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Use of fish pond waste water in bioremediation of degreaser contaminated open farmland

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Abstract
Environmental pollution in the Niger Delta has been of huge concern, and bacteria have proven to be of great benefit in the degradation of petroleum hydrocarbons. 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. Bioremediation monitoring was for a period of 56 days, analysis was carried out at an interval of 14 days: Nine (6) experimental plots were employed using a Randomized Block Design each having dimensions of 100 x 50 x 20 cm (Length x Breadth x Height) which were formed and mapped out on the 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 a degreaser contaminated site. Thereafter, the bio-stimulating agent (Fish pond effluent) were applied. Soil microbiological (THB, F, DUB, and DUF) and physicochemical characteristics before and after contamination (Temperature, pH, Nitrogen, potassium, phosphorus and Total hydrocarbon content) were monitored. The baseline microbiological analyses revealed that Total Heterotrophic Bacterial counts (THBC) ranged from 5.4±0.57 to 6.3±1.06 CFU/g, Degreaser Utilizing Bacteria (DUB) 2.1±0.07 to 4.1±0.64 CFU/g, Fungal counts (FC) 1.6±0.85 to 3.33±0.64 CFU/g, and Degreaser Utilizing Fungi (DUF), 1.6±0.21 to 2.5±0.35SFU/g. The results for the physicochemical parameters monitored in the duration of 56 days showed that mean pH value ranged between 5.0 to 7.38. Temperature 27.5 to 29.2ºC, Nitrogen concentration 43.0 to 9.9 mg/kg, Phosphorus 0.05 to 0.58 mg/kg, Potassium 0.07 to 3.4 mg/kg and the Total Hydrocarbon Content 253 to 3983mg/kg. Bioremediation efficiency was estimated from percentage (%) reduction of Total Hydrocarbon Content (THC) from day 1 to the residual hydrocarbon at day 56 of bio-augmented/ bio-stimulation plots with the control. Results revealed amount of remediated hydrocarbon and % Bioremediation efficiency at 56 days in the different treatment plots (Initial THC contamination value of 3984 mg/kg) in a decreasing order as follows: CS+FPE (76.6%) > CS (71.5%) > CTRL 1 –US (66.4%). The study showed that bioremediation of degreaser polluted soils with combination of organic nutrients is a better degradative option. Therefore, amendment with organic nutrients like fish pond effluent is recommended for degreaser contaminated soils due to their high nutrient content as substrates for biostimulation of indigenous and augmenting biodegrading microbes.

Keywords: Bioremediation, degreaser, bio-augmenting, bio-stimulating agents

1. Introduction
The environment is in danger of ecological damage due to the current rapid population growth, urbanization, and industrialization (Cherniwchan, 2012) [5]. Stated differently, the environment is under threat because of the ongoing damage caused by human activities (Goudie, 2013) [8]. There is a significant concern about the widespread pollution and contamination of natural elements, including aquatic and terrestrial ecosystems, caused by industrial production, excessive petroleum and its derivative use, polyethene, pesticides, and organic herbicides, which are primarily used to prevent fungal, insect, and weed attacks (Mrozik *et al.*, 2010; Tyagi *et al.*, 2010; Federici *et al.*, 2012; Schultz-Jansen *et al.*, 2016) [12, 22, 6, 18]. Gas stations, oil refineries, petrochemical and pharmaceutical companies, seeping water from agricultural lands treated with pesticides and herbicides, and gas stations are the main sources of these pollutants in the soil and water resources. Long-term persistence in the environment is a common characteristic of most of these pollutants (Srirangan *et al.*, 2012) [20].

The most dangerous category when it comes to toxic pollutants is the toxic and hazardous chemicals that are yearly released by industries. Industrial facilities all over the world release 310 kg of toxic chemicals into the air, land, and water every second.

This is equivalent to about 10 million tonnes annually (Tiefenbacher & Hagelman, 2013) [21]. Lubricant oil, which is often used to run various engines and machinery, is the next most dangerous material. These oils are hazardous, a build-up from the auto-mechanic workshops could cause major environmental issues in the future (Abdulsalam & Omale, 2009) [1]. Nrior and Odokuma (2015) [14] states that degreasers are chemical compounds that are used to remove water-insoluble materials from vehicle engine parts, including grease, oil, and paint.

According to Kumar *et al.* (2011) [11] and Sharma (2012) [19], bioremediation is a naturally occurring process that uses microorganisms, plants, and/or their derivatives (Enzymes or spent biomass) to break down or modify environmental contaminants while these organisms go about their daily lives. Compared to physicochemical methods, bioremediation is thought to be a more affordable, adaptable, effective, and environmentally friendly method of handling environmental pollutants (Kumar *et al.*, 2011; Sharma 2012; Jeon & Madsen, 2013) [11, 19, 10]. This can be done by excavating soils and sediments, transporting the contaminated media, and possibly containing or handling it under controlled conditions (Quintero *et al*., 2007; USEPA, 2003; Pino-Herrera *et al.*, 2017; Azubuike *et al.*, 2016) [17, 23, 16, 3]. The possibility exists that the contamination may spread to other environments. Azubuike *et al*. (2016) [3], additional pretreatment of contaminated media, such as crushing and drying, may be necessary, increasing the cost of the process.

2. Materials and Methods

2.1 Study Area and Sample Collection

The area used for this study is within the Rivers State University Demonstration farmland in Nkpolu-Oroworukwo, Mile 3 Diobu area of Port Harcourt, Rivers State. The piece of land is situated at Longitude $4^0$48'18.50''N and Latitude $6^0$58'39.12''E measuring 5.486m x 5.1816m with a total area of 28.4283m^2. At the Rivers State University Demonstration Farm in Port Harcourt, Rivers State, soil samples were randomly taken using a hand-held boring metal soil auger at a depth range of 0 to 15 cm.

Source of fish pond waste water

Fish Pond Waste Water used as organic nutrients for the experiment were obtained from a fish farm in Omoku, Rivers State. The waste water stayed for 7 to 10 days befor being changed in order to enrich the nutrient of the waste water

2.5 Bioremediation set-up

The soil was treated for bioremediation as described by (Williams & Inweregbu, 2019) [24]. In this method, 3 setups in triplicates each were made. Each basin contained;

1. 2500 g of soil which served as control 1 (Triplicates)
2. 2500 g of the soil + 250 ml of Degreaser which served as control 2 (Triplicates)
3. 2500 g of the soil + 250 ml of Degreaser + 75 ml of fish pond effluent (Triplicates).

2.7 Monitoring of the bioremediation potential

This bioremediation set up was monitored for selected microbiological and physicochemical parameters from day 1 to 56 days. The parameters include Total Heterotrophic Bacteria, Fungi, Degreaser Utilizing Bacteria, DUF, total hydrocarbon content (THC), nitrogen, potassium, phosphorus, temperature and pH at 14 days' interval. Eighty milliliter (80 ml) of sterilized water was added to the set up three times weekly and agitated for proper aeration and adequate distribution of microorganisms.

2.8 Physiochemical parameters

2.8.1 Determination of total hydrocarbon content (THC) in soil

A clean beaker was filled with five grams (5 g) of the soil sample, and 10 ml of xylene was added. The beaker was covered with a cork for thirty minutes. An aliquot of the extract was put into the analyzer of an infrared spectrophotometer. By comparing the THC value to a calibrated curve made from dilution of a stock solution of 1: 1 Bony light Degreaser. Using an ultraviolet light spectrophotometer set at 450 nm, the absorbance was determined.

The amount of pollutant remediated and the % remediation in the experiment were determined using the approach of Nrior and Mene (2017) [13].

For pollutant remediated

Pollutant remediated amounts are equal to Initial Pollutant Concentration (Day 1) minus Final Pollutant Concentration (Day 56).

$$Ba = Ic - Fc$$

Where:
Ba= Amount of pollutant remediated
Ic = Initial Concentration of pollutant (day1)
Fc = Final Concentration of pollutant in plot x (day56)

For percentage remediation

The percentage (%) remediation equals Amount of pollutant remediated divided by the Initial Concentration of pollutant (Day 1), multiplied by 100

$$\% \text{ remediation} = (Bc/Ic) \times 100$$

2.8.2 Determination of pH

A digital pH meter was used to measure the pH of the sediment sample, and it was set to stable for 15 minutes. A contaminated soil sample weighing forty grams (40g) was obtained following calibration and dried in an oven before being weighed in duplicates. After adding 40 milliliters of distilled water to the soil and letting it settle for 15 minutes, slurry was created. To calibrate the pH meter to pH 7, a buffer was utilized. A 20 ml water sample was placed in a beaker, and the pH meter was submerged in it for five minutes. pH was reported as the reading's outcome after the mean of the three values obtained was applied.

2.8.3 Determination of temperature

A calibrated thermometer was used to measure the temperature of the soil. Prior to examination, the readings of established standards were used to calibrate the conducting cells. At every stage of analysis, the probe cells were thoroughly cleaned with distilled water before the experiment's control was conducted. Following the lowering of the conducting cells into the analytes, a simultaneous measurement of the temperature in degrees Celsius was made.

2.8.4 Determination of moisture content
To prevent evaporation, 200g of the contaminated soil sample was taken out and put in a funnel that was lined with filter paper and covered with foil. Overnight, it was left to stand. After overnight (M1), an aliquot was weighed out and baked for 24 hours at 106 °C in a Gallen Kamp BS, 250, England, oven. The new weight (M2) was measured after cooling (APHA, 1998).

2.8.5 Determination of nitrogen
According to (APHA, 1998), the semi-micro Kjeldahl technique was applied. Zero point one grams (0.1g) of the sample and one tablet of selenium catalyst were added. The mixture was slightly moistened with distilled water. Five milliliters of concentrated H_2SO_4 were added, and the digesting block was covered. Over a fume cupboard, the sample was heated until it was digested. The digest for semi-micro distillation was prepared in a 50 ml volumetric flask. The MARKHAM distillation device was turned on, and ten milliliters of the digest were added to the distillation chamber. Distilling the material into 10ml of 4% boric acid required a gentle addition of 10ml of 45% NaOH. About 50ml distillate was collected and titrated with 0.02N H_2SO_4 to get back a pinkish-red end point.

2.8.6 Phosphorous
Phosphate was measured using Standard Method 4500-P B.5 and 4500-PE as described by APHA (1998).

Procedure: One hundred milliliters of the filtered and homogenized sample were pipetted into a conical flask. In addition, a separate conical flask was pipetted with the same volume of distilled water (which served as the control). One milliliter of 18M H2S04 and 0.89 grams of ammonium persulphate were added to each conical flask, and they were then gently heated for one and a half hours while keeping a volume of 25 to 50 cm3 with distilled water. It had finally cooled. After adding one drop of the phenolphthalein indicator, it was neutralized with the 2M NaOH solution until it took on a light pink hue. The pink hue vanished when 2M HCl was added drop by drop to a solution composed of 100 ml of distilled water. Twenty milliliters of the sample and ten milliliters of the mixed reagent were pipetted into test tubes for the colorimetric examination. Using 20 ml of pure water and 1 ml of the reagent as a reference, the absorbance at 690 nm was measured in a spectrophotometer after the tubes had been shaken for ten minutes.

2.8.7 Determination of Potassium
Digested samples from the Kjeldah assay were produced up to 50ml using distilled water. To calibrate the device and produce a graph of the standard ion concentration, a standard potassium ion concentration was inhaled into the burner chamber of the spectrometer. The wavelength that was used was 760 nm. Prior to aspirating the sample, water was pumped through the spectrometer's aspirator tube system. The potassium ion concentration in the sample was displayed right away on the spectrophotometer's screen.

2.9 Microbiological parameters
2.9.1 Total Heterotrophic Bacteria (THB)
Total heterotrophic bacteria were enumerated using spread plate method. An aliquot (0.1ml) from 10^{-5} dilution (Dilution (10^{-5}) was used as appropriate after dilution range finding test) from each of the set-ups were aseptically transferred unto properly dried nutrient agar plates in duplicate, spread evenly using flamed bent glass rod and incubate at 37 °C for 24 hours as described by Prescott *et al.* (2005). After incubation, the bacterial colonies that grew on the plates were counted and average taken. Total Heterotrophic Bacteria (THB) Counts was then taken and expressed as colony forming unit per millilitter using the equation below as adopted by (Williams and Ogolo, 2018) [26]. Dilution (10^{-4}) was used as appropriate after dilution range finding test.

$$\text{THB (cfu/ml)} = \frac{\text{Number of Colonies}}{\text{Dilution } (10^{-4}) \text{ x Volume plated (0.1 ml)}}$$

2.9.2 Fungi (F)
The total heterotrophic fungi in each of the setups were enumerated using spread plate method. An aliquot (0.1 ml) of the dilution of 10^{-2} dilution was aseptically transferred unto properly dried Sabouraud Dextrose Agar plates containing antibiotic (250 Tetracycline) to inhibit bacterial growth, in duplicate, spread evenly using bent glass rod and incubate at 35 °C for 3 days (This incubator temperature when using Sabouraud Dextrose Agar gives optimal clear growth in 3 days but ambient temperature of 28±0.2 °C in South South Nigeria stays for 5 days for optimal growth). Williams (2018) [27] fungal colonies that grew on the plate were counted and expressed as colony forming unit per milliliter using the below equation: Dilution (10^{-2}) was used as appropriate after dilution range finding test (Williams and Ogolo, 2018) [26].

$$\text{F (cfu/ml)} = \frac{\text{Number of colony}}{\text{Dilution } (10^{-2}) \text{ x Volume plated (0.1 ml)}}$$

2.9.3 Hydrocarbon utilizing bacteria and fungi (DUB and DUF)
An aliquot of 0.1 ml from 10^{-2} dilution of the respective set-ups were inoculated into Mineral salt agar which was formulated as adopted by Williams and Madise (2018) [28] for isolation of both hydrocarbon utilizing bacteria and fungi, in duplicate using spread plate techniques. Sterile filter papers placed in the cover of the Petri dishes were saturated with 1 ml of Degreaser. The plates were then incubated inverted at 28 °C for 5-7 days. The filter paper saturated with Degreaser served as a sole source of carbon Nrior and Odokuma (2015) [14]. Colonies formed in the respective plates were counted and the mean values were recorded and expressed as cfu/ml. The mineral salt agar used for enumeration of hydrocarbon utilizing bacteria was amended with fungizone lotion while for hydrocarbon utilizing fungi the medium was amended with 250 µg of tetracycline to inhibit the growth of hydrocarbon utilizing bacteria Odokuma and Nrior (2015) [14].

3. Results
The baseline physicochemical and microbiological properties of the soil before the application of various bioremediation treatments are shown in Table 1. The parameters determined were pH, temperature, potassium, phosphorus, nitrogen, and total hydrocarbon content (THC). The microbial properties determined were Total Heterotrophic Bacteria (THB), Hydrocarbon Utilizing

Bacteria (DUB) and Degreaser Utilizing Fungi (DUF). The baseline results revealed that the pH value of 5.8 for uncontaminated soil and 7.2 for contaminated soil, temperature was observed to be 27.8 °C for uncontaminated while the contaminated was 28.5 °C, Nitrogen was 13.3 mg/kg for uncontaminated whereas the contaminated soil was 9.6 mg/kg, potassium was 3.41 mg/kg and 2.38, phosphorus was 0.65 mg/kg and 0.46 mg/kg for uncontaminated and contaminated respectively and THC for uncontaminated was 754 mg/kg and contaminated value of 3984 mg/kg respectively.

The baseline analysis for microbiological parameters for THB, DUB, F, DUF for uncontaminated and contaminated showed the average counts of 6.3±1.06 cfu/g and 5.4±0.57 cfu/g for THB, 3.33±0.64 cfu/g and 1.6±0.85 cfu/g for F, 4.1±0.64 cfu/g and 2.1±0.07 cfu/g for DUB, 2.5±0.35 cfu/g and 1.6±0.21 cfu/g for DUF respectively.

Table 1: Baseline physicochemical parameters of experimental soil

Parameters	Uncontaminated Soil	Contaminated Soil
Temperature (°C)	27.8	28.5
pH	5.8	7.2
Nitrogen (mg/kg)	13.3	9.6
Phosphorus (mg/kg)	0.65	0.46
Potassium (mg/kg)	3.41	2.38
Total Hydrocarbon Content (THC) (mg/kg)	754	3984

Table 2: Mean Changes deviation showing physicochemical parameters during Bioremediation of Degreaser Contaminated Soil

Incubatin (Day)	Temperature (^{0}C)			pH			Nitrogen (Mg/Kg)			Phosphorus (Mg/Kg)			Potassium (Mg/Kg)			THC (Mg/Kg)		
Treatments	US	CS	CS+FPE	US	CS	CS+FPE	US	CS	CS+FPE	US	CS	CS+FPE	US	CS	CS+FPE	US	CS	CS+FPE
Day 1	27.8± 0.03^{ab}	27.5± 0.00^{a}	27.8± 0.00^{ab}	6.7± 0.01^{a}	5.0± 0.00^{a}	5.2± 0.01^{a}	9.1± 0.00^{a}	9.1± 0.00^{a}	9.1± 0.00^{a}	0.54± 0.00^{a}	0.58± 0.00^{b}	0.58± 0.00^{b}	3.4± 0.00^{a}	3.4± 0.00^{a}	3.4± 0.00^{a}	754± 0.07^{a}	3984± 0.07^{b}	3983± 0.21^{b}
Day 14	29.2± 0.07^{c}	29.2± 0.07^{c}	28.7± 0.01^{b}	7.26± 0.01^{g}	7.08± 0.01^{d}	7.06± 0.01^{c}	8.8± 0.00^{h}	9.9± 0.00^{i}	5.1± 0.00^{a}	0.21± 0.00^{c}	0.24± 0.01^{e}	0.25± 0.00^{f}	2.8± 0.00^{e}	2.5± 0.07^{c}	2.7± 0.01^{de}	347.50 ±0.07^{a}	3460± 0.07^{d}	3460± 0.07^{d}
Day 28	28.4± 0.07^{b}	28.5± 0.07^{bc}	28.4± 0.7^{b}	6.43± 0.01^{a}	6.76± 0.0^{b}	7.2± 0.14^{f}	8.6± 0.00^{h}	9.87± 0.00	5.3± 0.00^{a}	0.19± 0.00^{c}	0.23± 0.00^{e}	0.23± 0.01^{e}	2.4± 0.00^{c}	2.6± 0.00^{e}	2.5± 0.01^{d}	287± 0.07^{a}	2924± 0.07^{f}	2974± 0.07^{h}
Day 42	26.5± 0.07^{a}	26.6± 0.07^{b}	26.7± 0.7^{bcd}	5.58± 0.01^{a}	5.97± 0.01^{c}	6.46± 0.01^{d}	7.9± 0.00^{h}	8.3± 0.00^{i}	4.5± 0.00^{a}	0.07± 0.00^{a}	0.08± 0.01^{a}	0.09± 0.00^{ab}	2.3± 0.00^{f}	2.6± 0.00^{g}	1.8± 0.00^{e}	272± 0.07^{a}	2800± 0.07^{i}	2744± 0.07^{h}
Day 56	2.5± 0.07^{a}	26± 0.07^{a}	26± 0.7^{a}	5.0± 0.01	5.88± 0.01^{b}	6.94± 0.01^{g}	7.47± 0.00^{g}	8.07± 0.00^{h}	3.60± 0.00^{b}	0.06± 0.00^{b}	0.08± 0.01^{cd}	0.08± 0.00^{de}	1.4± 0.00^{f}	1.6± 0.00^{g}	1.7± 0.00^{h}	253± 0.28^{a}	1134± 0.07^{g}	934± 0.07^{b}

*Means with similar superscripts down the group showed no significant difference (P>0.05)

KEY: US – Unpolluted Soil, CS-Contaminated soil, FPE – Fish Pond Effluent

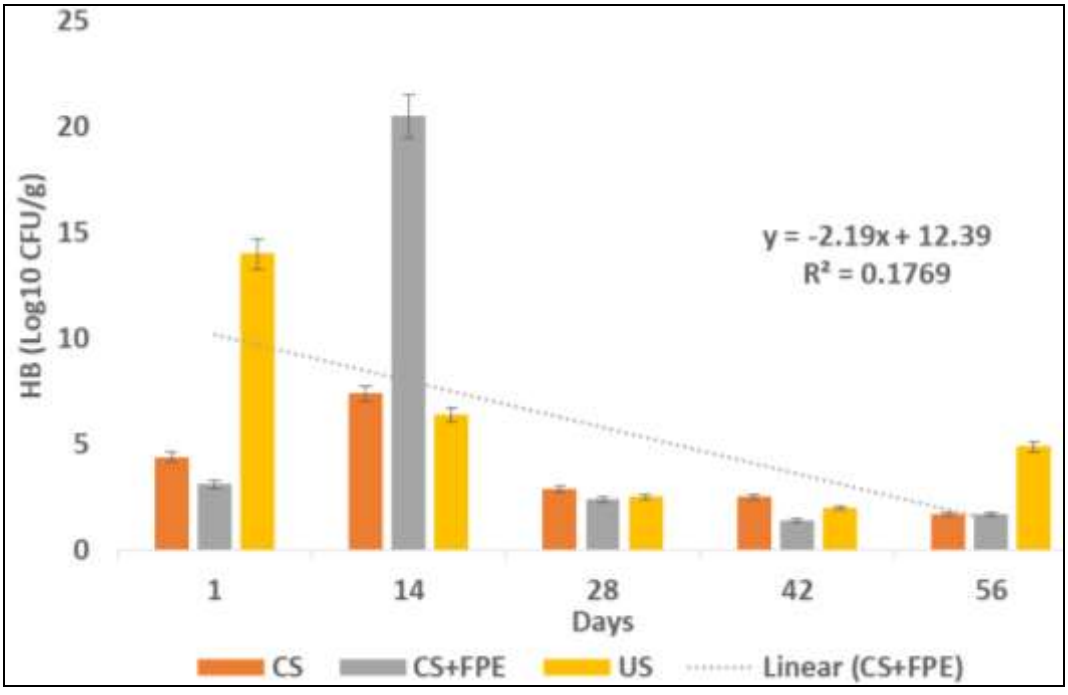

Fig 1: Changes in Total heterotrophic Bacteria (THB) During Bioremediation of Degreaser Contaminated Soil

KEY: US – Unpolluted Soil, CS-Contaminated soil, FPE – Fish Pond Effluent

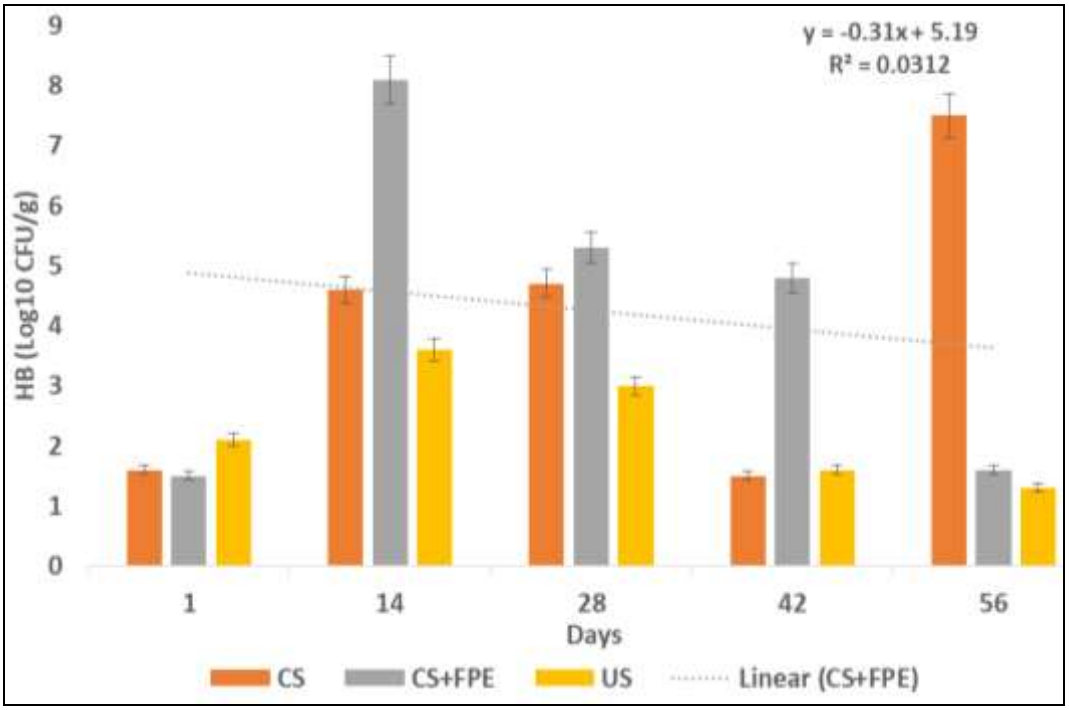

Fig 2: Changes in Degreaser utilizing Bacteria (DUB) (CFU/g) During Bioremediation of Degreaser Contaminated Soil

KEY: US – Unpolluted Soil, CS-Contaminated soil, FPE – Fish Pond Effluent

Table 9: Changes in Total Hydrocarbon Content (THC) (Mg/Kg) In Soil during Bioremediation Of degreaser Contaminated Soil Augmented with Microbes

Set-up code	Day 1	Day 14	Day 28	Day 42	Day 56	Amount Remediated	% Remediated
US	754	347.50	287	272	253	501	66.4
CS	3984	3460	2924	2800	1134	2850	71.5
CS+FPE	3984	3460	2974	2744	934	3050	76.6

KEY: US – Unpolluted Soil, CS-Contaminated soil, FPE – Fish Pond Effluent

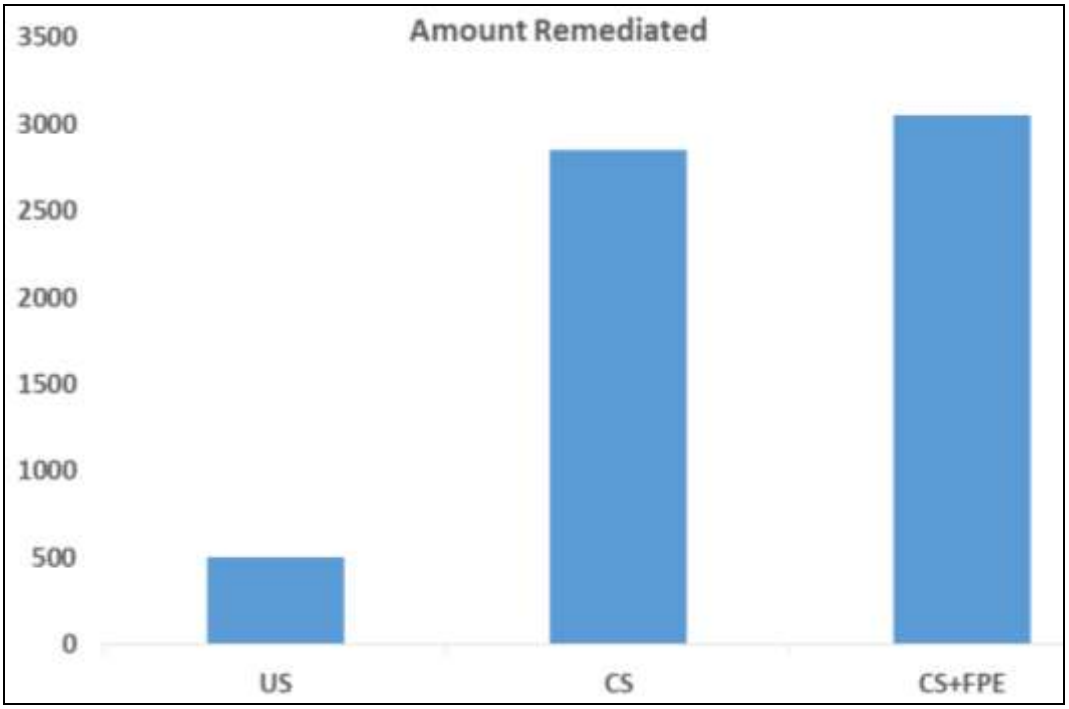

Fig 3: Amount of THC (mg/kg) Removed during bioremediation of degreaser polluted soil

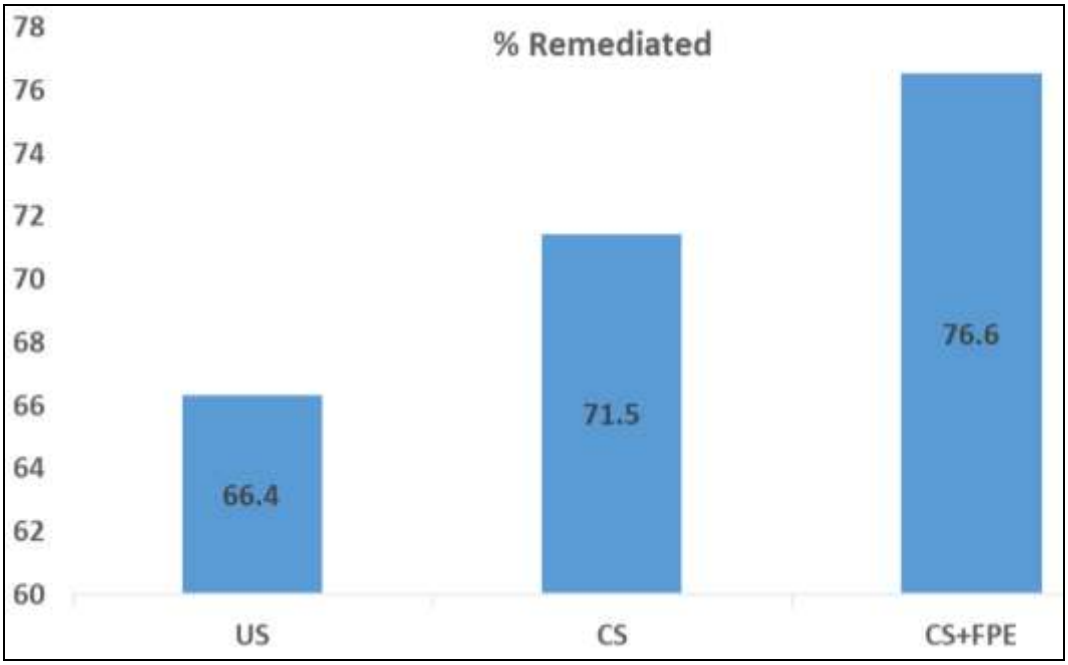

Fig 4: Percentage THC (mg/kg) Remediated during bioremediation of degreaser polluted soil

4. Discussion

The results of the microbial counts obtained during bioremediation of degreasers are total heterotrophic bacterial (THB) count sampled, are presented in Figure 1, and The Degreaser utilizing bacterial (DUB) count sampled are presented in Figure 2.

The Total heterotrophic bacterial (THB) revealed counts from the lowest to highest during monitoring for each set up and day to be: CS + FPE (3.1), day1 day1 US (14.0), Figure 1.

Degreaser utilizing bacterial (DUB) counts revealed were as follows: day14 US (3.6), day14 CS+FPE (8.1), Figure 2.

The mean changes of the physiochemical parameters of the polluted soil with the different treatment during the bioremediation study are shown in Tables 2 - 7. The parameters determined include; pH, temperature, nitrogen, potassium, phosphorous and total hydrocarbon content (THC).

pH values obtained from the various treatments during bioremediation are shown in Table 2. The highest values for eat treatment during monitoring were as follows: day14 US (7.26), day14 CS (7.08), and day28 CS+FPE (7.2).

Temperature values obtained during the study were relatively same between treatments. The highest values for eat treatment during monitoring were as follows: day14 US (29.2), day14 CS (29.2), day14 CS+FPE (28.7). Table 3.

Nitrogen concentration during monitoring of the polluted soil is shown in Table 4. day1 US (9.1), day14 CS (9.9), day28 CS+FPE (5.3).

Potassium concentration during monitoring of the polluted soil is shown in Table 5. day56 US (1.4), day14 CS (2.5), day14 CS+FPE (2.7).

Phosphorous concentration during monitoring of the polluted soil is shown in Table 6. day1 US (0.54), day14 CS (0.24), day14 CS+FPE (0.25).

Total hydrocarbon content (THC) during monitoring of the polluted soil is shown in Table 7. The amount of degreaser remediated and the percentage remediated after 56days of monitoring the various treatment are given in their increasing order as; US (501mg/kg; 66.4), CS (2850 mg/kg; 71.5%), CS+FPE (3050 mg/kg; 76.6%).

Results of the microbial population of the degreaser uncontaminated and contaminated soil showed that total heterotrophic bacterial count had the highest count when compared to the rest of the parameters. This was followed by the degreaser utilizing bacterial counts. This agreed with the study of Williams and Hakam (2016) who recorded an increase in the THB counts. The increase in the counts may be as a result of the abundance nature of bacteria in soil and their ability to utilize hydrocarbon substances as carbon source.

The main difference of degreaser biodegradation between the soil amended with fish pond waste and unamended soil treatment occurred during the 14-28 days, where biostimulation resulted in significant increase of degreaser biodegradation. The addition of nutrients stimulates the degradative capabilities of the indigenous microorganisms thus allowing the microorganisms to break down the organic pollutants at a faster rate.

The samples amended with Fish pond effluent degraded the degreaser contaminated soil more than the unamended. This may possibly be due to a higher nutrient level present in Fish pond effluent. Research has shown that organic waste harbors hydrocarbon utilizing bacteria (Agarry *et al*, 2012) [2]. The biodegradation recorded in the unamended soil sample could be due to non-biological factors such as evaporation, photo-degradation volatilization, adsorption, abiotic factors (Temperature and pH) (Onuoha 2013) [13]. Reduction of petroleum hydrocarbon in unamended sample has also been reported by previous studies (Idowu and Ijah, 2017) [9].

The results of the total hydrocarbon content (THC-mg/kg) of the bioremediation set up suggested that there was a reduction in the on the 56th day of monitoring

Conclusion

The study showed that bioremediation of degreaser polluted soils with combination with organic nutrients is a better degradative option. Therefore, amendment with organic nutrients like fish pond effluent is recommended for

degreaser polluted soils due to their high nutrient content as substrates for biostimulation of indigenous and augmenting biodegrading microbes. Natural bioremediation of degreaser pollutants by activation of naturally occurring microorganisms will be cost effective in cleaning up the environment to save the environment from hazardous effects of hydrocarbon on our agricultural ecosystem.

Bioremediation could be a feasible and efficient response to soil contamination with petroleum hydrocarbons. The selection of proper microbial strains is the key step to a successful bio-augmentation. For a pollutant to be eliminated, it is very important to select microbial inoculant isolated from contaminated sites.

From the study, it was observed that fish pond effluent have high nitrogen and phosphorus content which are known as most important nutrients needed by degreaser utilizing bacteria to carry out effective and efficient activities of biodegradation of xenobiotics in the soil environment.

5. Conflict of Interest

Not available

6. Financial Support

Not available

7. References

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