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Original Article

In Situ Bioremediation of Crude Impacted Soil Using Cow Dung, Compared To Natural Attenuation For Treatment

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Abstract - *This research investigated the effectiveness of cow dung versus natural attenuation in bio-remediating crude oil-contaminated soil. Twenty square meter (20m²) plot was created and divided into two portions, one was treated with 25 kg of cow dung (Portion A), and the other left for natural attenuation (Portion B). After the application of 25 litres of crude oil, soil samples were collected weekly for six weeks. Hydrocarbon content was analysed for Total Petroleum Hydrocarbon (TPH), Polycyclic Aromatic Hydrocarbon (PAH), Benzene, Toluene, Ethylbenzene, Xylene (BTEX), Gas Chromatography Flame Ionization Detection (GC-FID), and Gas Chromatography Mass Spectrometry (GC-MS) in the soil samples. The initial hydrocarbon content was 10,851.40 mg/kg, 13.81 mg/kg, and 0.28 mg/kg for Portion A, and 10,710.100 mg/kg, 13.90 mg/kg, and 0.276 mg/kg for Portion B, for TPH, PAH, and BTEX, respectively. The final hydrocarbon content analysis of the soil sample was 600.100 mg/kg, 1.50 mg/kg, and 0.01 mg/kg in Portion A, and 6,030.97 mg/kg, 6.90 mg/kg, and 0.05 mg/kg in Portion B for TPH, PAH, and BTEX, respectively. Analysis showed that initial hydrocarbon contents were similar for both portions, but the final results demonstrated significantly lower hydrocarbon levels in the cow dung-treated soil compared to the naturally attenuated soil, indicating that cow dung enhanced the degradation of contaminants and accelerated soil restoration.*

Keywords *– Bioremediation, Cow Dung Natural Attenuation, Crude oil, Hydrocarbon content.*

1. Introduction

Exploration of petroleum, pipeline sabotage, pipeline transportation, petroleum refining, storage, and operational failure have resulted in many oil spills in many parts of the world, especially in the Niger Delta region of Nigeria. These activities have generated large amounts of hydrocarbon spills that have adverse impacts on soil and groundwater quality. Many restorative techniques have been employed to restore the impacted soil and groundwater to their original state. Among these techniques is bioremediation. Bioremediation exploits the ability of certain microorganisms, usually heterotrophic bacteria and fungi, to degrade organic materials to harmless materials such as carbon dioxide, methane, water, inorganic salts, and biomass (Atlas, 1991).

$$C_7H_8 + 9O_2 \longrightarrow 7CO_2 + 4H_2O$$

Thus, when bioremediation is occurring, the concentration of inorganic carbon (represented by CO_2) should increase as the concentrations of hydrocarbon and oxygen decrease.

Microorganisms may derive the carbon and energy required for growth through the biodegradation of organic contaminants or transform more complex organics through fortuitous co-metabolism. Fortuitous co-metabolism is synonymous with the microbial metabolism of contaminants that are not growth substrates (Anderson et al;1995, and, Anderson &Walton 1992).

Bioremediation has been widely applied in the restoration of petroleum hydrocarbon-contaminated sites (Bento et al.,2005). It is known that hydrocarbons and weathered oils with carbon contents of about 20% degrade slowly (Adams et al.,2015). However, petroleum hydrocarbons in excess of 20,000 ppm can be tolerated without inhibition to microbial growth (Flathman et al., 1993). Efforts continue to improve the rates of degradation of these types of materials. The complexity of the bioremediation process varies at many sites due to a large number of environmental factors that impact the microbial populations and their abilities to degrade the contaminants present (Kauffman 1993, ASTM, 1998). This complexity has shown the remediation industry the need to carry out detailed laboratory analysis both chemically and biologically before the bioremediation process can be successfully applied in the field (Ibekwe et al., 2018). Laboratory treatability studies generate useful information for the design of full-scale systems and may be used to develop new processes or specific bacterial cultures that can be taken to the field. Efforts in the laboratory most

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often translate into successful field applications of bioremediation technology (Oludele et al., 2021, EPA 1995).

Contaminated sites are complex, dynamic in nature, and often several techniques are required for complete remediation. A range of factors is known to influence the biological transformation and degradation of contaminants. Parameters that may affect the rate and efficiency of biodegradation include temperature, moisture, salinity, nutrient availability, microbial species, and type and concentration of contaminants (Hinchee et al., 1995, Omorowou, 2019).

Nutrient availability, such as nitrogen and phosphorus, is often limited in petroleum-contaminated soils due to the excessive carbon input from the hydrocarbons. Therefore, nitrogen and phosphorus are usually identified as the limiting factors for biodegradation in soils (Chidiogo et al 2022, Brook et al 2001).

Other factors that can also affect the success of the bioremediation treatment of contaminants are climatic conditions, soil type, soil permeability, contaminant distribution and concentration, and drainage. As noted previously, the natural rate of microbial biodegradation of the petroleum hydrocarbons is usually constrained by limiting factors.

A variety of environmental factors may be rate-limiting in the microbial degradation of petroleum hydrocarbons. It is critical to identify and remove these rate-limiting factors for successful bioremediation of contaminated material. Western Research Institute (WRI), in conjunction with TechLink Environmental, Inc., and the U.S.

Department of Energy (DOE), conducted laboratory studies to evaluate major parameters that contribute to the bioremediation of petroleum-contaminated drill cuttings using land farming (Jin et al., 2006). The materials used in this investigation were collected from petroleum-impacted sites in eastern Egypt (Ras Shukheir facility). The investigations led to the development of a bench-scale biotreatment cell (solid-phase bioreactor), which then led to the design of a field-scale system.

In the past two decades, there have been recent advances in bioremediation techniques with the ultimate goal of effectively restoring polluted environments in an eco-friendly approach, and at a very low cost. Researchers have developed and modelled different bioremediation techniques; however, due to the nature and/or type of pollutant, there is no single bioremediation technique that serves as a 'silver bullet' to restore environments. Autochthonous (indigenous) microorganisms present in polluted environments hold the key to solving most of the challenges associated with biodegradation and bioremediation of polluting substances, provided that environmental conditions are suitable for their growth and metabolism. Environmentally friendly and cost-saving features are among the major advantages of bioremediation compared to both chemical and physical methods of remediation. Thus far, several good definitions have been given to bioremediation, with particular emphasis on one of the processes (degradation). Nevertheless, in some instances, the term biodegradation is used interchangeably with bioremediation; the former is a term that applies to a process under the latter. In this research, bioremediation is defined as a process that relies on biological mechanisms to reduce (degrade, detoxify, mineralize, or transform) the concentration of pollutants to an innocuous state. The process of pollutant removal depends primarily on the nature of the pollutant, which may include: agrochemicals, chlorinated compounds, dyes, greenhouse gases, heavy metals, hydrocarbons, nuclear waste, plastics, and sewage. Apparently, taking into consideration the site of application, bioremediation techniques can be categorized as ex-situ or in-situ. A contaminated site may be relatively stable but may pose a future threat if not remediated. If cleaning of such a site is attempted by excavation followed by, for example, mixing with a suitable matrix material and nutrients for composting, there is a risk of mobilizing the contaminant by volatilization or flushing. Therefore, remediation in situ by improving the conditions and/or the degradation potential in the contaminated soil layer should be preferred. (Walworth & Reynolds 1995).

The objective of this work is to show the efficiency of cow dung in the treatment of crude oil-impacted soil, compared to the natural attenuation process. *A* few researchers focused on the use of animal waste for bioremediation, but they did not compare it side by side with the use of the natural attenuation process, with respect to the time the contaminated land was restored to a near-pristine state.

2. Materials and Method

2.1. Material

Bonny light crude oil was used for this study; it was collected from 20" Kolo-creek, SPDC, location in Bayelsa State. It was transported to Rivers State. Cow dung was collected from the slaughter abattoir site as a stimulant for the bioremediation, measuring cylinder, tape rule, weighing balance, caution tape, recently calibrated GC-FID, GC-MS, laboratory standards, shovel, nose mask, sampling plate, spatula, sample bottle, vials, chain of custody, and a piece of uncontaminated land soil.

2.2. Method

This research was carried out for six (6) weeks, consisting of experimental consideration, analysis, and study, focusing on hydrocarbon content reduction on the polluted site. The exercise was carried out at a nearby space at 4, Nnaworlu Street, Woji Road, Rumuogba. Port Harcourt. The chemical analysis of the of chemical of concern (TPH, PAH, and BTEX) was carried out at Kemnoulli Nigeria Limited, a laboratory certified and recognized by the Nigeria Upstream Regulation Commission (NUPRC), National Oil Spill Detection Regulation Agency (NOSDRA), Rivers State Ministry of

Environment (RSME), and Federal Ministry of Environment (FME). Twenty square meters ($20m^2$) of uncontaminated portion of land was tilled to 0.5m with a clean shovel and divided into two portions. A trench of 1.5 m was made to avoid interaction between the control (Natural attenuation portion) and a portion with the application of cow dung. Also, a 3-inch width and a 3-inch depth were dug around the two portions of land to avoid interaction between the portions and the environment. 25 litres of Bonny Light crude oil were applied equally on the two prepared portions; the lithology of the two was mainly of silty clay. A clean shovel was used to bulk and homogenize the soil and the crude together. A thoroughly homogenized composite sample was taken from each portion of the experimental site for chemical analysis under a full chain of custody protocol.

Twenty-five kilograms (25kg) of dried cow dung, earlier analysed in a laboratory, was spread on portion A; the cow dung was homogenized and bulked with the tilled soil up to 0.5m, while portion B, which was to be used as a control experiment (Natural attenuation), was allowed to remain clean naturally. 20 litres of water were added equally to the portions.

Composite samples were taken from each portion weekly for chemical analysis in the laboratory using high-precision Agilent 7890 GC-FID and GC-MS equipment, using diesel range standard and a detection limit of 0.01ppm. The estimated cow dung to soil ratio is approximately **1**:156, having a bulk density of 1,300 *kg/m*

Table 1. Nutrient composition values

NUTRIENT	UNIT	VALUE
N	%	16.89
P	%	15.1
K	%	17.52

2.3. Samples and Sampling Technique

New 60ml amber sample bottles were used to collect samples for both TPH and PAH laboratory analysis, while BTEX samples were collected into a new vial bottle containing 10ml of methanol; this methanol was used to preserve the volatile hydrocarbon gas. Methanol is the most preferred because it is an effective solvent that helps to retain the BTEX Sample, prevent analyte losses, and also helps to dissolve the analyte effectively, ensuring the analyte is in solution.

Lastly, methanol is also compatible with the gas chromatography and GC-MS method of BTEX analysis. The BTEX samples were first sampled from the sample plate after homogenisation with a strong spatula and immediately covered before the other were sampled. This is due to the volatility of the hydrocarbon gas to be analysed; this is part of the precautions when sampling BTEX. The samples were kept at 4 °C inside a refrigerator before being taken to the laboratory. The chain of custody was filled out appropriately before submission to the laboratory.

2.4. Laboratory Extraction of THP for Gas Chromatography (GC) Analysis

A 10 g soil sample was weighed into a pan, and 10 g of anhydrous sodium sulphate was added. The mixture was blended with a spatula. The mixture was transferred to a 20 ml sample bottle, and 10 ml of DCM/Acetone (1:1) was added. 0.05 micro/litre of O-terphenyl was added, and the mixture was vortexed for homogenization.

The mixture was agitated intermittently for about 3 hours. The extract was transferred into 1.5 ml sample vials. The filtrate was allowed to concentrate to 1 ml in a fume hood. Finally, the sample vial was placed on the autosampler tray for TPH analysis on GC-FID.

2.5. Laboratory extraction of PAH for Gas Chromatography (GC) analysis

The laboratory procedure for the extraction method for semi-volatile Polycyclic Aromatic Hydrocarbons (PAH) in the laboratory is as follows: 5.0g of soil sample was weighed in a pan, 5.0g of anhydrous sodium sulphate salt was added to the pan, and they were mixed with a spatula. The mixture was transferred to a 20 mL sample bottle, and 10ml of DCM/Acetone was added to the mixture. 0.05 micro /litres of O-terphenyl were added to the mixture, and the mixture was placed on the vortex mixer for homogenisation. The mixture was agitated intermittently for about 45 minutes, and then filtered with glass wool and a glass funnel. The filtrate was allowed to concentrate to 1ml in a fume cupboard. Finally, the extract was transferred to a 1.5ml sample vial and placed on the sampler tray for PAH analysis on GC-MS.

2.6. Laboratory Extraction of BTEX for GC-FID Analysis

5g of soil sample was weighed into a glass vial, and 10ml of methanol solvent, the sample was capped with a screw-cap and Teflon. The sample was shaken vigorously, and the mixture was positioned in the headspace sampler tray for BTEX analysis on GC-MS.

3. Results

The following data were obtained from the laboratory analysis of soil samples from the chemical analysis using a recently calibrated high-precision GC-FID (Gas chromatography flame ionization detector) and GC MS (Gas chromatography mass spectrometer). The baseline Total Petroleum Hydrocarbon (TPH) concentration of the soil before application of bonny light crude oil was 3.1mg/kg, also, the baseline Polycyclic Aromatic Hydrocarbon (TPH) concentration of the soil before adding bonny light crude oil was below the detection level. Lastly,

the baseline BTEX (Benzene, Toluene, Ethylbenzene, and Xylene) concentration of the soil was also below the detection level**.** Table 2 below shows the initial value of chemicals of concern (TPH, PAH, and BTEX) in the soil before application of cow dung.

Table 2. The baseline concentration of the Chemical of Concern (CoC), after adding Bonny Light crude oil to the soil but before applying cow dung

PORTION	TPH (mg/kg)	PAH (mg/kg)	BTEX
Portion A	10,851.40	13.81	0.28
Portion B	10,710.100	13.90	0.276

The chemical analysis results for the period of six (6) weeks of research for Portion A (the one with the application of cow dung) and Portion B (The natural attenuation) are shown below.

Table 3. Chemical analysis results of the chemicals of concern with respect to weeks

Weeks	Portion A (Using cow dung)			Portion B (Natural Attenuation)		
	TPH (mg/kg)	PAH (mg/kg)	BTEX (mg/kg)	TPH (mg/kg)	PAH (mg/kg)	BTEX (mg/kg)
1	9,023.36	13.81	0.28	10,710.100	13.90	0.27
2	7,905.13	10.11	0.15	10,152.00	13.00	0.26
3	5,433.00	6.25	0.02	8,800.21	12.78	0.17
4	3,500.56	4.67	0.01	7,729.01	10.20	0.12
5	1,201.12	2.45	0.01	6,800.29	8.00	0.09
6	600.10	1.50	0.01	6,030.97	6.90	0.05

3.1. Discussion of Results

3.1.1. Total Petroleum Hydrocarbon

The graph of the TPH of portion A and portion B in Figure 1 shows a sharper decline in the TPH of portion A than in portion B. This is a yardstick to show the efficiency of the cow dung mechanism on portion A. The rate of microorganism reproduction that eats up the contaminant for its source of energy is shown in the TPH value decline. The calculation in Appendix A shows the percentage reduction of both portions A and B.

The percentage loss of TPH was calculated, and it can be deduced that the biodegradability of TPH, i.e., the breakdown of TPH to carbon and water in Portion A, is far higher than in Portion B. Portion A has a 94.468% reduction after 6 weeks, while Portion B has **a** 43.68% reduction after 6 weeks. As reported by Jin Song, Fallgren Paul, Brown, Terry [20]. The decrease in TPH from 8,000 mg/kg to 800mg/kg with the duration of six (6) weeks shows the efficiency of cow dung in bioremediation of impacted soil.

Also, Chidiogo M. Orji-Oraemesi and Kelechi L. Njoku [16] used a phytoremediation method to bioremediate crude-impacted soil; the result shows a slight decrease in TPH from 7,500mg/kg to 4,500mg/kg after week 6. This shows that using cow dung is more effective than plants to remediate crude-impacted soil

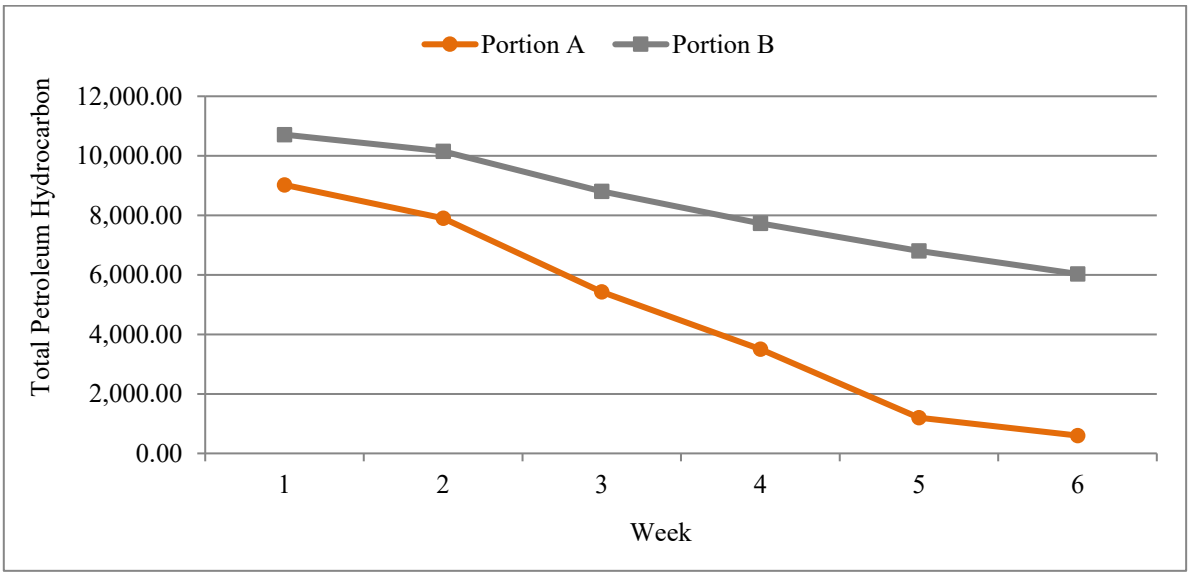

Fig. 1 Graph of TPH of portions A and B against weekS

3.1.2. Polycyclic Aromatic Hydrocarbon (PAH)

The graph of the PAH of portion A and portion B in Figure 2 shows a sharp declination in the PAH of portion A than in portion B. This is a yardstick to show the efficiency of the cow dung mechanism on portion A. The rate of microorganism reproduction that eats up the contaminant for its source of energy is shown in the PAH value decline. The control site (Natural attenuation) was designed to operate in its natural environment, where contaminants were broken down by natural factors. Here, some already existing microbes in the soil affect degradation aerobically and anaerobically. The microbes deplete the contaminant in the presence of oxygen and nutrients or in the absence of oxygen as a source of energy. Most of the previous works majorly analyses for TPH. The initial value of PAH 13.81mg/kg compared to the final value of 1.50mg/kg shows the effectiveness of cow dung.

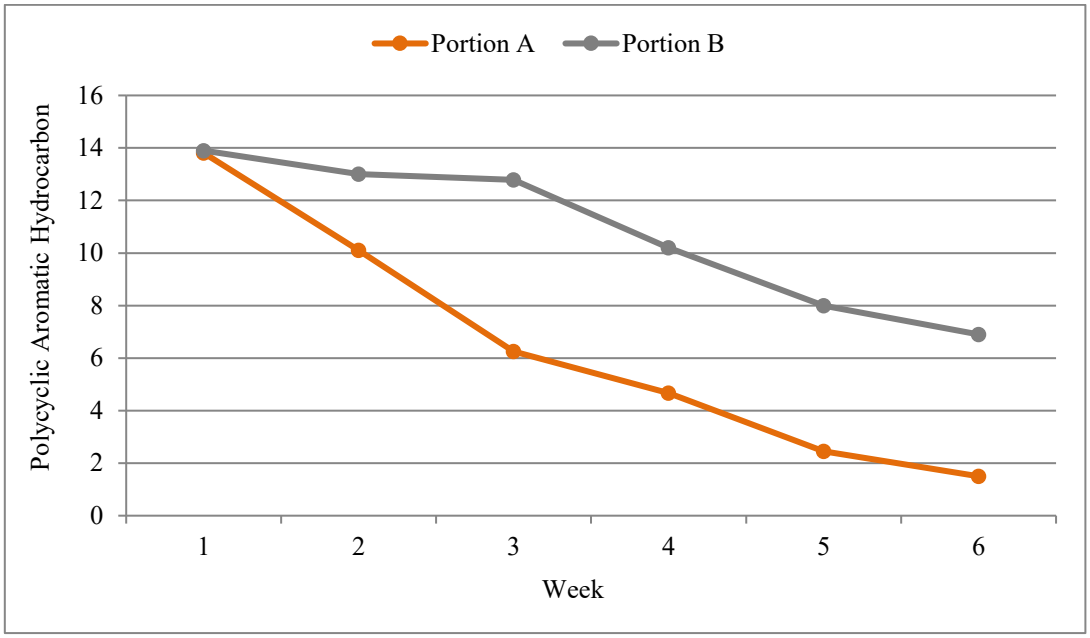

Fig. 2 Showing the graph of PAH of portions A and B against weeks

3.1.3. Benzene, Toluene, Ethylbenzene, Xylene (BTEX)

The rate of decline of BTEX was so high to the extent that it almost became BLD (Below detection Level) after the third week. The constant value of BTEX from the third week indicates the rate of hydrocarbon breakdown in portion A than in portion B due to the application of cow dung. In conclusion, none of the parameters went beyond the intervention level of EGASPIN and NUPRC after the sixth week, including BTEX; semi-volatile PAH breakdown has an indirect relationship with extremely volatile BTEX.

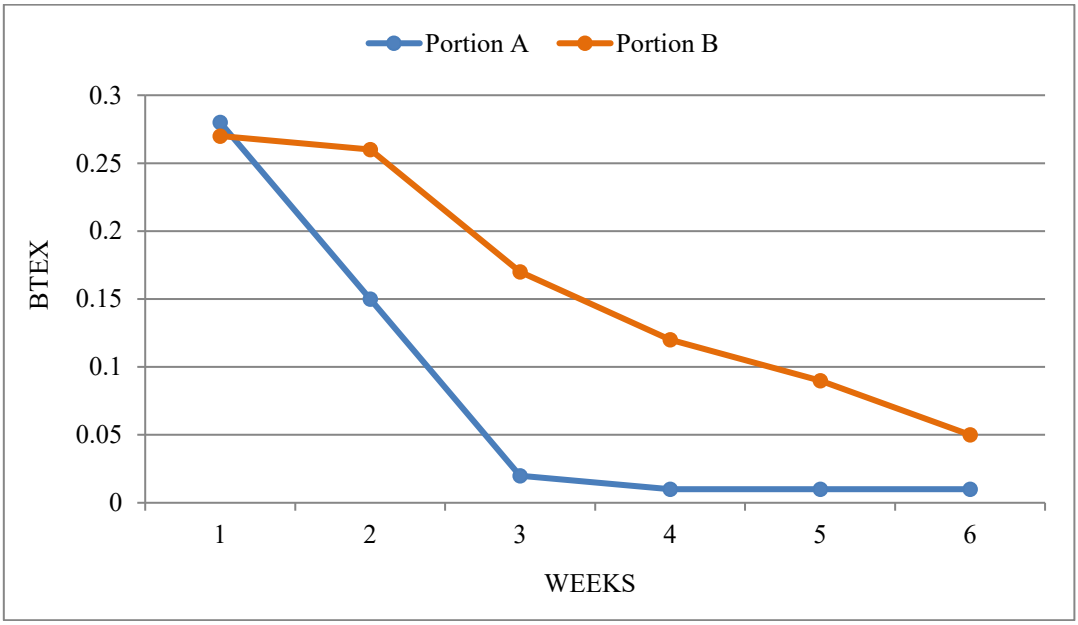

Fig. 3 Graph of BTEX in portions A and B against weeks

The declination results in the constituent of concern after a period of six weeks in portion A was majorly as a result of the application of cow dung coupled with exposure to sunlight; aeration occur, which promote the breakdown of hydrocarbon contaminants by naturally occurring microorganisms. The results of this study demonstrate that the application of cow dung in the bioremediation of crude oil–contaminated soil facilitates a faster rate of soil restoration than the natural attenuation process (Portion B). This finding indicates that cow dung is a more effective bio stimulant for accelerating the degradation of petroleum hydrocarbons and enhancing the recovery of contaminated soil.

4. Conclusion

The efforts of man to provide solutions to energy demand, create wealth, and develop their spaces has its own effect on the environment. Environmental pollution has become a major world concern. To combat world environmental pollution, the use of cow dung has emerged as a powerful tool to make the environment clean of impacted crude oil contaminants. Obviously, the use of cow dung has proven efficient in the biodegradation of the hydrocarbon content of the contaminant in the soil. From the analytical data and plots, one can obviously ascertain the rate of degradation of the TPH, PAH, and BTEX of the soil. The TPH, PAH, and BTEX were 10,851.40mg/kg, 13.81mg/kg, and 0.26mg/kg, respectively, for Portion A (with cow dung), while the TPH, PAH, and BTEX of Portion B (Natural attenuation) were 10,710.100mg/kg, 13.90mg/kg, and 0.276mg/kg, respectively. The contents of these chemicals of concern were reduced to near-pristine values of 600.10mg/kg for TPH, 1.50mg/kg for PAH, and 0.01mg/kg for BTEX in portion A, while TPH, PAH, and BTEX had 6,030.97mg/kg, 6.90mg/kg, and 0.05mg/kg, respectively, after 6 weeks of the experiment.

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Conflict of Interest

We declare that there is no conflict of Interest.

Author Contribution

Ademiluyi Oluwaseun Shina conducted the research and wrote the original draft, while Dr Yirakpoa Patience Nwambo reviewed and edited the manuscript**.**

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