



INVESTIGATION OF THE BIOREMEDIATION PERFORMANCE OF COW-DUNG AND WIRE-CROTON ON CRUDE OIL POLLUTED SOIL

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ABSTRACT

Crude oil exploration is associated with oil spillage causing environmental degradation including loss of soil fertility and biodiversity. Various technologies are available for remediating crude oil polluted soil, but, bioremediation seems to be more environmentally friendly and cost effective. Thus, the current work is on the bioremediation of crude oil polluted soil using cow dung and wire croton leave as the amendments. It was conducted as a batch process and was monitored for 42 days (six weeks). Separate cow dung and wire croton, and different quantities of cow dung and wire croton were blended to remediate the soil. The analysis conducted included the Total Petroleum Hydrocarbon (TPH), Hydrocarbon utilizing Bacterial (HUB) and the Total Organic Carbon (TOC) content of the soil. The result shows that wire croton and cow dung have the potential to be used as bioremediation nutrients. When these nutrients were mixed at different proportions, the combination involving 30 g of cow dung and 30 g of wire croton gave the least performance in terms of HUB, TPH and TOC across the duration of the study, and the maximum was achieved using 200 g of cow dung and 200 g of wire croton as shown by over 650 thousand HUB increment, 95 % hydrocarbon degradation, and 76.3 % total organic carbon obtained. This shows that appropriate combinations of cow dung and wire croton is required to obtain a higher performance using both nutrients for remediating a crude oil polluted soil.

KEYWORDS: Bioremediation; cow dung; wire croton; nutrients; degradation

1.0 INTRODUCTION

The exploration of crude oil usually generates large amount of environmental waste. These activities can cause severe environmental degradation known to affect both terrestrial and aquatic bodies which constitutes majorly as a source of human livelihood and home to several species (Oludele et al, 2021). Crude oil can be harmful to organisms including human and its degradation rate is extremely slow under normal circumstance. It is composed of dangerous chemicals including polycyclic aromatic hydrocarbons (PAH), total petroleum hydrocarbons (TPH) which are known to cause mutation and cancer. The crude oil spill penetrates to a depth of about 10 - 20 cm resulting to the loss of soil fertility and, initiation of environmental degradation (Ofoegbu et al, 2015). The process renders the soil impotent making it to lose its capacity to produce crops compared to the periods prior to the spill. Restoring the fertility of the soil after degradation is usually very difficult and time consuming through natural remediation. The various technologies in use for remediating the soil include evaporation, burying and dispersion (Zadaka-Amir et al., 2013). Most of these technologies are very expensive and thus, bioremediation is considered in this study. This involves the use of organisms (microorganism or plant) or their enzymes to return a polluted environment to its original condition. Bioremediation relies on bacteria, plants and fungi to degrade, breakdown, transform or remove contaminants or impairments of quality from the contaminated soil. When nutrients such as animal dung and croton leave are added, the microorganisms can rapidly degrade the oil, utilizing it as the carbon source. The microorganism produced masticates the excess Petroleum Hydrocarbon content present in the soil. Bioremediation is a promising treatment method for crude oil contaminated sites remediation owing to its cost effectiveness and tendency to achieve complete mineralization of organic contaminants into carbon dioxide, water, and inorganic compounds (Ehirim et al 2020). The Total Petroleum Hydrocarbons (TPHs) are one of the common contaminants in a crude oil polluted soil. They include a broad family of several hydrocarbon compounds that originally come from crude oil which is used to make petroleum products. Most petroleum hydrocarbons encountered in the environment are ultimately degraded or metabolized by indigenous bacteria because of their energetic and carbon needs for growth and reproduction, as well as the requirement to relieve physiological stress caused by the presence of petroleum hydrocarbons in the microbial bulk environment (Hazen et al., 2010; Kleindienst et al., 2015).



2.0 MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Virgin soil was collected from an uncultivated land at the back of the Centre for Nuclear Energy Studies' (CNES) building, University of Port Harcourt, Nigeria between 10 cm and 15 cm deep using a shovel. Cow dung was collected from Nkpogu slaughter market, Port Harcourt using a hand trowel. These materials were stored in polyethene bags separately. Wire-Croton leaves were collected from University of Port Harcourt using a knife and stored in a polyethene bag. The crude oil sample was obtained from Nigeria Agip Oil Company (NAOC). The cow dung and croton leaves were weighed and sun dried for a period of one week. The croton was blended to reduce the particle size. Cow dung and croton leaves were sieved to remove debris. The physiochemical properties of the soil and crude oil analysed after pollution were Total Bacterial count (TBC), Total petroleum hydrocarbon (TPH), Total Organic Carbon (TOC).

2.2 Methods

2.2.1 Assessing the effect of nutrients on the bioremediation of crude oil polluted soil

The experiments were conducted using 1.0 kg of soil and the quantity of materials used are presented in Table 1. The samples were contaminated with 200 mL of crude oil to achieve 16 % pollution of the soil, except in control (1). The contaminated soil was left to stay for one week to settle allowing the microorganisms to acclimatize before adding the amendments. The samples were mixed and observed for six weeks (42 days), and analysis carried out on each sample from day zero and every seven days. The parameters analyzed were total petroleum hydrocarbon, total bacterial count, and total organic carbon.

Table 1. Quantities of materials used for the bioremediation experiments

Batch reactor	Volum of crude Oil (mL)	Cow dung Weight (g)	Croton leaves Weight (g)
Control (1)	-	-	-
Control (2)	200	-	-
3	200	200	-
4	200	30	30
5	200	115	30
6	200	200	30
7	200	30	115
8	200	115	115
9	200	200	115
10	200	30	200
11	200	115	200
12	200	200	200
13	200	-	200

2.2.2 Determining the physiochemical properties of the soil and nutrients used for the polluted soil

(1). pH of the Soil and nutrients

The air dried soil was sieved to obtain a particle size of 2 mm, before 20 g was weighed into a beaker. 50 mL of distilled water was added and stirred with a glass rod thoroughly for about 5 min and kept for 30 min. Within this period, the pH meter was turned on and allowed to warm up for 15 min. The glass electrode was standardized using the standard buffer of pH 7 and calibrated with the buffer pH of 4. The electrodes are dipped in the beakers containing the soil water suspension with constant stirring. As the pH is recorded, the pH meter was switched to pH reading. After 30s, the pH value was recorded to the nearest 0-1 unit. The pH meter was put in standby mode immediate after recording, the electrodes was removed from the soil suspension and cleaned with distilled after. The electrodes were rinsed after each determination and carefully blotted dry with filter paper before subsequent determination. The glass electrodes were standardized after every 10 determinations, and were dipped in distilled water when not in use. This method was repeated using the cow dung and wire croton as the sample.

(2). Determining the electrical conductivity (EC)

The electrical conductivity meter was used to measure EC of the samples. The same procedure stated for pH measurement was used in the determination of EC. However, the EC electrode was thoroughly washed after each reading to avoid cross-contamination and error.

(3). Determining the hydrocarbon utilizing bacterial (HUB)

Microbiological analysis enumeration of heterotrophic bacteria and fungi was carried out by pour plating technique. This was done by inoculating 0-1mL tenfold serialing diluted sample onto nutrients agar (bacterial), acidified streptomycin (1 mg/100 ml) (fungal) and mineral salt agar (MSA) (hydrocarbon degraders). The mineral salt media of Miu et al (1978) as modified by Okpokwasili and Amanchukwu (1988) was used. The inoculated nutrient agar plates was incubated at 37 °C for 24 hr while the potato dextrose Agar plates was incubated at room temperature, counted and expressed as colony forming units per gram (Cfu/mL).

(4). Determining the total organic carbon (TOC)

TOC was determined using a method described by Umeda et al. (2017). Thus, 1.0 g of soil



sample was weighed into 250 mL beaker, while 10 mL of potassium dichromate solution was pipette into the beaker and vortexed gently to completely wet the soil sample. Thereafter, 20 mL of concentrated H₂SO₄ was added using automatic pipette, and gently vortexed for 1 min to obtain a uniform suspension, as well as for effective and more complete oxidation before allowed to settle for about 30 min on asbestos sheet. On settling, 100 mL of distilled water was added followed by 3-4 drops of 0.5 mL diphenylamine indicator. The solution was titrated with 0.5 N ferrous sulphate solution until the colour changes from violet to blue and finally bright green. The process was repeated on distilled water (blank titration), but without soil to standardize the dichromate. The procedure was repeated using cow dung and wire croton. The TOC was calculated using Equation 1.

$$TOC = Blank - \frac{\text{volume of soil sampletitre} \times 0.195}{\text{weight of soil sample}} \times 100\% \quad (1)$$

5). Determining the total petroleum hydrocarbon (TPH)

The TPH was analyzed using Gas Chromatography-Flame Ionization Detector (GC-FID). The soil sample was discharged into a 1 L separation funnels. 50 mL of methylene chloride was added to the sample bottle seal and vortexed for 30 s to raise the inner surface. The solvent was transferred to the separation funnel and extracted by shaking the funnel for 2 min with periodic venting to release excess pressure. The organic layer was allowed to separate from the water phase for a minimum of 10 min. The methylene chloride was extracted in a 250 mL flask. An additional 60 mL of methylene chloride was added to the sample bottle. The separation funnel and the column were rinsed with 25 mL of the solvent into the extract. The extraction procedure was repeated thrice and the output combined with the other extract in an Erlenmeyer flask. The combined extracts was poured through a drying column containing packed cotton wool, anhydrous sodium sulphate and silica. The extract was collected in the vial and concentrated by boiling it down with nitrogen gas to 1.0 mL. The remaining extract was mixed with 1.0 mL of the solvent and 1-0 mL was injected into the flame ionization detector gas chromatograph for the TPH analysis. The residual TPH at any time was calculated using Equation 2.

$$TPH_R (\%) = \frac{TPH_i - TPH_f}{TPH_i} \times 100\% \quad (2)$$

Where: TPH_R is the residual TPH percentage with time, TPH_i is the initial concentration of TPH and TPH_f is the concentration of TPH measured with time.

(6). Determining the Phosphorous Content

Phosphorus content was determined according to APHA method 4500 - PO₄³⁻ (APHA, 1998). 1.0 g of the representative soil sample was weighed into clean extraction flask and 10 mL of Bray P-1 extracting solution (0.025N HCl and 0.03N NH₄F) was added and vigorously agitated for 1 min before being filtered. 5 ml of the filtrate was pipetted into 25 mL volumetric flask and diluted to about 20 mL of distilled water, and then, by 4 mL of ascorbic acid solution (1.056 g ascorbic acid in 200 mL molybdate-tartarate solution), which was diluted. The diluted solution was allowed to settle for at least 30 min. The recording of data was done after a clear colour had been developed.

3.0 RESULTS AND DISCUSSION

3.1 Physiochemical properties of soil and treatments used for the polluted soil

The physiochemical properties of the soil, cow dung and wire croton were done using the experimental procedure described in section 2.2 of the Materials and Methods. The result obtained are presented in Table 2. The table shows that the pH of the soil, cow dung and croton were 5.11, 7.4 and 6.3. This shows that the soil is moderately acidic, cow dung is slightly alkaline and wire croton is slightly acid. The moderate acidic nature of the soil is probably due to the geographical location where the soil was collected. The soil was obtained in Obio-Akpor Local Government Area of Rivers State which is known to have a pH of 6.20 - 7.60 Ngah et al. (2017). As shown in Table 2, the electrical conductivity (EC) of the soil is 89.34µs/cm. This means that the soil can hold on to cations and so will not lose nutrients easily. The total Nitrogen content of the soil was 0.048 %, while that of the cow dung and croton were 21.2 % and 16.1 %. The result agrees with that of the total nitrogen content of soil obtained by Oludele et al (2021) and Ofoegbu et al (2015). The total hydrocarbon utilizing bacterial for the virgin soil was 1.03x10². The total organic carbon for the soil was 6.29 % while that of the cow dung and croton were 14.6 and 2.1 respectively. The result corroborates that obtained by Ofoegbu et al (2015) and Olawale et al. (2020). The total petroleum hydrocarbon obtained for the soil was 310.3 as the soil was not polluted with crude oil.

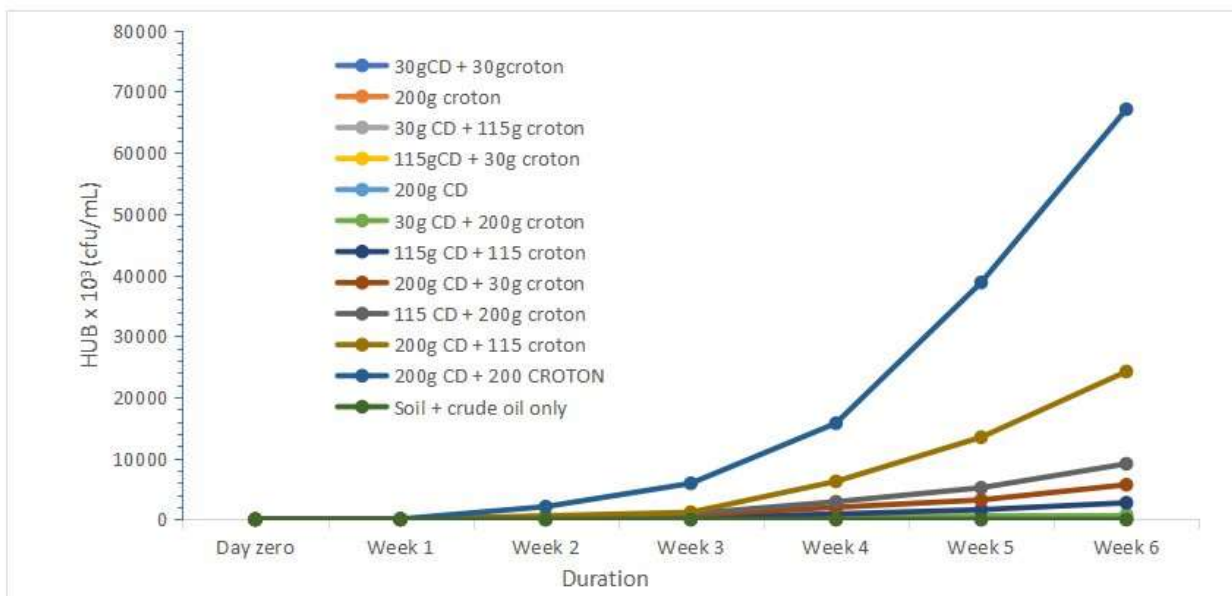


Table 2. Physiochemical Properties of the parameters used in the bioremediation of the crude oil polluted soil

Parameters	Soil	Cow Dung	Wire croton
pH	5.11	7.4	6.3
EC (µs/cm)	89.34		
HUB (Cfu/mL)	1.03x10 ²		
Total Organic Carbon (%)	6.29	14.6	2.1
TPH	310.3		
Phosphorus (mg/kg)	1.38		

3.2Determination of the total hydrocarbon utilizing bacterial (HUB)

The HUB content of the soil was assessed in order to determine the quantity of bacterial available for the degradation of the crude oil, as the higher the bacterial content, the higher the rate of degradation. The HUB content was determined using the pour plating technique. The experiment was carried out in six weeks using two different nutrients (cow dung and wire croton) and combined in different proportions. The results obtained are presented in Figure 1. The figure shows that in day zero, the soil with the mixture of 200 g cow dung and



Where CD is cow dung

Figure 1. effect of nutrients on the total hydrocarbon bacterial for a period of six weeks using varied proportions of cow dung (CD) and wire croton

The result show that the crude oil polluted soil containing no nutrient remained relatively unchanged throughout the duration of study (six weeks) as indicated by the zero HUB obtained. This is because, a long time is required for soil to remediate itself naturally and six weeks may not have been sufficient to achieve that (Khalilova, 2015). Between day zero and week 1, based on the zero HUB obtained, there was no appreciable change in the crude oil polluted soil as the microorganisms contained in the soil was beginning to acclimatized in their new environment and there was no addition of amendments to facilitate their growth. From week 1, the microorganisms were beginning to degrade the contaminated soil as shown by the increasing HUB values. However, the HUB increased progressively throughout the six weeks irrespective of the type of nutrient and their combination with the highest HUB obtained at the end of the six weeks and the least achieved at week 1. This is because bacterial increases with availability of nutrients and they multiply over time. Also, from Figure 1, the soil containing 200 g of cow dung plus 200 g of wire croton gave the highest HUB of 67100 x 10³ cfu/mL and the soil with 30g of cow dung and 30 g of wire croton gave the least HUB of 9.57 x 10³ cfu/mL. The result shows that the quantity of the nutrient has a direct relationship with HUB. This was evident with the soil containing 200 g of cow dung and 200 g of wire croton with more nutrient compared to the soil with 30 g of cow dung and 30 g of wire croton resulting to the highest HUB. This was expected as Oludele et al. (2019), and Thieman and Palladino (2009) obtained an increase in the HUB upon addition of nutrients as this will increase the amount of the microorganism leading to increase in their growth and then, rise in the biodegradation rate. This may be due to increased population of the microorganism leading to high HUB. Comparatively, the crude oil polluted soil without nutrient had the least amount of Hydrocarbon Utilizing Bacteria of 1.03 x 10³ cfu/ mL. All the soils with nutrients had higher amount of HUB present when compared with the crude oil polluted soil without



nutrient. At the end of the six weeks, HUB increase of over 650 thousand was achieved using 200 g of cow dung and 200g of wire croton.

3.3 Determination of the degradation of the Total Petroleum Hydrocarbon (TPH) of the polluted soil

This was done to determine the amount of TPH present in the soil. High amount of TPH in the soil can hinder the growth of crops in the soil. The TPH was analysed using Gas Chromatography-Flame Ionization Detector (GC-FID) and the experiment was carried out in six weeks with analysis done every seven days (weekly). The result obtained is as shown in Figure 2. As shown in Figure 2, the rate of degradation of the crude oil in the soil with

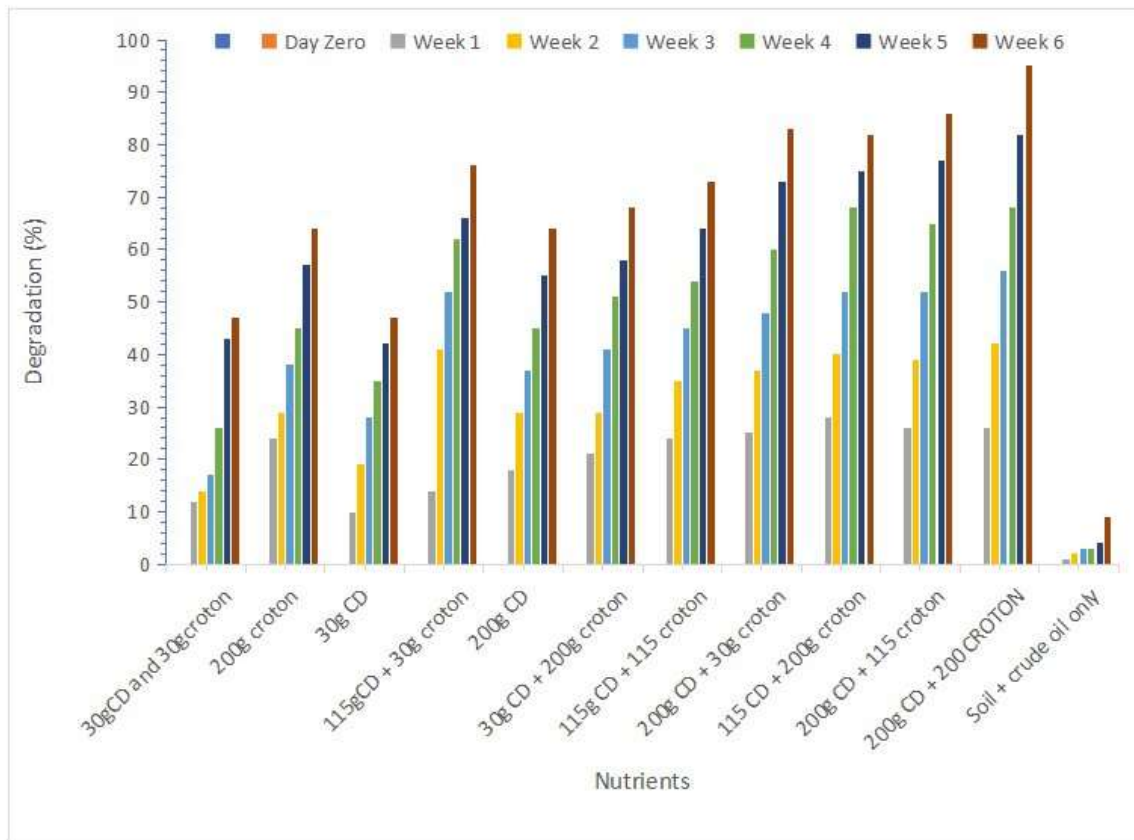


Figure 2. Illustration of TPH degradation in the different soil samples using various proportions of the nutrients within six weeks

was slow with a maximum of 9 % degradation on the week 6, probably as there was no addition of nutrients and the microorganisms were beginning to acclimatize in their new environment. The soils amended with single nutrients having equal quantity (200 g wire croton and 200 g cow dung) showed almost the same rate of degradation compared to the one with lesser quantity like 30 g cow dung. This shows that the degradation of the hydrocarbons component of the crude oil may depend more on the quantity of the nutrient compared to the type. The soils with mixed nutrients showed greater degradation than those with single nutrients. This may be due to the similarities in the composition shared by both cow dung and wire croton (Fulhage, 2000; Izionworu et al., 2020). Among the soils with mixed nutrients, the one with nutrient composition of 30 g plus 30 g croton gave the least degradation of the hydrocarbons of 14-47% between weeks one and six. The highest hydrocarbon degradation of 95 % was achieved with the soil composition having 200 g of cow dung plus 200 g wire croton across the six weeks, probably due to higher amount of nutrients composition which increases the hydrocarbon utilizing bacterial (HUB).

3.5 Determination of the Total Organic Carbon of the soil

The analysis of the Total Organic Carbon content of the soil was done to ascertain the organic content of the soil. The analysis was done for six weeks at a weekly interval using the method prescribed in Sub-section 2.2.2. The result obtained are presented in Figure 3 and it shows that the TOC for the soil increased from 6.29 % to 7.58 % upon the addition of the crude oil (see Table 2). This was expected as crude oil contains hydrocarbon which is a source of carbon and is known to increase the total carbon content of the soil (Schuster et al., 2002).

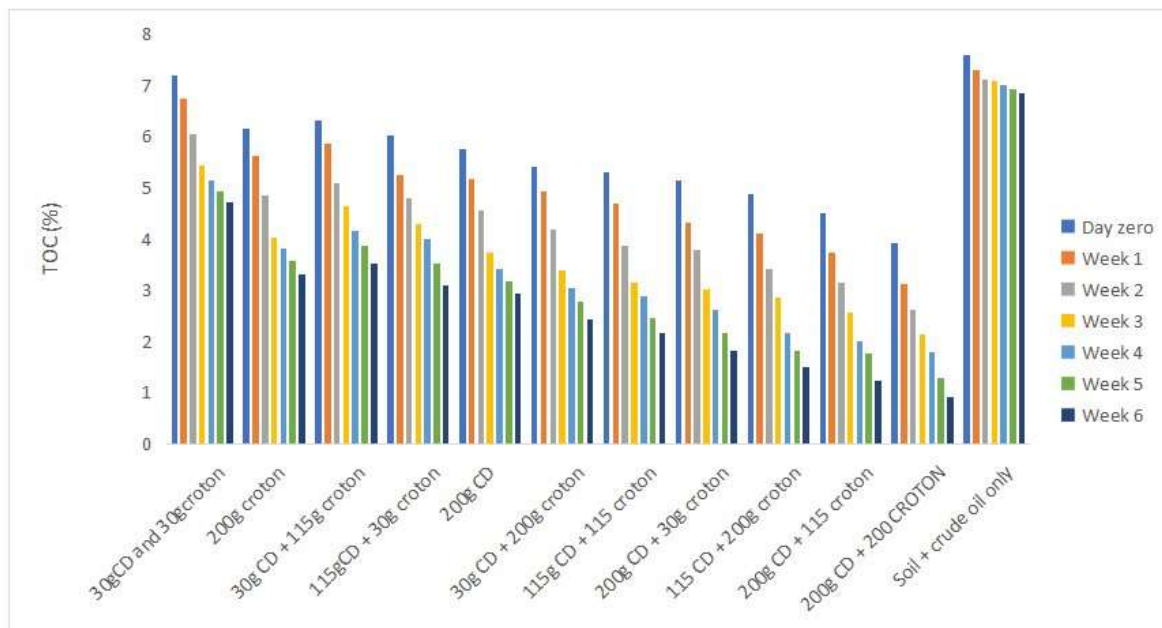


Figure 3. Effect of nutrients on the total organic carbon content of the soil during bioremediation of crude oil polluted soil.

Naturally, throughout the period of study, the carbon content of the soil was degraded by 9.8 % without the use of nutrients. Upon the addition of nutrients, the TOC decreased considerably across the six weeks of study. The single nutrients wire croton and cow dung performed well with the former degrading the carbon content by 46.3 % and the later by 48.6 % at the end of the sixth week using 200 g of each nutrient. This performance was further increased by mixing the nutrients using different ratios with 30 g cow dung plus 30 g croton giving 34.5 % carbon degradation which is the least obtained using nutrients at the end of week six. The highest hydrocarbon degradation was recorded between TOC of 3.92 % on day zero and 0.93 % on week six which is about 76.3 % using 200 g cow dung plus 200 g croton. As presented earlier, this high great performance might be due to high quantity of the nutrients used. The reduction in TOC as obtained in this work using nutrients is corroborated by Ojewumi et al. (2018) in which the researchers were able to achieve TOC decrease of less than 1 % at the end of 45 days using treated crude control sample (TCC).

4.0 CONCLUSION

The current work has demonstrated that nutrients such as cow dung and wire croton have the capability to bioremediate crude oil polluted soil as evidenced by the result of the total hydrocarbon utilizing bacteria, total petroleum hydrocarbon and the total carbon content. But, this capability can be enhanced by mixing the nutrients together as shown by the over 650 thousand HUB increment, 95 % hydrocarbon degradation, and 76.3 % total organic carbon obtained when 200 g of cow dung and 200 g of wire croton were combined. With the appreciable result obtained, the length of the bioremediation can be increased beyond six weeks (42 days) using higher combination of the nutrients in order to optimize and to assess the large scale application of the method developed.

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