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Original Research Article

Enhanced remediation of a long term crude oil polluted soil using soya bean and poultry residues

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The aim of this study is to evaluate the effect of soy bean and poultry residues and its applicability in the remediation of a long term crude oil contaminated soil. Crude oil contaminated and uncontaminated soil samples were randomly collected at 0-30 cm depth from subsurface horizon soil. The controlled experimental setups with different nutrient formulations were exposed to natural weather conditions for 365 days. Crude oil-contaminated soil samples were collected from Bodo West community in Gokana Local Government Area of Rivers State at sites. The Crude oil impacted soil sample was put into six vessels, four of which contained different nutrient amendments (25g of each nutrient formulation) and two untreated controls. Soybean, poultry droppings, NPK fertilizer and a combined nutrient formulation served as the sources of nutrients. The inorganic NPK fertilizer was observed to be more effective in the proliferation of total heterotrophic bacterial population. However, the application of these nutrient sources in combined form was found to be more effective than their individual application. All the sources of nutrient were observed to significantly ($p< 0.05$) induce bacterial proliferation and enhanced the natural attenuation of hydrocarbon pollutants in the soil, compared to the control sample. The efficacy of soy bean and poultry residues as organic nutrients as well as their application in enhancing natural attenuation has been determined from this study. The combined nutrient formulation was observed to be more efficient than the single application of the nutrient sources. These findings are crucial in developing a large scale strategy for field-based applicationsin this long termcrude oil polluted region. This research therefore provides crucial insight for future projects involving optimization of nutrient utilization and degrading abilities of indigenous microbial population.

Keywords: Polluted soil, enhanced remediation, soy bean, poultry residue, crude oil.

INTRODUCTION

Pollutants have been in existence since the inception of time, and life on the earth has always evolved amongst them. With pollutant analogues from volcanic and geothermal activities, comets, and space dust which are about 100 tons of organic dust per day, the earth will constantly be a polluted planet (Megharaj et al., 2011). Oil spills became a cause for international concern in 1967, when approximately 120,000 tons of crude oil was released by the Torrey Canyon super tanker into the English Channel. This first large-scale oil spill forced United Nations

Organization's (UNO's) International Maritime Organization to create in 1973 the International Convention for the Prevention of Pollution from Ships which was aimed at designing emergency protocols and strategies toward oil spills. Ever since then, there have been a number of significant marine oil spills. Oil spills are difficult to avoid during the petroleum processing and delivery (Fuentes et al., 2014).

The soil represents a repository for many hydrocarbons, which is a concern as a result of its adverse impact on human health and also environmental persistence (Towell et al., 2011). The increasing dependence of the Nigerian economy on exploration of hydrocarbon and its extraction has resulted to severe pressures on the environmental components and other receptive systems (Ite et al., 2013), resulting from accidental and incidental discharge of hydrocarbons and their products into the environment. Soil contamination in the Niger Delta has become widespread and assumed international concern (UNEP, 2011), affecting local fishermen and farmers whose major source of livelihood is dependent on rivers and fertile soil. The deleterious effects of crude oil contamination on the flora and fauna of the impacted media usually result in biodiversity loss, as crude oil pollution reduces bacterial population in the affected media, leading to loss in species diversity (Sampson et al., 2016a).

Microbial communities are the bedrock of ecological sustenance by virtue of their ability to govern biogeochemical cycles, plant productivity as well as environmental health in pristine ecosystems (Desai et al., 2009). During the process of bioremediation, microorganisms may convert the chemical contaminants to less toxic compounds or completely degrade the toxic hydrocarbon compounds to CO_2 and H_2O as opposed to simply transferring them from one phase to another (Megharaj et al., 2011; Rahman et al., 2003). The biodegradability of hydrocarbons and their level of persistence in natural environments are influenced by different factors, most crucial of which are: the chemical structure of the hydrocarbons, the availability of viable microbial population able to degrade them and environmental conditions suitable for microbial degradation (Stroud et al., 2007). The microbial degradation of hydrocarbons may be driven by energy needs, or a need for detoxification, or may be fortuitous in nature (co-metabolism). Some unique qualities of microorganisms which include: their ubiquity, (Curtis et al., 2002), wider diversity in their catalytic mechanisms (Chen et al., 1999; Paul et al., 2005), as well as their ability to function in the absence or scarcity of oxygen and other extreme conditions (Mishra et al., 2001; Watanabe, 2001); have warranted the search for pollutant-degrading microorganisms.

An understanding of the microbial community structure of contaminated sites and how it is affected by factors which may be limiting hydrocarbon degradation will enable optimization of remediation activities and also give a broader insight of contaminated sites (Powell et al., 2010).

Hydrocarbon-degrading microorganisms have been found in a wide range of polluted and non-polluted environments including soil, sediment, groundwater, sea-water and sea-ice. As the study of these organisms progressed, it has become evident that the ability to degrade hydrocarbons is not restricted to a narrow range of microbes nor is it mediated by a few enzymes (Rojo, 2009). Biodegradation of total petroleum hydrocarbons is particularly limited by the low availability of contaminant compounds due to their low water solubility and strong absorption in inorganic and organic soil components. This phenomena is the resultant effect of a notable decrease in the mass transfer rate as most carbon sources are unavailable to the cells and is regarded as a major bottleneck in the efficiency of biodegradation (De la Cuvera et al., 2016). The ability of soils to release pollutants determines its susceptibility to microbial degradation, thereby influencing effectiveness of the bioremediation process (Megharaj et al., 2011).

Several organic and inorganic substances have been applied to enhance bioremediation process. These inorganic fertilizers include: compost materials, poultry residues and periwinkle shell (Orji et al., 2012). While the application of inorganic fertilizers as nutrient sources have been extensively explored, the challenges associated with soil and plant toxicities, likelihood of eutrophication as well as huge cost during remediation still exists (Nwankwegu et al., 2016)

The propensity to metabolize hydrocarbons is displayed by a variety of microbes. By understanding the factors that influence microbial diversity, it is expected that they can be utilized to enhance bioremediation of contaminated sites. Soil microbial degradation capacity is usually limited to bioavailability of the hydrocarbon and environmental factors such as availability of carbon substrates, nitrogen or other nutrients, aeration conditions, temperature and pH (Wu et al., 2008).

The aim of this study was to evaluate the biostimulation potentials of soya bean and poultry residues in the remediation of an aged crude oil contaminated soil.

MATERIALS AND METHODS

Description of the area under study

Bodo is a host community to the shell Petroleum Development Company's oil operations(Figure 1). An estimate of about 20-km^2 network of creeks as well as inlets in Bodo community have been distressed by crude oil spills especially due to leaking pipelines and sabotage (The Guardian 2018).

Sample collection and preparation

Crude oil contaminated and uncontaminated soil samples were randomly collected from Bodo West Community in Gokana Local Government Area of Rivers State, at 0 – 30cm depth from subsurface horizon soil using hand auger, from

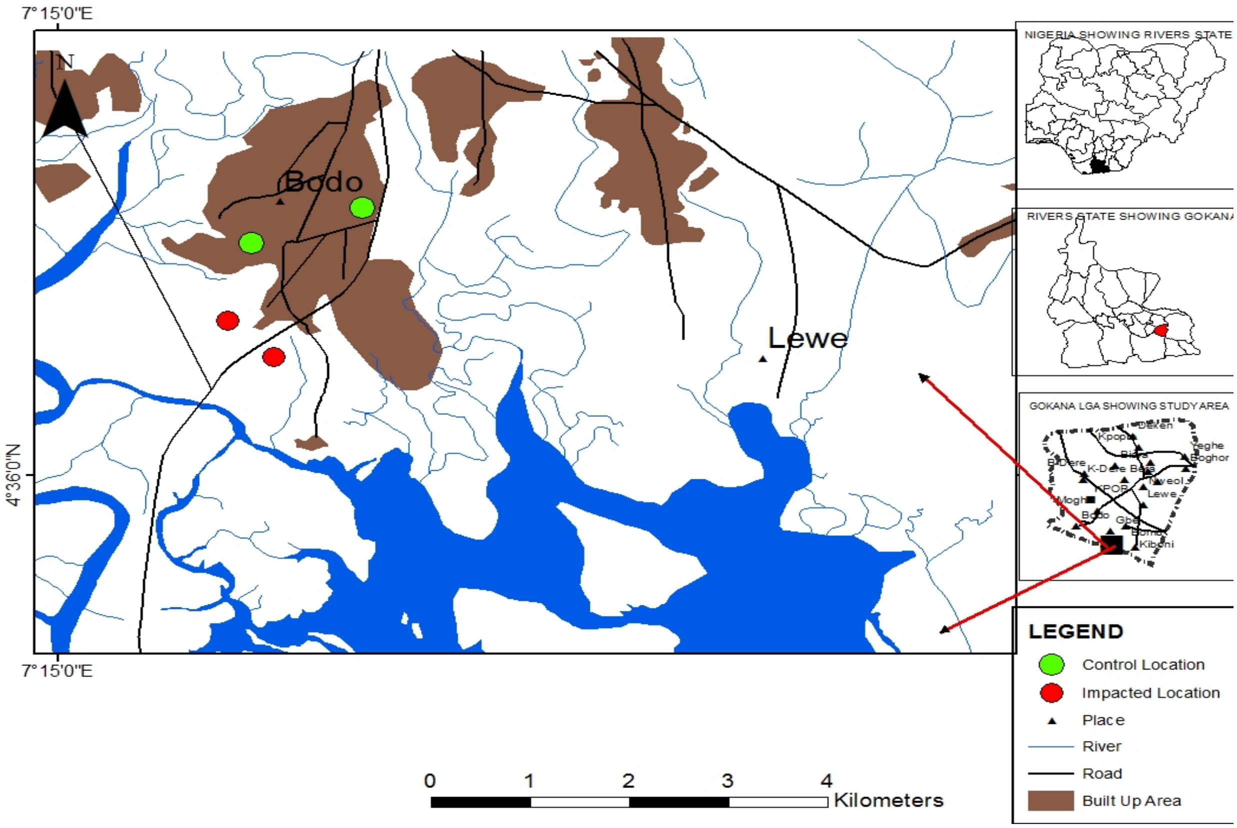

Figure 1: Sampling locations

sites/points geospatially located within latitude 7°15 53.552E and 4°37 0.18N, 7°46 10.195E and 4°36 46.164N. The samples were aseptically transported to the laboratory for further analysis and preserved as described (Sojinu et al. 2010). Soybean waste and poultry droppings were incorporated as the organic nutrient sources. The soybean waste was sourced from a local soybean milk processing site at Bori Camp, while the poultry droppings used was obtained from a poultry farm at Mgbuoba, all in Port Harcourt, Rivers State, Nigeria. A commercial NPK fertilizer (10:10:10) by Spring Field Agro LTD was applied as the source of inorganic nutrient.

Experimental Procedure

The crude oil impacted soil sample was amended using four different nutrients (organic and inorganic types) and kept exposed to natural weather conditions for 365 days. Four kilograms (4kg) of the hydrocarbon contaminated soil was put in vessels and 0.6% of nutrient formulation was added with an exception of the combined nutrient treatment that contained 8.4 g of each of the nutrients. The different vessels were in triplicates (except the contaminated control and pristine soil) as described in Table 1. Tilling was employed weekly to ensure the distribution of oxygen in the setups except the unammended setup.

Enumeration of Total heterotrophic bacterial counts and Hydrocarbon utilizing bacterial counts

Hydrocarbon utilizing bacteria count in soil was determined using vapour phase transfer. A serial 10-fold dilution of the soil sample was carried out by weighing 1 g of the soil sample into a sterile test tube containing 9ml of sterile physiological saline. A serial ten-fold dilution was carried out and 0.1ml was inoculated unto Bushnell Haas agar (Sigma-Aldrich, USA) which was incubated for 7 days at 30°C.

The population of heterotrophic bacteria was enumerated using the spread plate technique. From each dilution, 0.1 ml was inoculated on nutrient agar plates (petri dishes). Duplicate plating of each dilution was employed and media were incubated at 30°C for 24 hours.

Monitoring Bioremediation

Measurement of pH and Total Petroleum Hydrocarbons (TPH)

Physicochemical parameters such as soil pH, total organic carbon, phosphate as well as nitrate contents were determined using the methods from APHA (2008). Total petroleum hydrocarbons (TPH) and polycyclic aromatic

Table 1. Bioremediation setup

Treatment	Composition
Sample A	4kg soil + 0.6% soy bean waste (SBW)
Sample B	4kg soil + 0.6% NPK fertilizer (NPK)
Sample C	4kg soil + 0.6% poultry dropping (PD)
Sample D	4kg soil + 0.2% (SBW, PD and NPK)
Sample CS	4kg unamended crude oil contaminated soil (CS)

Table 2. Physicochemical and microbiological properties of the soil samples

Parameters	Contaminated soil	Pristine soil
pH	7.6 ± 0.1	6.9 ± 0.3
Total organic carbon (%)	0.95 ± 0.02	0.70 ± 0.04
TPH (ppm)	48,501 ± 0.50	105 ± 0.60
PAH (ppm)	16,320 ± 0.44	16.5 ± 0.3
Nitrate (mg/kg)	1.39 ± 0.02	11.5 ± 0.05
Phosphate (mg/kg)	2.16 ± 0.11	12.4± 0.6
Moisture (%)	29.6 ± 0.25	25.2 ± 0.2
Silt (%)	2.28 ± 0.22	3.51 ± 0.05
Sand (%)	91.7 ± 0.55	95.3 ± 11.05
Total heterotrophic bacteria (cfu/g)	3.6×10^7	5.9×10^7
Hydrocarbon utilizing Bacteria (cfu/g)	6.1×10^6	8.5×10^6

Parameter values are presented as mean ± standard deviation of triplicates

hydrocarbons (PAH) were determined using a gas chromatograph-flame ionization detector (GC-FID) as described by ASTM (2010).

Statistical Analysis

One-way Analysis of variance was done using Minitab Statistical software (version 17, Minitab Inc., USA) to check for significant difference in the values of the various treatment options. A 2-tailed Pearson's Moment Correlation coefficient ρ was used to determine degrees of relationship between the various parameters.

RESULTS AND DISCUSSION

Physicochemical and microbiological properties of the soil samples

From the physicochemical and bacteriological analysis of the pristine and the crude oil-contaminated soil, the pH of the pristine soil was 6.9 ± 0.1 while the initial pH of the polluted soil was 7.6 ± 0.3 (Table 2). This shows that pristine soil and crude oil impacted soil had different degrees in the level of acidity and alkalinity. Although different factors influence the pH of a system, the acidity or alkalinity of soil plays an important role in bioremediation. The values of nitrates, phosphates, silt and sand as presented in Table 2 revealed that the pristine and crude oil polluted soil had variations in the their physicochemical and bacteriological characteristics. These variations are attributable to the impact of crude oil contamination on soil microbiota and physicochemical properties.

pH changes during bioremediation

The initial pH of the crude oil-contaminated soil prior to biostimulation (in August 2017) was 7.6 ± 0.1. The pH changes was monitored quarterly (Dec 2017, April and June 2018) and a slight decline to 7.3 was observed in the unammended by the fifth month (December, 2017). All the biostimulated samples still maintained neutral pH range in the fifth month with exception of the combined treatment sample which had a pH of 6.9 in the soil. In the last month of sampling, all the amended samples attained acidic pH concentrations with the combined treatment plot having the most acidic pH of 5.9 in soil.

The pH values obtained from this study is similar to the report of Linden and Palsson (2013) that carried out an extensive chemical analysis of over 40 sites in Ogoni land and reported an average pH of 6.1. Soil pH of 7.5 has been previously reported to be optimal for biodegradation by hydrocarbon utilizing bacteria. The gradual decline in pH over time in this study (Figure 2) suggests biodegradation of hydrocarbons by hydrocarbon utilizing microorganisms. An increase in total heterotrophic bacterial count (THBC) was observed in the soybean treatment plot by the fifth month from 5.2×10^5cfu/g to 7.6×10^5cfu/g by the ninth month with a corresponding decline in pH from 7.0 to 6.6. According to Sihag et al. (2014) *in situ* microorganisms at a crude oil-contaminated site may not only be tolerant to the site conditions, but may have the propensity to utilize

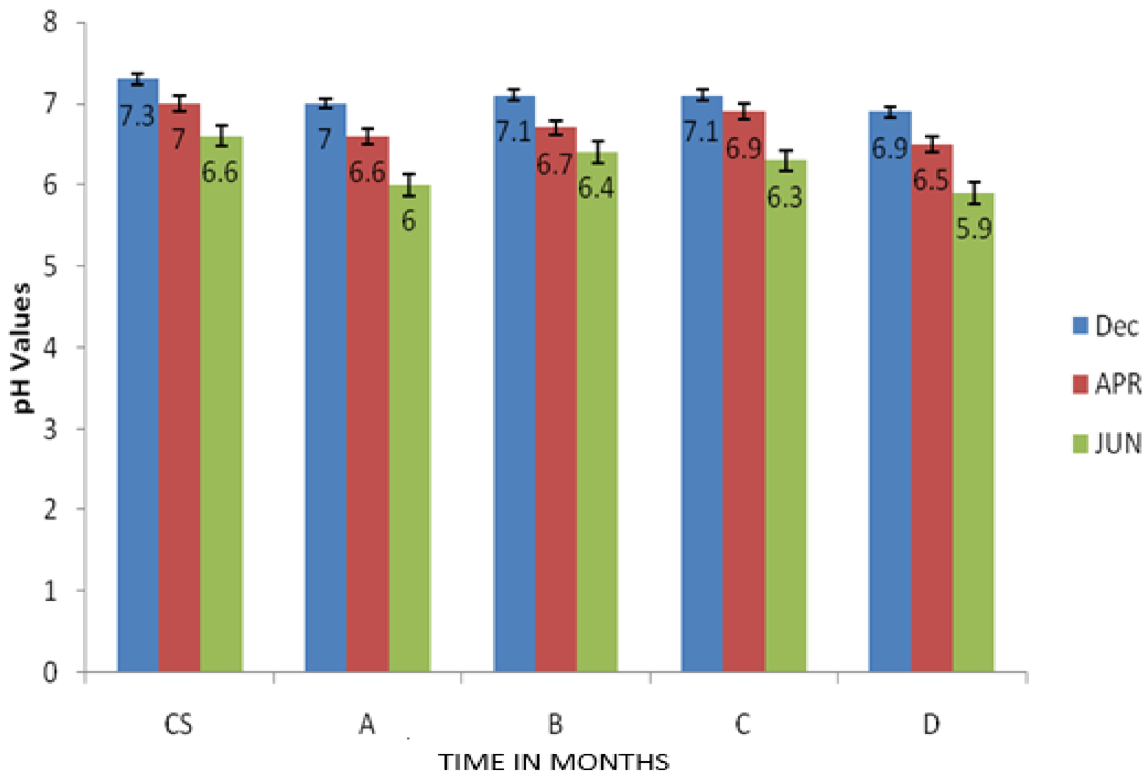

Figure 2: Changes in pH concentration with time in soil samples obtained from various treatment set-ups

hydrocarbons in sub-optimal conditions (in the case of high pH).

Bacterial population dynamics in soil during bioremediation

The baseline bacterial population assessed prior to bioremediation setup were determined to be 33.6 x 10^7cfu/g and 6.1 x 10^6 cfu/g for total heterotrophic bacterial and hydrocarbon utilizing bacteria, respectively. The changes in bacterial population were evaluated bi-monthly to cover the months of October 2017, December 2017, February, April and June 2018 as presented in Figure 3. The heterotrophic count of bacteria in the NPK amended soil sample after 2months was 4.58×10^7cfu/g and varied from 1.72x10^6 to 2.90×10^7cfu/g over the period of study. The bacterial load showed a continual decline as the degradation progressed probably due to the depletion of nutrients.

In the contaminated soil, the bacterial count drastically reduced from 5.60x10^6cfu/g to 1.7×10^6cfu/g after 8months of study indicating a negative effect of crude oil on the soil bacterial flora. In the soils treated with poultry dropping, the bacterial count was 2.09×10^7cfu/g after 2months of study. It decreased to 8.60 x10^5cfu/g after 4months and then reduced further to 2.6×10^5cfu/g after 6months. The count was however observed to increase after the 6th month. This could have been attributed to the ability of the organisms becoming resistant to the hydrocarbons and utilizing them as carbon sources. A similar trend was observed for the soybean amended soil sample as well as the treatment combinations. The count after 6 months reduced to 3.3×10^5cfu/g and 4.30×10^6cfu/g from 1.57×10^7cfu/g and 7.4×10^7cfu/g in soil microcosms containing soybean and treatment combinations respectively.

The counts further rose after the 6months as was also observed for the poultry dropping biostimulated soil. It was observed, that the combined nutrient setup stimulated bacterial proliferation more than individual application. Also, the sources of nutrient applied as single treatment regimen stimulated bacterial growth in the order NPK > poultry dropping > soybean residue (Figure 3). This difference is attributed to the physicochemical properties of the nutrients. NPK is a compound fertilizer composed of nitrogen, phosphorus (P_2O_5) and potassium (K_2O), and this property could be responsible for the higher efficiency of NPK.

Poultry and soybean waste are organic in nature and organic manures are believed to be slow release in nature and may be affected by the early stage nutrient deficiency phenomena as stated by Sampson et al., (2016). It follows that at the early state the nutrients are released at rates that do not support much microbial growth necessary for high microbial proliferation, compared to the direct inorganic fertilizer application. Inorganic fertilizers like

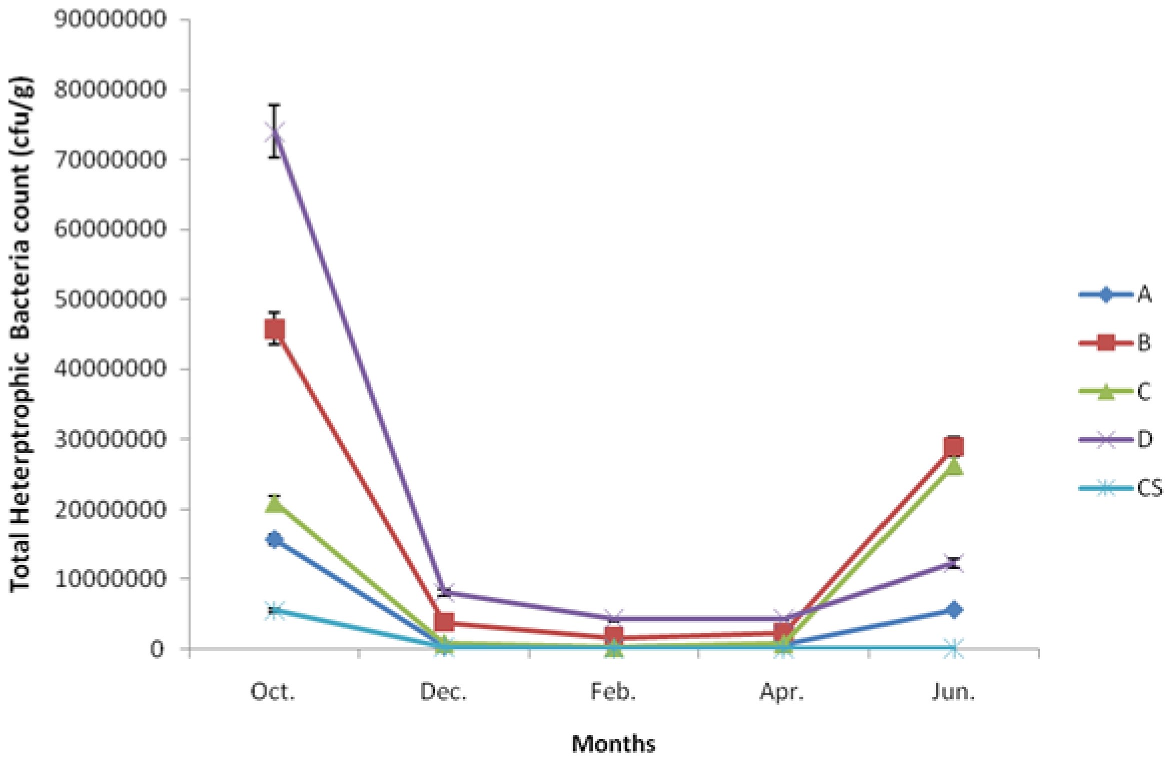

Figure 3: THB counts and distribution as remediation progressed according to treatments in the soil samples

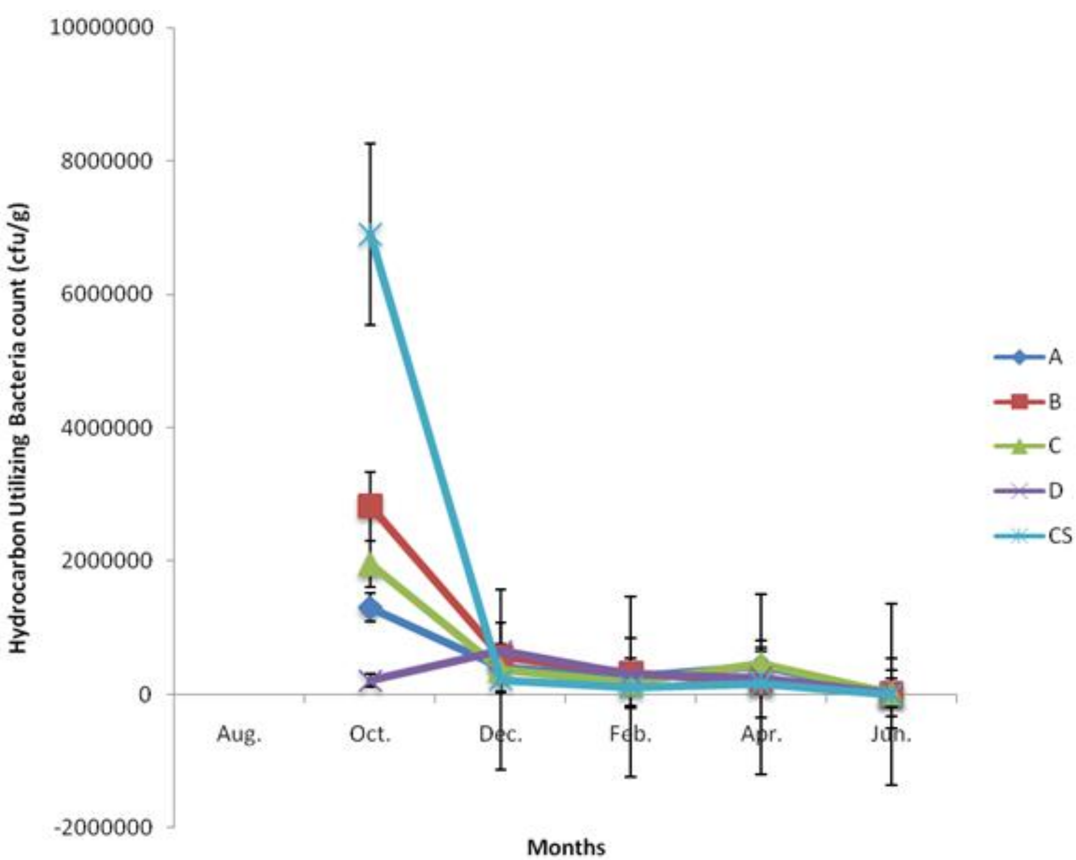

Figure 4: HUB counts as remediation progressed according to treatments in the soil samples

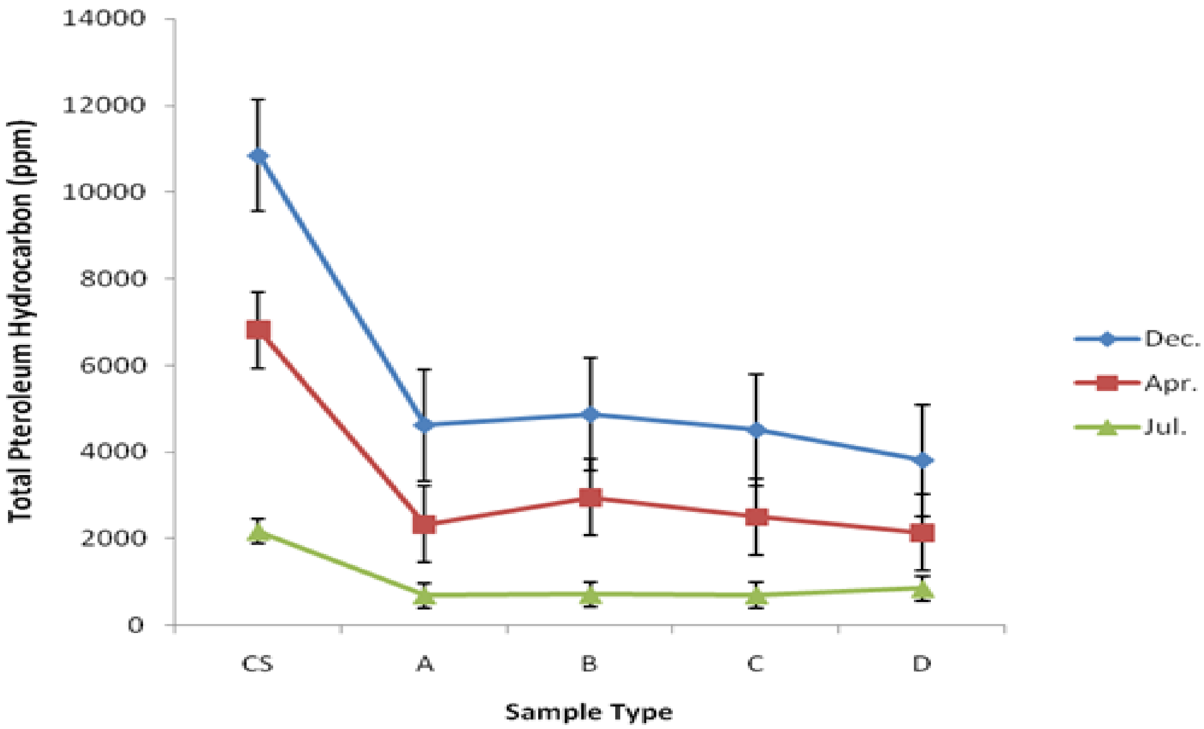

Figure 5: Degradation of TPH in the various soil sample

NPK are associated with leaching and the need for frequent application that eventual results in underground water pollution. However, this research has provided a guide in developing bioremediation strategies that targets the optimization of bio-stimulating potentials of organic and inorganic nutrient formulations. The findings from this investigation show that a combined application of organic and inorganic nutrients in the appropriate ratio will cause a faster rate microbial growth and a concomitant removal of pollutants from the environment.

Nutrient amendment and the fate of Petroleum Hydrocarbon in soil

The TPH contents in the contaminated and pristine soils were found to be 48501ppm and 105ppm, respectively. TPH levels in the soil samples were higher than the global average permissible limit of TPH for soil (1000ppm), indicating high Petroleum Hydrocarbon pollution. The concentration of TPH observed for the contaminated soil in this investigation was however higher than that of Pathak et al. (2011) where they observed high concentrations of 11149 mg/kg and 14244 mg/kg TPH in soils contaminated with PHC and engine oil respectively as compared to uncontaminated soils (614 and 700 mg/kg)(Figure 4). They suggested the probability of reduced microbial population in these polluted soil samples. Uche et al. (2011) also reported high TPH concentration (>200 mg/kg) in surface and sub-surface soil samples collected from crude oil polluted sites which far exceeded the 50 mg/kg compliance baseline limit set for petroleum industries in Nigeria.

The changes in TPH was monitored quarterly (Dec 2017, April and June 2018) and it was discovered that the total petroleum hydrocarbon content reduced significantly ($p < 0.05$) as during the study period (Figure 5). The addition of poultry droppings to the oil contaminated soil in this study reduced up to 98.6% of total petroleum hydrocarbons (TPHs) within 11months of the study. This result is in agreement with earlier findings of Chorom et al., (2010). Soybean waste on the other hand has also been used as a source of organic nitrogen in the bioremediation of several oil-contaminated soils (Diab, 2013). The addition of Soybean waste has been shown to enhance hydrocarbons bioavailability (Diab and Sandouka, 2012). Previous studies showed an increase in the degradation of motor oil to reach 60–90% upon the addition of soybean waste (Diab, 2013). Soybean waste was also used in the bioremediation of palm oil mill effluent (Ibegbulam-Njoku and Achi, 2014). The reduction observed in this study was in agreement with that obtained in a related study by Aghalibe et al. (2017). Increased oil degradation rates by the soybean biostimulated sample treatment could be attributed to the active microbial community in the treatment.

Successful bioremediation events as was observed from this investigation have been reported for various hydrocarbon contaminated sites, wherein, amendment of appropriate inorganic nutrients (N and/or P) resulted in enhanced growth and activity of efficient indigenous microorganisms, thus, expediting bioremediation (Smith et al., 2015). Nutrient (in the form of nitrate) amendment has been found to be one of the most efficient biostimulation approach, owing to thermodynamic favorability of nitrate which facilitates efficient oxidation of carbon substrates, allowing bacterial growth as well as hydrocarbon catabolism (Dashti et al., 2015; Bell et al., 2016).

Addition of substrates favorably alleviates the nutrient deficient state of high organic carbon-rich sludge and helps in stimulating an array of biogeochemical transformations (Mason et al., 2014).

CONCLUSION

The bioremediation enhancement potentials of soy bean, poultry residues as well as NPK inorganic fertilizer has been determined in this study. The biostimulation strategy involved the single and combined direct application of these sources of nutrients with the sole target of restoring a long term crude oil impacted soil to its pristine state.

From the analysis, the treatment options varied in their degree of biostimulation of total hydrocarbon utilizing bacteria, with the combined nutrient treatment performing better than NPK> poultry waste > soy bean residues after the fourth period of analysis. There was also a concomitant and significant ($p < 0.05$) reduction in the concentration of residual petroleum hydrocarbon at the end of the investigation.

It can thus be inferred that bioremediation remains a slow process especially in long term polluted areas, and requires technological approach to enhance this process. By implication, natural attenuation can be enhanced by harnessing the hydrocarbon utilizing potentials of autochthonous bacterial population, while taking into cognizance their nutrient requirements; to improve on their bioremediation efficiencies.

This research therefore provides important data set for future studies involving the optimization of the nutrient utilization and degrading capabilities of indigenous microbial population.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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