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Two-Way ANOVA for Comparison of Remedial Nutrient Solution and Enhanced Natural Attenuation Using SPSS for Treating Petroleum Contaminated Soils

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Abstract

Statistical comparison of two remediation methods: Remedial nutrient solution and enhanced natural attenuation were analyzed in terms of TPH of different soil samples collected from Khana Local Government Area of Rivers State, Nigeria at different locations and placed inside sample bottles labelled A to D and replicated into two, one for each of the above treatment technique. The TPH of the soil was determined using GC analyzer after solvent extraction was carried out using hexane/dichloromethane mixture. Three batches of treatment were performed on the samples at every interval of eight weeks for a duration of six months. The result obtained was analyzed using a two-way ANOVA factorial experimental design to test the significance of the various sources of variation. From the result obtained, source of variation for sample and interactions were non-significantly different from each other which means that irrespective of the number of samples analyzed or the combination of both samples and batches of treatment, they will still not be significantly different from each other. The source of variation for batch and replications were significantly different from each other and this means that irrespective of the batches of treatment applied or the number of replications (methods of treatment used), they will always be significantly different from each other. The individual comparison of each sample showed that the efficiency of the Remedial Nutrient Solution method was better than Enhanced Natural Attenuation method.

Keywords

SPSS, ANOVA, Attenuation, Nutrient, Remediation, TPH, Soil, Contaminant

1. Introduction

Consequent upon many health problems caused by hydrocarbons pollution of the environment, the need to proffer solution to the problems has been of great concern as it affects everyone directly or indirectly [1]. Synesthetic manures are quite expensive to purchase especially in a 3rd world economy like Nigeria, so to overcome these challenges, researchers have ventured into new technologies that can produce less expensive means of soil remediation [2]. Some of the causes of oil spillage on the environment can be attributed to failure of equipment, accidents resulting tankers, vandalization of high-pressure pipelines etc. [3].

The application of biological treatment to clean polluted soils is known as bioremediation. Through this process, the toxicity of the environment is brought to lower levels acceptable by international standards [4]. One of the commonly used bioremediation techniques is the *in-situ* technique and it involves the treatment of polluted soils right on the site where the contamination is present [5]. For the effective utilization of hydrocarbon by bacteria, certain nutrients needed by the soil must be in place along with other factors such as: oxygen, temperature and pH etc. [6]. The three methods involved in natural attenuation are: bio-stimulation, bio-augmentation and tilling of polluted soil and spreading it to allow for oxidation of the soil [7].

Crude oil spills have degraded most agricultural lands in the Niger Delta due to destruction of soil nutrients inducing a decrease in soil fertility. This development has forced many farmers to abandon their farm lands and seek non-existent alternative means of livelihood. The soil which is a very important component of the environment upon which the lives of microorganisms, vegetation, living organisms and humans largely depend is the most impacted by crude oil spills with oil hydrocarbons leaching to depths below the topsoil surface. Owing to the pivotal role of the soil in the ecosystem, soil contamination and pollution by oil hydrocarbons is dangerous to biodiversity and threatens the very essence of our collective survival [4] [5] [6] [7]. Providing a lasting solution to this environmental menace has been on the front burner for decades by Stakeholders in industry and government. This drive led to calls by host communities, environmental monitoring directorates and agencies as well as other stakeholders in industry and government that oil multinationals prevent/contain crude oil spills, clean up all oil impacted sites in the Niger Delta and remediate the soil to its natural attenuation. In response to these agitations, Government of Nigeria, in consultation with many of the relevant actors, United Nations Environment Programme (UNEP) was invited to consider undertaking an assessment of oil pollution in Ogoni land. This produced the infamous UNEP Report which was

to serve as a template for the Cleanup of the entire Niger Delta. Currently, many scientific techniques and technology have thus far been developed to degrade oil spills from oil impacted sites including physical, chemical, thermal and biological methods. But with dwindling revenues and high cost of technology, there is need to employ a remediation technology that would not only be aptly efficient in degrading the hydrocarbon contaminants but also be cost-effective as well. Moreover, with Oil multi-nationals having to pay billions of dollars in compensation to host communities, there has been a focus on adopting a remediation technology with minimal disruptive impact on the ecosystem as well as little or no secondary contamination. Bioremediation technology to a large scale, is the best suited for these purposes [8] [9]. Crude oil, a mixture of many thousands of organic compounds, can vary in composition from one source to another. This suggests that the effects of crude oil spill will vary from source to source. However, details of the potential biological damage will depend on the ecosystem where the spill occurred. In this case, the polluted material is removed and degraded in special facilities outside the incident site. After excavation, the polluted soil is transported elsewhere for treatment. The selection of an ex-situ bioremediation technique is usually made on the basis of the following aspects: operating costs, extent and depth of contamination, type of contaminant, location and geological features of the contaminated site. Ex-situ techniques allow better control of environmental conditions, leading to an increase in the biodegradation rate compared to in-situ treatment techniques. Moreover, soil excavation leads to an increase in the mobility of pollutants and exposure for faster degradation [9] [10].

2. Materials and Methods

2.1. Materials

The materials used includes: 1) Glass bottles; 2) Conical flask; 3) GC analyser; 4) Hexane/dichloromethane; 5) Papaya peel; 6) egg shell; 7) water; 8) N.P.K fertilizer.

2.2. Methods

The methods adopted includes:

2.2.1. Determination of Soil TPH

Experimental Procedures for Determination of TPH

10 g of sample was weighed and put into a 120 ml glass bottle for extraction. The solvent used for the extraction was hexane/dichloromethane mixture. The solvent was then added to the glass bottle containing the soil sample. The glass bottle was shaken to stir the mixture for a period of 1 hour to allow the sample to dissolve in the solvent. After the extraction the solvent was separated from the sample via filtration. The solvent phase was then transferred to a clean flask for analysis. The solvent phase was further concentrated to smaller volume to increase the sensitivity of the analysis through a rotary evaporator. Gas chromato-

graphy (GC) technique was applied to analyze the concentrated solvent phase to determine the total petroleum hydrocarbon in the sample (TPH).

2.2.2. Nutrient Preparation

100 kg of papaya peels (green waste product) + 30 kg of raw eggshell was dried and grinded into powdered form, weighed and mixed with 10 kg water in a 2:1 ratio. The slurry mixture was further mixed with 10 liters of water and mixed thoroughly to give a homogeneous solution. This homogeneous solution was called "Remedial Nutrient Solution". This was then transferred into four sample bottles contaminated with crude oil and collected from different locations in Khana Local Government Area of Rivers State, Nigeria. The sample bottles were labelled A1, B1, C1 and D1. (The ones represent the first method of treatment). This treatment was done in three batches after every eight weeks interval totaling a duration of six months for the entire remediation period.

2.2.3. Enhanced Natural Attenuation (ENA)

Enhanced natural attenuation is a process that relies on naturally occurring microorganisms to degrade contaminants in soil. The experimental process is done as follows:

1) Site Characterization: the first step was to characterize the site to determine the extent of contamination and the type of contamination present in the polluted soil and these includes sampling of the soil to determine the concentrations of contaminant as well analyzing the physicochemical properties of the soil. The next step is the selection of amendment to enhance the natural attenuation process as follows:

2) Oxygen: Aerobic degradation is known to be faster than anaerobic for many organic compounds, especially petroleum Hydrocarbons hence it is often the preferred method of treatment. The dissolved oxygen concentration can either be supplied continuously using a diffuser (external source), or introduction of oxygen (air) can be achieved through the method of mixing, e.g. tumbling. Tilling was the method used to expose the sample to oxygen.

3) Nutrients Availability: To degrade any substrate, microorganisms need essential nutrients, such as nitrogen and phosphorus. Nitrogen and phosphorus are important nutrients for microorganisms. N.P.K fertilizer was added to the contaminated crude polluted soil samples to promote the growth of microorganisms. This was then transferred into four sample bottles contaminated with crude oil and collected from different locations in Khana Local Government Area of Rivers State, Nigeria. The sample bottles were labelled A2, B2, C2 and D2 (The two's represent the second method of treatment). This treatment was done in three batches after every eight weeks interval totaling a duration of six months for the entire remediation period.

3. Results and Discussion

The results obtained at the end of the experiment are presented as follows:

3.1. TPH Results

Table 1 shows the results obtained in terms of TPH of four samples that were collected and treated in three batches for two different methods namely: Remedial nutrient solution and Enhanced natural attenuation.

As shown in Table 1, the three batches of treatment is seen to reduce the TPH values of the soil for sample A after the third batch of treatment, the TPH for the first and second method were 323.38 mg/kg and 7432.46 mg/kg showing that method 1 is more efficient than method 2. for sample B after the third batch of treatment, the TPH for the first and second method were 508.43 mg/kg and 6202.59 mg/kg showing that method 1 is more efficient than method 2. For sample C after the third batch of treatment, the TPH for the first and second method were 420.62 mg/kg and 8200.52 mg/kg showing that method 1 is more efficient than method 2. for sample A after the third batch of treatment, the TPH for the first and second method were 650.45 mg/kg and 9024.13 mg/kg showing that method 1 is more efficient than method 2.

3.2. SPSS Result Using Factorial Experimental Design

Factorial designs refers to a specific way in which treatments are done and it consist of all possible combinations of the selected levels in two or more factors varying simultaneously. Table 2 shows the ANOVA table for the factorial design from SPSS.

From table two factorial design for a two way ANOVA has five sources of variation which Factor A (Samples), factor B (Batches of treatment), interactions (combination of factor A and factor b), replication (numbers of methods applied for treatment) and error (from human or instrument). The test for significance is if the value of alpha is less than the p value (0.05), and when it is greater than 0.05 it is said to be non-significant. The source variation due to the various samples, (A to D) is not significantly different from each other since the value of alpha (0.416) is greater than p value (0.05). The source of variation due to different

Table 1. Experimental result for sample and batches of treatment in terms of TPH.

Sample	TPH (mg/kg)		
	Batch 1	Batch 2	Batch 3
A	6520.74	2004.88	323.38
	10,220.64	8324.46	7432.46
B	7222.34	2508.45	508.43
	9600.34	6900.43	6202.59
C	6820.01	2220.97	420.62
	10,400.22	9202.74	8200.52
D	7010.45	2850.45	650.45
	11,003.30	10,500.31	9024.13

Table 2. SPSS Result output.

Source of Variation	Sum of Squares	d.f	Mean Square	F	Sig (Alpha)
Samples	6,107,163.389	3	90,237,090.94	1.031	0.416
Batch	84,433,236.96	2	2,035,721.130	21.387	0.000
Interactions	1,094,022.747	6	42,216,618.48	0.092	0.996
Replication	192,388,930.2	1	182,337.124	97.464	0.000
Error	21,713,462.90	11	192,388,930.2		
Total	1,194,795,645	23	1,973,951.173		

batches of treatment *i.e.* (Batch 1 to 3) is significant since the alpha value (0.000) is less than the p value (0.05). The source of variation due to interactions is not significant since its alpha (0.996) value is greater than the p value (0.05). Finally the source of variation due to replication is significant since the alpha (0.00) value is less than the p value (0.05).

3.3. Comparison of the Efficacy of Treatment Methods for Different Soil Samples

The four samples that were labelled A1 to D1 and treated for the first method which is Remedial Nutrient solution (RNS) while the samples labelled A2 to D2 were treated using the second method of treatment which is Enhanced Natural Attenuation (ENA). The plots for the samples are shown in Figures 1-4.

3.3.1. Comparison of Both Treatment Methods in Terms of Samples A1 and A2 Respectively

Figure 1 shows the plot of TPH (mg/kg) versus time for the two treatment methods RNS and ENA respectively.

From Figure 1, the initial TPH concentration of the crude contaminated soil before any batch of treatment was carried out was 8276.67 mg/kg and 12,567.87 mg/kg for A1 and A2 respectively and after carrying out three batches of treatment on both samples at every eight week interval the final concentration of A1 and A2 were 323.38 mg/kg and 7432.46 mg/kg at the end of the 24 weeks duration. Hence the efficiency of RNS method if greater than of ENA method for samples A1 and A2.

3.3.2. Comparison of Both Treatment Methods in Terms of Samples B1 and B2 Respectively

Figure 2 shows the plot of TPH (mg/kg) versus time for the two treatment methods RNS and ENA respectively.

From Figure 2, the initial TPH concentration of the crude contaminated soil before any batch of treatment was carried out was 9134.12 mg/kg and 10,234.15 mg/kg for A1 and A2 respectively and after carrying out three batches of treatment on both samples at every eight week interval the final concentration of A1 and A2 were 508.43 mg/kg and 6202.59 mg/kg at the end of the 24 weeks duration.

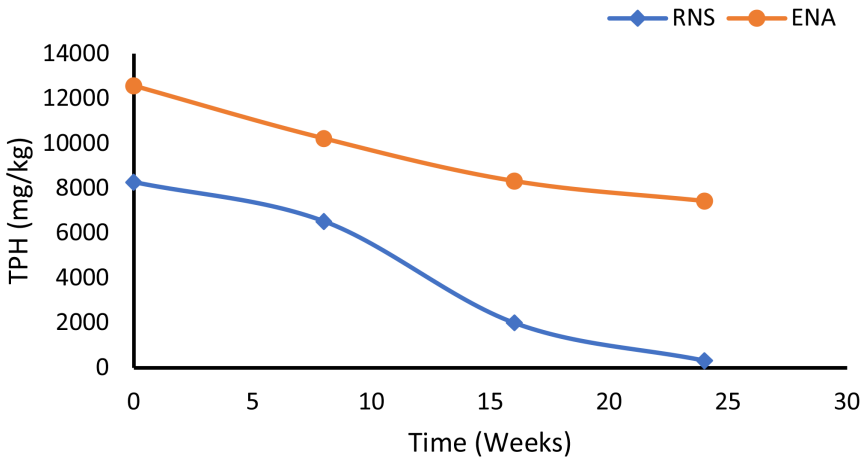

Figure 1. RNS and ENA methods in terms of samples A1 and A2.

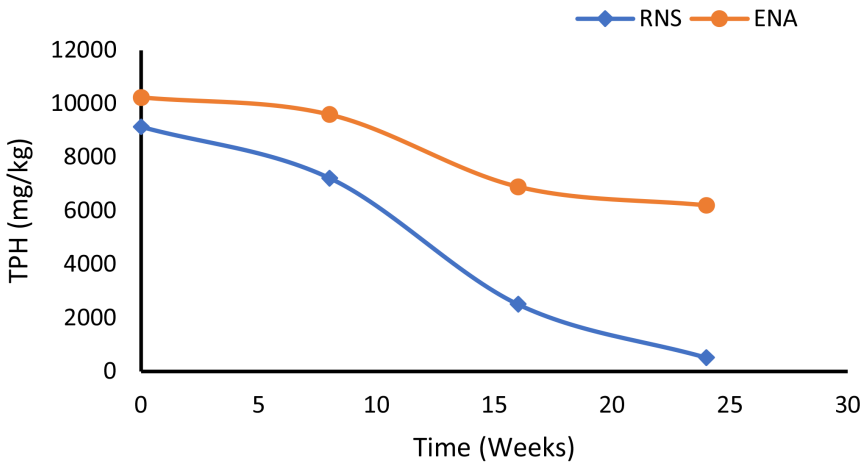

Figure 2. RNS and ENA methods in terms of samples B1 and B2.

Hence the efficiency of RNS method if greater than of ENA method for samples B1 and B2.

3.3.3. Comparison of Both Treatment Methods in Terms of Samples B1 and B2 Respectively

Figure 3 shows the plot of TPH (mg/kg) versus time for the two treatment methods RNS and ENA respectively.

From Figure 3, the initial TPH concentration of the crude contaminated soil before any batch of treatment was carried out was 7659.13 mg/kg and 11,123.45 mg/kg for A1 and A2 respectively and after carrying out three batches of treatment on both samples at every eight week interval the final concentration of A1 and A2 were 420.62 mg/kg and 8200.82 mg/kg at the end of the 24 weeks duration. Hence the efficiency of RNS method if greater than of ENA method for samples C1 and C2.

3.3.4. Comparison of Both Treatment Methods in Terms of Samples B1 and B2 Respectively

Figure 4 shows the plot of TPH (mg/kg) versus time for the two treatment methods

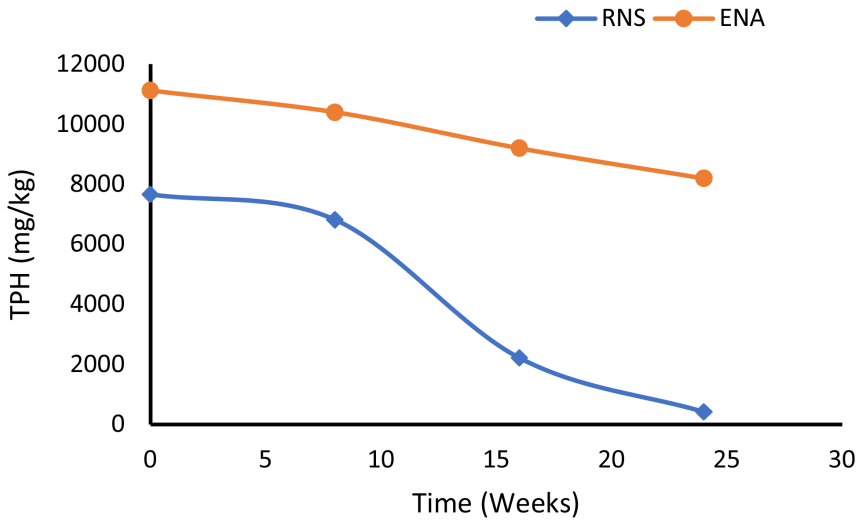

Figure 3. RNS and ENA methods in terms of samples C1 and C2.

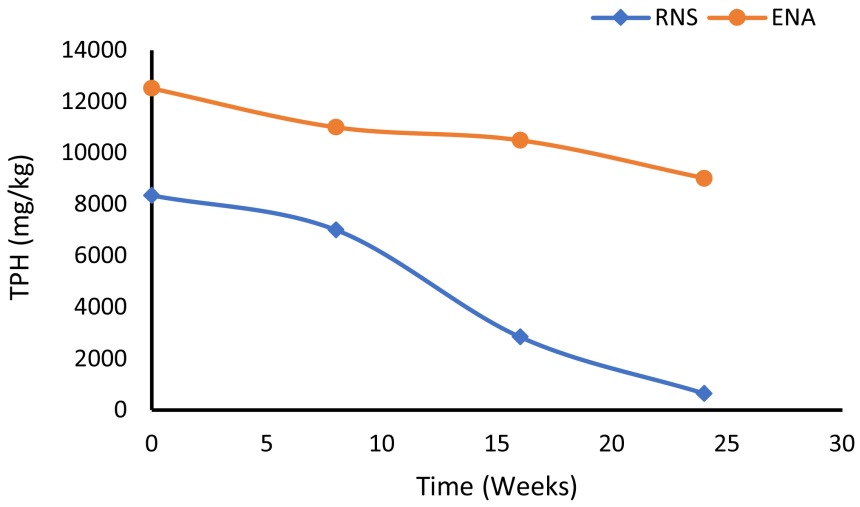

Figure 4. RNS and ENA methods in terms of samples D1 and D2.

RNS and ENA respectively.

From **Figure 4**, the initial TPH concentration of the crude contaminated soil before any batch of treatment was carried out was 8350.67 mg/kg and 12,520.23 mg/kg for A1 and A2 respectively and after carrying out three batches of treatment on both samples at every eight-weeks interval the final concentration of A1 and A2 were 650.45 mg/kg and 9024.13 mg/kg at the end of the 24 weeks duration. Hence the efficiency of RNS method if greater than of ENA method for samples D1 and D2.

4. Conclusion

Bioremediation of environment plays a vital role in reducing the toxicity of the soil and several methods to achieve this feat have ventured into by a lot of researchers to know which method is more efficient and cost-effective. This research followed similar trend by comparing between Remedial nutrient solution

and Enhanced Natural Attenuation techniques. The result obtained was analyzed using a two-way ANOVA factorial experimental design to the significance of the various sources of variation and from the result obtained source of variation for sample and interactions were non-significantly different from each other which means that irrespective of the number of samples analyzed or the combination of both samples and batches of treatment, they will still not be significantly different. The sources of variation for batch and replications were significantly different from each other and this means that irrespective of the batches of treatment applied or the number of replications (methods of treatment used), they will always be significantly different from each other.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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