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Laboratory Scale Bioremediation of Soils from Automobile Mechanic Workshops Using Cow Dung

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Abstract The environment is constantly degraded with contamination from the petroleum industry and their by-products, dealing with this problem in most cases further compounds the problem as environmentally unfriendly technologies such as in situ burning have been adopted previously. The efficiency of cow dung for the remediation of automobile mechanic workshops was studied on a laboratory scale. The purpose of the study was to evaluate the nutrient availability and presence of total petroleum degraders in cow dung and efficiency for use as a 'green' technology in our environment. The study was carried out in perforated buckets to allow for leachate collection. A total of 7 buckets were filled with 2.5 Kg of soil and mixed with cow dung in a ratio of 10%-90%, 20%-80%, 30%-70% in two replicates while the final bucket was filled with control soil not amended with cow dung. The study period was for 56 days (8 weeks). 'Analytes' were obtained from each of the buckets labelled CD 10%, CD 20%, CD 30% and the control on a weekly basis to check for pH, Total Organic Carbon (TOC), Total Organic Nitrogen (TON), Total Petroleum Hydrocarbon (TPH) and Total Petroleum Hydrocarbon Utilizers (bacterial and fungal) and some organisms capable of petroleum degradation were identified in the samples. The pH ranged between 6.8 and 8.2 for the period of study which was not significantly (<0.05) different from the control soil samples. TOC ranged between 2.0 mg/kg and 2.49 mg/kg throughout the period of study and not significantly different from the control samples. TON increased significantly in the soils amended with cow dung by up to 21% compared to control soils which were significantly lower. TPH Degradation observed was 79.38%, 79.03% and 81.72% respectively. The values were significantly higher than the control which had 35.70% reduction. Total Hydrocarbon Utilizers identified included Bacillus Sp., Staphylococcus Sp,. Pseudomonas Sp., Flaviobacterium Sp., Arthobacter Sp., Enterobacter Sp., Trichoderma Sp., Mucor Sp. and Aspergillus Sp. The use of cow dung showed good prospects in bioremediation of automobile workshop soils contaminated with spent oil. The research can further be implemented in a pilot scale study and subsequently on spent oil contaminated sites.

Keywords: *cow dung bioremediation, TPH Degradation, Hydrogen Ion concentration (pH), Total Organic Carbon, Total Organic Nitrogen and Total Petroleum Hydrocarbon Utilizers*

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1. Introduction

The quality of life on earth is connected to the overall quality of the environment. Releases of recalcitrant, bioaccumulative and toxic chemicals into the environment have a negative impact on human health, species and the environment. These contaminants find their way into the tissues of plants, animals and human beings by the movement of, and absorption of hazardous constituents in the environment. Contaminated lands generally result from past and present industrial activities with little or no awareness of the health and environmental consequences of their production processes and waste disposal methods. The problem is worldwide, but more severe in the developing countries such as Nigeria where there are no effective regulatory policies on the environment, thus encouraging unwholesome industrial practises.

Petroleum hydrocarbons are some of the most widely distributed pollutants resulting from oil exploration, spills, tankers, ballast water, fuels, mechanic sites and garages (Okerentugba and Ezeronye, 2003). Oil pollution can cause severe effects on the environment and human health. The presence of these pollutants in the terrestrial and aquatic environments constitutes health problems and socioeconomic hazards (Makut and Ishaya, 2010). Apart from this, used engine oil renders the environment unsightly and constitutes a potential threat to humans, animals and vegetation (Chwukuma et al., 2010).

Various organics are generated as by-products from various industries (such as petroleum and petrochemical, pulp and paper, chemical industries and others), which may be released into the environment, or are accidentally spilled. Aromatics and their chlorinated derivatives - which are difficult to biodegrade and are toxic - are of primary concern. Aromatics and their chlorinated

derivatives are generated in chlorine bleaching of cellulose pulp (e.g., dioxins), pesticide and herbicides (e.g., chlorophenols), moth repellents and air deodorant (e.g., p-dichlorobenzene), petroleum and petrochemicals (e.g., naphthalene), transformer oils (e.g., polychlorinated biphenyls (PCB), chemical, plastics, iron and steel industries (e.g., phenols), wood preservation (e.g., pentachlorophenol's (PCP), used motor oil and grease among others. As the above chemicals are toxic and are proven carcinogens, their release to water and soil is prohibited. If, however, they do appear in the environment, they must be treated and detoxified (Chukwuma et al., 2010).

Since the late 1980s, after the chemical and mechanical treatments of lands and water bodies and thermal treatment (incineration) of hazardous wastes proved economically and environmentally unsustainable, focus shifted towards the biological methods which are cost-effective as well as environmentally sustainable and also socially acceptable. Bioremediation is a soft bioengineering technique to clean up contaminated lands/sites using microbes (bacteria or fungi), plants (terrestrial and aquatic) and earthworms. It is also a technique to stabilize the eroded lands and prevent soil erosion. Bioremediation works carried out by the microorganisms are called 'micro-remediation' while those performed by plants are called 'phyto-remediation' (Perfumo et al., 2007).

Contaminants can absorb to soil particles, rendering some contaminants unavailable to microorganisms for biodegradation. Thus, in some circumstances, bioavailability of contaminants depends not only on the nature of the contaminant but also on soil type. Hydrophobic contaminants, like petroleum hydrocarbons, have low solubility in water and tend to adsorb strongly in soil with high organic matter content. In such cases, surfactants are utilized as part of the bioremediation process to increase solubility and mobility of these contaminants. The existence of thermophilic bacteria in cool soil also suggests that high temperatures enhance the rate of biodegradation by increasing the bioavailability of contaminants (Perfumo et al., 2007).

Automobile workshops are an important component of the service sector industry. The most significant environmental impact associated with the existing workshops is the seepage of used engine oil and washed water into the soil. Contamination of the soil by oil causes it to lose its useful properties such as fertility, water-holding capacity, permeability and binding capacity (Odjegba and Sadiq, 2002).

In some developing countries, used engine oil is discharged into the environment. In Nigeria, about 20 million gallons of waste engine oil are generated annually from mechanic workshops and discharged carelessly into the environment (Onwurah, 1999; Taiwo and Otolorin, 2009). According to USEPA (1996), a litre of used engine oil is enough to contaminate one million gallons of freshwater. Used engine oil also renders the environment unsightly and constitutes a potential threat to humans, animals, and vegetation (Adelowo et al., 2006). As the usage of petroleum hydrocarbon products increase, soil contamination with diesel and engine oils is becoming one of the major environmental problems, as a result, environmental pollution with used engine oil continues to generate interest.

Engine oil is a complex mixture of hydrocarbons and other organic compounds including some organo-metalic constituents (Butler and Mason, 2000) that is used to lubricate the parts of an automobile engine in order to avoid excessive wearing out (Rahman et al., 2009). Used (also called spent or waste) motor engine oil contains metals and heavy polycyclic aromatic hydrocarbons (PAHs) and these could contribute to chronic hazards including mutagenicity and carcinogenicity (Hagwell et al., 2002; Boonchan et al., 2000). As it is inevitable for the efficient and effective functioning of the automobile engines, soil contamination with used engine oil is becoming one of the major environmental problems (Mandri and Lin, 2006), mainly due to uncontrollable disposal, particularly in developing economies. The widespread ability of microorganisms to assimilate these hydrocarbons is of great significance and when it occurs in the natural environment, the process is known as biodegradation. Hydrocarbons, including PAHs, have been long recognized as substrates supporting microbial growth.

Research on the isolation of bacteria associated with engine oil contaminated soil was carried out by Ugoh and Moneke in 2011. They selected different mechanic sites in a town within the Northern part of Nigeria. They suggested that Bacillus sp. is most adapted to conditions present in soils contaminated with used engine oil and hence could be exploited in bioremediation activities.

Bioremediation of benzene was carried out using cow dung microflora in a bioreactor by Akinde, (2008). The bioremediation of benzene under the influence of cow dung microflora was found to be 87% and 67.5%, at initial concentrations of 100 mg/l and 250 mg/l within 72 hours respectively. Pseudomonas putida was isolated from cow dung microflora as potential benzene degrader and its ability to degrade benzene at various concentrations was also evaluated. The data showed 74%, 81% and 65% degradation at the concentrations of 50 mg/l, 100 mg/l, and 250 mg/l within the time period respectively.

This study was carried out to investigate the efficiency of air dried cow dung in the remediation of automobile mechanic sites contaminated with spent engine oil.

2. Statement of Problem

The deleterious effect of pollutants on the environment is alarming and this gives room for concern. Spent oil is discharged indiscriminately in open free land, sewers and gutters and this is channelled to inland/coastal water bodies. Engine oil contaminated site is aesthetically unpleasant, it affects the growth of plants and microbial diversity and when it seeps to groundwater, it renders it polluted and unfit for purpose. Aquatic lives are also not spared of this trend as there abound a tendency for drop in fish population, bioaccumulation and biomagnification.

3. Limitation of Study

This study was conducted ex situ in a laboratory setup and sample size collected was rather small due to logistic problems associated with managing larger quantities of contaminated soil sample, this prevented a more detailed

insight to the problem. Laboratory analytical methods for individual strain identification was not available, therefore efforts were made to thoroughly investigate the species present in the experimental matrix. Also, because already contaminated soil samples were excavated, it was impossible to get desired level of initial TPH concentration. Subsequent studies can focus on in situ or pilot scale trials while attempting to set optimum initial TPH concentrations of the sampling soil. It might be necessary to vary the concentrations so as to have an idea of the ideal range of concentrations that can effectively be remediated using this technology.

4. Materials and Methods

Engine oil polluted soil samples for the experiment were collected with a spade at the surface soil (loamy clay) between the depths of 0-15±0.2 cm from a mechanic workshop at the Federal Government Girls College Road Junction, Ugbowo, Benin, City, Edo State, Nigeria. The soil samples were bulked together, and transported to the laboratory in a polythene bag. Cow Dung was obtained from a slaughter house along Benin Technical College Road, Benin City, Edo state. The air dried cow dung substrates were used as amendments and stimulants for the bioremediation of engine oil polluted soil sample.

5. Methods

5.1. Process Description

In the laboratory, the soil sample was homogenized; impurities removed and weighed (42 KG). Soil pH, carbon, nitrogen and TPH measurements were taken immediately. The pH, TON, TOC, Total Microbial Count (Bacterial and Fungal) and TPH content of cow dung were also measured immediately in the Laboratory. 2.5 kg of the soil sample was measured into seven (7) perforated glassware buckets labelled Soil + Cow Dung 10% (CD 10%) 1, Soil + Cow Dung 10% (CD 10%) 2, Soil + Cow Dung 20% (CD 20%) 1, Soil + Cow Dung 20% (CD 20%) 2, Soil + Cow Dung 30% (CD 30%) 1, Soil + Cow Dung 30% (CD 30%) 2 and Control respectively.

Measurements for pH, percentage nitrogen, and percentage carbon were taken on a bi-weekly basis while that of TPH was taken on a weekly basis.

5.2. Microbiological Parameters

5.2.1. Sterilization of Materials

Glass-wares such as petri dishes, test tubes, glass rod, pipette, measuring cylinder, beakers and conical flasks required for this investigation were soaked and washed in detergent and rinsed with distilled water. They were wrapped with aluminum foil paper and dried in the oven in an inverted position at 160°C for 45-60 minutes. All the glass wares used were manufactured by Pyrex (England).

5.2.2. Preparation of Culture media

All media were prepared according to manufacturer's instruction. The media used included Nutrient Agar, Potato Dextrose Agar and mineral salt agar. Total microbial analysis was carried out on the soil by weighing 10 g of soil sample into 90 ml of distilled water and serially diluting to obtain a tenfold diluent, one drop was inoculated unto nutrient agar and potato dextrose agar to culture bacteria and fungi respectively. The culture plates were incubated for 24 and 78 hours respectively. On completion of the culture, microbial species were identified using biochemical tests such as Urease, Catalase, Coagulase, Gram staining, and Indole. Stock cultures of the identified organisms were also prepared and preserved.

a. Nutrient agar

Exactly 28 g of the medium was dissolved in 1000 ml of distilled water. The suspension was first dissolved completely by shaking and then sterilized by autoclaving at 121°C for 15 minutes. The molten medium was allowed to cool at 45° C before dispensing into sterile petri dishes.

b. Potato dextrose agar

Thirty-nine (39 g) of the medium was dissolved in 1000 ml of distilled water. The suspension was first dissolved completely by shaking and then sterilized by autoclaving at 121°C for 15 minutes. The molten medium was allowed to cool at 45°C before dispensing into sterile petri dishes.

c. Mineral salt agar

The medium was used for isolation of degrading microorganisms. The composition of the basal mineral salt medium used in this study was as follows (g/L): $NaNO_3$ 4.0, NaCl 1.0, KCl 1.0, $CaCl^2{\cdot}2H_2O$ 0.1, KH_2PO^4 3.0, $Na_2HPO^4{\cdot}12H_2O$ 3.0, $MgSO^4$ 0.2, $FeSO^4{\cdot}7H_2O$ 0.001; 2 ml trace element stock solution composed of (g/L): $FeCl^3{\cdot}6H_2O$ 0.08, $ZnSO^4{\cdot}7H_2O$ 0.75, $CoCl^2{\cdot}6H_2O$ 0.08, $CuSO^4{\cdot}5H_2O$ 0.075, $MnSO^4{\cdot}H_2O$ 0.75, H_3BO^3 0.15, $Na^2MoO^4{\cdot}2H_2O$ 0.05. Exactly 22.7 g of the medium was dissolved in 1000 ml of distilled water. The suspension was first dissolved completely by shaking and then sterilized by autoclaving at 121°C for 15 minutes. The molten medium was allowed to cool at 45°C before dispensing into sterile petri dishes.

5.2.3. Total Heterotrophic Bacterial and Fungal Counts

Samples were enumerated by making ten-fold dilutions of the soil samples from 10^1 to 10^3. Aliquot 0.1 ml of the 10^{-3} dilution was transfer plated in nutrient agar amended with nystatin (0.5-1 µg/ml) for isolation of bacteria while potato dextrose agar amended with streptomycin (0.02-1 µg/ml) was used for the isolation of fungi. The plates were prepared and inoculated in duplicates. The inoculated nutrient agar plates were incubated at 37°C for 24 hours while the potato dextrose agar plates were incubated at 28°C for 72 hours. After incubation, the colonies of the isolates were counted and expressed in CFU/g. Isolated colonies were further purified by sub-culturing and identified using bio-chemical tests and microscopy.

5.3. Isolation of Degrading Microorganisms

The culture medium used for the isolation of hydrocarbon degrading bacteria was mineral salt agar which is the enrichment medium for the isolation of hydrocarbon degrading bacteria. Aliquot of 0.1 ml of the 10^{-3} dilution was plated in the medium and the plates were incubated at 30°C for 5 days. Discrete colonies that developed were counted and expressed in CFU/g.

5.4. Identification of Isolates

Each isolate was examined for its size, shape, margin, consistency, elevation, pigmentation, Gram reaction and cell morphology; they were also cross matched with Bergey's Manual of Determinative Bacteriology for bacterial cells. The isolates were characterized as described by Holt et al., (1999). Biochemical tests which were carried out included production of catalase, coagulase, indole and oxidase enzymes. Spore production and oxidation/fermentation of sugars were also examined.

5.5. Chemical Parameters

5.5.1. pH

Soil pH was determined using the method of Bates (1954).

5.5.2. Nitrogen

Total Organic Nitrogen was determined by the Kjeldahl method described by APHA (1998).

5.5.3. Total Percentage Carbon

This was determined using the Walkey Black method (APHA, 1998).

5.5.4. Total Petroleum Hydrocarbons (TPH)

A solvent 50:50 mix of acetone and methylene chloride was prepared. 10gram aliquot of well-mixed sample was measured into a solvent rinsed beaker. 50ml of the solvent mix was added to the samples and spiked with 1ml of the surrogate-ortho-terphenyl (OTP) and mixed. Sample was placed in sonicator and sonicated for about 10-15 minutes at about 70°C. About 10g of anhydrous sodium sulphate was added to the sample until a clear extract developed. The extract solvent was poured into a round bottom flask. The solvent, hexane was concentrated, exchanged and re-concentrated to 1-3ml. The total extracts were combined and absorbance was quantified using a CE 1020 (1000 Series) UV Spectrophotometer at 400 nm. Oil and Grease contents were extrapolated from a standard curve of absorbance (A400 nm) against concentration. The machine was calibrated before reading was taken. This was done by injecting a series of normal alkane standards. The volume injected was 1µL.

5.6. Statistical Analysis

Data collected from the study were analyzed using general descriptive statistics, one Way Analysis of Variance (ANOVA) at 95% probability level of significance. If significant differences were found, Duncan's multiple range tests was used to compare the different experimental groups. Computer software Statistical Package for Social Scientists (SPSS) and Microsoft Excel were used for the statistical analyses.

6. Results and Discussion

6.1. Microbiological Parameters

The use of microorganisms has gained tremendous application in bioremediation of contaminated soils. However, it is known that hydrocarbon biodegradation in soil can be limited by many factors, such as microorganism type, nutrients, pH, temperature, moisture, aeration, soil properties, and contaminant concentration (Semple et al., 2001; Wilias et al., 2005; Mohan et al., 2006; Rahman et al., 2009).

Biotics analysis showed that the isolated microorganisms upon biochemical tests included some bacteria and fungi species such as Bacillus spp., Staphylococcus Sp, Corynebacterium Sp, Streptococcus Sp, Pseudomonas Sp, Flavobacterium Sp, Arthobacter Sp, Enterobacter Sp, Micrococcus Sp, Klebsiella Sp, Escherichia Coli Sp, Aspergillus Sp, Trichoderma Sp, Penicillum Sp, and Mucor Sp. Petroleum hydrocarbon utilizers included Bacillus Sp, Staphylococcus Sp, Pseudomonas Sp, Flavobacterium Sp, Arthobacter Sp, Enterobacter Sp, Trichodema Sp, Mucor Sp and Aspergillus Sp. Table 1 shows the microorganisms that were isolated and identified during the experiment and under the laboratory conditions previously stated. While the hydrocarbon utilizing bacteria identified during the experiment are presented in Table 2.

Table 1. Microbial Isolates

Bacteria	Fungi
Bacillus Sp.	Aspergilus flavus Sp.
Staphylococcus Sp.	Trichoderma Sp.
Corynebacterium Sp.	Penicillum Sp.
Streptococcus Sp.	Mucor Sp.
Pseudomonas Sp.	Aspergilusniger
Flavobacterium Sp.	
Arthobacter Sp.	
Enterobacter Sp.	
Micrococcus Sp.	
Klebsiella Sp.	
Escherichia coli Sp.	

Table 2. Total Petroleum Hydrocarbon Utilizers

Bacteria	Fungi
Bacillus Sp.	Trichoderma Sp.
Staphylococcus Sp.	Mucor Sp.
Pseudomonas Sp.	Aspergillus Sp.
Flavobacterium Sp.	
Arthobacter Sp.	
Enterobacter Sp.	

Both bacteria and fungi are heterotrophic in nature and related to a large number of taxonomic genera which are capable of utilizing hydrocarbons as sources of energy and carbon for their growth (Rahman, 2003). In this study, known bacterial and fungal petroleum hydrocarbon degraders such as Bacillus Sp., Staphylococcus Sp., Pseudomonas Sp., Flavobacterium Sp., Arthobacter Sp., Enterobacter Sp., Aspergilus Sp, Mucor Sp., and Trichoderma Sp. were identified in contaminated soil, poultry litter and cow dung. Several researchers such as Vidali, 2001; Kourkoutas and Petsas, 2001, Bhattacharya et al., 2003 also reported similar findings which made their application for bioremediation efforts suitable. There was an increase in the quantity of bacterial cells present as the quantity and duration of treatment increased. This could be because of the simple reason that the growth and proliferation of microbial cells depend on time and quantity of nutrients available.

6.2. Chemical Parameters

6.2.1. Hydrogen Ion (pH)

Results for pH obtained for the analysis are shown in Figure 1.

The results revealed that the average pH was 7.8 and 8.1 for the cow dung and polluted soil respectively before the experiment. During the experiment, pH ranged between 6.8 (week 1 of CD 30%) and 8.2 (week 7 of CD 30%). It increased with time in all the treatments and there was no significant difference (P<0.05) in pH within the CD treated soils. All the treatments had higher pH values when compared with the control although the difference was not significant (<0.05)

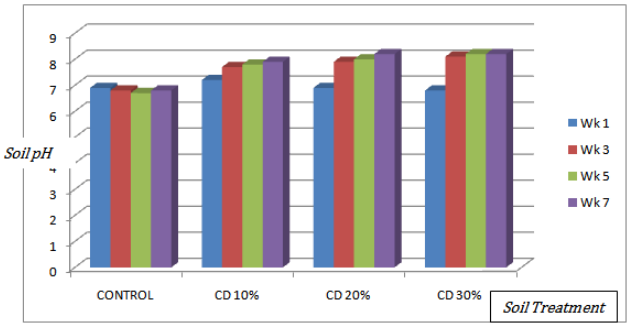

Figure 1. Hydrogen Ion (pH) of samples during bioremediation

Microbial growth and activity are readily affected by pH and subsequently, growth of microorganisms impact the pH of the test matrix. In microorganisms, biochemical reactions are catalyzed by enzymes. Although microorganisms have been also isolated in extreme conditions, most of them grow optimally over a narrow range, so it is important to achieve optimal conditions. In this study, pH ranged from 6.2 to 8.7. Boonchan, (2000) and Joanne et al., (2008) reported that optimum pH for bioremediation is between 6.0 and 8.9. They opined that changes from initial levels of pH could be as a result of the release of acidic and alkaline intermediates and final products during biodegradation of hydrocarbons which has an effect on the pH.

6.2.2. Nitrogen

Results for nitrogen are presented in Figure 2.

Nitrogen level (%) of sampled specimen before treatment was 4.59 mg/kg for polluted soil and 10. 2 for cow dung. After bioremediation, nitrogen in control soil was as low as 0.22 (week 5 and 7). In CD treated soils, nitrogen (%) values ranged between 2.25 (week 1 of CD 10%) and 2.88 (week 7 of CD 10%). There was an increasing trend (with time) in nitrogen values observed in the treated soils. There were significant differences (>0.05) between all the treatments and control.

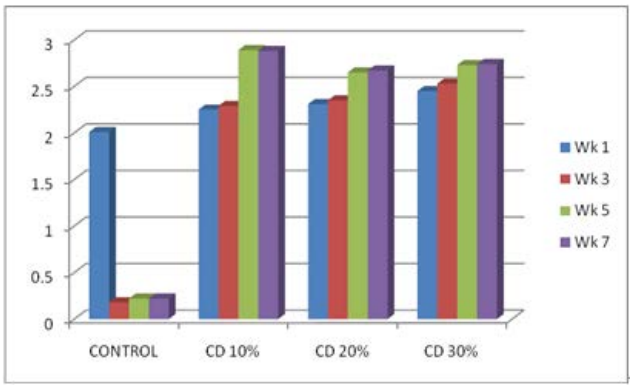

Figure 2. Nitrogen (%) content of samples during bioremediation

Microorganisms thrive better in nutrient enriched soils and the degradation of petroleum hydrocarbon is encouraged by the presence of such nutrients.

Nitrogen is an essential nutrient for all forms of life. It is a structural component of amino acids from which proteins are synthesized. Animal and human tissue (muscle, skin, hair, etc.), enzymes, and many hormones are composed mainly of proteins. Nitrogen deficiency is a major factor limiting oil biodegradation rate (Brookes, 2005, Jain et al., 2001). In this study, it was observed that nitrogen presence was significantly higher in treated soils than in untreated (control soils). This is definitely linked to the presence of nitrogen in the substrate (cow dung) used as amendment as reported by Ugoh and Moneke, (2011).

6.2.3. Carbon

Before commencement of the experiment, percentage carbon was 1.73 in polluted soil and 12.9 in cow dung. The results as shown in Figure 3 indicate that the highest carbon value in control soil was 1.05 (week 2). In CD treated soils, carbon (%) was between 2.0 (week 1 of CD 30%) and 2.49 (week 7 of CD 30%). Carbon content in CD treated soils increased with time in all the treatment ratios. All values showed no significant difference (P<0.05) within the groups but showed significant difference (P>0.05) when compared with the control.

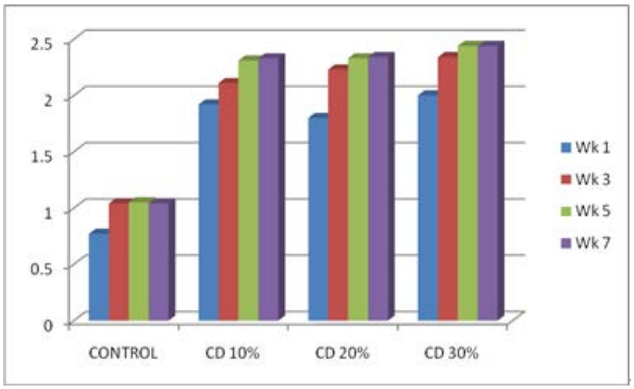

Figure 3. Carbon (%) content of samples during bioremediation

In this study, carbon reduced from the initial values present in substrates used for the experiment. This is probably due to the utilization of the nutrients in these substrates as energy source. When compared with the control (untreated soils), the values in the treatment categories were higher, and this might be as a result of residual carbon present after the degradation of TPH which was low in untreated soils.

6.2.4. Total Petroleum Hydrocarbon

At the beginning of the experiment, TPH in mg/kg was below detectable limit (BDL) in cow dung and was 2910 in soils polluted with engine oil. However, after bioremediation, in the control soil, TPH reduced from 2,910 to 1,870 mg/kg (35%). In the CD treated soils, TPH reduced to 545 (week 5 of CD 30%- 81%) there was more reduction in TPH in the first three weeks of the experiment when compared with the values of the fourth to eight week of the experiment. There was not a detectable trend in TPH reduction over time especially from the fourth week of the experiment. There was no

significant difference (<0.05) within the treatments, but there were significant differences between all the treatments and the control.

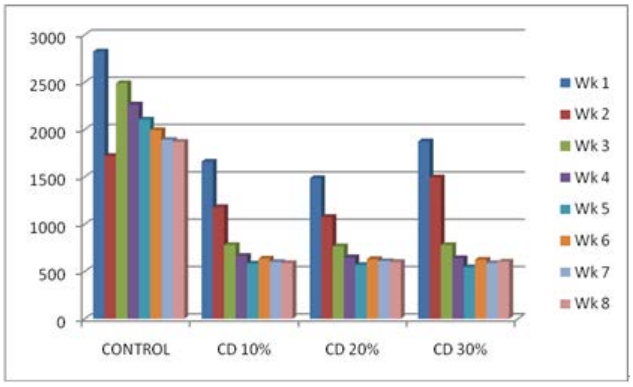

Figure 4. Total Petroleum Hydrocarbon reduction during bioremediation

Used engine oil has been found to alter soil biochemistry, soil microbial properties, pH, Oxygen and nutrient availability. In this study, there was more reduction in TPH observed in the first three weeks when compared with the final five weeks. This is in agreement with the findings of Anyasi and Atagana, (2011) who reported that degradation of crude oil was highest in the first two week of their experiment, and generally slowed down as time progressed and independent of the quantity of microorganisms present. They suggested that this could have been due to the presence of highly reactive intermediates present after hydrocarbon degradation that could hinder and slow down microbial activity. TPH was reduced significantly (>0.05) by up to 81% using cow dung. These surpassed results of similar researchers (Hamme and Ward, (2000), Mandri and Lin, (2006), Adelowo, et al., (2008), Akinde et al., (2008), and Udeani (2009).

7. Conclusions

From the results of this research, the following conclusions can be drawn: The percentage composition of nutrients present was directly proportional to the quantity of substrates added. The degradation of TPH was dependent on the microbial consortia present within the contaminated soil and substrates used for the treatment. The duration of exposure also played a significant role in determining the quantity of hydrocarbons degraded. CD 30% gave the best degradation percentage although the rate was not significantly different from other treatment ratios.

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