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Bioremediation of Crude Oil Contaminated Soil Using Goat Droppings

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Abstract
The ability of goat droppings to remediate a crude oil contaminated soil was studied. The soil used was obtained in Evergreen street, Aluu, while the goat droppings used were collected from an animal farm in Egberu-Ndoki. A crude oil contaminated soil was simulated in the laboratory by mixing the soil with bonny light crude oil (4% of Soil mass) obtained from Ogu refinery in Rivers State. Three sets of experiments were set-up using the contaminated soil sample. The first set-up called the Control (C) contained the contaminated soil alone. The second set-up called treatment one (CG) contained the contaminated soil and 100g grinded goat droppings. The third set-up called treatment two (CGG) contained the contaminated soil and 150g grinded goat droppings. Each experiment was set-up in quadruplicate and destructively sampled and analysed every two weeks for two months for moisture content, pH and total hydrocarbon content. The results obtained showed that the total hydrocarbon content and moisture content of C, CG and CGG decreased with time. The soil pH decreased with time for C but increased with time for CG and CGG. The percentage of total hydrocarbon degradation was found to be 92.6% for CGG, 70.5% for CG and 24.7% for C. It was therefore concluded that goat droppings could effectively remediate a crude oil contaminated soil.

Key words: *Bioremediation; Crude oil; Contamination; Soil; Goat manure*

1. Introduction

Soil contamination by crude oil has been a major issue in the world. The extraction, processing and use of crude oil and its refined products present many opportunities for soil contamination. The threat to human, animal and plant life posed by soil contamination cannot be over-emphasized and its impact on environment is of local and global concern. The United State produces, distributes, and consumes large quantities of oil every year to fuel their factories, power plants, homes and transportation. From the production, storage, transport and use of oil, 10-25 million gallons of oil spill each year. This oil released threaten public health and safety by contaminating drinking water, causing fire and explosion, diminishing air and water quality, destroying available agricultural lands and recreation areas, and wasting non-renewable resources. Crude oil spills also have a severe environmental impact on ecosystems by harming or killing wildlife, plants and destroying habits (USEPA, 2012).

Oil spills are common events in Nigeria (Baird, 2010). Half of all spills occur due to pipeline leakages and tanker accidents (50%), other causes include sabotage (28%), oil production operations (21%) and inadequate or non-functional production equipment (1%) (Nwilo and Badejo, 2006). The Nigerian National Petroleum Corporation places the quantity of petroleum jettisoned into the environment yearly at 2,300 cubic meters with an average of 300 individual spills annually (Dare, 2013). In Nigeria, the soils in the Niger Delta are more polluted with crude oil. Soil is the basic component that supports life and productivity in the ecosystem. Flora and fauna depend on the soil for support and effective functioning. The effects of petroleum spills on mangroves are known to acidify the soils, halt cellular respiration, and starve roots of vital oxygen (Limson, 2002). Mangrove forest is a major source of wood for local people and habitat for species like manatee and pygmy hippopotamus. Therefore, there is every need for remediation of crude oil polluted site.

Remediation of crude oil polluted sites is of paramount importance because of environmental degradation and health risk associated with this

form of pollution (Obiakalaije et al., 2015). Remediation refers to processes or methods for treating contaminants in soils such that they are contained, removed, degraded or rendered less harmful. Remediation involves four principal approaches, namely natural attenuation, bioaugmentation, phytoremediation, and bioremediation. The Environmental Protection Agency defines natural attenuation as a variety of physical, chemical or biological processes that under favourable conditions, act without human intervention to reduce the mass, toxicity, mobility, volume or concentration of contaminants in soil. Bioaugmentation refers to the inoculation of soil using micro-organisms with enhanced ability to degrade the organic contaminant. Phytoremediation involves using deep-rooted trees to degrade or volatilize contaminants in the soil. Bioremediation is the use of biological systems, consisting of compost (plant and animal wastes) and micro-organisms in the waste, for the reduction of pollution in the terrestrial ecosystem (Bañuelos et al., 1997).

Bioremediation is the most preferred method of remediation because the biological systems that are needed for its implementation are readily available and cheap. Also, bioremediation increases soil fertility as well as solve the problem of waste management with regards to plant and animal wastes. Consequently, this research investigated the effectiveness of goat manure as a remediating agent for a crude oil polluted soil.

2. Materials and methods

2.1. Crude oil

The crude oil used in this study was the bonny light crude oil obtained from Ogu refinery in Bolo Local Government Area, Rivers State. The properties of the crude oil were as follows: colour (greenish-yellow), specific gravity (0.85 kg/l), API gravity (35.4) and viscosity (2.90 P).

2.2. Soil

The soil used in this study was loamy soil obtained from Evergreen Street in Aluu Community, Obio-Akpor Local Government Area, Rivers State. Evergreen Street soil was used because the inhabitants are mostly farmers and this study focused on treating crude oil contaminated soil used for agricultural purpose. The loamy soil was gotten from the top soil not exceeding a depth of five inches after cleaning the vegetative cover. The collected soil was sun-dried for 48 hours. The dried soil was sieved using a 5mm sieve to remove debris and large stones. The loamy soil sample has a moisture content of 11.1%, bulk density of 1.3 g/cm^3, particle density of 2.1 g/cm^3 porosity of 38.9% and a pH of 6.2.

2.3. Remediating agent

The remediating agent (goat droppings) was obtained from an animal farm in Egberu-Ndoki, Oyibo Local Government Area, Rivers State. The goat droppings were hand-picked with the aid of hand gloves. The goat droppings were sun-dried for two weeks, sieved using 2mm sieve and grinded using hand grinder. The grinded goat droppings were then taken to the laboratory for analysis.

2.4. Contamination of the soil by crude oil

Twelve perforated plastic containers were each filled with exactly 0.5kg of the loamy soil sample. The containers were perforated to enhance aerobic conditions for the degrading micro-organisms. The perforated containers were twelve in number because the experiment was projected to last for two months and it involves destructive testing for an interval of two weeks. Each loamy soil sample in the perforated container was mixed with 4% by soil mass of concentrated crude oil following Deuel et al. (1994) who observed that soil contaminated with more than 5% of crude oil by weight do not readily degrade. The crude oil was thoroughly mixed with the soil and was watered to enhance effective biodegradation.

The set-ups were designated by the symbols: $C_1 – C_4$, $CG_1 – CG_4$ and $CGG_1 – CGG_4$ as shown in Fig. 1. Where C_1 - C_4 are the crude oil contaminated soils without treatment (Control), CG_1 - CG_4 are the crude oil contaminated soils mixed with 100g of goat waste, and CGG_1 - CGG_4 are the crude oil contaminated soils mixed with 150g of goat waste. The pH, moisture content and total hydrocarbon content (THC) of the twelve cells were measured every two weeks throughout the duration of the experiment. A temperature of 35℃ was maintained throughout the duration of the experiment.

2.5. Laboratory analysis

Soil samples from the experiments were analysed for moisture content, pH and total hydrocarbon content (THC). The moisture content, pH and THC were determined using oven, pH meter and gas chromatograph with a flame ionization detector, respectively. A distance biplot showing the projection of the different parameters measured onto a 2-dimensional factor space was used to illustrate the correlation and significance of the measured parameters with the time period and with the different samples.

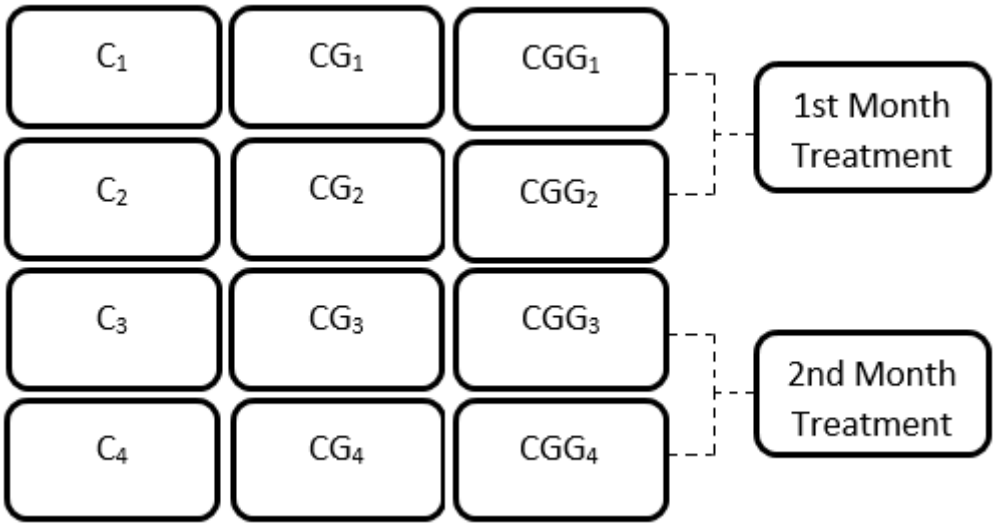

Fig. 1: Experimental layout

2.5.1. Determination of moisture content

The moisture content (MC) of the uncontaminated loamy soil was determined in the laboratory by weighing the container with a weighing scale. The soil was transferred into the container and weighed. The container with the sample was then placed in an oven at 105ºC for 24 hours and thereafter placed in a desiccator and cooled for one hour and weighed afterwards. The same procedure was followed to determine the moisture content of the control and the treatments. The moisture content was calculated using Equation (1):

$$MC = \frac{w_2 - w_3}{w_3 - w_1} \times 100 \qquad (1)$$

where w_1 is the mass of the container, w_2 is the mass of the container and wet soil, and w_3 is the mass of the container and oven-dried soil.

2.5.2. Determination of pH

The pH meter was first standardized, then 20g of the soil was transferred into 50ml beaker containing 20ml of distilled water. The mixture was allowed for 30 minutes while stirring occasionally with a glass rod. The electrodes of the pH meter were inserted into the partially settled suspension and the pH was measured.

2.5.3. Determination of THC

The 0.5 kg soil sample from the plastic container was transferred into a one-litre separatory funnel. Exactly 50ml of methylene chloride was added to it and shook for 30 seconds. The sample was extracted by shaking the funnel for two minutes with periodic venting to release excess pressure. The organic layer was allowed to separate from the water phase for a minimum of ten minutes, and then the methylene chloride extract was collected in a 250ml flask. The extraction procedure was repeated the second and third time, and the extracts were combined in an Erlenmeyer flask. The combined extract was poured through a drying column containing packed cotton wool with anhydrous sodium sulphate and silica and collected in a vial. It was then concentrated by boiling it down to 1ml using a Bunsen burner. The concentrated 1ml extract was mixed with 1ml soil sample from the separatory funnel, and $1\mu l$ of the mixture was injected into the flame ionization detector gas chromatography for THC analysis.

2.5.4. Determination of soil bulk density, particle density and porosity

Oven-dried soil sample was poured into a container of known volume. The container was weighed empty and later weighed with the oven-dried soil in it. The difference in weight gave the weight of the dry soil. Bulk density was calculated using Equation (2).

$$Bulk\ Density = \frac{Mass\ of\ the\ dry\ soil}{Volume\ of\ the\ soil} \qquad (2)$$

Approximately 60ml of water was added to a 100ml graduated cylinder. Exactly 50g of dry soil was transferred into the cylinder and stirred to remove the trapped air. The difference in the volume was the volume of soil particle. Particle density was calculated using Equation (3). Percentage solid space was calculated using

Equation (4) and percentage porosity was calculated using Equation (5).

$$Particle\ Density = \frac{Dry\ mass\ of\ Solid}{Volume\ of\ Soil} \quad (3)$$

$$\%\ Soild\ Space = \frac{Bulk\ Density}{Particle\ Density} \times 100 \quad (4)$$

$$\%\ \text{Porosity} = 100 - (\%\ \text{Solid Space}) \quad (5)$$

2.6. Remediation efficiency

The remediation efficiency (RE) which shows the percentage effectiveness of the goat droppings was calculated using Equation (6).

$$RE = \frac{THC_{ci} - THC_{ti}}{THC_{ci}} \times 100 \quad (6)$$

where THC_{ci} is the total hydrocarbon content in contaminated soil without treatment, and THC_{ti} is the total hydrocarbon content with treatment at a given time (t).

2.7. Biodegradation rate (Hydrocarbon loss)

Average biodegradation loss rate of hydrocarbons under different treatments were estimated according to Yeung et al. (1997):

$$\Delta HL = \frac{(HC_{ini} - HC_{end})}{t} \quad (7)$$

where ΔHL is the average hydrocarbon loss, HC_{ini} is the initial hydrocarbon content in the soil, HC_{end} is the hydrocarbon content at the end of the experiment, and t is the degradation time.

3. Results and discussion

3.1. pH

The pH of the Control and Treated soils from the beginning to the end of the experiment is presented in Fig. 2. The soil pH decreased from 6.3 to 5.6 in the Control(C) but increased from 6.3 to 6.5 in 100g remediating agent treatment (CG) and to 7.9 in 150g remediating agent treatment (CGG) at the end of the experiment. The observed pH range falls within the reported range for optimum bioremediation (Vidali, 2001) and is consistent with the report of previous studies (Onyeiwu, 2008). The decrease in pH observed in the Control could be due to the production of carboxylic acids during degradation process (Atlas, 1984) while the increase observed in the treated soils could be due to the degradation of the crude oil. In corroboration, studies have shown that degradation of crude oil increases with increasing pH and that optimum degradation occurs under slightly alkaline condition (Dibble and Bartha, 1979; Foght and Westlake, 1987).

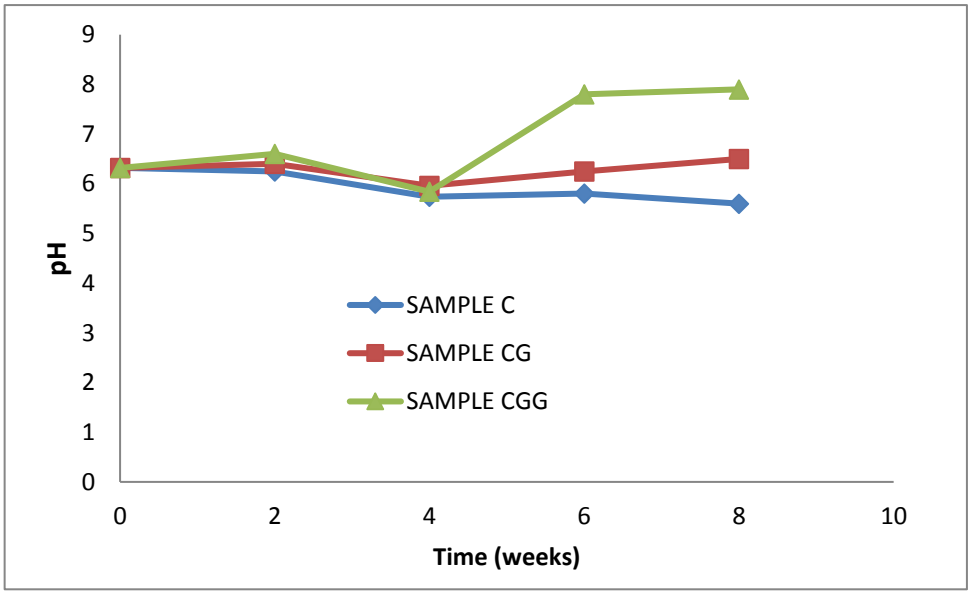

Fig. 2: pH of control and treated soils

The pH biplot of samples and weeks is presented as Fig. 3. It is observed that weeks 0 and 2 are closely linked to Sample C. This indicates that the pH of Sample C in weeks 0 and 2 are correlated and begin to change in the other weeks. The Sample CGG is associated with weeks 6 and 8 indicating a correlation or similarity in the pH in those weeks and also a difference in the pH measured in the previous weeks. The pH in the Sample CG is not linked to any week which

indicates that it is either similar for all weeks or changing continuously across the different weeks.

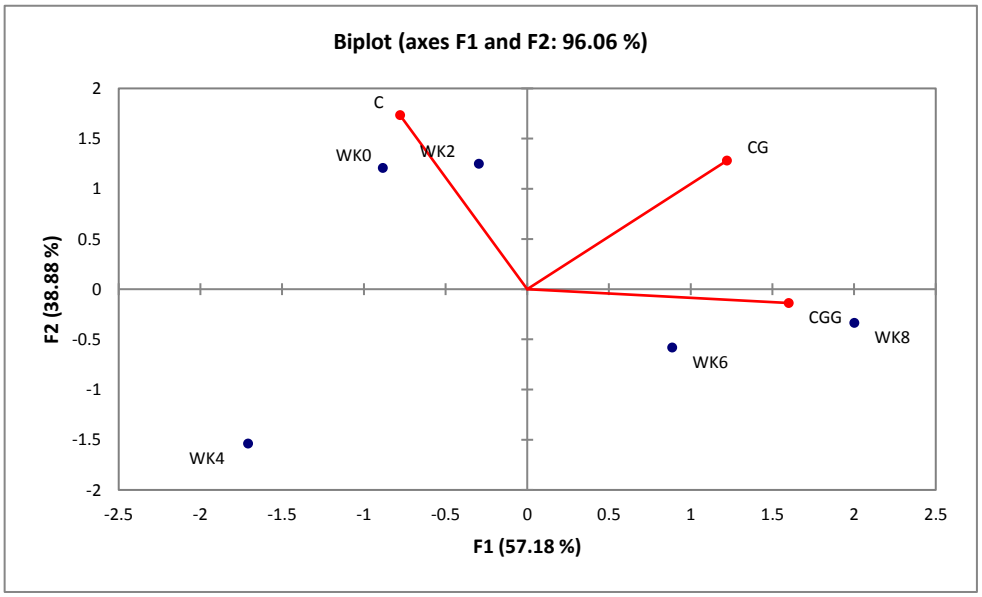

Fig. 3: pH biplot of samples and weeks

To determine how significant the observations made in the biplot is, a two-way analysis of variance is done for the measured parameter. The result is presented in Table 1 which shows that the p-value for weeks is greater than 0.05 therefore a null hypothesis is accepted. This means that the time period has no significant effect on the pH when considering all three samples. The p-value for the three samples is observed to be slightly greater than 0.05 therefore a null hypothesis is also accepted. This indicates that the pH between samples were not significantly different during the period of observation.

Table 1: pH analysis of variance

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Weeks	1.282067	4	0.320517	0.963665	0.477147	3.837853
Samples	2.313453	2	1.156727	3.477814	0.081873	4.45897
Error	2.660813	8	0.332602			
Total	6.256333	14				

3.2. Moisture content

Fig. 4 presents the moisture content of the Control and Treated soils from the beginning to the end of the experiment. The soil moisture content decreased generally after crude oil contamination and continued throughout the duration of the experiment especially for the contaminated soil with treatments and agrees with similar previous study (Ayotamuno et al., 2006). The soil moisture content decreased by 2.8% for C, 11.7% for CG and 20.6% for CGG. The decrease was expected because in polluted soil, water droplets adhere to hydrophobic layer formed which prevents wetting of the inner part of soil aggregate. The further decrease in moisture content observed throughout the remediation process could be because of the metabolic activities of the microbes utilizing the crude oil as well as evaporation and aerobic environmental conditions. The Control having the highest moisture content could be because of small metabolic activities of the microbes since there was no source of nutrient for the microbes.

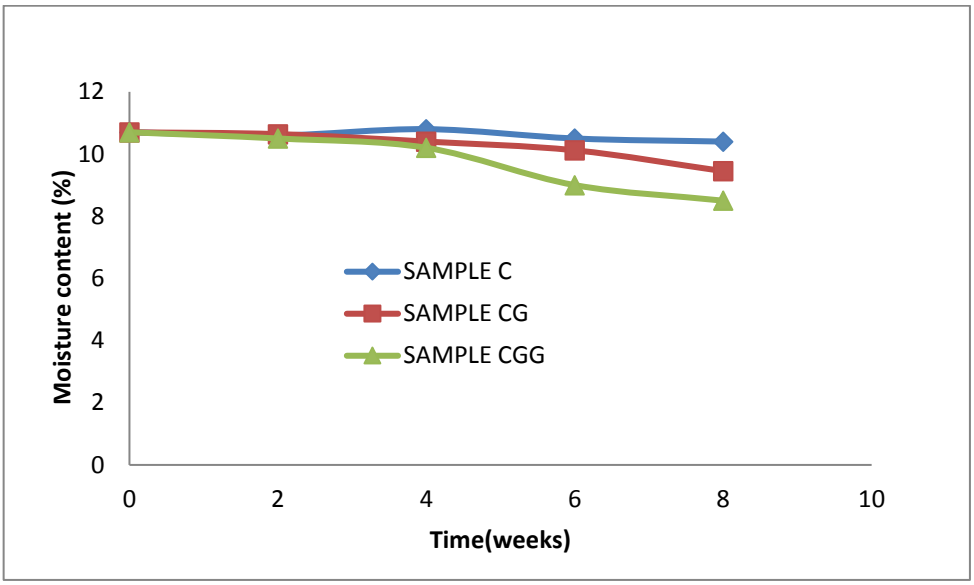

Fig. 4: Moisture content of control and treated soil

The moisture content biplot of samples and weeks is presented as Fig. 5 which shows a clustering of CG and CGG and they are closely linked to weeks 0 and 2. This indicates that the measured moisture contents of these samples in weeks 0 and 2 are correlated and begin to change significantly in the other weeks. The moisture content in Sample C is linked to week 4 but with considerable distance between them, indicating a weak correlation which could change after that week.

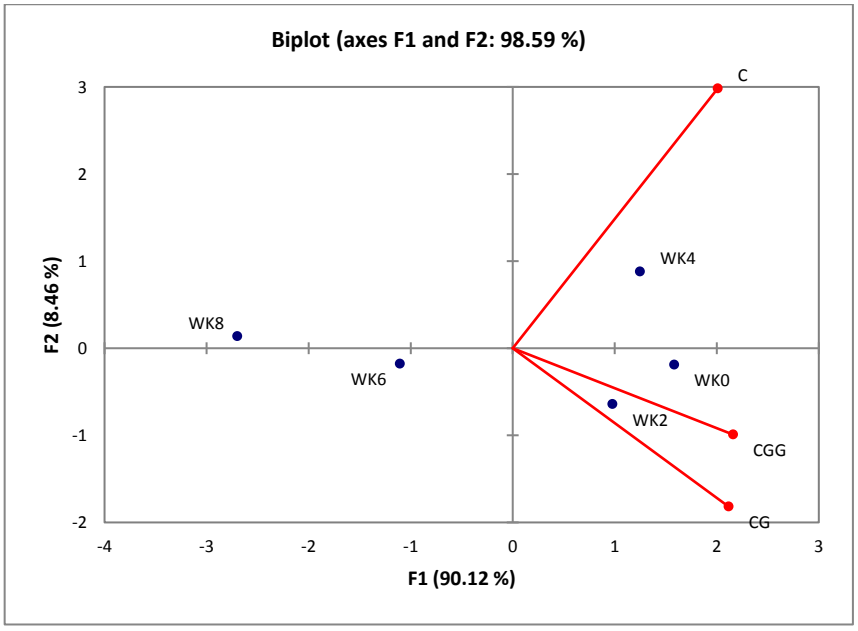

Fig. 5: Moisture content biplot of samples and weeks

A two-way analysis of variance is done for the moisture content to determine how significant the observations made in the biplot is. The result is presented in Table 2 and shows that weeks has a p-value of 0.034 which is less than 0.05 indicating that a null hypothesis is rejected, and the alternate hypothesis accepted. This means that the time period has a significant effect on the moisture content of the samples as more biological processes take place. The p-value of the samples is slightly less than 0.05 indicating that a null hypothesis is rejected, and the alternate hypothesis accepted. The

change in moisture content varies significantly across each sample. This is so because each sample has different treatment applied to it which resulted to different metabolic activities of the microorganisms in the soil sample.

Table 2: Moisture content analysis of variance

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Weeks	3.401227	4	0.850307	4.475141	0.034263	3.837853
Samples	1.69828	2	0.84914	4.469001	0.049764	4.45897
Error	1.520053	8	0.190007			
Total	6.61956	14				

3.3. Total hydrocarbon content

The total hydrocarbon content (THC) of the Control and Treated soils from the beginning to the end of the experiment is shown in Fig. 6. Generally, the THC of the Control and Treated soils decreased consistently throughout the duration of the experiment. The rate of decrease of THC was found to be in the order of CGG (92.6%) > CG (70.5%) > C (24.7%), representing a degradation rate of 238.1 ppm/day, 181.4ppm/day and 63.6ppm/day for CGG, CG and C, respectively. The THC decrease in the Control is expected because of natural degradation processes such as evaporation and degradation by indigenous microorganism taking place. However, such reduction in THC is usually limited because of the high carbon content of crude oil and the low level of nutrients essential for microbial growth and activities (Leys et al., 2005). In contrast, the effective degradation process observed in the treated soils could be because of the availability of nutrients (nitrogen, phosphorus and potassium) from the goat droppings. In agreement, previous studies have opined that the availability of nitrogen and phosphorus generally become the basic factors for crude oil degradation (Atlas, 1984; Leachy and Colwell, 1990). Thus, goat droppings could be an effective remediating agent for the remediation of crude oil contaminated soil.

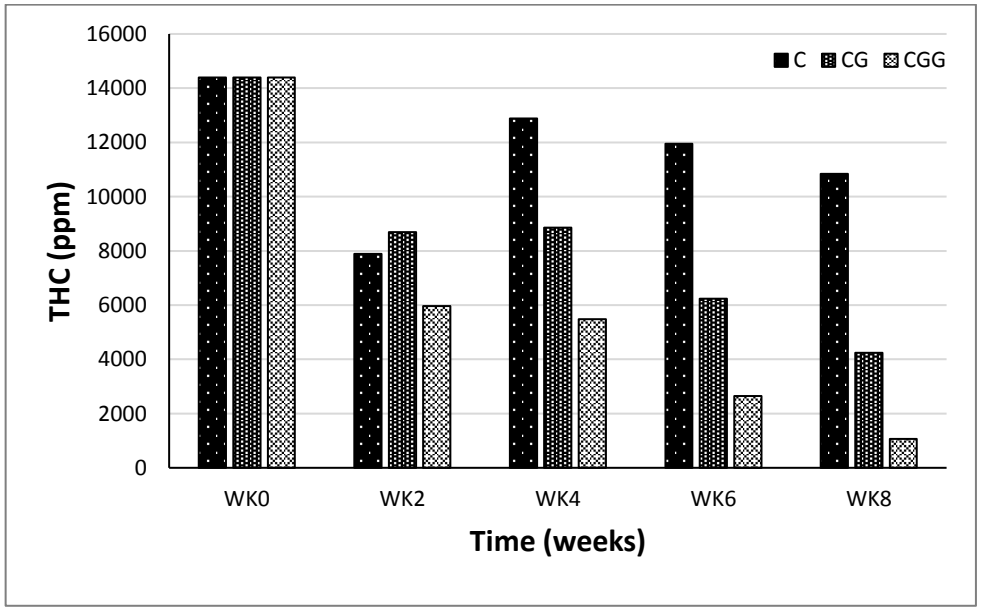

Fig. 6: Total hydrocarbon content of control and treated soils

The THC biplot of samples and weeks is presented as Fig. 7 from which a clustering of CG and CGG is observed and they are closely linked to week 0. This indicates that the measured THC of these samples in week 0 is correlated and begins to change significantly in the other weeks. The THC

in the Sample C is closest to week 4 but with significant distance between them, indicating that week 4 might be a pivotal week for this sample. The measured values show that week 4 was the point after which there was a significant drop in THC for Sample C.

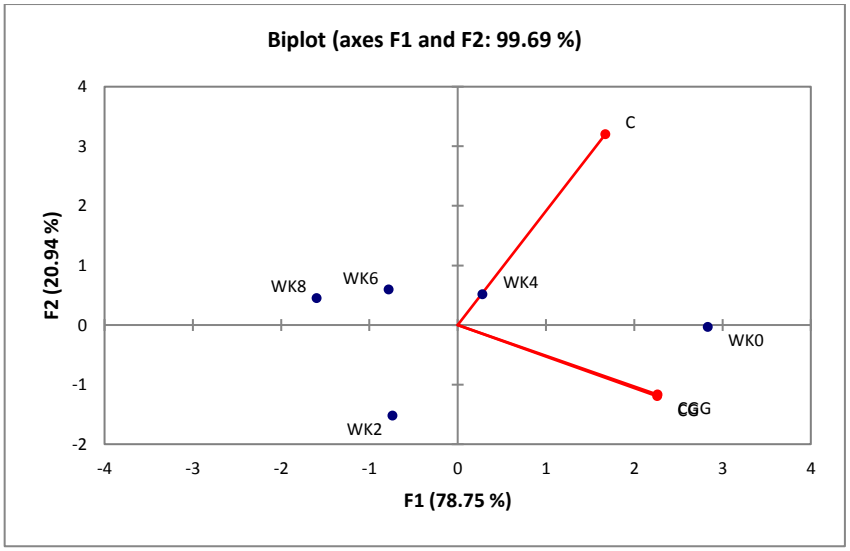

Fig. 7: Total hydrocarbon content biplot of samples and weeks

A two-way analysis of variance is done for THC to determine how significant the observations made in the biplot is. The result is presented in Table 3 and shows that weeks has a p-value of 0.012 which is less than 0.05. Hence, a null hypothesis is rejected, and the alternate hypothesis accepted. This means that the time period has a significant effect on the THC of the samples as degradation proceeds. The p-value of the samples is also less than 0.05 which means that a null hypothesis is rejected, and the alternate hypothesis accepted. THC changed significantly across each sample as remediation improves across the samples from sample C to CGG.

Table 3: THC analysis of variance

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Weeks	1.44E+08	4	36094894	6.524363	0.012307	3.837853
Samples	80913439	2	40456719	7.312789	0.01563	4.45897
Error	44258595	8	5532324			
Total	2.7E+08	14				

4. Conclusions

Bioremediation of crude oil contaminated soil using goat droppings has been studied. This study was driven by the need for proper utilization of agricultural wastes. The results obtained showed that the percentage degradation of the total hydrocarbon content of the treated crude oil contaminated soil was 92.6% for 150g remediating agent (CGG), 70.5% for 100g remediating agent (CG) and only 24.7% for the control experiment (C), representing a degradation rate of 238.1 ppm/day, 181.4ppm/day and 63.6ppm/day for CGG, CG and C, respectively. The results also lead to the conclusion that the pH of the treated soil is most significant after 6 weeks of treatment, the moisture content is seen to change significantly after 2 weeks of treatment and the THC is expected to reduce after the first week of treatment. Thus, it is concluded that goat droppings could be an effective remediating agent for crude oil contaminated soil. Since this study was conducted using a light crude oil contaminated soil, it is recommended that further studies should be carried out on the effectiveness of goat droppings on heavy crude oil contaminated soil.

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