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Potentials of Sugarcane Bagasse and Poultry Manure in the Remediation of Spent Motor Oil Contaminated Soil

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Authors' contributions

This work was carried out in collaboration between both authors. Author IOI managed the literature searches, designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript while author AYS corrected the first and final draft. Both authors read and approved the final manuscript.

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ABSTRACT

This study sought to investigate the potential of sugarcane bagasse, poultry manure and their combination in the remediation of spent motor oil contaminated soil. About 1.5 L of spent motor oil was sprinkled on plots measuring 0.5 m x 0.5 m dimension in 12 replicate plots to simulate a major spill. Poultry dung (PD), Sugarcane bagasse (SB) and hybrid of bagasse and Poultry dung (SB - PD) were applied as organic amendments and bulking agents. The amendments were randomly administered twice within two weeks to treatment plots except the control at rate of 24tons/ha. The soil was tilled to enhance aeration and watered twice a week for 56 days. A significant decrease in Total Petroleum Hydrocarbon, TPH of 61, 53, 46 and 23% for PD, SB - PD, SB and Control plots respectively was observed. TOC and TN decreased in all the plots except the control. Soil moisture contents between 21 and 24% while soil pH within the range of 6.9 to 7.5 enhanced biodegradation process. The Total Heterotrophic Bacterial Counts (THBC) varied in all the plots while heavy metals content remained unchanged during the study. The application of these biostimulants gave promising results on hydrocarbons removal from contaminated soil.

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1. INTRODUCTION

Industrialization and urbanization have resulted in dramatic changes in the nature and composition of wastes from Nigerian cities [1]. This can be attributed to economic activities largely driven by the use of petroleum products. The emergence of urban sprawls has led to population explosion and unprecedented wastes generation. Refined oil namely lubricant, engine and transmission oils are largely used for maintenance of equipment and systems in the automobile and industrial sectors. In Nigeria, attention has largely been shifted to the use of imported used vehicles, machines and spare parts due to hike in price of new ones. As a result, frequent breakdown of machines and oil discharges have characterized most Nigerian environment.

Spent oil is the already used lubricant drained from engine parts and is often mixed with water or impurities which could adversely affect its viscosity. Spent motor oil contains heavy metals (e.g. lead, arsenic, cadmium, zinc) and polycyclic aromatic hydrocarbon (e.g. benzene, ethylene, toluene and xylene) which are generally believed to be mutagenic and carcinogenic [2]. Because of the health risk it poses to soil and water bodies, concerted efforts are required globally to salvage the environment from the menace. Oil spills from auto mechanic service centres, heavy and light duty machineries is a major contaminant of the environment. According to United States of Environmental Protection Agency [3], 1L of spent motor oil is capable of polluting one million gallons of freshwater. It is estimated that about 300million litres of lubricating oils are consumed annually in Nigeria and close to 80% of this is largely exposed to the environment [4]. Improper disposal of spent motor oil in drains or on land can endanger the environment as well as pollute ground and surface water supplies.

Large dosage of inorganic hydrocarbon in soil can create carbon-nitrogen imbalance. The growth of micro-organism and the utilization of carbon source can also be inhibited when oxygen is in short supply. Measures designed to alleviate the challenges of contaminated soils include the physical, chemical and thermal methods. Recently, researchers have started exploring the suitability of biological stimulation methods. It focuses on the use of micro-organism present in the soil to degrade hydrocarbon pollutants through reactions that takes place as part of their metabolic processes [5]. It involves the addition of organic and inorganic fertilizers which have multiplier effects on the microbial population thereby using them as part of catabolytic enzymes to accelerate the breakdown of contaminants [6]. Biostimulation processes can be accelerated by using additives such as sawdust, peat, waste-cotton, organic and inorganic fertilizers [7].

Agricultural residues are in abundance, non toxic and generally eco-friendly. They have a role to play in solving environmental problems. The use of agricultural residues and animal waste namely sugarcane bagasse and poultry manure is wide-spread among studies in the remediation of contaminated soils because bacteria and fungi can thrive in them. Bagasse particle is the remnants coagulated after the solid sucrose had been extracted. It is burned as fuel in the sugar mills to produce steam and electricity. The efficacy of sugarcane bagasse in the removal of weathered hydrocarbon from contaminated tropical soils has been reported [8,9]. Poultry manure has similar biodegradability potential and is often used to improve soil fertility. Its efficacy in promoting plant growth in hydrocarbon polluted soils has also been reported [10]. This study is aimed at investigating the potential of sugarcane bagasse, poultry manure and their combination in the remediation of spent motor oil contaminated soil. It also sought to examine the effect of the biostimulants on the physico-chemical properties of spent motor oil contaminated soil.

2. MATERIALS AND METHODS

2.1 Site Description

The study was conducted on a demonstration plot at the Department of Agricultural and Environmental Engineering, Faculty of Technology, University of Ibadan, Nigeria. Each plot was measured and divided into three sub plots each of dimension 0.9 m by 0.9 m. All adjacent plots were constructed with bricks and separated by 0.5 m gap. The bricks served as buffers and were raised to depth of 20 cm below and 10 cm above ground surface. The aim was to minimize interaction between the plots and sub plots as well as check runoff. The buffers were used to prevent leaching of contaminants and thus forestall possible pollution of adjacent land.

2.2 Sample Collection and Preparation

The bagasse residues were collected from Ojoo market in Ibadan and sun dried. The dried residues were crushed into fine dust form by ball milling. Poultry manure was obtained from a private farm in Ibadan. Spent motor oil was obtained from the auto-mechanic service centre of the Maintenance Department, University of Ibadan. Soil without prior history of contamination was excavated between 0 – 20 cm depths around the Computer Based Test (CBT) Centre of the University and transported to the study site.

2.3 Experimental Design

Four triplicate beds that consist of three sets of triplicate treatment and a triplicate control beds were constructed in all the sub plots in a random manner using a square wooden box. The wooden box of dimension 0.5 m length, 0.5 m width and 0.2 m height was used to raise the 12 replicate plots into beds which allowed the control of depth and exposed surface area of soil for nutrient concentration. The gap left between the beds and the buffer (about 20 cm on both sides of the bed) was to give room for sufficient aeration during tilling operation. They were labelled randomly as plots A - F, P - R, and Control O1 – O3.

2.4 Soil Treatment

Condition of a major spill was simulated by the application of 1.5 L of spent motor oil on each replicate bed. The application was done by sprinkling the oil through a perforated cylindrical can on the bed surface. The plots were left alone for a period of two weeks before the commencement of soil sampling. The objective was to achieve proper acclimatization between the soil and the oil. Sub plots were scheduled to receive Bagasse dust (SB), Bagasse dust-Poultry manure (SB - PD) mix and Poultry manure (PD). The nutrients were applied at 100% SB, 100% PD and 50-50% SB - PD concentration. Three kilograms of the specified amendments of each mix were incorporated into the soil twice within the first 28 days. Water application rate was done at a level of 2 L of water twice weekly as recommended in literatures. Tilling was done daily on all beds except the control plot using shovel and hand trowel. This provided maximum aeration, even distribution of nutrients, microbes and water supply in the contaminated soil. The control O1 – O3 received no nutrient application and was only rain fed.

2.5 Determination of Physicochemical Parameters of Soil

Soil particle size distribution, bulk density, porosity, moisture content were determined using procedures described in [11]. Electrical conductivity and total nitrogen content of the soil was determined using the Kjeldahl method [12]. The total organic carbon content and organic matter was determined by the Walkey-Black method [13]. Soil phosphate was determined using methods described in [14]. The total heterotrophic bacterial (THB) count was performed on nutrient agar (Oxoid) using the spread plate method [15]. The residual Total Petroleum Hydrocarbon, TPH remaining in the soil after incubation period was determined using the method described by [16]. The percentage degradation of TPH was calculated as follows:

$$\%\ \text{Degradation} = \left(\frac{C_o - C_t}{C_o}\right) \times 100 \qquad (1)$$

C_o: Initial concentration in g/kg, C_t: final concentration in g/kg

2.6 Statistical Analysis

Two-factor analyses of variance (ANOVA) and correlation coefficient were used to analyse experimental data for all physico-chemical parameters investigated. This was used to investigate the significance amongst the various types of amendments applied during the study period. All statistical analysis was carried out using Excel 2010 Microsoft tool. Differences was set as significant at $p<0.05$.

3. RESULTS AND DISCUSSIONS

The results of the physico-chemical characteristics of the soil sample before contamination are shown in Table 1.

3.1 Changes in Moisture Content

There was a sharp increase in moisture level in all the plots after two weeks of treatment as shown in Fig. 1. After four weeks of treatment, a significant ($P<0.05$) drop in moisture content was noticed in all the plots. Such drop can be attributed to the rapid use of water by microbes during mineralization while carrying out enzymatic activities. This translated into rapid multiplication of THB counts during the period. In this study, moisture removal rate was highest in the fourth week of remediation at a level of 21.3% and 24.8% in plots treated with poultry manure and control respectively. Molina-Barahona et al. [8] reported that high TPH removal can be obtained when soil moisture content are kept at about 20% using crop residues.

It appears that for biodegradation to occur in a spent motor oil polluted soil, a moisture level of between 22 and 28% is required to sustain microbial survival especially in a tropical environment. In the last week of the remediation, there was an increase in moisture in all the plots. This is attributed to increase in precipitation during the period and may result in slowing down biodegradation process by a decrease in microbial count.

Table 1. Physico-chemical characteristics of soil sample

Parameter	Values	Parameter	Values
Sand (%)	79.2	Electrical conductivity (µS/cm)	49.1
Silt (%)	10.0	Total nitrogen (%)	0.78
Clay (%)	10.8	Total organic matter (%)	6.6
Soil texture	Sandy Loam	Phosphate(mg/kg)	55.5
Porosity	0.88	TPH(mg/kg)	19.5
Bulk density(g/cm3)	1.79	Organic carbon (%)	3.85
pH	6.4	C/N ratio	4.9
Moisture (%)	33.5	THBC(CFU/g)*10^7	8.3

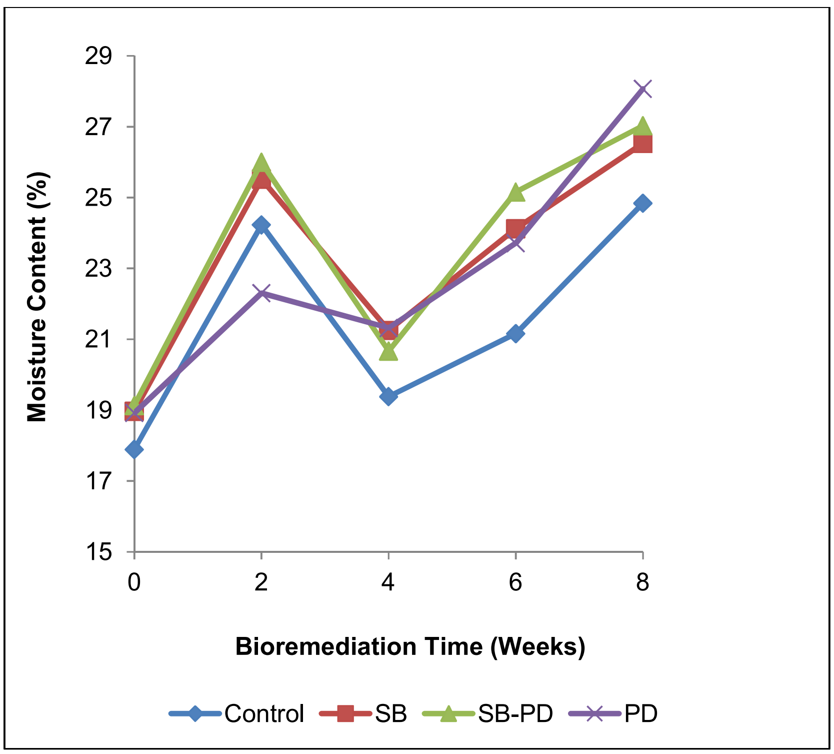

Fig. 1. Variation in moisture content during remediation

3.2 Soil pH

Following the addition of nutrients, soil pH increased in all the plots as shown in Fig. 2. This is in line with observations reported by [17] where increase in pH was recorded after two weeks of remediation. This was attributed to the breakdown of hydrocarbons and the release of ammonium ions. The rise in pH could also be due to microbial adjustment to a newly found environment induced by the supplementation of nutrient and oxygen thus resulting in the secretion of enzymes necessary for metabolism. After 28 days of treatment, there was a general drop in the pH with time in all the treatment cells. The possible reason for pH drop can be the degradation of organic matter and hydrocarbons which was accompanied by the production of acidic intermediates and CO_2 [18]. The pH value that enhanced the removal of hydrocarbon from polluted soil ranges from 6.9 to 7.5. From the study, it can be inferred that the range of pH observed can support degradation by metabolites and enzymatic activities by microbes as recommended by [19].

3.3 Total Organic Carbon

After 14 days of simulation, there was noticeable increase in organic carbon in all plots. Petroleum derivatives such as crude oil are known to contain between 84 and 87% carbon [20]. Two weeks into the remediation period, there was a marginal increase in the TOC in all the treatment cells as shown in Fig. 3 with the possible exception of the PD plot. The increase might be due to trapped leachates and the organic carbon aggregation occasioned by rapid evaporation within plots [21]. Such development may have been predicated by the microbial mineralization of hydrocarbons as previously observed by Obasi et al. [22].

The increase in SB and SB - PD plots could be due to carbohydrate release from bagasse. As bioremediation progressed, total organic carbon decreased with time. On average, the decreases in total organic carbon were in the order of 15, 40, 46 and 48% in Control, SB, PD and SB - PD plots respectively. When the data for TOC and TPH loss during the period are compared, a significantly positive correlation was observed. This is contrary to the observation by Odokuma et al. [23]. This may be due to longer period required by the microbes to utilize the organic amendments for an appreciable decomposition. The reduction in TOC led to decrease in the amount of TPH concentration in the amended plots.

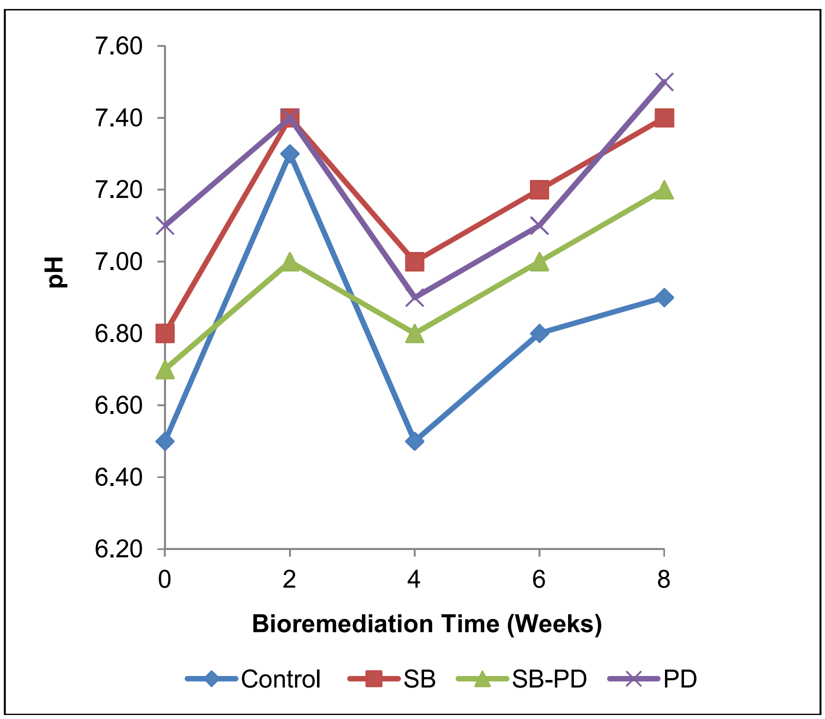

Fig. 2. Variation in pH during remediation

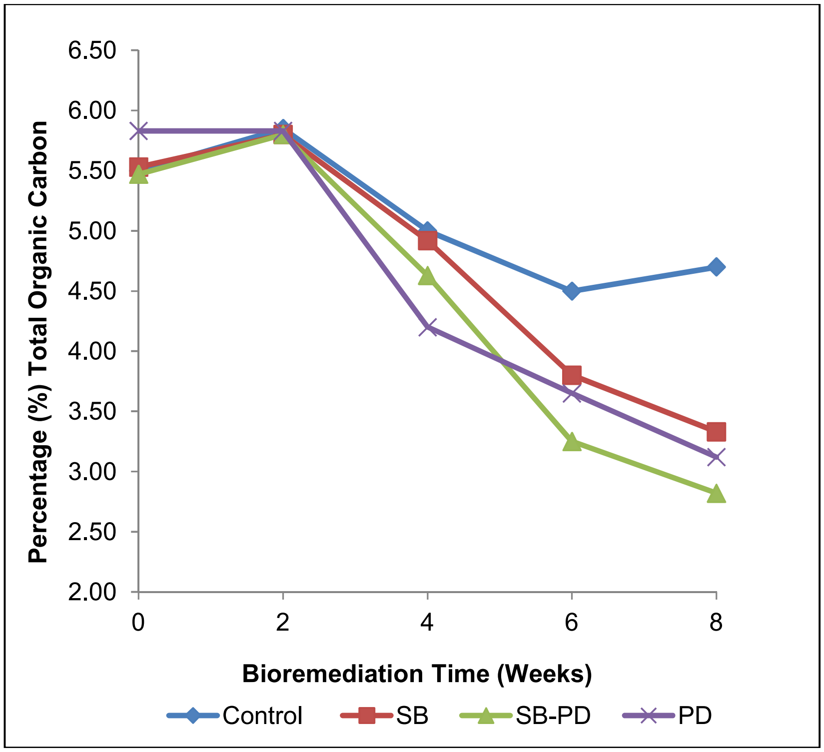

Fig. 3. Percentage (%) total organic carbon during remediation

3.4 Changes in Total Nitrogen

Two weeks after the application of nutrients, there was a marked variation in nitrogen level especially the Control and PD plots (Fig. 4). The bagasse and hybrid simulated plots (SB - PD) did not record any change in nitrogen concentration. This was not expected considering that nutrients was added to the contaminated soil and apparently would have decreased the nitrogen level in the plots. It might largely be that concerned plots harbour some limited concentration of specialised nitrogen fixing micro-organisms.

After the second application of nutrients, nitrogen level decreased significantly as remediation progressed in all plots except the Control O which had elevated levels. This trend is in agreement with [24] when inorganic fertilizer was used in the remediation of hydrocarbon polluted soil. Statistically ($P<0.05$) there is no significant difference in the level of nitrogen reduction among the biostimulated plots. It can be deduced that the indigenous microbes present in the contaminated sediment repeatedly utilised nitrogenous nutrients supplied by the addition of organic supplements. This is evidenced in the progressive decrease in TOC. The reduction in the level of nitrogen encountered as bioremediation progressed can be attributed to nitrogen loss or release to the atmosphere. In this situation, nitrate ions are converted to nitrogen gas by denitrifying bacteria such as *Pseudomonas, Bacillus and Micrococcus* [25].

3.5 Variation in Total Heterotrophic Bacterial Growth

The THB count of the contaminated soil met the minimum threshold of THBC (10^5 cfu/g) required to activate biodegradation of hydrocarbon polluted soil as recommended by [26]. The result of the THBC detailed in Fig. 5 indicates a significant decrease after pollution. This suggests that spent motor oil has inhibitory effect on microbial growth. However, following the addition of nutrient there was a marginal increase [PD (2.9- 3.2 x 10^7cfu/g), SB - PD (3.2- 3.7 x 10^7 cfu/g) and SB (3.2- 4.2 x 10^7cfu/g)] in all the biostimulated plots while a reduction in microbes was observed in the Control plot. The slow microbial response to nutrient supplementation might be due to inability of some microbes to adjust rapidly to a newly found environment. It could also be due to low substrate level initiated by inadequacy of nutrient that resulted to secretion of necessary enzymes for growth and thus signalled slow rate of degradation in biostimulated plots.

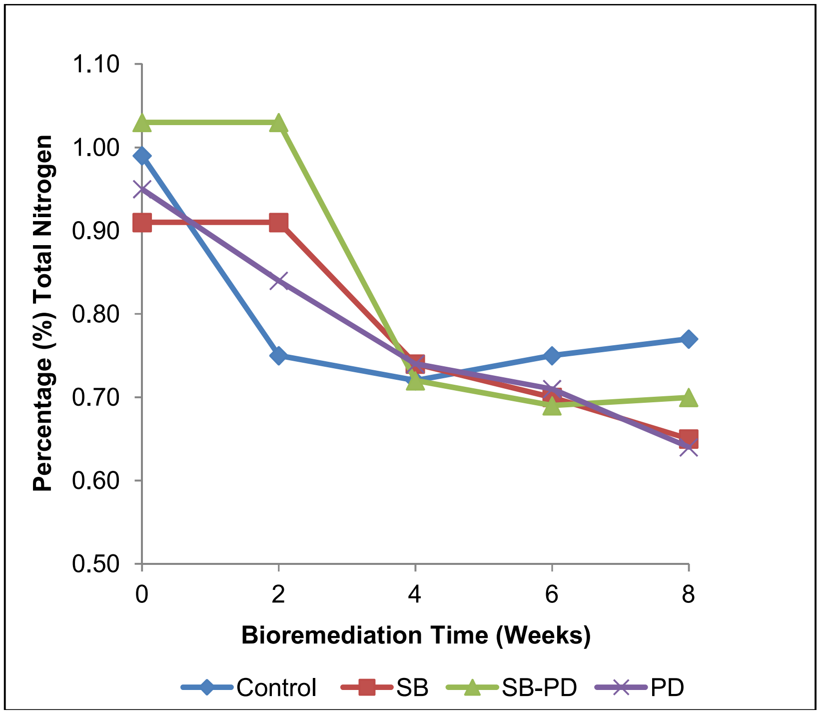

Fig. 4. Percentage (%) total nitrogen during remediation

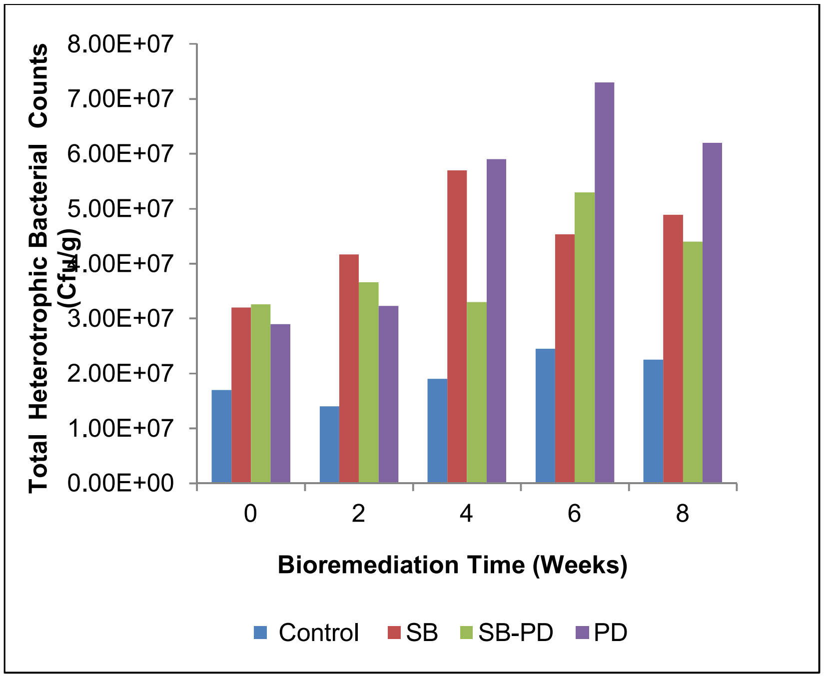

Fig. 5. Variation in THBC during remediation period

The addition of more amendment led to an increase in microbes in all the treated plots except SB - PD where a reduction in the number of microbes was observed. It is possible that some specialised micro-organisms adjusting to varying substrates and increasing their metabolic activities will restrain the growth of autochthonous micro-organisms [27]. Another possible reason could be that there was unhealthy competition for food among the microbes for nutrients which led to their death in SB - PD plots. The rise in micro-organisms in plots SB and PD can be adduced to the fact that large supply of carbon and nitrogenous substrates resulted in an increased growth [28]. These rises in microbial counts in SB and SB - PD further corroborate the fact that micro-organisms utilized sugar present in the bagasse amended plots for growth and secretions of enzymes [29].

Evidently, poultry dung has an intrinsic potential for microbial proliferation that enhanced the removal of hydrocarbon [30,31]. However, this cannot be said of the bagasse treated plots where biostimulation had no effect on degradation between 28 and 42 days. This might be due to inability of some microbes to produce metabolites that will breakdown the hydrocarbon chains owing to nutrient limitation in terms of bioavailability [32]. There was gradual increase in the number of microbes in the control plot and this was responsible for the slow rate of hydrocarbon depletion. During the last week of remediation, there was a decrease in the number of micro-organisms in the PD and SB - PD plots which signal the depletion of nutrients in the plots.

The micro-organisms identified from the contaminated soil are *Bacillus spp*, *Pseudomonas sp*., *Micrococcus sp* and *Flavobacterium sp.* These micro-organisms have been mentioned in the removal of hydrocarbon from contaminated soils [33,34]. During the bioremediation, *Bacillus spp*, *Pseudomonas sp*., *Micrococcus sp*. may be responsible for the significant TPH reduction because of their consistencies. It was postulated that *Bacillus spp.* are more liberal to high degrees of hydrocarbons in soil due to their resistant endospores [35]. As a result, *Bacillus spp.* may be a major microbial driver in biochemical processes that resulted in mineralization of hydrocarbons though this was not investigated. It would appear that an appropriate amount of microbial biomass with unique biodegradation capabilities and favourable environmental conditions is vital to decomposition of contaminated soils whether by natural attenuation or biostimulation.

3.6 Effect of Biostimulants on Total Petroleum Hydrocarbon

There was a marked increase in TPH concentration after 14 days of simulation. The values of TPH recorded in all the plots namely Control, SB, SB - PD, and PD were 22.18, 21.98, 20.38 and 20.81 g/kg respectively. Following the addition of nutrients, there was a slight reduction in TPH levels after 14 days. This marginal decrease in TPH was due to the complex nature of chemical hydrocarbon and high viscous characteristics of the contaminant which might adversely affect bioavailability [36]. It is also possible that the nutrient level in all the biostimulated plots were yet to attain the maximum threshold to kick start microbial activities for rapid mineralization of pollutants.

After 42 days of biostimulation, there was a significant reduction ($p<0.05$) in the quantity of TPH (as shown in Fig. 6.) especially in SB - PD and PD plots. The increase in nutrient level supplied in the form of nitrate enhanced microbial population and activities. This was evident in reduced nitrogen levels of the plots. However, a strange occurrence was observed in the SB plot between day 28 and 42, where there was a trace increase in the amount of hydrocarbon. This interesting trend has been reported in previous studies for lubricating oil [37] and was attributed to production of biosurfactants by oil degrading micro-organisms and possible changes in interaction between the hydrocarbons and soil particles that led to increased level of hydrocarbon and extraction efficiency.

On average, the TPH level of the contaminated soil dropped by 62, 53, 46 and 23% respectively in plots PD, SB - PD, SB and Control. The percentage degradation in SB - PD was higher than SB plot and thus corroborates the claim that combined nutrients show high biodegradation potentials than single nutrient deployment [38]. The percentage decrease in SB - PD plots depicted that bagasse and poultry manure may have complemented well in creating a favourable medium for microbial attack during the biodegradation process especially between week 4 and 6. The use of sugarcane bagasse as crop residue reaffirms the potentials of agro-industrial waste in the mitigation of hydrocarbon

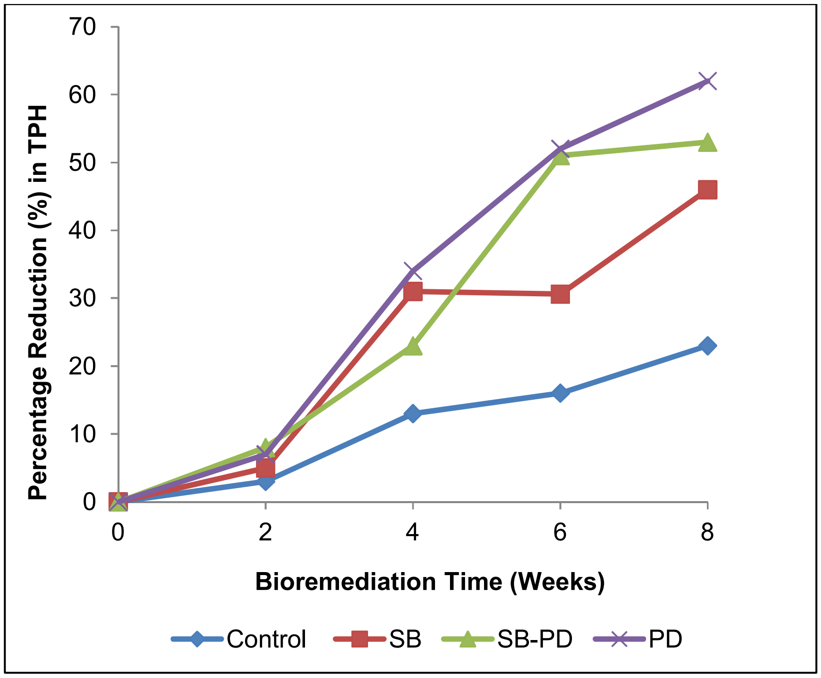

Fig. 6. Percentage reduction of TPH in soils with bioremediation period

contaminated soils because it provides a better platform for aeration and nutrient availability. Also, the 46% TPH reduction recorded using bagasse residue as a bulking agent is in line with [28] when a percentage decreases between 39 and 58% was recorded using sawdust and woodchips. The removal efficiency was lower in the naturally attenuated soil (Control O). However, the 23% recorded in this study is in contrast to 43% for untreated soil reported by [39]. The disparity might be caused by the soil type and varying environmental factors which fluctuated towards the end of the bioremediation period.

3.7 Heavy Metals Concentrations in Contaminated Soil

The heavy metals investigated viz: Cd, Pb, As, Zn and Cu were generally low before, during and after remediation. The total metal concentration is put at 0.70mg/kg before treatment. The metal concentration is considered to be at a level that is neither toxic nor suppress microbial proliferation [40]. After remediation, there was no significant effect of biostimulants on the metal concentration in all plots. The low metal concentrations found may be attributed to the source and composition of the spent motor oil.

4. CONCLUSION

The use of organic amendments such as sugarcane bagasse and poultry manure in the restoration of spent motor oil contaminated soil has been investigated. These organic amendments have promising potential in hydrocarbon degradation. The hybrid treatment substantiates the fact that combined nutrient supplementation such as sugarcane bagasse and poultry manure has a synergistic effect on hydrocarbon reduction in contaminated soils. Following the decrease in TPH in the poultry manure treated plot, poultry manure is a reliable amendment in the treatment of hydrocarbon owing to the fact that it contains large dosage of nitrogen as a result of presence of protein and amino acids for microbial growth. However, more research is needed using this source of organic treatment to investigate the exact quantity, rate of nutrient supplementation and favourable environmental conditions that will yield optimal results before deployment in field scale application.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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