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# REMEDIATION TRIALS OF CRUDE OIL CONTAMINATED SOIL USING DIFFERENT SAWDUST AND DETERGENT COMBINATION LEVELS

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## ABSTRACT
*A 3 month remediation trial of the use of detergent and sawdust in different combination forms in the restoration of a crude oil contaminated tropical soil was investigated. 8 remediation treatments labeled A – H in addition to the control (I) were used in 10 kg soil artificially polluted with 300 ml crude oil each. Remediation treatments were: A (20 g detergent), B (40 g detergent), C (100 g sawdust), D (200 g sawdust), E (20 g detergent + 100 g sawdust), F (20 g detergent + 200 g sawdust), G (40 g detergent + 100 g sawdust), H (40 g detergent + 200 g sawdust) and I (polluted soil without an amendment) arranged in a completely randomized design. Soil chemical parameters such as total hydrocarbon content, total organic carbon, Total organic matter, nitrogen, nitrate and pH were evaluated. The resulted indicated that the amendments (either in single or combined form) reduced soil electrical conductivity and Total Hydrocarbon Content (THC); and increased soil pH, Nitrate, Nitrogen, Total Organic Carbon (TOC) and Total Organic Matter (TOM). There was also significant increase in the soil microbial counts in the amended soil as compared to the control. Therefore, detergent and sawdust in single or combinations can be used to restore crude oil polluted soil with the ability to reduce the toxic effect with treatment A (20 g detergent) and C (100 g sawdust) and D (200 g sawdust) performing best. Though caution should be taken in their application (especially detergent), as not to cause any adverse effect.*

**Keywords**: remediation, detergent, sawdust, pollution, crude oil, soil.

## INTRODUCTION
Soil is an important natural resource upon which environmental sustainability largely depends (Adenipekun, 2008; Onuh *et al*., 2008; Srivastava *et al*., 2016). Several factors arising from natural and anthropogenic activities have rendered the soil impotent; since the soil is a repository of many wastes; thus, making the soil incapable of performing its natural endowed functions. In the Niger Delta region of Nigeria, one of such anthropogenic activities is crude oil exploration and exploitation by multinational oil companies. The invention of the internal combustion engine as a means of transport led to the increase use of crude oil as a source of energy (Chorom *et al*., 2010) with a concomitant increase in its production demand and transport (Difiglio, 2014).

These activities resulted in minor and major oil spillages into the environment which have caused severe damage to the ecosystem especially the soil. Apart from the spillages, the oil waste discharged into water bodies and soil from petroleum refining (Voulvoulis and George, 2015), and artisanal automobile workshops (Anyanwu *et al*., 2014) also contribute significantly to oil pollution problem.

Petroleum hydrocarbon pollution can occur in both terrestrial and aquatic ecosystems and it is currently on the increase. This is disturbing due to its negative consequences. Crude oil has been known to affect soil physico-chemical properties such as aeration, pH, capillarity, organic/inorganic nutrient contents and biota (Kayode *et al*., 2009, Gighi *et al*., 2012). These soil properties contribute to the sustainability of plants (Verma and Agarwal, 2007). As a result, so many food crops are at considerable risk. This will have negative effect on crop production and economic livelihood of the local communities affected by the pollution (Inoni *et al*., 2006). Zhang *et al*. (2016) attributed the toxicity effect of crude oil to its low volatility and aqueous solubility. Amro (2004) reported that the high viscosity of oil limits the penetration of oil into soil due to its reluctant to flow. The hydrophobic compounds of crude oil bind to soil particles and block soil pores thereby preventing water and air flow into the soil. Prince (1993) observed that hydrocarbon compounds are incomplete food sources for microbial growth since they do not contain significant amounts of major nutrients, such as nitrogen and phosphorus, even though they are excellent source of carbon and energy for the microbes. The toxicity of crude oil in the environment depends on the degree of contamination, season and oil type that is spilled (Pezeshki *et al*., 2000; Sarkar *et al*., 2005) A heavily crude oil polluted soil may remain unsuitable for the growth of plants for several years. Tanee and Albert (2015) observed that the total hydrocarbon content of soil more than 15 years after pollution was still above the acceptable limit with a significant reduction in the phytodiversity in an unremediated crude oil polluted soil at Kwawa, Ogoni Nigeria.

Due to the undesirable socio- economic and ecological consequences often associated with crude oil pollution (Panel, 2013); remediation of crude oil polluted soil becomes inevitable for good and efficient agricultural productivity, and proper and sustainable use of soil. Since natural attenuation of crude oil polluted soils may take a long time to accomplish and because of high demand for cultivated land, it may be inappropriate to allow polluted soils to be rehabilitated naturally.

Several approaches have been adopted to decontaminate or restore a contaminated environment. Depending on the approach used, the remediation can be done either *in-situ* or *ex-situ*. It is *in-situ* when the remediation is done on the polluted site and *ex-situ* when it is done outside the polluted site. Some of these approaches include physical/mechanical (burning, excavation, spray, vapor extraction, stabilization, solidification); chemical (detergent, surfactant), and biological (biostimulation and bioaugmentation) methods. The choice of method to be used depends on the circumstances and the suitability of the method in each case. Among the current approaches used for restoration or decontaminating crude oil polluted soils, bioremediation and chemical remediation appear to be taken the lead, although they

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have their limitations. Bioremediation could be biostimulation (ie addition of nutrient to stimulate the biodegradation process) and biouagmentation (addition of biodegrading organisms). The rationale behind biostimulation is to remove nutrient limitations in soil usually associated with crude oil pollution. The chemical method involves the application of chemicals such as dispersant to dislodge the hydrocarbon chains and provide a good surface area for biodegradation (Couto *et al*., 2010). Though, the extensive application of this method has some reservations because of the fear of its toxicity and long-term environmental effect (USEPA, 1999).

This study is carried out to test the suitability of single and/or combined application of organic and chemical agents in the remediation of crude oil polluted soil using sawdust and detergent as remediation materials. Results obtained from this study will give us a wider view on suitability of different methods of restoring crude oil contaminated habitat as to achieve as effective and eco-friendly remediation.

## MATERIALS AND METHODS

### Description of experimental site

The experimental site was the experimental field of Centre for Ecological Studies in the Department of Plant Science and Biotechnology, University of Port Harcourt, Choba, Rivers State of Nigeria. The study site is located between Latitude 4 $^0$N and 5 $^0$N, and Longitude 6 $^0$E and 7 $^0$E of the tropical rainforest belt of Nigeria.

### Sources of materials

Soil for the experiment was collected randomly with a spade from an old Agricultural Demonstration Farm at the University environment. Surface soil of 0 – 15 cm depth was collected, bulked and homogenized. 10 kg of soil was weighed into each experimental bag with a 10 cm allowance at the top of the bag for proper watering. The bags were also perforated at base for drainage. A total of 54 bags filled with experimental soil were used for the experiment.

The sawdust used was collected from timber mill at Rumuosi, Port Harcourt. The detergent was bought from a supermarket at Eleme. The crude oil (bonny light) was obtained from Nigerian National Petroleum Corporation (NNPC), Eleme, Port-Harcourt.

### Experimental design

Crude oil was applied to the soil as the pollutant. 300 ml of crude oil was added to each bag containing the 10 kg of soil, representing 3 % v/w contamination level; and thoroughly mixed with the soil and allow for 5 days. This was done to obtain homogeneity and mimic the normal crude oil spill pollution situation. After the elapse of the 5 days, the 54 bags were separated into 9 sets. Different remediation materials were applied to 8 set labeled A – H, while the 9$^{th}$ set acted as the control with no remediation. The remediation materials used were sawdust and detergent in different combinations. The different ratios of sawdust and detergent used were in the following order: A (0 g sawdust + 20 g detergent), B (0 g sawdust + 40 g detergent), C (100 g saw dust + 0 g detergent), D (200 g sawdust + 0 g detergent), E (20 g detergent + 100 g saw dust), F (20 g detergent + 200 g sawdust), G (40 g detergent + 100 g saw dust), H (40 g detergent + 200 g sawdust) and I (no amendment). The remediation treatments were mixed thoroughly with the soil in each bag. Thus a Completely Randomized Design (RCD) comprising a control and 8 remediation treatments with each replicated 6 times was adopted. The remediation trial was monitored for 3 months.

**Soil collection and Analysis**

Soil samples were collected from the different treatment bags at two different times (initial and final) for the analysis of the soil physicochemical parameters. Initial analysis was done 5 days after pollution of the soil with crude oil and the final analysis at the end of the experiment (three months later). The following soil parameters were analyzed for the remediation trial; pH, electrical conductivity, nitrate, total organic carbon (TOC), total hydrocarbon content (THC), nitrogen and total organic matter (TOM).

**Determination of measured parameters**

The electrical conductivity and pH of the soil were determined electronically using a glass electrode pH metre and conductivity metre (HANNA HI Series), respectively. Brucine method (USEPA, 1971) and Kjeldahl Method (Stewarte *et al.* 1974) were used for the determination of soil nitrate and nitrogen contents, respectively.

Total Hydrocarbon Content of the soil samples was determined using Soxhlet Extraction Method (APHA, 1995) and; Nelson and Somners approach (1982) was used for the determination of Total Organic Carbon (TOC) while total Organic Matter (TOM) was derived using the method of Osuji and Adesiyan (2005) as

% Organic matter = % Organic carbon x 1.724

Soil microbial (Total Heterotrophic Fungi and Total Heterotrophic Bacteria) analysis was done with 1 g of soil sample, weighed into 9 ml sterile diluents (0.85 % Nacl) under aseptic conditions. 0.1 ml Aliquot of inoculums (after vigorous shaking and serially diluted) were inoculated at room temperature on Potato Dextrose Agar (PDA) acidified on 0.1 % Lactic acid for 7 days (for Total fungi) and on Nutrient Agar (NA) surface for 24 hours (for Total Heterotrophic Bacteria). Thereafter the numbers of visible colonies were enumerated. Hydrocarbon Utilizing Bacteria (HUB) and Hydrocarbon Utilizing Fungi (HUF) were analyzed by inoculating soil sample in Mineral Salt Agar (MSA) using the spread plate technique for 5 days and 7 days, respectively.

**Statistical evaluation**

Statistical evaluation such as means, standard error means (SEM) and Least significant difference (LSD) were calculated from the data obtained using excel version 10. Results were presented in bar graph of mean ± SEM.

**RESULTS**

Remediation of the artificially crude oil contaminated soil altered the physico-chemical characteristics such as total hydrocarbon content, total organic carbon, Total organic matter, nitrogen, nitrate, pH and carbon-nitrogen ratio of the soil after 3 month of remediation.

It was observed that soil electrical conductivity in the different treatments were significantly different (P = 0.05). Each treatment also showed significant difference between the initial (before remediation) and the final (at the termination of the experiment). All the treatments showed significant reduction in the electrical conductivity except in treatment I (no pollution and no remediation) in which the conductivity increase at the end of the experiment (Fig. 1). Highest reductions were observed in treatment B > F > G > H > A.

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Amending the contaminated soil with sawdust and detergent in single or combined form is shown to cause significant reduction in the soil total hydrocarbon content (THC) of the different treatment options (Fig. 2). Between the treatment options, treatment A (0 g sawdust + 20 g detergent), C (100 g saw dust + 0 g detergent) and D (200 g sawdust + 0 g detergent) showed the highest reduction in THC.

There were alterations in the pH of the soil in the different remediation treatment options of sawdust and detergent (single or combine application) as presented in Fig. 3. At the end of the 3 month, the remediation treatments raised the soil pH from neutral to alkaline especially in treatments A (0 g sawdust + 20 g detergent), B (0 g sawdust + 40 g detergent), and C (100 g saw dust + 0 g detergent) (p=0.05). The only exception was in treatment H (40 g detergent + 200 g sawdust) in which there was a decrease in the pH level after remediation.

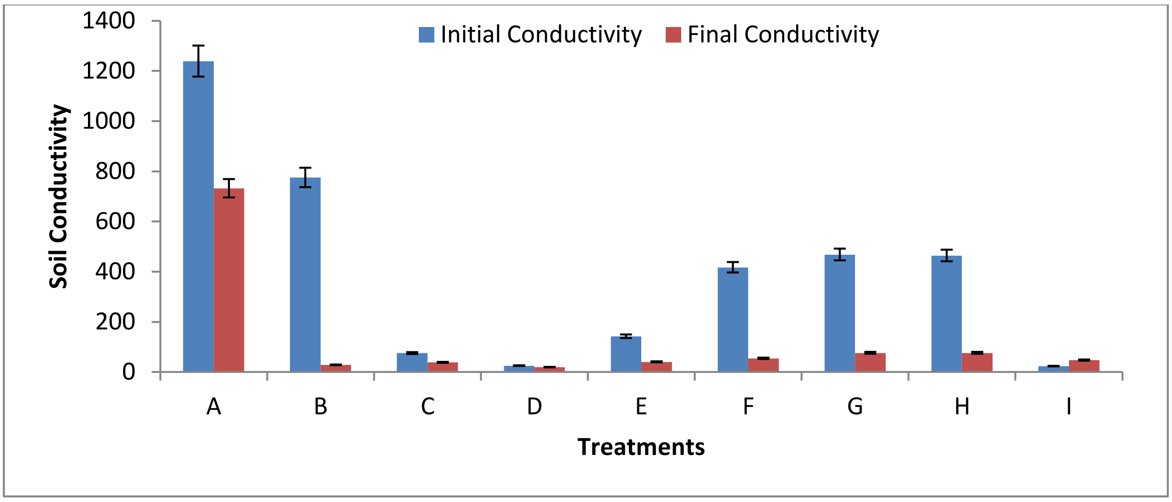


**Fig. 1:** Effects of Treatments on the Soil Conductivity

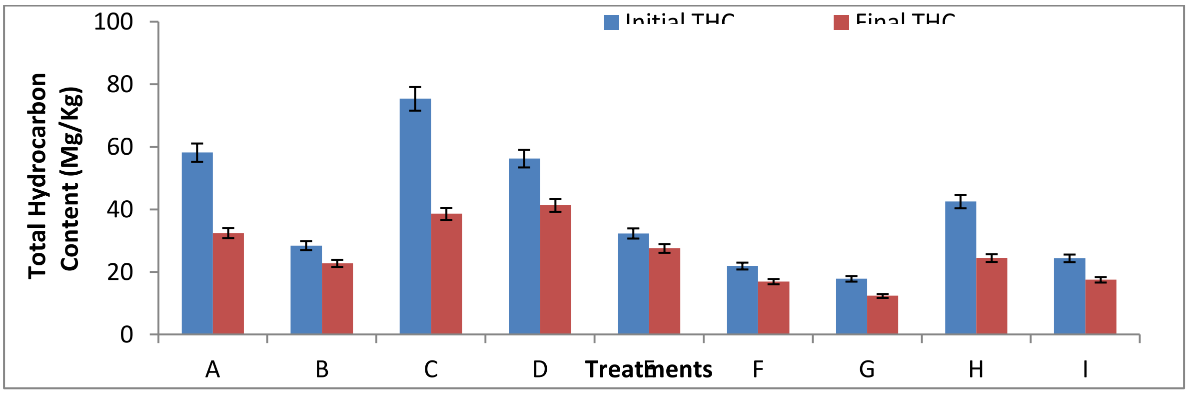


**Fig. 2:** Effects of Treatments on the Soil Total Hydrocarbon Content (THC)

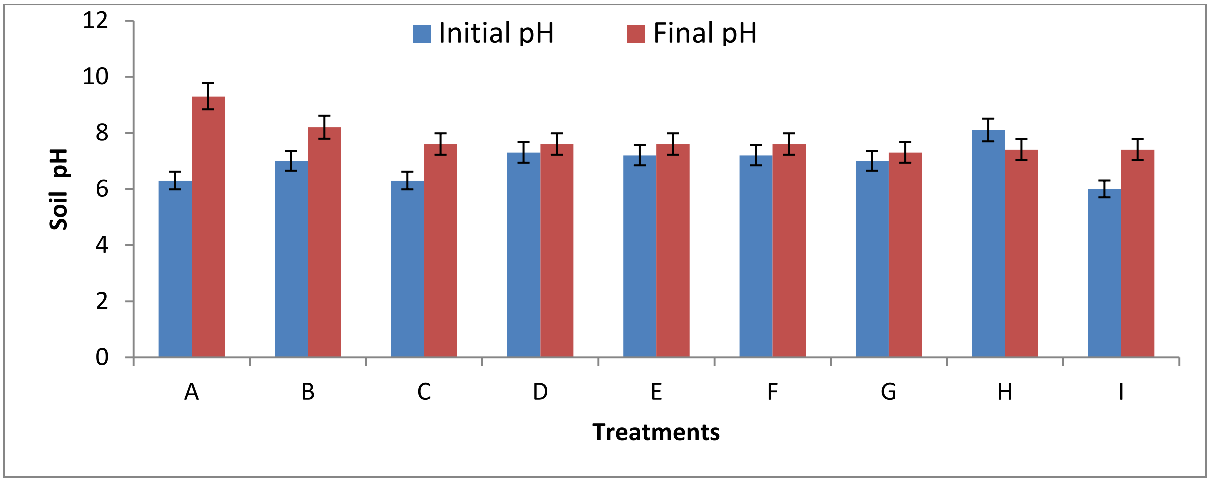


**Fig. 3:** Effects of Treatments on the Soil pH

Fig. 4 and 5 showed that remediating the contaminated soil with the different sawdust and detergent combination options affected the soil nitrogen and nitrate. The Total nitrogen increased in all the treatment options from the initial concentration (before remediation) to a higher concentration (final) at the expiration of the experiment (Fig. 4). Though significant increase (p=0.05) was only observed at treatments A, B, G and H. There was also improvement in soil nitrate content after remediation when compare with the initial (before remediation). Significant increase in soil nitrate was observed in treatments A, F, G and I. Whereas, treatment B, and H showed significant decrease in soil nitrate as compared with the initial. Treatments C, D and E showed no change (Fig. 5).

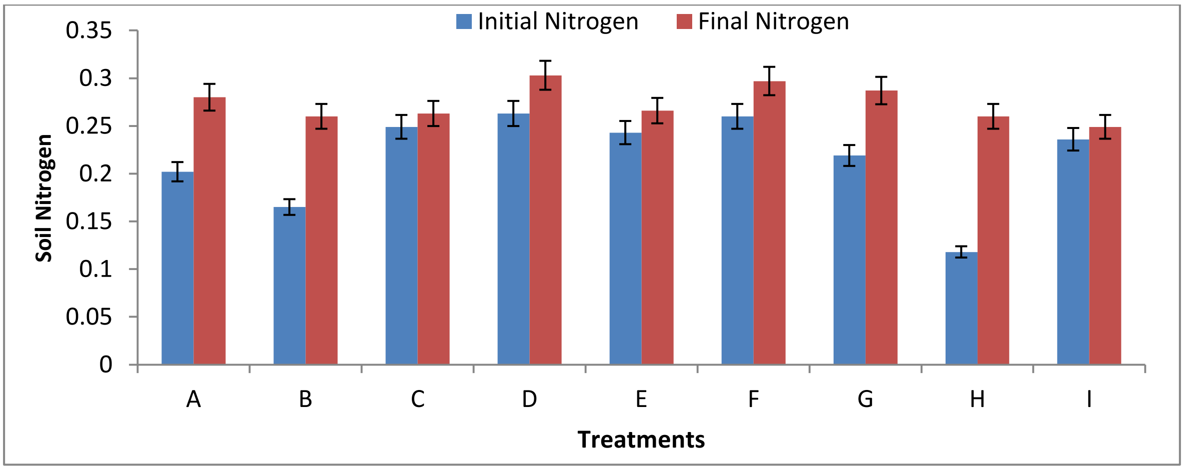


**Fig. 4:** Effects of Treatments on the Soil Nitrogen

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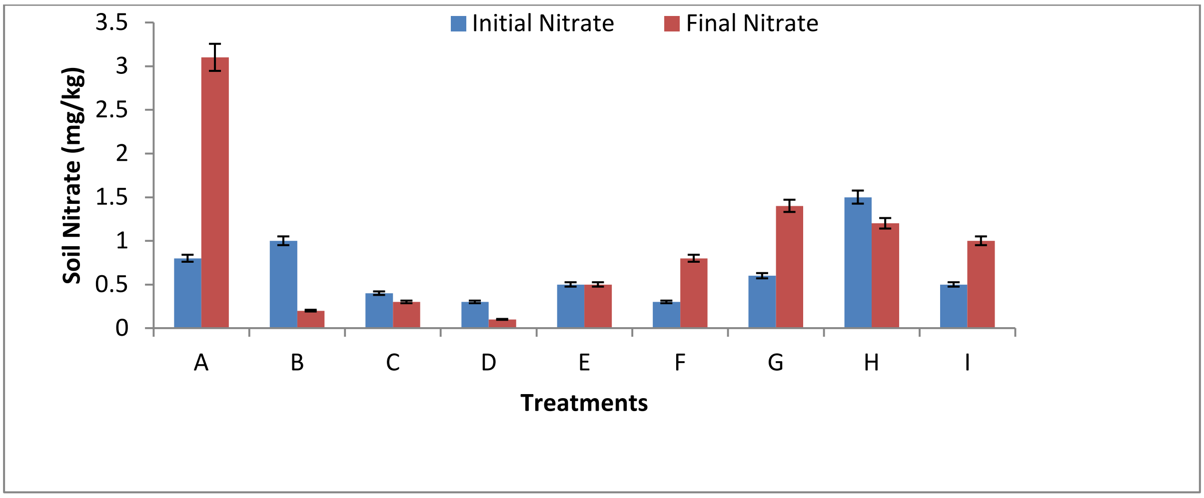


**Fig. 5:** Effects of Treatments on the Soil Nitrate

Results of TOC (Fig. 6) and TOM (Fig. 7) followed the same trend. The total organic carbon (TOC) and total organic matter (TOM) contents of all the treatment options at the end of the experimental trial were greater than the initial (before remediation). Highest significant increases in the both parameters were observed in treatments A, B, G and F.

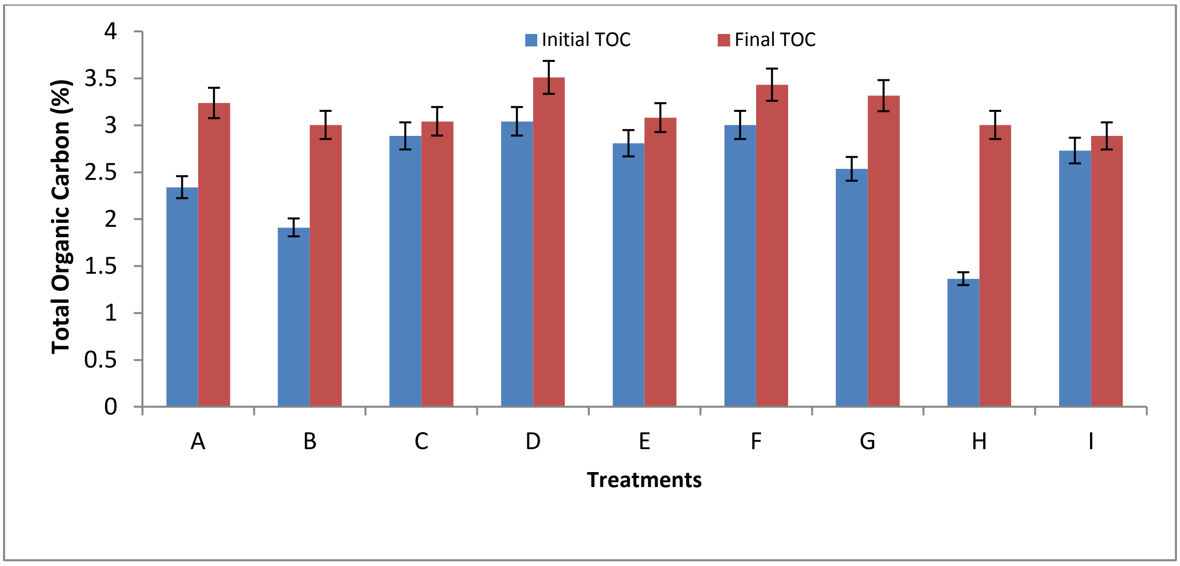


**Fig. 6:** Effects of Treatments on the Soil Total Organic Carbon (TOC)

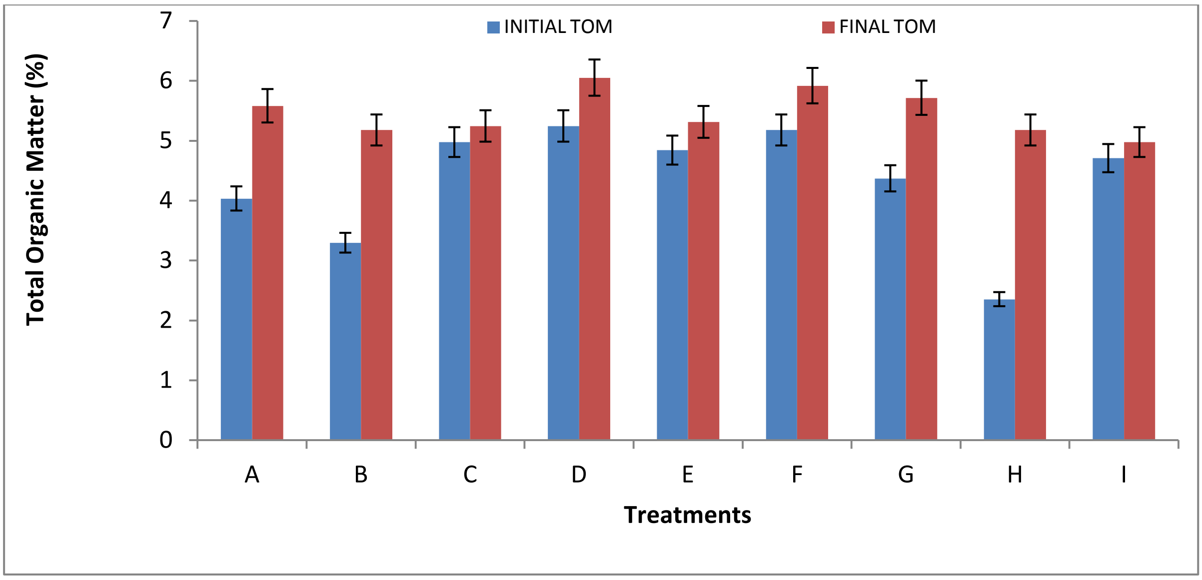


**Fig. 7:** Effects of Treatments on the Soil Total Organic Matter

Table 1 showed the result of the microbial analysis. Treatment options A and B showed significant increase in all the different microbial populations (THB, HUB, HUF, THF) from the initial (before amendment) to the final (expiration of the experiment). Apart from A and B, increment in microbial populations were also observed in other treatments; THB (treatments C, D, and H); HUB (treatments E and I); HUF (treatment G); THF (treatments G and H). There was also significant increase in microbial populations in the treatment options when compared with the control with few exceptions.

**Table 1:** Microbial Parameters of the different treatment options

| **Treatments** | **Microbial population counts** (cfug$^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **THB** | | **HUB** | | **HUF** | | **THF** | |
| | **Initial** | **Final** | **Initial** | **Final** | **Initial** | **Final** | **Initial** | **Final** |
| A | 7.2x10$^6$ | 7.7x10$^7$ | 1.6x10$^6$ | 6.0x10$^6$ | 3.3x10$^4$ | 2.9x10$^5$ | 3.0x10$^4$ | 1.6x10$^5$ |
| B | 5.8x10$^6$ | 3.4x10$^7$ | 9.2x10$^4$ | 1.3x10$^6$ | 1.5x10$^4$ | 2.0x10$^5$ | 4.9x10$^4$ | 2.0x10$^5$ |
| C | 4.8x10$^5$ | 6.0x10$^6$ | 17.9x10$^5$ | NIL | 3.1x10$^4$ | 1.05x10$^4$ | 6.0x10$^4$ | 3.9x10$^4$ |
| D | 3.9x10$^5$ | 5.9x10$^6$ | 12.5x10$^5$ | 3.2x10$^6$ | 3.5x10$^4$ | 3.8x10$^4$ | 8.9x10$^4$ | 4.8x10$^4$ |
| E | 3.7x10$^6$ | 2.1x10$^6$ | 9.0x10$^4$ | 1.0x10$^5$ | 8.1x10$^4$ | 3.5x10$^4$ | 8.9x10$^4$ | 4.2x10$^4$ |
| F | 5.1x10$^6$ | 4.1x10$^6$ | 21.5x10$^5$ | 4.8x10$^5$ | 5.3x10$^4$ | 4.5x10$^4$ | 4.8x10$^4$ | 1.10x10$^4$ |
| G | 3.9x10$^6$ | 2.3x10$^6$ | 26.8x10$^5$ | 8.4x10$^5$ | 3.3x10$^4$ | 4.3x10$^4$ | 2.8x10$^4$ | 6.0x10$^4$ |
| H | 4.8x10$^5$ | 7.0x10$^7$ | 13.0x10$^7$ | 4.1x10$^5$ | 4.3x10$^4$ | 4.0x10$^4$ | 3.9x10$^4$ | 6.1x10$^4$ |
| I | 7.5x10$^6$ | 3.5x10$^6$ | 13.9x10$^5$ | 7.1x10$^6$ | 7.8x10$^4$ | 2.5x10$^4$ | 4.8x10$^4$ | 2.1x10$^4$ |

*THB =Total Heterotrophic Bacteria; HUB = Hydrocarbon Utilizing Bacteria; HUF = Hydrocarbon Utilizing Fungi; THF =Total Heterotrophic Fungi*

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**DISCUSSION**

The detrimental effect of crude oil pollution on the environment has been well documented. Addition of ameliorating agents to crude oil contaminated soil help to reduce crude oil toxicity and improve the physical and chemical characteristics of soil. Results showed that addition of sawdust and detergent either in single or combine forms to crude oil polluted soil reduced the hydrocarbon content and improve soil physico-chemistry. There was reduction in the electrical conductivity in the remediated soil indicating that the remediating agents (sawdust and detergent) are capable of reducing the high conductivity of crude oil contaminated soil (Sedat and Sahriye, 2011). The initial electrical conductivity in the different treatments was found to differ. This might be attributed to biological and physic-chemical changes in the soil from the time of polluted and remediation.

The reduction of THC in all the treatment options especially in treatment C (100 g sawdust) and D (200 g sawdust) confirms the result of Tanee and Albert, (2011) that sawdust is a good bioremediating agent in crude oil polluted soil. Agarry and Jimod, (2013) have also reported the use of plant derived organic waste in the bioremediation of soil contaminated with petroleum hydrocarbons This could be inferred that sawdust acts as absorbent, thus removing petroleum hydrocarbons from the soil (Pala, *et al*., 2005; Trejo-Hernandez, *et al*., 2006). It might also be that the sawdust provides the necessary nutrients for microbial degradation since nutrient limitation is a major hurdle to crude oil biodegradation. Also the reduction in THC in treatment A (20g detergent) and B (40 g detergent) could be attributed to the fact that detergent dislodges oil from the soil part for the oil to be acted upon easily by microorganisms (Couto *et al*., 2010; Millioli *et al*., 2009).

There was an increase in pH in most of the remediation treatments. Though, the observed pH range (6 – 9) was within the tolerance range of oil degrading microorganisms (Mckee and Mendelssohn, 1995) thus providing a favourable environment for biodegradation. The final nitrogen and nitrate contents of the soil at the end of the experiment were higher than the initial contents in most of the treatments especially with sawdust amended soil. This increase may be as a result of anthropogenic inputs of this nutrient source from the organic manure (sawdust) because organic manure has been reported as being capable of increasing soil nutrients by supplementing the limiting nutrients (Tanee and Kinako, 2008). Thus the increase in the percentage nitrogen content of the amended soils by the organic manure enhances its fertility which may accelerate the biodegradation process of the crude oil polluted soil. Soil with high nitrogen value was observed high pH value. This showed that there is a relationship between soil pH and soil nitrogen in oil-spill soil (Capuzzo, 1987).

Total organic carbon (TOC) and total organic matter showed significant increases in all the treatments at the end of the experiment as compared to the initial. This agrees with Asuquo *et al.,* (2001); Tanee and Kinako (2008) and; Tanee and Albert (2011). The increase in organic carbon and organic matter content of the soil could be attributed to the decomposition of the amendment agents and / or hydrocarbons by microbial actions. Crude oil has been known to contain high amount of carbon (Speight, 2014). Mbah *et al*. (2009)

observed that organic carbon/organic matter from waste can influence the ability of micro organisms to degrade pollutants. This increase in organic carbon and organic matter buttress the reason for the reduction in THC in the amended soil.

The results of the microbial parameters observed are in agreement with Nwandinigwe and Onyeidu, (2012) who observed similar trend when crude oil contaminated soil was treated with poultry manure. The increase could be attributed to the increase organic carbon and organic matter contents by the remediating agents (sawdust and detergent) to the contaminated soil which increases oxygen diffusion, availability of nutrients as well as carbon (energy) source quality and physical support for microbial adaptation, growth and reproduction (Molina-Barahona *et al.*, 2004). The increased growth of hydrocarbons degrading microorganisms consequently lead to the increased THC reduction that was observed. Cellulosic wastes such as sawdust has been observed to enhance the growth of fungi in petroleum hydrocarbon contaminated soils (Embar *et al*. 2006; Minai-Tehrani and Herfatmanesh, 2007).

From this study, there is ample evidence to show that the use of detergent and sawdust (either single or combine applications) for remediation can achieve the degradation of petroleum hydrocarbons in agricultural soils by modifying soil physical, chemical and biological properties. This is demonstrated by the significant reductions in Total Hydrocarbon Content (THC) with improvement in nutrient contents observed in all the amended soils. Best biodegradation performances were recorded in treatment A (20 g detergent), C (100 g sawdust) and D (200 g sawdust) suggesting that single treatments performed better than combined treatments. It was observed that biodegradation performance was more at lower detergent concentration than at higher concentration while the reverse was the case for sawdust. Therefore, caution should be taken in the use of detergent in remediation to avoid detrimental effect.

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