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REMEDIATION OF PETROLEUM IMPACTED FARMLAND USING BLENDED BIO-STIMULATORS AND AUGUMENTATORS (BIOFERTILIZER)

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Abstract

The study investigated the impact of blended fermented melon pulp and chicken manure (Biofertilizer) in remediation of gasoline polluted farmland at Umuimo municipality, in Osisioma Local Government Area of Abia, Nigeria. The impacted farmland has no vegetation growth on it compared to areas around it that was not impacted, it indicates low plant nutrients. The investigation is to reinstate the plant nutrients by using bio stimulators and augumentators sourced locally. Pulverized fermented melon pulp and chicken manure were blended at 1:1 ratio. The gasoline polluted soil was mixed with the blended sample at 10:1, 5:1 and 3:1 ratios. The physiochemical and microbial properties were determined at interval of 15 space days. The control sample's physicochemical average results were: Total Petroleum Hydrocarbon (TPH) 0.08mg/kg, Potassium (K) 10.89mg/kg, Nitrogen (N) 1.56mg/kg, Phosphorous (P) 4.3mg/l, pH 5.8 whereas the polluted farmland were: TPH 216.28mg/kg, K 5.74mg/kg, N 0.65mg/kg, P 1.86mg/kg, pH 6.2. Microorganisms determined the control sample were Bacteria: Bacillus sp, Corynbacterium sp, Micrococcus; Fungi: Aspergillus, Mucor sp, Staphyloccus sp, Rhizopus sp whereas the microorganism in the polluted soil sample were Bacteria: Bacillus subtilus, Micrococcus, Corynbacterium, Acinetobacter, Bacillus, Pseudomonas, Spirillum; Fungi: Fusarium sp, Rhizopus sp, Asperigillus sp, Fusarium sp, Muco sp, Staphyloccus sp. The microbial count of the polluted soil were - Total heterotrophic bacteria: 2.76 x 105 Colon forming unit per gramme (cfu/g), Total hydrocarbon utilizing bacteria: 2.54 x 105 cfu/g, Total hydrocarbon utilizing fungi: 1.85 x 105cfu/g. The identified microorganism content of the blended sample were Citrobacter sp, Klebsiella sp, Corrynebacterium sp, Bacillus sp, Escherichia sp, Micrococus sp, Lactobacillus, Saccharomyces, Cervisae. The identified nutrients were nitrogen, potassium, phosphorous. The remediated soil showed gradual reduction of TPH as days increased, the concentrations of Nitrogen, Phosphorous and potassium decreased within the first 15 days and later increased within the remaining 15 space days. The outcome of Statistical Package for the Social Sciences(SPSS) confirmed huge variation between polluted and remediated soils. SPSS indicated that the outcome of different blends varied but was not pronounced.

Keywords: gasoline pollution, soil physicochemical analysis, microbial analysis, total petroleum hydrocarbons.

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1.0 INTRODUCTION

Oil production, transportation and distribution activities have consequences of environmental pollution. Crude and processed petroleum are transported from the producing points to different locations and the activities are prone to leakages, explosions and fire outbreak. Vandalization of petroleum facilities is a major cause of oil spillage. In 12th October, 2018, a farmland at Umuimo municipality in Osisioma Local Government Area in Abia State, was polluted by a petroleum hydrocarbon due to gasoline pipeline explosion. Many lives, residential buildings, economic trees and vegetation were destroyed.

Gasoline is hydrocarbon with many components such as inorganic compounds, straight chain, branched chain, cyclic chain aromatic hydrocarbon. Significant quantity of petroleum products in environment has adverse effect organisms, including humans (Alexander 1994). Some routes of environmental pollution are through spillage, flow leakage, effluents from anthropogenic activies (Chikere, Chijioke–Osuji 2006). Gaseous components volatilize polluted surface and leaves the non-volatile components as residues (Odu, 1977). Physical and chemical nature of soil is affected by oil pollution (Minai-Tehran, Herfatmanesh 2007). Some adverse impact of Oil pollution on economic and environment are enormous; damage to vegetation, soil fertility, microorganisms and so on (Nwachukwu, Ugorji 1995). Toxicity of the pollutant varies depending on the type of oil, additives used during processing as well as the biota of spillage (Reddy, 2001).

Bioremediation is a biological degradation process that relies on microorganisms activities. It transform pollutants to another state where the remnants or concentrations are either undetectable or detectable but within the acceptable by appropriate regulatory agencies (Wami, E.N. et al. 2008). Bioremediation technologies assist microorganism's growth and increase microbial populations by creating optimum environmental conditions for them to detoxify the maximum amount of pollutants; this is achieved by giving them access to a variety of material and conditions that help them thrive and build more cells (Fogel, M.M. et al. 1986). Bioremediation of polluted soil requires adjustment in water content, pH, oxygen, temperature and nutrients (phosphorus, nitrogen, potassium, trace minerals, etc). These adjustments are more achievable in reactors than insitu. Bioremediation has higher probability of generation hazardous gases and leachable residues (Ola, S.A. et al. 2008).

The efficiency of bioremediation depends on soil, environment, degradable material and its concentration as well as the population of microorganism at the site (Oyoh, K. B. et al. 2007). Bioremediation is effective in the remediation of hydrocarbon based pollutant such as petroleum, Polychlorinated biphenyls (PCBs), pesticides, chlorinated solvents. Applicable methods are in-situ and ex-situ. The microorganisms feed and breakdown the degradable pollutants in the soil. Bioremediation technologies are phytoremediation, mycoremediation, bioventing, bioleaching, landfarming, bioreactor, composting, bioaugumentation, rhizofiltration and biostimulation.

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Biostimulation and bioaugumentation technologies were applied in this study. Biostimulation is a method of enhancing degradation by stimulating the growth of existing microorganism capable of feeding and breaking down degradable materials. Addition of Oxygen and nutrients (Nitrogen, Phosphorus, Potassium) aid in the growth of microorganism in the site (Dubey, 1996). Engineered in situ bioremediation accelerates the desired biodegradation reactions by encouraging growth of more microorganisms via optimizing physicochemical conditions. Biological breakdown of Hydrocarbon can be influenced by many factors such as nutrients, pH, temperature, moisture, oxygen, soil and contaminant characteristics (Atagana, 2008).

Perfumo et al. (2007) stated that biostimuation is incorporation of nutrients, oxygen to the polluted site so as to boost the population or activity of existing microorganisms. Margesin etal. (2001) also stated that it is the remediation that can enhance pollutant breakdown by efficiently controlling operations like aeration, nutrients inputs, pH and temperature adjustments. The advantage is that the method stimulates the existing or native microorganisms at the environment. The main disadvantage of the method is that the geology of the site affects proper distribution of the nutrients and other operations. Impermeable and fractured subsurface hinder evenly distribution of additives. Also addition of nutrients can as well aid to the growth of microorganism which are not hydrocarbon degraders, thereby creating competition.

Fermented melon pulp and chicken manure are biofertilizers. A biofertilizer contains microorganisms and plant nutrients which when applied to seeds, plant surfaces promotes growth by increasing the supply of nutrients to the host plant. The term biofertilizers denote all the natural nutrient inputs for plant growth (Subba Rao, 1982). Subba Rao (1982) stated that biofertilizers microbial inoculants that adds nutrients through nitrogen fixation, solubilizing phosphorous, and restore the soil's natural nutrient cycle and build soil organic matter.

Nicholson, F. A. et al. (1996). stated that chicken excreta is an organic fertilizer. The nutrient contents of the individual excreta varied although all had similar concentration of nutrients (N,P,K, Magnesium(Mg),Sulphur (s)) on a dry basis. Typically N:P:K ratios were 3:1:1 for layer excreta and 6:2:3 for broiler/turkeyexcreta. Fresh chicken excreta contains 0.8% potassium, 0.4% to 0.5% phosphorous and 0.9 to 1.5% nitrogen. A chicken discharges about 3.62kg – 4.98 kilogram (kg) of excreta monthly (Pollen et al. 1980).

Chikere (2012) identified in poultry droppings about fifty six hydrocarbon degrading microorganisms namely Staphylococcus spp., Pseudomonas spp., Citrobacter sp., Klebsiella sp., Micrococcus spp., Corynebacterium spp., Bacillus spp., Rhodococcus spp., Alcanivorax spp., Serratia spp., Arthrobacter spp., Nocardia spp., Flavobacterium sp., Escherichia sp., Acinetobacter sp., Proteus sp. The study reviewed that TPH was polluted soil was reduced by 95.35%.

Offonry, S.U. et al.(1998) identified microorganisms such as Bacillus subtilis, B. polymyxa, Lactobacillus fermentum, L. brevis and Streptococcus faecalis in the process of retting of melon pulp and melon seeds extraction. The process involved natural fermentation of melon pod.

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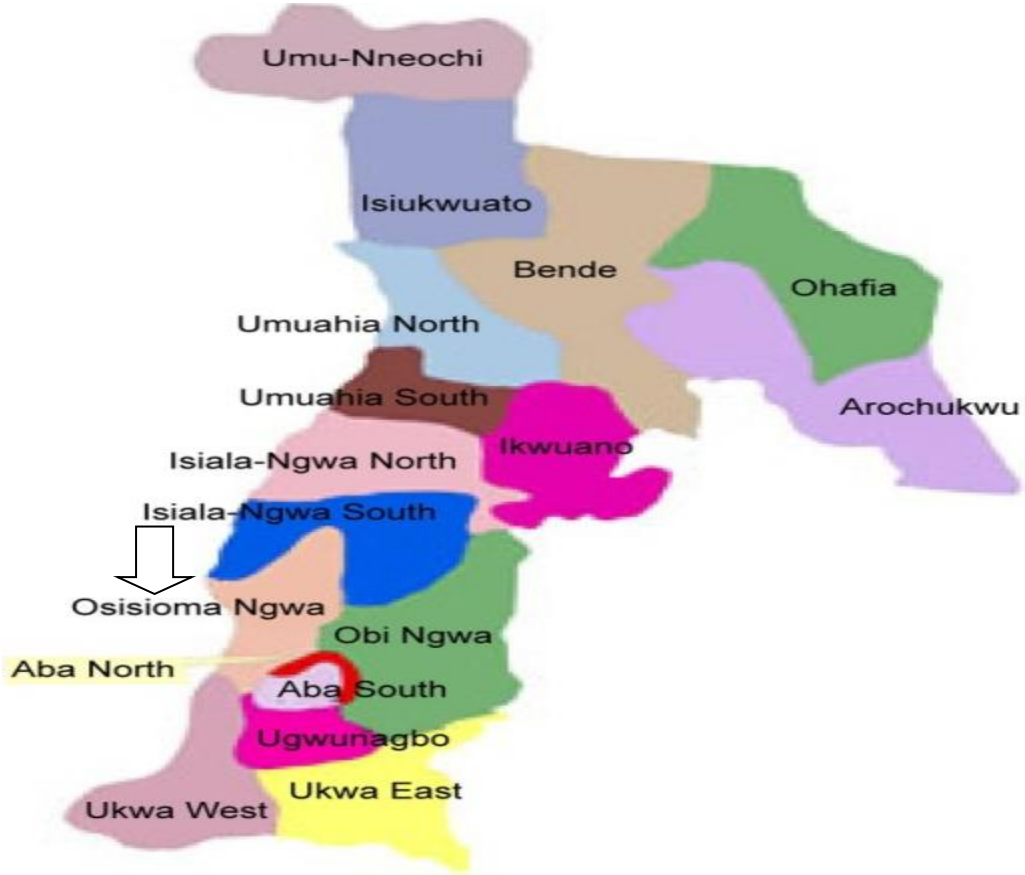
Bioaugumentation simply involves addition of microorganisms to a polluted site for feeding and breaking down the pollutants. Fungi have been found to be better degraders of petroleum than bacteria (Al-Nasrawi 2012). Microbial biomass influences rate of bioremediation.

Soil with appreciable microbial biomas has high potential to be rich in nutrients, and also cycle more nutrients through the ecosystem (Torstensson et al. 1998). Bahuguna et.al (2011) stated that environmental factors such as pH, nutrients, soil moisture and existing or native microorganisms as well as the nature of the pollutant have impact on the efficiency and effectiveness of bioaugumentation.

This study investigated remediation properties of equal blended ratio of fermented melon pulp and chicken manure on gasoline impacted farmland. The fermented melon pulp and chicken manure are biostimulators and augmentators because they contain nutrients such as N,P,K and microorganisms such as bacteria and fungi. The equal blend of melon pulp and chicken manure were mixed with gasoline impacted soil at 10:1 5:1and 3:1 ratios. The physicochemical and microbial tests were performed at space interval of 15days – 15, 30 and 45days.

1.1Study area



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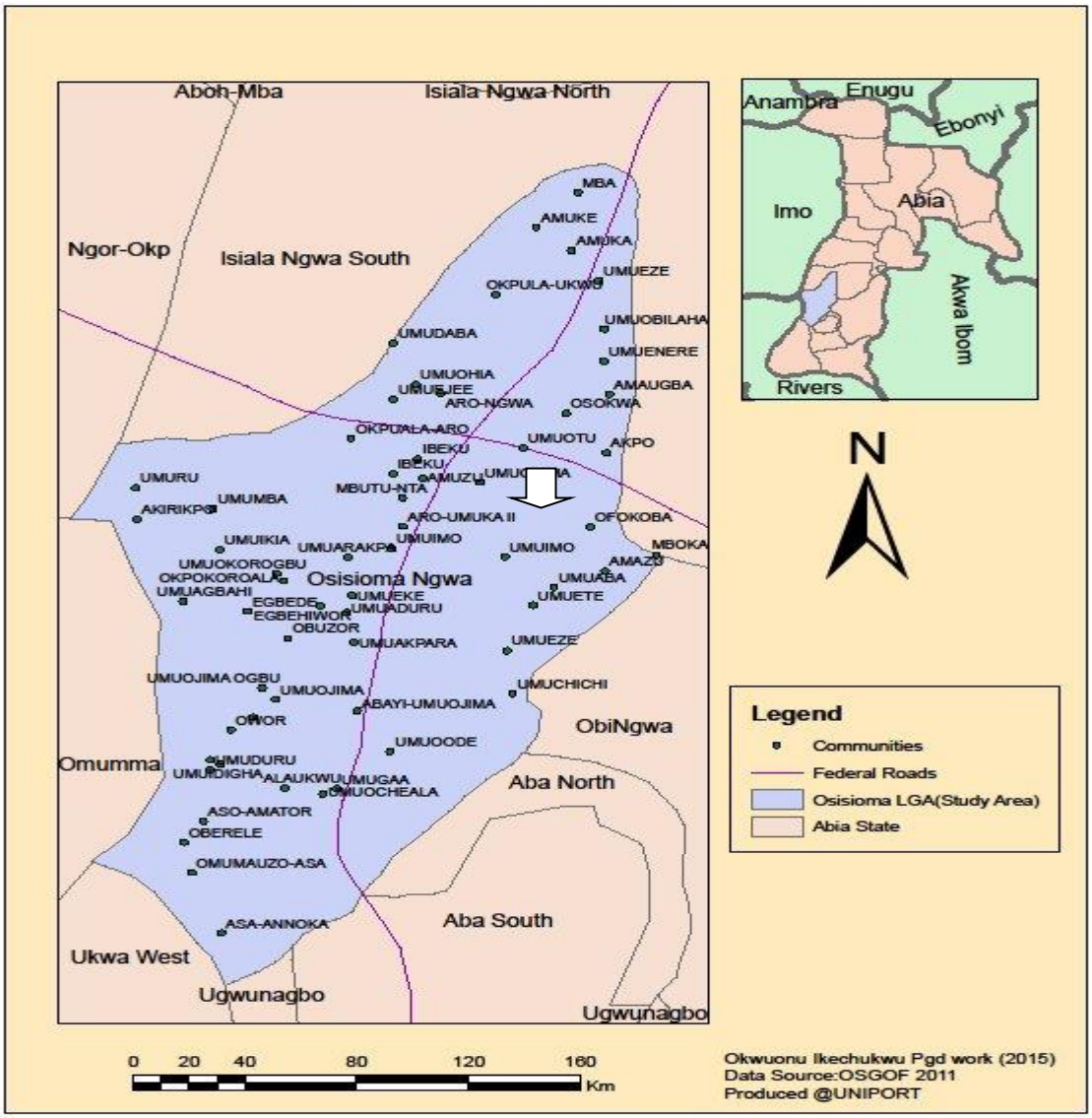

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Fig 1.0 Cross section of the polluted site

Fig1.1 Cross section of the polluted site

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Umuimo municipal is in Umueze Autonmous community in Osisioma Local Government Area (LGA). It is located along Aba-Enugu Expressway. Osisioma Ngwa LGA covers about 198Km^2 with coordinates of $5^0$8'59''N, $7^0$19'49''E, and a population of 219,632 (2006 census). It has table topography and its soil profile grades from fine silty sands to fine gravel sand. The general soil characteristic is dominated by silts, sands and sandy clay in different proportions. Osisioma Ngwa shares boundaries with Ukwa West and Umunagbo in the South, Aba South LGA and Aba North LGA in the East, Isiala Ngwa South LGA in the North. Aba-Port Harcourt Expressway cuts through Osisioma Ngwa.

2.0 Materials and method

Sampling of Soil samples

Soil was sampled at surface and subsurface at about 15cm from five different locations tagged A, B, C, D, E at the polluted site. Characteristic of oil pollution was considered in choosing sampling points. The oil spill was about two years at the time of sampling. The reference point was about 100m away from the polluted farmland. Stainless knife and I litre plastic bottles were used in the soil sampling. Distilled water and 10% nitric acid were used for washing and disinfecting of the apparatus.

2.1 Soil physicochemical properties

Kjeldahl digestion, Flame photometer and Maiti (2003) procedure were applied to determine total Nitrogen, Potassium and phosphate respectively.

2.2 Total petroleum hydrocarbons

2g of soil was introduced into sterilized extraction container.

Addition of 10ml of chloroform/dichloromethane (1:1, v/v), the mixture was vigorously shaked and allowed to settle. Filtration of the mixture through a Buchner funnel fitted with filter paper was performed.. The extracts were concentrated to 2ml and then transferred for cleanup/separation. The concentrated aliphatic fractions were transferred into labeled vials with Teflon caps for gas chromatograph analysis.

2.3 Total Heterotrophic bacteria count.

The pour plate technique was applied. 1 ml of the soil suspension was added aseptically into sterile Petri-dishes which were in triplicates, about 15ml of sterilized nutrient agar (Oxoid) was introduced into each plate and whirled, thereafter cooled. Incubation of the plates at 30^0C for duration 1to 2 days was done. Thereafter, colonies that formed on the plates were enumerated and designated in colony forming units.

2.3.1 Total Hydrocarbon degrading bacteria

The pour plate method was used using oil agar medium. 1ml of the soil suspension was introduced aseptically into sterile Petri-plates (in duplicates) and sterilized oil agar was poured aseptically into the plates and gently swirled and were then allowed to gel before they were

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incubated at 30^0C for 5-7 days. Colonies were seen on the plates and then counted. Hydrocarbon was the main source of carbon.

2.4 Isolation and Staining of bacteria

Bacterial colonies formed on the oil agar plates were collected based on their morphological differences and the collected colonies were cleansed by streaking on nutrient agar plates. Purity of the cultures was obtained by further sub-culturing. Pure culture of the isolates was Gram stained as described by Collins and Lyne (1984). A lean smear was made on a clean grease-free slide and heat-fix. The smear was then stained with 2 drops of crystal violet, the primary stain for half minute. Thereafter treatment with 2.5% Gram's Iodine solution which acts as mordant for 60 seconds. The iodine boost the stain of the cell strongly. Ethanol (95%) was applied in decolorizing the smear till the violet coloration disappeared. The slide was rinsed, and then counter-stained for 30-40 seconds with 2 drops of safranin. The film was dried with blotting paper, and observed with microscope. The bacteria that take stain and appear dark violet or blue black are called Gram-positive bacteria. Colonies that formed on nutrient agar plates were grouped according to their colonial morphology.

2.5 Experimental Method

2.5.1 Bio fertilizer preparation

Melon pulp was pulverized into paste. Water was sprinkled on it, thereafter; it was covered by perforated wooden sheet. The compost was allowed for 21days for it to decompose.

2.5.2 Experimental procedure

The pulverized melon pulp was blended with chicken mature at equal ratio.

The blend was mixed with the polluted soil at the ratio of 10:1, 5:1 and 3:1.

The experiments are denoted as follows

RE1 denotes the 10:1 blend

RE2 denotes the 5:1 blend

RE3 denotes the 3:1 blend

The physicochemical and microbial tests were performed at intervals of 15 space days.

3.0 Results and discussion

The study ratifies the claim that microorganism growth is stimulated by petroleum hydrocarbon. The pH of controlled soil and hydrocarbon polluted soil were 5.8 and 6.2 respectively. The pH of the polluted soil tended to neutrality. The result is similar to Atlas (1981) report that neutral pH boost degradation activity of bacteria. Fig 1 showcases that hydrocarbon degrading bacteria increased as days increased. RE1 experiment displayed that hydrocarbon utilizing bacteria

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(HUB) increased from about 1.2x 10^6 cfu/g in 15days to about 2.0 x 10^6 in 30days, and in 60days the population was about 2.2 x 10^6 cfu/g.

RE2 experiment, the population increase was about 2.7, 2.8 , 2.9 x 10^6 cfu/g in 15days, 30days and 45days respectively. Also in RE3 experiment the growth pattern was 2.4, 2.8, 3.0 x 10^6 cfu/g in 15days, 30 days and 45daysrespectively. This result confirmed the result obtained by Offonry S.U. et al(1998) in their remediation experiment. The result was in conformity to the outcome of Roling et. al (2002) investigation on bacterial dynamics and crude oil degradation after nutrient amendment which stated that nutrient enhancement increased bacterial count which impact significantly with hydrocarbon attenuation.

Fig 2 indicates decreasing trend of Total Petroleum Hydrocarbon in all the experimental samples as days increased. The numerical increase of hydrocarbon degrading microorganisms may lead to the decreasing trend of hydrocarbon because they feed on the hydrocarbon. This establishes the result of Agarry et.al experiment which reported that 73% and 50% TPH loss for hydrocarbon polluted soil treated with poultry manure and goat manure respectively.

Physiochemical properties in the bioremediation experiments revealed that within 15days of experiment, the nutrients (N, P, K) diminished in all. It might be due to numerical growth of microorganisms which likely fed on the nutrients. The nutrients increased progressively between 30 and 60 days. Refer to Fig 1 to Fig 5. The microorganism nutrient recycle activities might be the cause of the increment. Some microorganisms act on complex minerals, proteinous materials and releases potassium and phosphorous and other elements in available forms. There are also atmospheric nitrogen fixing microorganisms, besides, some soils contain phosphate solubilizing microorganisms, examples are species of Pseudomonas, Bacillus, micrococcus, Asperigillus, Penicillium, Fusarium etc.

From Table 1.2 the $p< 0.05$, the null hypothesis did not hold. Thus different biofertilizer application has appreciable difference in the quality of the remediated soil samples and polluted soil sample. The multiple comparisons indicates appreciable difference between pairs of polluted soil and any remediated soil as all the *p*-values are less than the alpha level, $p< 0.05$. The multiple comparisons also indicates no significant difference between any pair of the remediated soil samples as all the *p*-values are greater than the alpha level, $p> 0.05$.

From Table2.2 the $p> 0.05$, the null hypothesis is upheld. Thus the application of different biofertilizers does not have significant difference in the quality of the remediated soil samples. The multiple comparisons indicate no significant difference between any pair as all the *p*-values are greater than the alpha level, $p> 0.05$.

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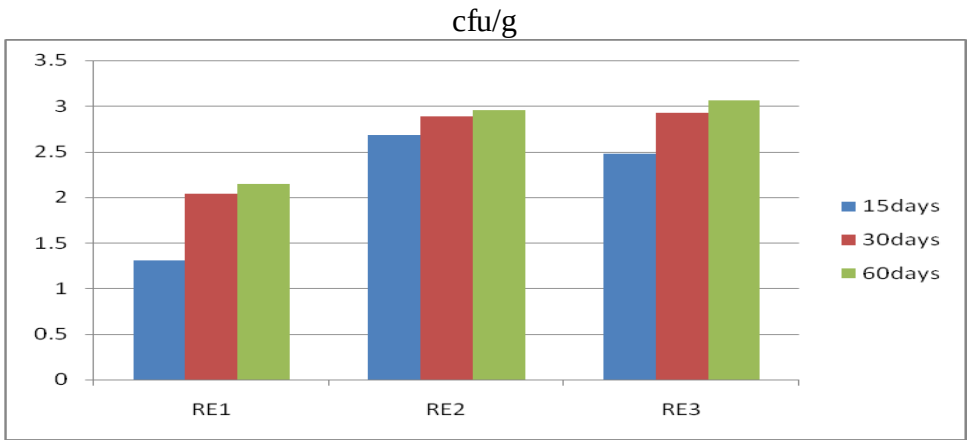

Fig 1 Total Hydrocarbon utilizing bacteria count (x10^6) cfu/g

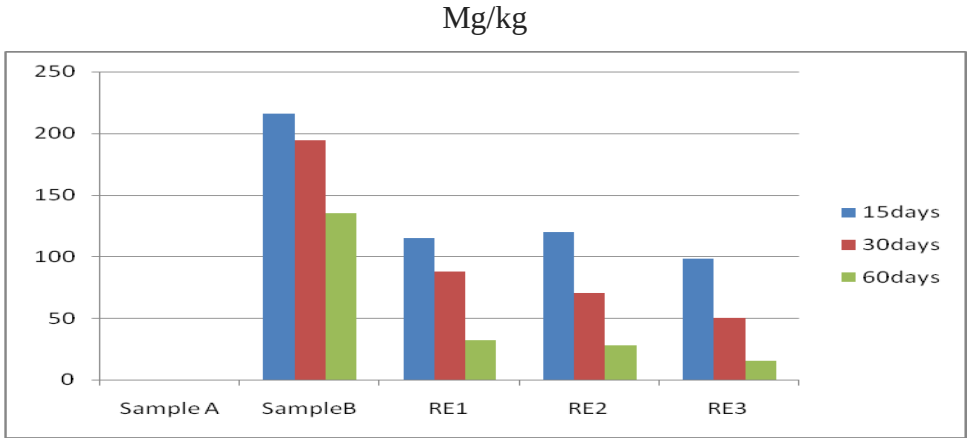

Fig 2 Change in the concentration of Total Petroleum Hydrocarbon

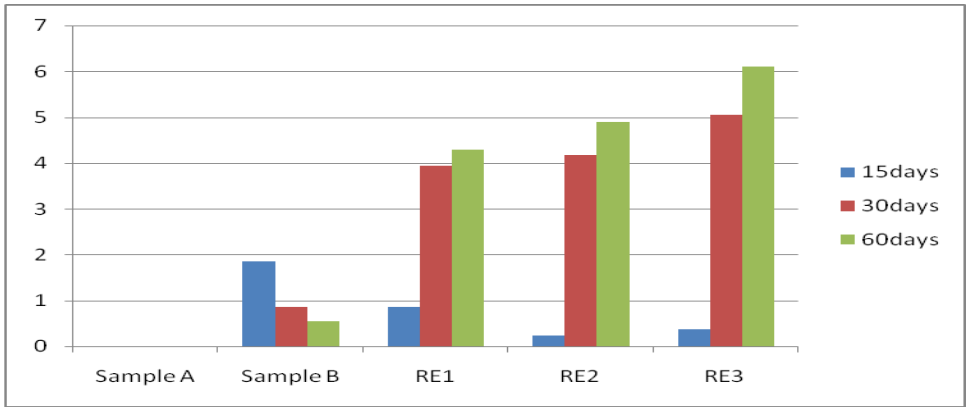

Fig 3 Change in the concentration of Potassium

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Mg/kg

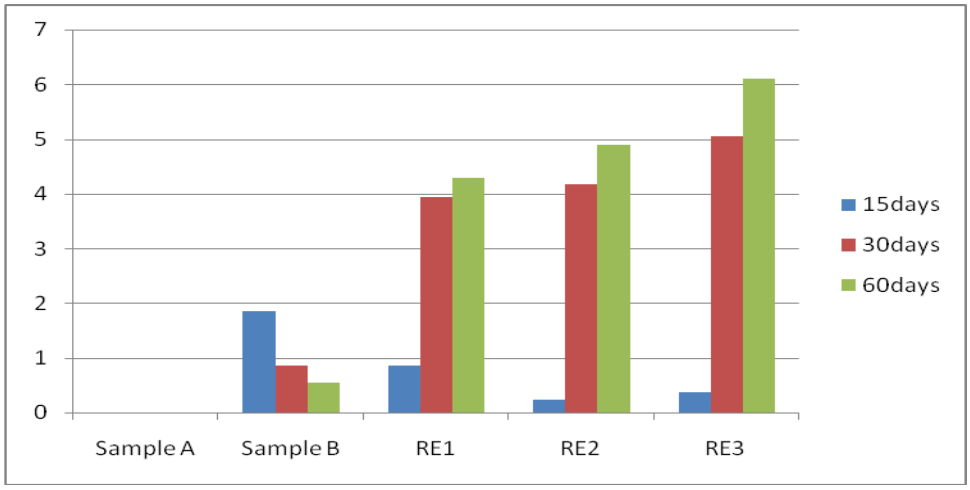

Fig 4 Change in the concentration of Nitrogen

Mg/kg

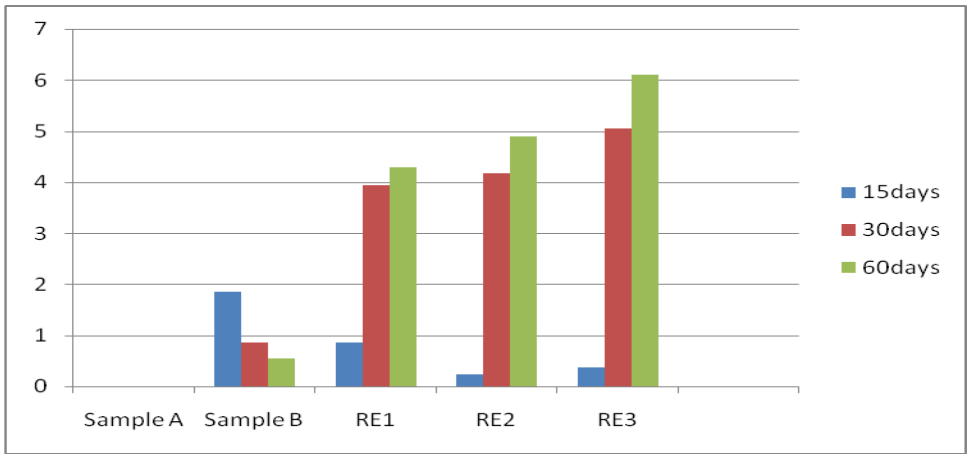

Fig 5 Change in the concentration of Phosphorous

3.1 Hypothesis testing

Hypothesis 1

H_0 There is no significant difference between the quality of the polluted soil and the quality of the individual remediated soil sample.

H_1 There is significant difference between the quality of the polluted soil and the quality of the individual remediated soil sample.

Oneway-ANOVA

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Table 1 Descriptives table for polluted soil sample and remediated soil samples

Descriptives								
VAR00002								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Polluted	9	181.8467	36.27786	12.09262	153.9610	209.7323	135.21	216.28
RE 1	9	106.6400	48.21939	16.07313	69.5753	143.7047	54.42	165.23
RE 2	9	102.7933	48.36943	16.12314	65.6133	139.9734	48.94	160.45
RE 3	9	86.5267	51.13005	17.04335	47.2246	125.8287	31.15	148.74
Total	36	119.4517	57.94827	9.65805	99.8448	139.0585	31.15	216.28

Table 1.1

ANOVA					
VAR00002					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	48769.475	3	16256.492	7.565	.001
Within Groups	68760.610	32	2148.769		
Total	117530.085	35			

Post Hoc Tests

Table 1.2 Multiple comparisons of polluted soil sample with remediated soil samples

Multiple Comparisons						
Dependent Variable: VAR00002						
Scheffe						
(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Polluted	RE 1	75.20667*	21.85187	.017	10.7405	139.6729
	RE 2	79.05333*	21.85187	.011	14.5871	143.5195
	RE 3	95.32000*	21.85187	.002	30.8538	159.7862
RE 1	Polluted	-75.20667*	21.85187	.017	-139.6729	-10.7405
	RE 2	3.84667	21.85187	.999	-60.6195	68.3129
	RE 3	20.11333	21.85187	.838	-44.3529	84.5795
RE 2	Polluted	-79.05333*	21.85187	.011	-143.5195	-14.5871
	RE 1	-3.84667	21.85187	.999	-68.3129	60.6195
	RE 3	16.26667	21.85187	.906	-48.1995	80.7329
RE 3	Polluted	-95.32000*	21.85187	.002	-159.7862	-30.8538
	RE 1	-20.11333	21.85187	.838	-84.5795	44.3529
	RE 2	-16.26667	21.85187	.906	-80.7329	48.1995
*. The mean difference is significant at the 0.05 level.						

Hypothesis 2

H_0 Application of different blends(Polluted soil and biofertilizer) does not have significant difference in the quality of the remediated soil.

H_1 Application of different blend (polluted soil and biofertilizer) has significant difference in the quality of the remediated soil.

Oneway for Comparing the values of the experiments with different biofertilizers

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Table 2 Descriptive table for remediated soil samples with different biofertilizers

Descriptives

VAR00002								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
RE 1	4	17.5950	24.91159	12.45580	-22.0449	57.2349	1.02	54.42
RE 2	4	16.3700	22.05216	11.02608	-18.7199	51.4599	1.68	48.94
RE 3	4	12.9300	12.95148	6.47574	-7.6787	33.5387	2.43	31.15
Total	12	15.6317	18.75845	5.41510	3.7131	27.5502	1.02	54.42

Table 2.1

VAR00002		**ANOVA**			
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	46.795	2	23.398	.055	.947
Within Groups	3823.878	9	424.875		
Total	3870.674	11			

Post Hoc Tests

Table 2.2

Multiple Comparisons

(I) VAR00001		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RE 1	RE 2	1.22500	14.57524	.996	-41.3012	43.7512
	RE 3	4.66500	14.57524	.950	-37.8612	47.1912
RE 2	RE 1	-1.22500	14.57524	.996	-43.7512	41.3012
	RE 3	3.44000	14.57524	.973	-39.0862	45.9662
RE 3	RE 1	-4.66500	14.57524	.950	-47.1912	37.8612
	RE 2	-3.44000	14.57524	.973	-45.9662	39.0862

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4.0 Conclusion

Gasoline polluted farmland was treated with " fermented melon and chicken excreta" blend so as to restore and improve its nutrient content. The hydrocarbon content was highly reduced and plant nutrients were appreciably restored. There was an increase in the numerical strength of microorganisms in both polluted and remediated soil samples. Some authors notion that hydrocarbon polluted soil attracts microorganisms natural especially the hydrocarbon degraders was established. It was established that microorganisms feed and breakdown petroleum hydrocarbon.

Physiochemical properties in the bioremediation experiments showcased that within 15days of experiment the nutrients in the soil diminished because microorganisms fed them. Whereas between 30 and 60 days, there was a progressive increment of these nutrients(N, P, K). This was attributed to nutrient recycle activities of microorganism and nitrogen fixing capability of some microorganisms. In addition, some microorganisms have phosphate solubilizing ability. Therefore, blended biostimulator and bioaugumenetator provides both plant nutrients and large number microorganisms which degrade TPH and as well introduce nutrients which boost agricultural value of soil.

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