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Bioremediation of Diesel-Contaminated Soil Enhanced By Banana Peels.

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Abstract
This research was carried out to evaluate the effectiveness of banana peels on bioremediation of diesel-contaminated soil. Total Petroleum Hydrocarbon (TPH), Microbial Count, pH, Electrical Conductivity(EC), Total Organic Carbon (TOC) were investigated on the diesel-contaminated soil and the amended soil samples for a period of 42-days. The pH and (EC) were determined using the pH meter, while the TPH was determined by Improved Gravimetric Method. The TOC was determined by Loss on Ignition Method, while the Microbial Count was determined by Dilution and Plating Techniques. At the end of the study, the result showed that the initial pH of the soil sample which was 7.15 increased in alkalinity to 8.41, 8.47, 9.02 and 9.44 for samples A (Neutral soil), B (contaminated soil), C (soil amended with dry biostimulant) and D (soil amended with wet biostimulant) respectively; EC increased from 27.2mV to 81.3mV, 89.6mV, 121.5mV and 147.9mV; the removal efficiency of contaminant (TPH) was higher in soil sample D when compared to sample C. The initial weight of the contaminant in the unamended soil was 1.146g while the removal efficiency of contaminant in the soil samples were 0.787g and 0.663g for soil samples C and D respectively. It was also found that the microbial count ($35x10^5$cfu/g and $23x10^5$cfu/g) was abundant in the soil samples C and D which were amended respectively. TOC increased from 3.22% to 7.01% in sample B due to addition of nutrients. For the amended soil samples C and D, TOC decreased from 12.66% and 8.77% to 12.25% and 7.04% for samples C and D respectively. The statistical analysis of the TPH and %TOC using one-way ANOVA showed that, there was significant difference ($p<0.05$) in TPH reduction and %TOC since F-values (16.28 and 20.51) falls outside the upper critical region (5.49). Hence, the reduction in TPH proved the effectiveness of banana peels in remediation of diesel-contaminated soil.

Keywords*: Bioremediation, Biostimulation, Banana peels, Organic carbon sources, microbial growth*

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I. Introduction

The contamination of the environment, either the land, water or air with crude oil and its related products poses a threat to the life of aquatic and terrestrial organisms living in such polluted environment. This is as a result of the potential toxicity of petroleum products when present in a very high concentration. These potential threats have driven man into the search for environmentally friendly approaches to reclaim the polluted environments particularly crude oil polluted soil.

Crude oil is known to contain a complex mixture of naturally occurring hydrocarbons such as aliphatics, alicyclic and aromatics which can be refined into diesel, gasoline, heating oil, jet fuel, kerosene and other products (petrochemicals) of economic importance (1).

Contamination of the soil with crude oil and its product can affect the soil properties and crop performance. Hence, there is need to employ an approach which can be environmentally friendly in order to combat the risk which these contaminants pose to the environment and as well as retain soil texture and microbial activities (2).

Bioremediation is a cost effective and environmentally friendly method, which involves the use of microorganisms to degrade contaminants from soil and water. It is the use of naturally cropped up microorganism by humans to decontaminate man-made contaminants. It is a non-invasive, cost effective technique which conserves soil texture and characteristics. The success of its application depends on the nature of the contaminants in the soil and the environmental conditions such as temperature, oxygen concentration, pH, moisture content, presence alternate carbon sources and microbes with degradation capability, soil property and nutrient availability. It is therefore necessary to provide nutrients, oxygen and suitable temperature to maximize growth of microorganisms which help to facilitate bioremediation (3).

It has been reported that the use of organic wastes enhances bioremediation of crude oil polluted soil by facilitating soil aeration and raising the water holding ability of the soil (4).

It is paramount that, in any polluted environment, appropriate nutrient concentrations especially Nitrogen(N) and Phosphorus(P) are maintained in the optimal ratio in order to offset the imbalance caused by high carbon content of crude oil during pollution which may retard the growth of microbes (1).

Over the years, synthetic fertilizers have been applied as biostimulants for enhanced bioremediation of petroleum hydrocarbon pollution sites. Its excessive application resulted in negative consequences such as atmospheric pollution, eutrophication and others. Synthetic fertilizers are costly in developing countries like Nigeria due to their high demand as essential agricultural input. Hence, these challenges increased the quest for environmentally sustainability through search for organic substrates which could be used as alternatives to synthetic fertilizers to enhance bioremediation (1).Lack of essential nutrients such as Nitrogen(N) and Phosphorus(P) is one of the major factors that affects biodegradation of hydrocarbon by microorganisms in the soil and water environment (5).

Therefore, the addition of organic nutrients (biostimulation) or inorganic nutrients rich in Nitrogen is an effective means to enhance the bioremediation process (6).

Currently, nitrogen rich organic substrates are being used as biostimulants and they have proven to be useful in enhancing the rate of bioremediation. Some of these nitrogen-containing organic substrates which have been used as biostimulants include corn residues, sugarcane bagasse, yam peel, carrot peel, saw dust, spent brewing grain, rice husk, coconut shell, cow manure, pig manure and poultry manure (7).

Hence, this study was aimed at evaluating the effectiveness of banana peels in bioremediation of diesel-contaminated soil. The banana peels were prepared as wet and dry biostimulants to ascertain their effectiveness.

Global increase in the use of petroleum products has led to an increasing contamination of the environment with hydrocarbons. Hydrocarbon contamination of the natural environment attracts public attention due to its carcinogenic, toxic and mutagenic effects. Prolonged exposure to high concentration of hydrocarbon containing compounds may cause the development of liver or kidney disease, possible damage to bone marrow (8).

The soil which is a home for most living organism can be contaminated by the hydrocarbon (diesel oil) by spillage and when this happens, it affects the performance of crops and some organisms in the soil.

According to a study carried out by Cleusa et al (9) on the effect of soil contaminated by diesel oil on germination of seeds and growth of *SchinusterebinthifoliusRaddi* seedlings. The soil samples were analyzed at different times after contamination of the soil. The experiment was conducted with four treatments. At the end of the study, the development of the plants were affected in all the treatment, but the toxic effect decreased over the time.

Hence, there is need to eliminate these contaminants in the soil, and this can only be achieved by bioremediation of the soil.

Principle of Bioremediation

Microorganisms are ideally suited to destruction of contaminants due to the possession of enzymes that allow them to use environmental contaminants as food and because they are so small that they are able to contact contaminants easily.

The goal in bioremediation is to stimulate microorganism with nutrients and other chemicals that will enable them to destroy the contaminants. These microorganisms are supplied with nutrients and optimum conditions for their growth. Microbial transformation of organic contaminants occurs because the organisms can use the contaminants for their own growth and reproduction (10). Hence, the organic contaminants provide a source of carbon which is one of the building blocks of new cell constituents and they provide electrons which the organisms can extract to obtain energy.

Generally, bioremediation depends on having the right microorganism under the suitable environmental conditions in order for degradation process to occur successfully. The microorganism metabolizes the contaminants into harmless compounds such as carbondioxide, water, methane or biomass (11).

Approaches to Bioremediation

There are two major approaches to bioremediation namely; biostimulation and bioaugmentation.

Biostimulation: It is the addition of limiting nutrients to encourage/support microbial growth.

Bioaugmentation: it is the addition of living cells capable of degradation.

According to a research conducted by Godleads et al (12), on a review on bioremediation, stimulation and bioaugmentation, at times, nutrient application alone or augmenting with microbes is not sufficient enough for remediation. Recent studies show that, a combination of both approaches (biostimulation and bioaugmentation) is equally feasible but not explicitly more beneficial. Hence, a combination of biostimulation, bioaugmentation and environmental parameters such as temperature in combination with duration of exposure is required.

A study by Sari et al (10) showed that cold conditions delay bioremediation of oil hydrocarbons. Comparison of means to stimulate biodegradation of diesel oil hydrocarbons in contaminated soil were carried out. Different combinations of nutrients, bulking agent, aeration and microbial inocula were examined in lab simulation, and

effective combination were tested in field conditions. Efficient degradation was attained when slow-release nutrients and aeration were used simultaneously. Bacteria inocula did not advance soil remediation or bacterial densities.

At the end of the study, biostimulation via optimization of nitrogen and supply of oxygen significantly improved bioremediation of oil-contaminated soil, while bioaugmentation had no additional effect.

Banana peels and its chemical composition

Musa sapientum is a herbaceous plant of the family of musaceae. It originated from the tropical region of Southern Asia. The fruit is protected by its peel which is discarded as waste after eating the inner fleshy fruit.

Banana has been reported to prevent anaemia by stimulating the production of haemoglobin in the blood and many other health benefits. They are used as food for animals, in water purification, manufacture of several biochemical products. Ripe banana peels contain up to 30% free sugar.

Studies have also shown that banana peels can be useful in bioremediation processes, that is, biostimulation of Hydrocarbon contaminated soil.

According to Anhwange (13), the result of mineral content in banana peel shows the concentration of potassium to be highest (78.10 mg g-). The concentration (mg 100 g-) of calcium, sodium, iron and manganese were 19.20, 24.30, 0.61 and 76.20, respectively. The concentration of bromine, rubium, strontium, zirconium and niobium are 0.04, 0.21, 0.03, 0.02 and 0.02 respectively. The nutritional composition includes protein 0.90, crude lipid 1.70, carbohydrate 59.00 and crude fibre 31.70.

II. Materials

pH meter was used to determine the pH and Electrical conductivity. The muffle furnace was used to determine the total organic carbon present in the soil samples. The Incubator and colony counter were used for microbial count. The weighing balance, Oven, Mechanical Shaker (120rpm), N-hexane and Sodium Sulphate were used for determining the reduction in Total Petroleum Hydrocarbon in the soil samples.

III. Methods

3.1. Sample Collection

The soil sample was collected from P.T.I. Church Village, Effurun, Delta State. The banana peels were bought from Effurun Market, Delta State.

3.2 Soil sample preparation

The soil samples were prepared adopting the method by Aigbodion and Ekperusi (14). The soil was sun-dried and sieved with 10mm and 20mm mesh.

400g of the sun-dried soil was weighed into four (4) plastic containers labeled A, B, C and D. 50ml of diesel was measured using the measuring cylinder into the four (4) soil samples. Afterward, the soil was moistened with deionized water to the water holding capacity of the soil. The contamination with the diesel was allowed to stay for twenty-one (21) days in the laboratory. Thereafter, 40g of the dry and wet biostimulants were added to the contaminated soil samples C and D respectively. The soil samples and the biostimulants were mixed twice thoroughly per week to allow for proper aeration for a period of forty-two (42) days. The soil samples were kept under a controlled temperature of 29^0c and pH range of 6.5 to 8.5 which were the optimal range for microbial growth and reproducibility.

The analysis on the soil samples were conducted intermittently at seven (7) days intervals.

3.3 Banana peels preparation

The banana peels were prepared into wet and dry biostimulants.

The dry biostimulant were prepared using the method described by Omoni et al (15) while the wet biostimulant was the modified method of Omoni et al 2015.

The dry biostimulant was prepared by sun-drying the banana peels for twenty-one (21) days and thereafter ground into powder. Same were kept in a tight container for further use.

For the wet biostimulant, the banana peels were collected in a cellophane bag and allowed to stand for twenty-one (21) days to decay and allow microbial activities to take place.

3.4 EXPERIMENTAL ANALYSIS

3.4.1 Determination of pH and Electrical conductivity

The soil samples for pH and Electrical conductivity determination were prepared using the method adopted by Adams et al (2). The soil pH and Electrical conductivity were determined using the pH meter.

The soil samples were prepared by weighing 5g of soil into a beaker. 10ml of deionized water was measured using the measuring cylinder and added to the soil in the beaker. The mixture was stirred and kept for thirty minutes. Afterward, the pH and conductivity of the mixture was read from the pH meter when the electrode was dipped into the mixture of the soil-water slurry.

3.4.2 Determination of Total Petroleum Hydrocarbon (TPH)

The total Petroleum hydrocarbon in the soil samples was extracted and determined by an improved general gravimetric method as described by Mario et al (15).

The soil samples were sieved using 10mm sieve followed by 20mm sieve and dried to 105^0c for two (2) hours. 10g of the soil samples was weighed accurately using the digital weighing balance into round bottomed flasks previously dried at 105^0c to constant weight. Afterward, 10g of anhydrous sodium sulphate was added to the flasks and 35ml of N-hexane was added to the flask for extractions in a mechanical Shaker at 120rpm for one hour. The extracts were filtered. Same was washed down with additional 25ml of N-hexane to complete 60ml in the final liquid extract for analyzed.

Thereafter, the hexane was evaporated in a rotary evaporator, followed by drying of the flask outer walls with lint-free absorbent paper and evaporation of the remnant hexane took place.
The residue was weighed in an analytical balance and designated as Total Petroleum Hydrocarbon.

3.4.3 Determination of Total Organic Carbon (TOC)

The total organic carbon (TOC) in the soil samples was determined by loss on ignition method as described by Andre et al (16).
Empty furnace crucibles with caps labeled A, B, C and D were weighed using the weighing balance and their respective weights recorded as **W1**. Afterward, 10g of each of the four soil samples labeled A, B, C and D were weighed into the four crucibles according to their labelling and their weights were taken as **W2.**
Thereafter, the muffle furnace was connected to power supply and turned on. The temperature was set between 550^oc to 600^oc, after which the four samples were introduced into the furnace and the temperature increased until it got to 600^oc at which TOC detection occurred. The temperature was held for about 5hours which ensured that all organic carbon was combusted.
Thereafter, the soil samples were removed from the furnace with the help of the furnace tong and placed in the desiccator for cooling. Upon cooling, the weights were taken severally until constant weights were obtained as **W3**.
The difference in weight before and after ignition were designated as the TOC. Hence,

$$\%TOC = \frac{W2-W3}{\text{Weight of soil}} \times 100$$

Where W2=Weight of soil + crucible before ignition
W3=Weight of soil + crucible after ignition
Weight of sample= 10g

3.4.4 Determination of Microbial count

The microbial count of the soil sample was determined by dilution and plating techniques. This method utilized Agar as a medium for bacterial growth. A small sample of soil was serially diluted in water prior to being plated on Agar within a petri dish.

Preparation of soil dilutions

10g of soil sample was weighed using the digital weighing balance and added to 95ml of deionized water in a beaker. The suspension was agitated properly and labeled as "A". 1ml of the suspension was removed with a sterile pipette and transferred to a 9ml deionized water blank in a test tube. This was labeled "B".

Afterward, the dilution step was repeated thrice each time with 1ml of the previous suspension and a 9ml deionized water blank. These tubes were labeled sequentially as tubes C, D and E. These resulted in serial dilutions of 10^{-1} through 10^{-5} grams of soil per ml.

Making spread plates for bacterial culture

To grow bacterial colonies, a sterilized pre-prepared Agar plate was taken and labeled as E. The suspension in test tube E was agitated properly and 0.5ml was pipetted onto the Agar plate. The plate was opened quickly holding the lid close by, while the nutrient agar was quickly poured into the plate. The plate lid was further replaced.

Furthermore, the process was repeated with soil samples B, C and D. The bacteria plate was incubated at room temperature for 48hours. The plates were inverted during incubation to prevent drop of moisture as a

result of condensation from falling onto the agar surface. Thereafter, the number of bacterial colonies were counted and recorded using the colony counter.

$$\text{Number of CFU} = \frac{1}{\text{Dilution factor}} \times \text{Number of colonies}$$

IV. Results And Discussions

4.1 RESULT

The result for the analysis of pH, EC, TPH, %TOC and Microbial count are summarized in Table 1 below:

TABLE 1

Week	Samples	pH	Temp (^{O}c)	E.C (mV)	TPH(g)	TOC (%)	Microbial Count (CFU/g)
1	A	7.15	29.2	27.5	0.239	3.62	-
	B	7.23	29.5	33.1	1.146	3.22	-
	C	7.41	28.2	27.3	0.988	12.66	$35x10^5$
	D	8.61	28.4	100.3	0.980	8.77	$23x10^5$
2	A	7.71	26.5	46.5	0.237	0.76	$2x10^5$
	B	7.73	27.6	49.7	0.955	8.66	$3x10^5$
	C	8.69	26.8	99.0	0.875	13.89	$5x10^5$
	D	8.98	27.1	124.4	0.665	6.7	$10x10^5$
3	A	8.41	30.1	81.8	0.139	1.48	$3x10^5$
	B	8.47	31.0	89.6	0.754	7.01	$2x10^5$
	C	9.02	30.8	121.5	0.787	12.25	$3x10^5$
	D	9.44	31.2	147.9	0.663	7.04	$7x10^5$

V. Discussion Of Result

5.1. pH

Figure 1 shows that the initial pH of the soil sample was 7.15, which is quite neutral but tended towards alkalinity during the study period of three weeks after remediation. In the 1st week, the pH was 7.15, 7.23, 7.41 and 8.61 for samples A, B, C and D respectively with samples C and D being the amended soil samples. However, there was increase in alkalinity to 7.71, 7.73, 8.69 and 8.98 in the 2nd week, while in the 3rd week, the pH further increased in alkalinity to 8.41, 8.47, 9.02 and 9.44 for samples A, B, C and D respectively.

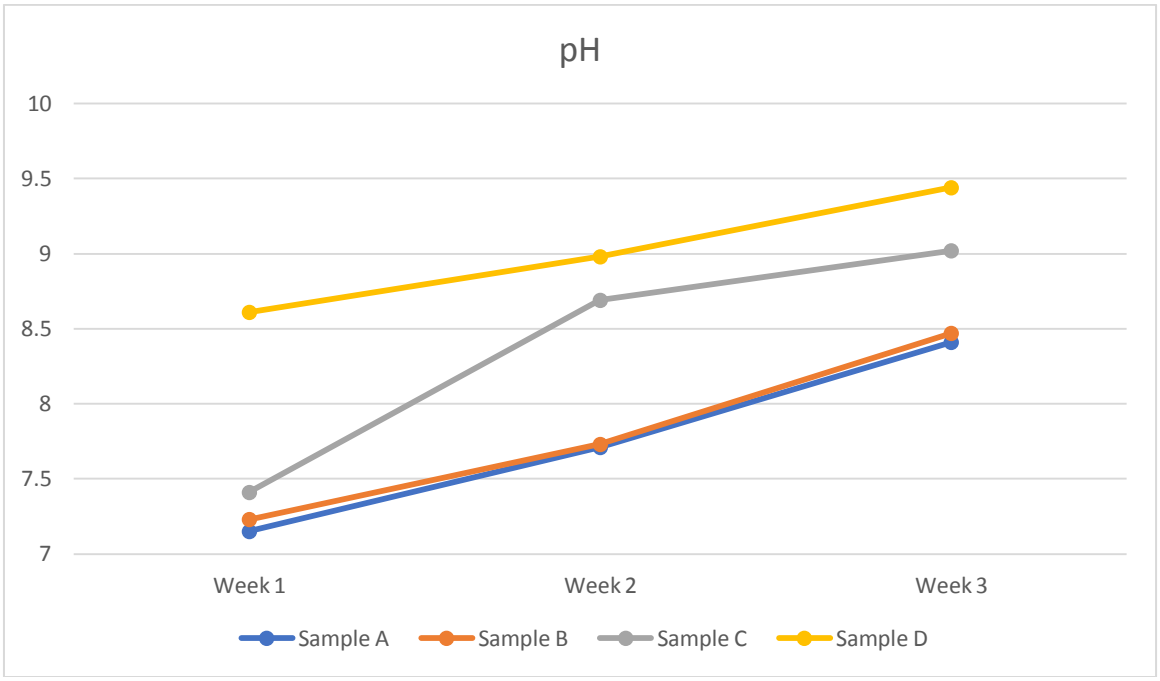

Figure 1 showing the result for increase in pH towards alkalinity for samples A, B, C and D in week 1, week 2 and week 3 after remediation

Sample C showed a significant increase in alkalinity in the 2nd week, that is, from 7.41 to 8.69, whereas, sample D remained the highest in alkalinity throughout the study period. The increase in alkalinity could have been as a result of aeration and watering of the soil samples which could have led to an increase in metabolic activities of microbes present in the soil resulting in the synthesis of intermediary metabolites as described by Adams et al (2, 17).

5.2 Electrical Conductivity

Figure 2 shows the result for Electrical conductivity of the soil samples throughout the study period. From the result, there was an increase in Electrical conductivity of the soil samples throughout the study period.

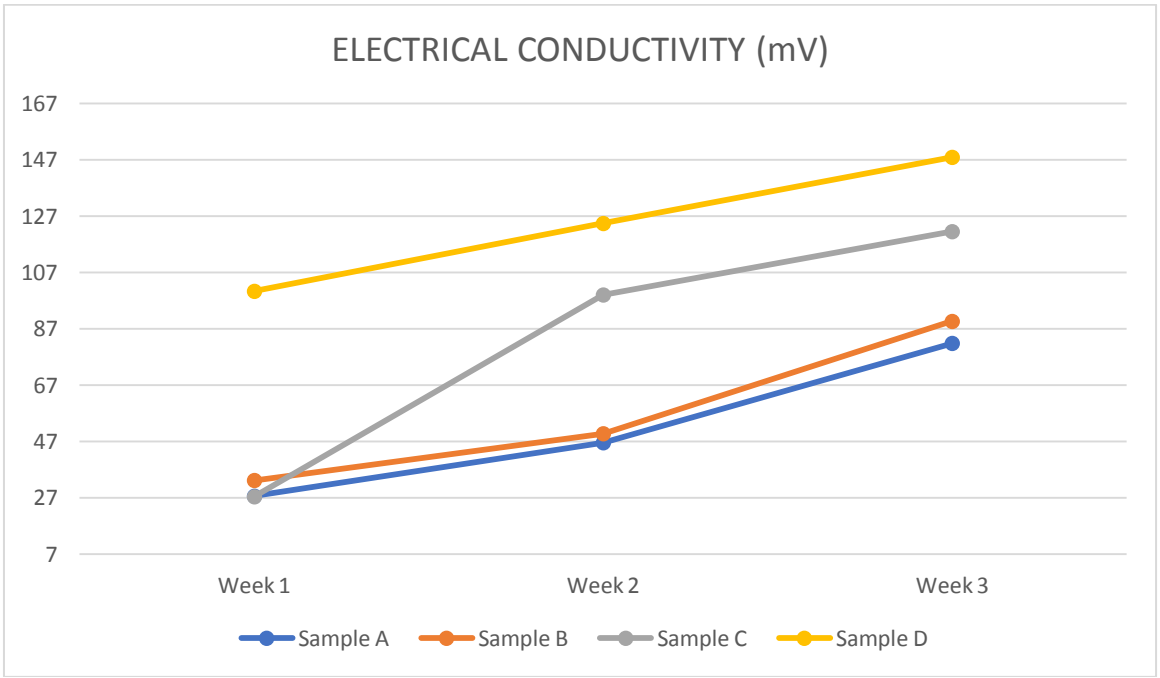

Figure 2 showing the result for increase in EC for samples A, B, C and D in week 1, week 2 and week 3 after remediation.

In the 1st week, the Electrical conductivity were 27.2mV, 33.1mV, 27.3mV and 100.3mV for samples A, B, C and D respectively, although, the Electrical conductivity increased to 46.5mV, 49.7mV, 99.0mV and 124.4mVIn the 2nd week, while in the 3rdweek, the Electrical conductivity further increased to 81.3mV, 89.6mV, 121.5mV and 147.9mV for samples A, B, C and D respectively. Samples D showed the highest Electrical conductivity value throughout the study period which were 100.3mV, 124.4mV and 147.9mV for week 1, week 2 and week 3 respectively.

According to Adams et al (2), the increase in Electrical conductivity could have been due to the availability of nutrients in the soil samples. The higher the nutrients, the higher the Electrical conductivity and vice versa as described by Acharya et al (18). Sample D was amended with wet biostimulant while sample C was amended with dry biostimulant. Hence, the nutrient and moisture content of sample D could have contributed to the high EC of sample D when compared to samples A, B, and C.

5.3. Total Petroleum Hydrocarbon (TPH)

Figure 3 shows the TPH degradation of the soil samples all through the study period of three weeks. In the 1st week, there was a significant and rapid reduction from 1.146g being the initial weight of the contaminant to 0.988g and 0.980g for the amended soil samples C and D respectively, with sample C amended with dry biostimulant and sample D amended with wet biostimulant. In the 2nd week, there was further reduction to 0.875g and 0.665g although in the 3rd week, there was a lower rate of reduction to 0.787g and 0.663g for samples C and D respectively.

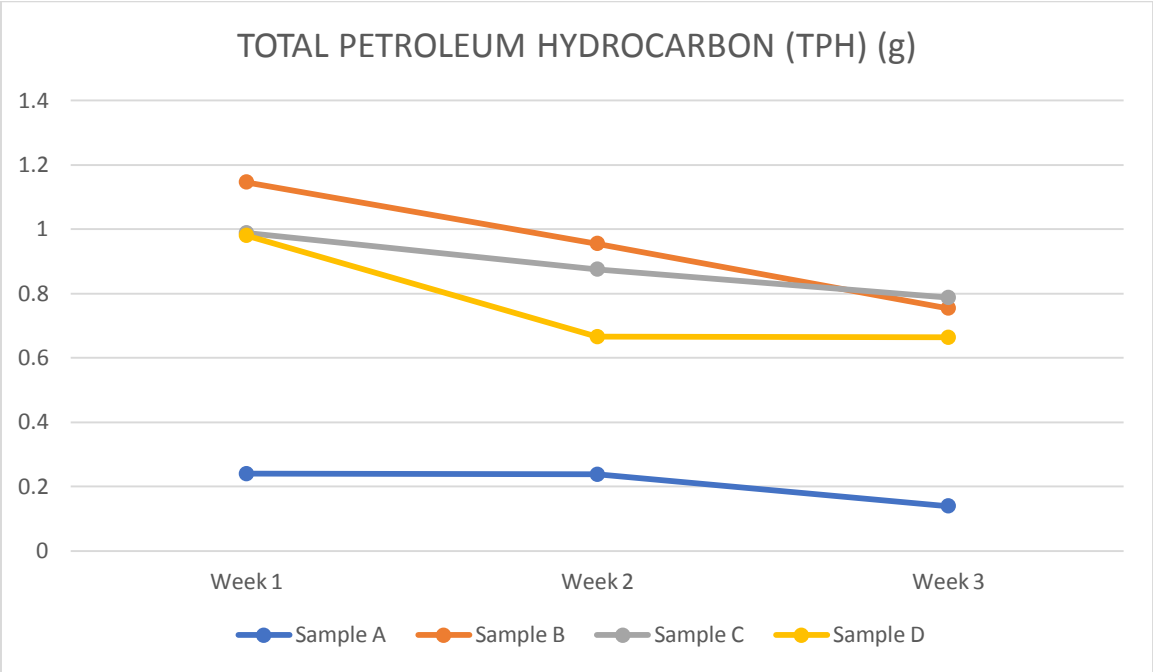

Figure.3 showing the result for TPH reduction in samples A, B, C and D in week 1, week 2 and week 3 after remediation.

All through the study period, sample D showed a higher rate of reduction, followed by sample C. This could be attributed to biodegradation by microorganisms and availability of nutrients in the soil sample D amended with wet biostimulant, unlike the dry biostimulant which supplied mostly nutrients to the indigenous microorganisms in sample C as described by Fu et al (19).

Also, the increase in pH towards alkalinity was due to the addition of organic waste, decreased hydrocarbon contaminant as reported by Nwogu et al (1).

According to Fu et al (19), the result proved that hydrocarbon biodegradation can be improved by aeration, nutrient addition and microbial activities.

5.4. Total Organic Carbon (TOC)

Figure 4 shows the percentage total organic carbon present in the soil samples throughout the study period.

From the result, there was variation in the %TOC present in the soil samples. The initial %TOC present in the soil was 3.62% for sample A, which is the uncontaminated soil. Upon contamination, the %TOC

decreased to 3.22%; then upon amendment with dry and wet biostimulant in samples C and D respectively, the %TOC increased to 12.66% and 8.77%.

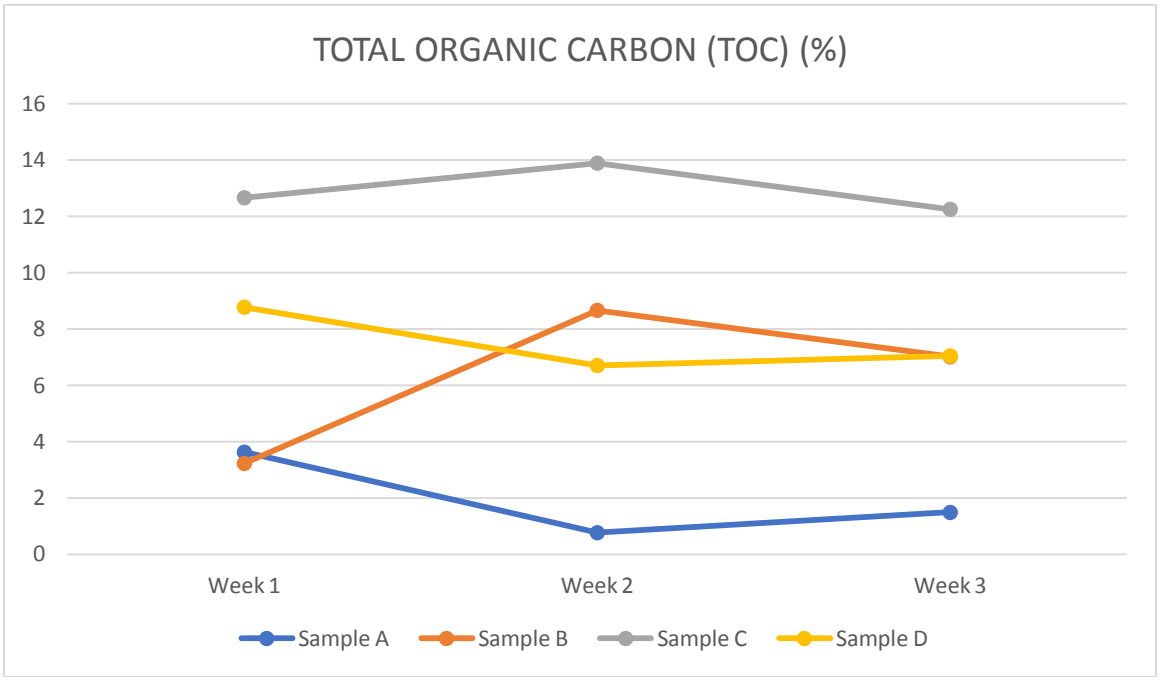

Figure 4 showing the result for %TOC in samples A, B, C and D in week 1, week 2 and week 3 after remediation.

The increase in %TOC could have been as a result of biostimulation, which is the addition of nutrients obtained from organic matters. Also, the increase in %TOC is likely to have been as a result of increase in hydrocarbon contaminant which releases additional carbon to the soil (20, 21).

In the 2nd week, the %TOC were 0.76%, 8.66%, 13.89% and 6.7% while in the 3rd week, the %TOC were 1.48%, 7.01%, 12.25% and 7.04% for samples A, B, C, and D respectively.

The reduction in %TOC in samples A and D in the 2nd week and samples B and C in the 3rdweek, is likely to have been due to increased microbial activities and nutrient utilization by the microbes as energy source (20).

According to Andre et al (16), the variation in the result obtained could have been attributed to factors such as position of samples in the furnace, exposure time, sample size and laboratory measurement.

5.5. Microbial Count

Figure 5 shows the result for microbial count in the soil samples throughout the study period of three weeks. From the result, there was an initial increase in microbial count but later a significant drop occurred with time.

In the 1stweek, samples A and B had no growth while samples C and D which are the amended soil had microbial growth of $35x10^5$cfu/g and $23x10^5$cfu/g respectively. In the 2nd week, there was a drastic drop in growth to $2x10^5$cfu/g, $3x10^5$cfu/g,$5x10^5$cfu/g and $10x10^5$cfu/g for samples A, B, C and D respectively, while in the 3rd week, a further gradual drop to $3x10^5$cfu/g, $2x10^5$cfu/g, $3x10^5$cfu/g and $7x10^5$cfu/g for samples A, B, C and D respectively was observed.

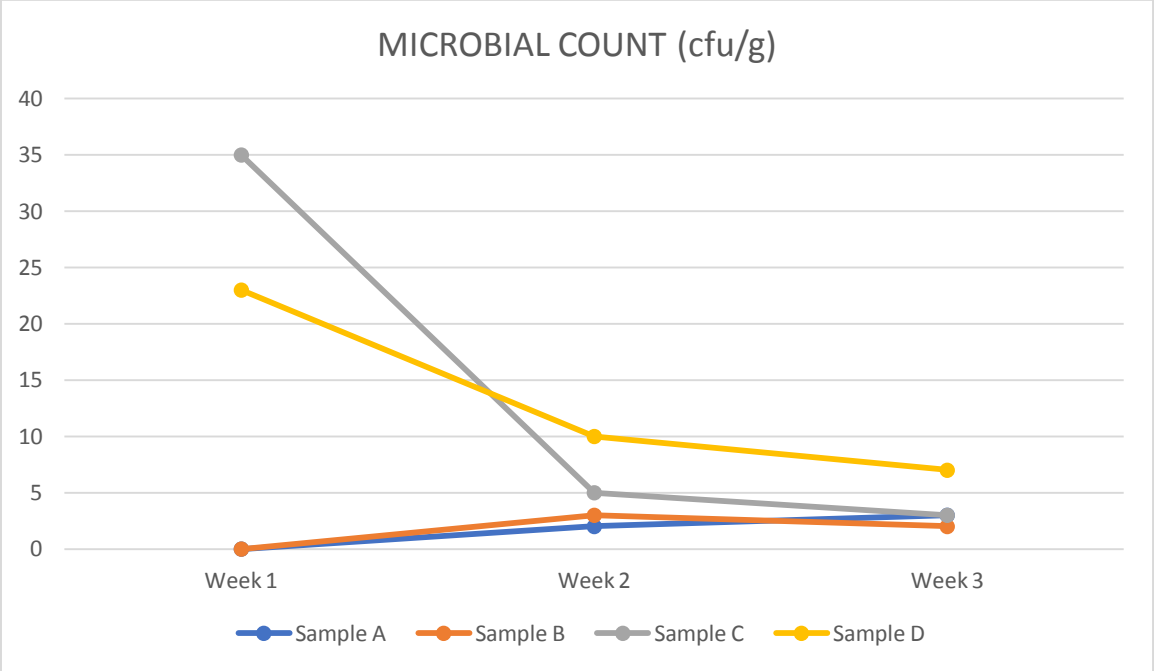

Figure 5 showing the result for microbial count in samples A, B, C and D in week 1, week 2 and week 3 after remediation.

From the study, the microbial count in the amended soil samples C and D were higher than that of the unamended soil samples A and B. Towards the end of the study, there was a decrease in the microbial count.

The increase in microbial count for the amended soil samples C and D in the 1st week could have been as a result of biostimulatiion, which is the addition of nutrients and oxygen to assist indigenous microorganisms whereas, the absence of microbial growth in samples A and B was as a result of unavailability of nutrients, aeration and moisture to activate the indigenous microbes as described by Fu et al (19). The availability of nutrients, oxygen, water, aeration, optimum temperature and pH accelerates and improves microbial growth and activities. Hence, the nutrients help microbes to create enzymes necessary to breakdown hydrocarbon contaminants as described by Ms. Madhavi and Ms. Mohini (22).

The drastic drop in microbial growth for samples in the 2nd and 3rd week is possible to have been due to depletion in nutrients and decreased bioavailability of hydrocarbons to the indigenous microbes. Also, short supply of nutrients can result to competition for nutrients within the microbial communities which may limit overall microbial growth and slow down contaminant removal. as reported by Nwogu et al (1).

VI. Conclusion

From the result obtained from bioremediation of diesel-contaminated soil enhanced by banana peels, there was significant reduction in the hydrocarbon contaminant as the contaminant in the soil sample was analyzed using the improved gravimetric method with extraction method.

There was significant reduction from 1.146g of contaminant in the contaminated soil to 0.787g and 0.663g in the soil samples remediated with dry and wet biostimulants respectively.

Hence, the reduction in the weight of contaminants in the remediated soil samples after extraction with N-hexane is a proof of the effectiveness of banana peels in biodegradation of the hydrocarbon contaminant (diesel).

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