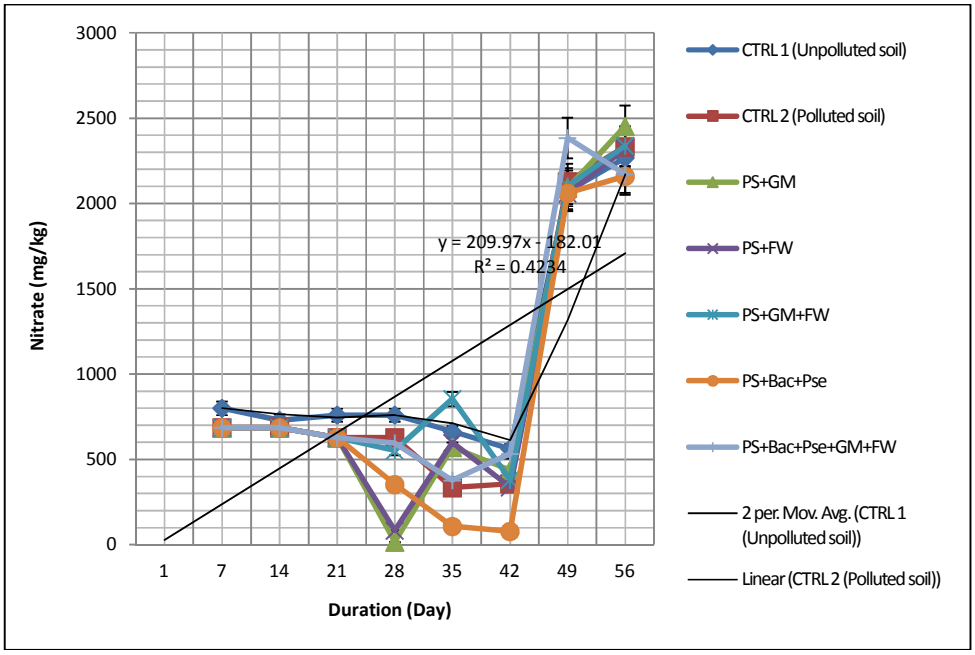


**Fig. 9. Variation in nitrate (mg/kg) during bioremediation crude oil polluted soil using *Bacillus amyloliquefaciens* FJAT-45825 and *Pseudomonas aeruginosa strain CL-9* with nutrient amendment organics goat manure and fish waste**

*(CTRL = Control, PS = Polluted soil with crude oil, GM = Goat manure, FW = Fish Waste, Bac = Bacillus amyloliquefaciens FJAT-45825, Pse = Pseudomonas aeruginosa strain CL-9)*

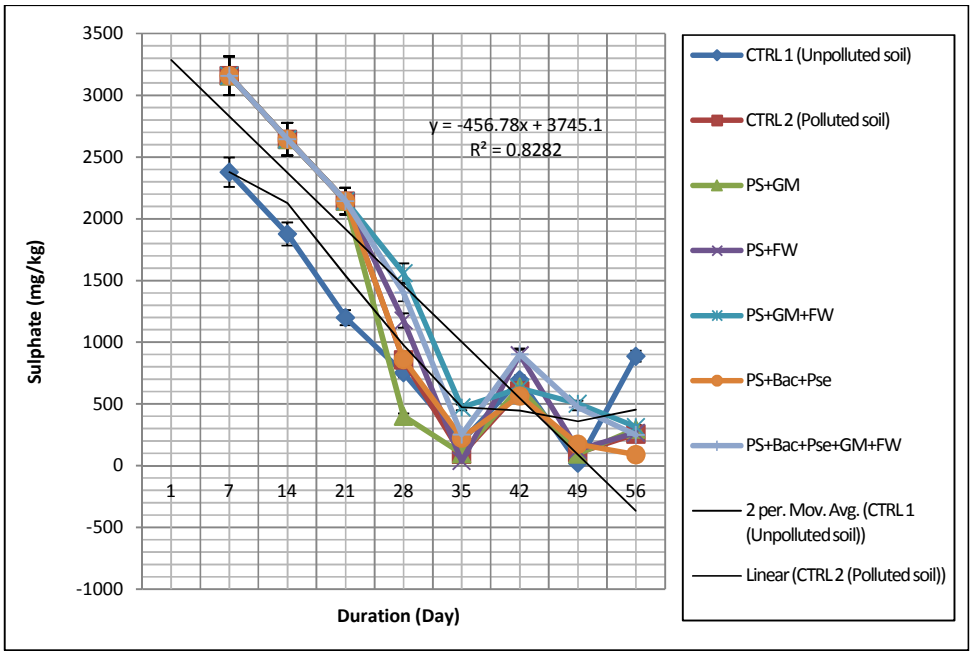


**Fig. 10. Variation in sulphate (mg/kg) during bioremediation crude oil polluted soil using *Bacillus amyloliquefaciens* FJAT-45825 and *Pseudomonas aeruginosa strain CL-9* with nutrient amendment organics goat manure and fish waste**

*(CTRL = Control, PS = Polluted soil with crude oil, GM = Goat manure, FW = Fish waste, Bac = Bacillus amyloliquefaciens FJAT-45825, Pse = Pseudomonas aeruginosa strain CL-9)*

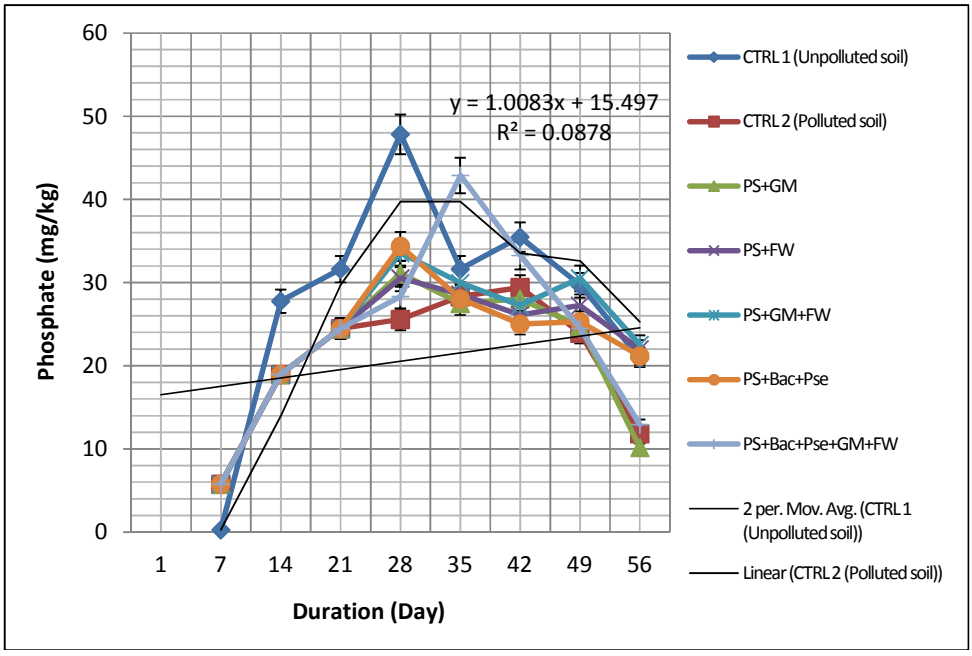


**Fig. 11. Variation in phosphate (mg/kg) during bioremediation crude oil polluted soil using *Bacillus amyloliquefaciens* FJAT-45825 and *Pseudomonas aeruginosa strain CL-9* with nutrient amendment organics goat manure and fish waste**

*(CTRL = Control, PS = Polluted soil with crude oil, GM = Goat manure, FW = Fish waste, Bac = Bacillus amyloliquefaciens FJAT-45825, Pse = Pseudomonas aeruginosa strain CL-9)*

## 4. CONCLUSION AND RECOMMENDATION

The use of bacterial isolates as bio-augmenting agents singly has shown to increase the bioremediation of crude oil-contaminated soil. However, a combination strategy of bacterial treatment with nutrient amendment organics in the bioremediation process produced more effective and faster bioremediation, achieving a greater reduction in petroleum hydrocarbon. It was further observed that microbial counts decreased with time in treatments with augmenting organisms alone but increased considerably in treatments supplement with organics.

It is therefore recommended that bioremediation of crude oil-polluted soil using bio-augmenting microorganism should always be supplemented with efficient nutrient organics such as fish waste or in combination with goat manure.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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