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Evaluation of the Role of Limiting Factors of Bioremediation Based on a Bench Scale Study Utilizing Biostimulation and Phytoremediation Approach

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Abstract:

With continuous occurrence of crude oil spill incidents; remediation has become an unavoidable switch that must be triggered often for restoration of the environment. Different remediation approach exists such as biostimulation and phytoremediation. There is no straight line to the evolvement of remediation approaches over the years; this is because, treatment of a single site will generally require a treatment train where more than one treatment technologies are combined to produce expected outcome and the choice of methods will depend on the result of the assessment or treatability studies of the site in question. Adoption of a remediation method is based on the findings of the treatability studies and not necessarily on advanced method criteria. Biostimulation is widely deployed in the Niger delta for treatment of crude oil impacted soils and it involves stimulation of indigenous microorganisms that are capable of bioremediation; this is mostly done by adding limiting nutrients such as nitrogen, phosphorous and potassium to enhance the biological activity of the microbes. Phytoremediation makes use of plants in removing contaminants of concern from the impacted environment. Regardless of the adopted bioremediation approach, high biodegradation of crude oil cannot be achieved if limiting factors of remediation are not taken into consideration in the deployment of the remediation approach. The limiting factors of bioremediation include temperature, oxygen, pH, nutrients, moisture content, and the microbial population present in the contaminated soil sample. Enhanced remediation is optimized when these limiting factors are maintained within allowable range. The study was carried out on a bench scale and the set ups were categorized to enable achievement of the research objective. While the deployed biostimulation and phytoremediation approach was applied in some set ups, some were subjected to conditions outside the acceptable range required for effective biodegradation (category C). The percentage mean TPH reduction in category C set ups was approximately 1.1% during TPH reduction monitoring and research close out against 63% at monitoring and 79% at close out that was observed in the biostimulation set ups and 73% at monitoring and 79% at close out observed in the phytoremediation approach as well as 90% at monitoring and 58% of the remaining TPH at close out observed in set ups involving combination of biostimulation and phytoremediation. This study has shown that the limiting factors of biostimulation and phytoremediation such as oxygen supply, water content, temperature, etc. play a vital role in the bioremediation process as category C set ups which were subjected to conditions that are outside acceptable range witnessed only minor reduction in TPH.

Keywords **—Bioremediation, Biostimulation, Phytoremediation.**

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1.INTRODUCTION

Crude oil pollution has become an uncontrollable variable considering that awareness campaign on the negative impact of pipeline vandalism, crude oil theft, and illegal refining activities has failed to put a stop on these activities leading to increased crude oil spill incidents (Uzochukwu, 2022). With continuous occurrence of crude oil spill incidents; remediation has become an unavoidable switch that must be triggered often for restoration of the environment. Remediation originated because of the need to keep the environment free from every form of pollutant. Different remediation approach exists such as biostimulation and phytoremediation. Remediation activities began as far back as 1989; biostimulation, for example, was done onPrince William Sound Shorelines in 1989 after the Exxon Valdez spillfollowing physical clean-up. Usually, conceptual reviews of methods indicate modification of existing methods to improved and technologically advanced methods that are more effective than the previous. This, however does not apply in the conceptual review of remediation. When it comes to remediation, there is no straight line to its evolvement over the years; this is because, treatment of a single site will generally require a treatment train where more than one treatment technologies are combined to produce expected outcome and the choice of methods will depend on the result of the assessment or treatability studies of the site in question. Data requirements such as soil particle size distribution, soil homogeneity and isotropy, soil pH, soil moisture, soil permeability, humic content of soil, bulk density of soil, oxygen content of soil, and hydrocarbon degrading microbes present in the soil obtained during the treatability studies is very essential as it guides the selection of remediation technologies unique to a specific site. For example, in low permeability soils or permeability variation in different soil layers, remediation technologies such as soil flushing and Soil Vapour Extraction (SVE) may not be feasible considering that the ability of soil flushing fluids to contact and remove contaminant will be lowered in such soils and there is hindrance to air and vapour movement through such soils. Loamy soils (with high humic content) will reduce the effectiveness of bioremediation as it will increase the oxygen demand; except the experimental design will include oxygen addition plan, bioremediation may not be effective in such soils.

In a nutshell, adoption of a remediation method is based on the findings of the treatability studies and not necessarily on advanced method criteria. Modifications to remediation technology design is based on site specific characteristics.

Biostimulation involves stimulation of indigenous microorganisms that are capable of bioremediation. This is mostly done by adding limiting nutrients such as nitrogen, phosphorous and potassium to enhance the biological activity of the microbes. Biostimulation is usually deployed in the remediation of environmental media such as soil impacted by crude oil spill. The advantage biostimulation has over bioaugumentation(addition of microorganisms capable of degradation) is that bioremediation will be undertaken by indigenous microorganisms that are well adapted to and well distributed in the environment.

Biostimulation is widely deployed in the Niger delta for treatment of crude oil impacted soils. Massive crude oil spills impacted soils remediation projects is ongoing in Ogoni undertaken by both HYPREP (Hydrocarbon Pollution Remediation Project) and SPDC (Shell Petroleum Development Company of Nigeria) following the United Nations Environmental Programme's (UNEP's) report of 2011 on the environmental assessment of Ogoniland and the adopted remediation approach in most parts (except where the terrain does not allow such as in Bodo creek) is RENA which is simply biostimulation aided by landfarming of the impacted soils.

Different people have researched on the effectiveness of certain bacteria to accelerate the degradation of crude oil. Varjani et al (2015) (after Xingjian, et al, 2018) constructed a halotolerant hydrocarbon utilizing bacterial consortium (HUBC) consisting of the bacterial isolates Ochrobactrumsp., Stenotrophomonas maltophilia, and Pseudomonas aeruginosa that was found to be good at degrading crude oil with a degradation percentage as high as 83.49%. Tao, et al (2017) (after Xingjian, et al, 2018) utilized a defined co-culture of an indigenous bacterial consortium and exogenous Bacillus subtilis to effectively accelerate the degradation of crude oil. Wang, et al (2018) (after Xingjian, X., et al, 2018) found that an aboriginal bacterial consortium based on Penglai 19-3 oil spill accident in China had higher oil spill degradation efficiency to individual bacteria and demonstrated that this indigenous consortium had the potential for bioremediating crude oil dispersed in the marine ecosystem. A field study showed that bioaugmentation with an artificial consortium containing Aeromonas hydrophila, Alcaligenes xylosoxidans, Gordoniasp, Pseudomonas fluorescens, Pseudomonas putida, Rhodococcusequi, S. maltophilia, and Xanthomonas sp. Contributed to high biodegradation efficiency (89%) in a 365 days treatment of diesel oil contaminated soil (Szule et al, 2014).

However, high biodegradation of crude oil cannot be achieved if limiting factors of remediation are not taken into consideration in the deployment of the remediation approach. The limiting factors of bioremediation include temperature, oxygen, pH, nutrients, moisture content, and the microbial population present in the contaminated soil sample. Enhanced remediation is optimized when these limiting factors are maintained within allowable range.

According to the United States Environmental Protection Agency (USEPA), bacterial growth is a function of temperature. Microbial activity doubles for every 10^0C within the range of 10^0C to 45^0C, slows down below and above this temperature range and ceases below 5^0C (EPA, 510-B-17-003). For phytoremediation utilizing maize and cowpea, a temperature range of 15^0C to 35^0C is required for their optimum growth (Burns, 2018; Davis, et al., 2020). Initial step of aerobic biodegradation by microorganisms involves oxidation of the substrate for which oxygen is required. Microbes use oxygen to oxidize part of the carbon in the contaminant (HC) to give rise to CO_2 while the rest of the carbon is used to produce new cell mass. This process of destroying organic compounds with the aid of oxygen is called aerobic respiration and its by products are CO_2 and water as shown in the equation below;

$4HC + 5O_2 \longrightarrow 4CO_2 + 2H_2O$ ……………………………………Equation 1

The above equation implies that 5 moles of oxygen is required to biodegrade 4 moles of hydrocarbon. Therefore, if measured total petroleum hydrocarbon (TPH) is one gram for instance, you need to find out how many moles it amounts to and what number of moles of oxygen is needed to biodegrade the contaminant. To calculate the number of moles equivalent to 1 gram of TPH, the mass of HC (1gram) is divided by the molecular mass of HC, this is illustrated below;

Number of moles = mass of HC / molecular mass of HC = 1/13 = 0.08 moles. ……Equation 2

If 4 moles of HC = 5 moles of O_2, then 0.08moles of HC = (0.08 X 5) / 4 = 0.1 moles of O_2. Thus, mass of O_2 equivalent of 0.1 moles of O_2 is number of moles of oxygen multiplied by the molecular mass, that is 0.1 x 32 = 3.2grams. 1000mg/kg of HC will therefore require 3,200mg/L of O_2 (O_2 : HC – 3 : 1). In an effective remediation process, while CO_2 is expected to increase, oxygen is expected to decrease if there is no oxygen replenishment. When the available oxygen is used, the bioremediation process will stop. Moreover, experiment has shown that when there is insufficient oxygen, there could be incomplete combustion which could lead to formation of toxic carbon monoxide as a by-product. This is an environmental aspect of the aerobic bioremediation process that can impact the environment negatively if control measures are not put in place. This implies that after analyzing samples to ascertain the contaminant of interest and oxygen content of the contaminated sample, a balanced equation of the contaminant and oxygen should be written to know how many moles of oxygen is needed to degrade Xmole of the contaminant. It is also important to note that there are inorganic oxidation processes that consume dissolved oxygen. If Fe^{2+}, NH^{4+}, and

Mn^{2+} are present in the contaminated sample, they will compete with the microbes (as shown in the equations below) for available oxygen limiting the ability of biodegrading microbes to effectively implement enhanced aerobic bioremediation.

Iron oxidation: $1/4O_2 + Fe^{2+} + H^+ \longrightarrow Fe^{3+} + 1/2H_2O$ ………………….…………..Equation 3

Nitrification: $O_2 + 1/2NH^{4+} \longrightarrow 1/2NO_3^- + H^+ + H_2O$ ………………….………………Equation 4

Manganese oxidation: $O_2 + 2Mn^{2+} + 2H_2O \longrightarrow 2MnO_{2(S)} + 4H+$ ………………Equation 5

When these three are present in the contaminated sample, they should be accounted for in the oxygen replenishment plan. They can either be removed where possible, for example Abdulrazak, (2013) has shown that corn can extract Fe^{2+} off the environment. Where removal is not possible, their oxygen requirement should be calculated as illustrated in equation 2 and added to the quantity of oxygen the microbes require to biodegrade the contaminant of interest. Where oxygen is not sufficient, it can be replenished by the use of oxygen releasing compound (ORC) such as calcium peroxide (CaO_2). Calcium peroxide naturally decomposes in the presence of water to form calcium hydroxide and oxygen as shown in the equation below;

$2CaO_2 + 2H_2O \longrightarrow 2Ca(OH)_2 + O_2$……………………………….…Equation 6

From the balanced equation, you can determine how many moles of calcium peroxide is required to produce the needed moles of oxygen. Note, however, that Oxygen Releasing Compounds (ORC) may raise the pH even higher than the allowable range which can be fatal to the microbes (EPA, 510-B-17-003). Hence, when using ORC, pH should be monitored to avoid rise in pH above allowable range.

Optimum pH for bacterial growth is approximately 7, but enhanced aerobic bioremediation can be effective over a pH range of 5 to 9. Maize and cowpea germinate and grow optimally within a pH range of 5.5 to 6.8 (Burns, 2018; Davis, et al., 2020).

The activity of microbes as well as plant growth depend on the availability of inorganic nutrients such as nitrogen, potassium and phosphorous to support cell growth and sustain biodegradation processes. Nutrients may be initially available in sufficient quantities, but with time they may need to be supplemented with additional nutrients to maintain adequate microbe population and plant growth. However, excessive amount of certain nutrients like phosphate or sulfate can re-press bio-metabolism (EPA, 510-B-17-003).Following USEPA (EPA, 510-B-17-003), quantity of nitrogen and phosphorous to be added is determined based on the oxygen-hydrocarbon (O_2 – HC) ratio obtained from their balanced equation. To avoid over-application of nutrients, it is important to understand how much carbon can be metabolized based on oxygen limiting condition. If the balanced O_2 - HC equation gave an O_2 – HC ratio of 3.1:1 as illustrated under oxygen limiting condition, amount of carbon that can be metabolized is taken to be 1 since ratio of HC is one in the equation. This implies that when using USEPA recommended nutrient ratios, to obtain the required nitrogen and phosphorous to be added, calculated values of nitrogen and phosphorous will be multiplied by the value of carbon that can be metabolized. According to USEPA (EPA, 510-B-17-003), the carbon: nitrogen: phosphorous ratios necessary to enhance biodegradation fall in the range of 100:10:1 to 100:1:0.5. That is, the range of 90.09:9.01: 0.90 to 98.52: 0.99: 0.49. If carbon that can be metabolized is 1, required nitrogen and phosphorous is in the range of 0.99 to 9.01 and 0.49 to 0.90 respectively. However, if carbon that can be metabolized is more than 1, required nitrogen and phosphorous range will be a multiplication of the original range ratios by the carbon number.

Research have shown that certain microorganisms are best suited for bioremediation (Xingjian, et al., 2018). Therefore, there is need to ascertain the microbes present in the contaminated media to be sure that they are hydrocarbon utilizing microbes. Also, population count of the microbes needs to be high to enhance bioremediation. Total microbial count range of 10^4 to 10^5 colony forming units (cfu) and hydrocarbon degrader count range of 10^3 to 10^5cfu per gram of soil indicates that the soil contains a significant naturally occurring microbial population (Hanson, 1999). In the Niger Delta,

because of occurrence of several oil spill incidences, hydrocarbon utilizing microbes are widespread. However, where possible, there is need to narrow down the microbes aiding the bioremediation process so that where bioaugmentation may be required such as in an area with no oil spill history, one can be informed on the microbe to source for.
Optimum soil moisture for effective crude oil remediation is 10% to 15% (Hanson, 1999). Moisture content needs to be checked and re-adjusted if required for enhanced bioremediation.
This study seeks to evaluate the role these limiting factors of bioremediation play in the remediation of crude oil impacted soils.

2. MATERIALS AND METHODS

The study was carried out on a bench scale. The soil samples were obtained from three locations in the Niger Delta (Gio, Eliozu, and Kpoghor). Plastic bowls each consisting of 4kg of soil was used as research set ups. Each container had two replicas for error correction. The soil samples were spiked with crude oil. The set ups were divided into two categories to enable achievement of the research objective. One of the categories was subjected to limiting conditions outside acceptable range while the other category was regularly monitored to ensure that the required conditions for effective biodegradation of crude oil are maintained and sustained within the acceptable range. The phytoremediation approach utilized maize and cowpea while the biostimulation approach made use of NPK fertilizer.

There were 12 control set-ups divided into 2 groups. Biostimulation and phytoremediation were not performed in the controls, however, whatever disturbance that was done in the experimental set-up was also performed on group one control set-up while group two was made to sit undisturbed. This implies that group one control set-up involved only enhanced natural attenuation while group two control set-up involved only natural attenuation. Both the experimental and control set-up were exposed to the same natural environmental conditions. The essence of the control set-up was to accurately rule out the contribution of enhanced natural attenuation and natural attenuation process in the experimental result and arrive at a precise conclusion on how much input proposed biostimulation and phytoremediation approach made in remediating the spill simulated impacted soil.

Parameters of interest were measured based on approved methods in EGASPIN and APHA standard. Contaminated soil sample (spiked soil) used in this study was first analysed for parameters of interest before commencement of the remediation process (initial analysis). The soil samples were analysed again following the monitoring plan and after the remediation process.

Methods of Sample Analysis

Parameters and measurement methods are shown in table 1 below.

Table 1: Analysed parameters and sampling methods

	PARAMETER	METHODS
NUTRIENTS		
1	Nitrogen	APHA 4500-N C (Persulfate method)
2	Phosphorous	APHA 4500 – P E (Ascobic method)
3	Potassium	NA
ORGANICS		
4	TPH	EPA 3550C (USEPA, 2007) EPA 8015C (USEPA, 2007) GC-FID RemScananalysing method
MICROBIOLOGY		
5	Total bacteria count	APHA 9215B
6	Total hydrocarbon utilizing bacteria	APHA 9215B
CATIONS		
7	Fe^{2+}	APHA 3111C
8	Mn^{2+}	APHA 3111C
9	NH^{4+}	APHA 3111C
OTHERS		
10	pH	APHA 4500-H^{+}B

		(Electrometric method)
11	Temperature	APHA 2550B (Thermometric method)

3. RESULTS AND DISCUSSION

pH values for all analysed soil samples fall in the range of 5 to 7 which is within the acceptable range required for bacterial and plant growth. The results of the initial analysis of soil samples for temperature showed that non is below or above 100C to 450C and according to the United States Environmental Protection Agency (USEPA), microbial activity doubles for every 100C within the range of 100C to 450C, slows down below and above this temperature range and ceases below 50C (EPA, 510-B-17-003). Also, maize and cow pea grow optimally within temperature range of 150C to 350C (Burns, 2018; Davis, et al., 2020). To ensure that temperature of the soil samples is maintained within the acceptable range, temperature was regularly checked with a thermometer following the research monitoring plan. Prior to research commencement, all samples (after crude oil spiking and proper homogenization) were analysed for nutrients (specifically phosphorous, potassium, nitrogen), and presence of iron, ammonia and manganese that could compete with the microbes for available oxygen via inorganic oxidation processes. Excessive amount of certain nutrients like phosphate or sulfate can re-press bio-metabolism (EPA, 510-B-17-003); hence, it is important to ascertain the exact quantity of required nutrients already present in the soil sample so that it can be subtracted from the calculated nutrient quantity required for the bioremediation process. With this in mind, before the commencement of the research, all samples were analysed to establish baseline of these parameters. However, because the results came in with oxygen not analysed for as the laboratory could not analyse for oxygen in soil but for only oxygen in water, not much was done with the result except to check that available quantity is at least enough to fuel the biodegradation process and that the iron, ammonia and manganese present is in minimal quantity to not disrupt the remediation process. Soil samples were analysed for the presence of hydrocarbon bacteria (THB). Considering that certain microorganisms are best suited for bioremediation (Xingjian, et al., 2018), the soil samples were further analysed for the presence of hydrocarbon utilizing microbes. Obtained population count of microbes in the samples ranged from 4.0 x 104 cfu/g to 3.1 x 107 cfu/g while the hydrocarbon degrader count range is from 1.1 x 103 cfu/g to 3.2 x 103 cfu/g. According to Hanson, (1999), total microbial count range of 104 to 105 colony forming units (cfu) and hydrocarbon degrader count range of 103 to 105 cfu per gram of soil indicates that the soil contains a significant naturally occurring microbial population and can therefore enhance bioremediation. Thus, the result of the analysis confirmed that the bioremediation process as the research progress will not be limited by required microbes. However, for Gio soil samples with over 90% sand sized particles (based on the particle size distribution analysis), the hydrocarbon degrader count range was observed so be somewhat below the lowest required limit for enhance bioremediation. Soil samples were checked for moisture content, the percentage ranged from 10% to 15% which is within the acceptable range for an effective and enhanced bioremediation according to Hanson, (1999). All samples were analysed for Total Petroleum Hydrocarbon (TPH) using both GC-FID and RemScan analytical equipment. RemScan results usually gives consistently higher TPH values, this can be attributed to the fact that RemScan dictates TPH higher than C40 while laboratory analysis will likely not be able to dictate the heavier fraction, in other words, the consistently higher RemScan values may indicate that the contaminant in the samples contains material heavier than what the laboratory analytical method can dictate. In the course of the research, the proposed monitoring plan in various aspect of the research was revised as follows;

1. Nutrient addition

Because the research is focused on soil alone, Oxygen parameter was not analysed for; thus, there was no way of ascertaining available oxygen content of the soil which is a required parameter in the estimation of required nutrient to be added as explained in the introduction section. Nutrients were, therefore, added in multiplies of 10ml and the progress was monitored for informed decision on its application. Analysis for nitrogen, phosphorous, and potassium (N P K) was therefore eliminated from the initial monitoring plan.

2. Checking for presence of Fe2+ (iron), NH4+ (ammonia), and Mn2+ (manganese)

Because of inability to analyse for Oxygen, Fe2+ (iron), NH4+ (ammonia), and Mn2+ (manganese) were subsequently not accounted for following the revised monitoring plan.

3. Hydrocarbon degrading / utilizing bacteria

Hanson, 1999 established that total microbial count range of 104 to 105 colony forming units (cfu) and hydrocarbon degrader count range of 103 to 105 cfu per gram of soil indicates that the soil contains a significant naturally occurring microbial population for enhanced bioremediation. Before research commencement analysis (initial analysis) of soil samples for Total Hydrocarbon Bacteria (THB) and Hydrocarbon Utilizing Bacteria (HUB) indicated that THB ranged from 4.0 x 104 cfu/g to 3.1 x 107 cfu/g while HUB ranged from 1.1 x 103 cfu/g to 3.2 x 103 cfu/g implying that the soil samples have significant microbial population for enhanced bioremediation.

Hydrocarbon degrading bacteria is expected to increase as bioremediation progress. Nwogu, T.P et al, 2015 conducted research on 'enhanced bioremediation of soil artificially contaminated with petroleum hydrocarbons after amendment with goat manure' and noted that the microbial population increased from 8.5 x 105 cfu/g to 2.7 x 106 cfu/g in sample amended with nutrient and from 8.5 x 105 cfu/g to 1.78 x 106 cfu/g in control sample (sample unamended with nutrient). Considering that both THB and HUB are expected to increase in the bioremediation process and that initial analysis of THB and HUB indicated presence of sufficient amount for enhanced bioremediation, further analysis of THB and HUB was eliminated from the monitoring plan.

4. Moisture content

To ensure that the moisture of the soil samples is maintained within the acceptable range of 10% to 15% (Hanson, 1999), soil moisture was regularly checked with a soil moisture meter following the research monitoring plan.

5. pH

To ensure that pH of the soil samples have not come below or above the acceptable range, the pH was checked regularly with a soil pH meter following the research monitoring plan.

6. Total Petroleum Hydrocarbon (TPH)

Following the revised monitoring plan, RemScan analysis only was utilized for monitoring of TPH reduction and the reduced TPH values compared to TPH values obtained before research commencement indicates that both bioremediation and phytoremediation is effectively taking place.

Set ups exposed to factors outside acceptable range

For the set ups included to demonstrate the importance of the limiting factors of bioremediation, visual observation during the close out sampling was the same as it was at the commencement of the research as well as during TPH reduction monitoring sampling; the black and brown oil on the surface was still present.

While crude oil was still very visible in these set ups, other set ups have undergone significant TPH reduction such that there was no longer visible crude oil impact in them and grasses as well as edible leaves (water leaves) that were not planted in them had begun to grow. The percentage mean TPH reduction in category C set ups was approximately 1.1% during TPH reduction monitoring and research close out against 63% at monitoring and 79% at close out that was observed in the biostimulation set ups and 73% at monitoring and 79% at close out observed in the phytoremediation approach as well as 90% at monitoring and 58% of the remaining TPH at close out observed in set ups involving combination of biostimulation and phytoremediation.

These findings and observations made in this study emphasize the importance of the limiting factors of bioremediation. To enable bioremediation as well as enhanced bioremediation, all factors that can limit the process such as availability of oxygen, nutrients, pH, water content (moisture), and temperature must be maintained within acceptable range.

4. CONCLUSION

This research has showed the effectiveness of both biostimulation and phytoremediation approach to remediating spill impacted soils; biostimulation reduced Total Petroleum Hydrocarbon (TPH) content of the impacted soils, 63% of the initial or starting TPH was degraded at monitoring and up to 79% of the remaining TPH was degraded at close out even in the absence of proper calculation of required nutrient application while phytoremediation reduced initial Total Petroleum Hydrocarbon (TPH) content of the impacted soils by up to 73% at monitoring and 79% of the remaining TPH (following TPH reduction monitoring) at close out even though the plants did not progress to the reproductive stage. The research, however, indicated that petroleum hydrocarbon biodegradation is more effective and enhanced when an integrated approach involving combination of biostimulation and phytoremediation is employed as this was found to reduce Total Petroleum Hydrocarbon (TPH) content of the impacted soils by up to 90% of the starting TPH at monitoring and up to 58% of the remaining TPH at close out. Following through the research hypotheses that engine oil is a valid substitute to ascertain a remediation approach' ability to remediate crude oil impacted soils; the research concluded that the limiting factors of biostimulation and phytoremediation such as oxygen supply, water content, temperature, etc. play a vital role in the bioremediation process as category C set ups which were subjected to conditions that are outside the acceptable range witnessed minor reduction in TPH with mean difference of just 117 mg/kg (1.1% of the initial or starting TPH) at TPH reduction monitoring and 1.1% of the remaining TPH at close out.

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