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Enhanced Natural Attenuation Technique, Edaphic and Microbiological Changes in Oil-Impacted Soil of Odhiaje Community, Rivers State

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ABSTRACT

Oil spills in the Niger Delta could exert environmental pressures on the soil component. We investigated the impacts of oil spills and the effect of the Enhanced Natural Attenuation (ENA) remediation method on contaminated soil and resident microbial populations in the Odhiaje community in Rivers State, Nigeria. Soil samples for microbiological studies were collected weekly during a 17-week remediation period, while those for edaphic parameters were taken before and after remediation, all at 4 sampling points (SPs). Serial dilution of the oil-impacted soils for microbial density enumeration was carried out according to standard methods. Results revealed that mean concentrations of Total Petroleum Hydrocarbon Contents (THC) ($Sig._{value}$ = 0.009), SO_4^{2-} ions ($Sig._{value}$ = 0.001), and sand compositions ($Sig._{value}$ = 0.045) all differed markedly across the sampling points at $p<0.05$. Mean levels of EC (Sig.tvalue = 0.039) and ΣN (Sig.tvalue = 0.058) & K^+ ions (Sig.tvalue = 0.004) differed significantly before and after the remediation exercise at the 95% confidence interval. Application of nutrients was rapidly accompanied by microbial population increases, leading to the consumption of oil contaminants in soils to levels comparable to control over the remediation period. Total Heterotrophic Bacteria counts correlated with pH (r = 0.501) and SO_4^{2-} ions (r = 0.500) ($p<0.05$), and K^+ ions (r = -0.800) ($p<0.01$); Total Heterotrophic Fungi correlated with pH (r = 0.520) ($p<0.05$), and Mg^{2+} ions (r = 0.820) ($p<0.01$); Hydrocarbon Utilizing Bacteria correlated with available P (r = 0.530) and silt composition (r = -0.504) ($p<0.05$), and K^+ (r = 0.626) and Mg^{2+} ions (r = 0.733) ($p<0.01$); and Hydrocarbon Utilizing Fungi correlated with K^+ (r = 0.500) & Mg^{2+} ions (r = 0.506) ($p<0.05$). Results indicate improvement in C/N ratios and effectiveness of the current cost-effective bioaugmentation technique in the restoration of arable soil productivity in the Odhiaje community.

INTRODUCTION

Crude oil drives the bulk of the Gross Domestic Product (GDP) of Nigeria, as the product currently generates the bulk of the country's foreign exchange and serves as an energy source as well as industrial raw materials used in producing several products and services. The extraction process of this natural resource in the environment of the Niger Delta region of the country could be very damaging. As a result, and over the last decade, oil exploration and exploitation have impacted harmfully on the socio-physical environments of oil-bearing communities in the Nigerian Delta, largely threatening their subsistent peasant economy, the environment, livelihood, and hence the basic survival of the people (Eni & Okpiliya 2011).

Incidence of oil spills in the Niger Delta areas have become rampant, and according to Bob-Manuel & Johnson (2001), they are mainly from fractured pipelines due to corrosion or company operational errors in the environment, as well as from sabotage of pipelines by locals for economic and political reasons. Further, Ebuehi et al. (2005) have identified other minor causes of spills, including the low level of technological know-how, the weakness of our laws and their feeble enforcement, the callousness of multinational enterprises participating in the oil business in the country, and the carelessness of various personnel within and outside the industry.

Oil spills have impacts, and their effects on the environment on the biota are also diverse. Additionally, widespread spillages on soil in rivers, creeks, ponds, and wells in the riverine areas of the country have rendered arable soils and good drinking water scarce, and many victims of the pollution have suffered from diarrhea and dysentery (Albert et al. 2018). Joseph et al. (2021) reported that the impact has severely degraded borehole water samples in a partially remediated oil spill site. Many products from oil spills are

toxic to wildlife, which, when incorporated into the food chain, will also be poisonous to humans. This knowledge has increased scientific interest in studying the distribution, fate, and behavior of oil and its derivatives in the environment (Semple et al. 2006). Fishing and farming, which are the traditional means of livelihood of the people of the oil-producing communities, are adversely affected. According to (Ogbuagu et al. 2019), deaths of fishes, crustaceans, and other aquatic organisms, which form the main sources of animal protein in the areas, have been reported.

Natural attenuation is the monitoring of natural processes in environmental segments that act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants (Jorgensen et al. 2011). It could be utilized as a bioremediation method to treat polluted environments, in which case microorganisms will contribute to pollutant degradation without deliberate human interventions. However, where site evaluations need rapid removal of pollutants, enhanced natural attenuation bioremediation, classified into biostimulation (addition of nutrients and chemicals to stimulate innate microorganisms) and bioaugmentation (inoculation with exogenous microorganisms), can be applied. Of the available remediation techniques, the enhanced natural attenuation is the least expensive in environmental management, because the technique could be practiced with little or no expertise and in a natural environment. The problem of oil spillage in the oil-producing areas of Nigeria has proved as challenging as the inability the recover the spill and remediate the impacted environment. In this regard, Mafiana et al. (2021) reported that over 73% of oil spills are unrecovered. Mafiana et al. (2021) identified non-supplemented in-situ remediation as a potentially cost-effective method for mitigating the impact of oil spillage in oil-producing communities and other impacted sites. Ebuehi et al. (2005) had earlier reported that remediation by enhanced natural attenuation (RENA) with spiking and tilling could be used for the reclamation of oil spill-impacted farm settlements in Rivers State, Nigeria. Chikere et al. (2017) reported successful remediation of oil-impacted soil in Bayelsa State, Nigeria, using enhanced natural attenuation with a significant reduction in total petroleum hydrocarbon (TPH) and polyaromatic hydrocarbon (PAH) as well as a spike in hydrocarbon utilizing bacteria (HUB) during remediation.

The current work evaluated the efficiency of the Enhanced Natural Attenuation (ENA) remediation technique on oil-impacted soil of Odhiaje communities in Rivers State, Nigeria. The main focus was on key indicator physicochemical and microbiological parameters of the impacted soil over a timeline. The objectives included the assessment of some edaphic variables in contaminated soil and population dynamics of relevant resident microbiological organisms before, during, and after the remediation exercise.

STUDY AREA

An Overview of the Study Area

Odhiaje community is located at latitudes 0532‘11°” and 0415‘60°” N and longitudes 0630‘32°” and 0625‘40°” E (Fig. 1) and is within the tropical rainforest zone of Nigeria, with much rainfall and thick vegetation. Presently, the vegetation is dwindling due to population growth, persistent farming, and rapid socio-economic development, including but not limited to mineral exploitation. The climate, typical of the tropics, has an average rainfall of 200 mm, mean ambient temperature of 28°C, and relative humidity of between 88 and 98 % (Shell Petroleum Development Company of Nigeria 2002). The wet season lasts between March and November, while the dry season lasts the remaining four months. The soil is mainly of sandy loam, and economic trees such as *Elaeis guineensis* (oil palm) and *Hevea brasiliensis* (rubber) grow well in the area. Annual crops, mainly cassavas and maize, as well as pineapples, okra, and other vegetables, are also grown extensively in farmlands.

Crude oil exploration and exploitation activities are ongoing in the area, and currently, several oil wells and pipelines are traversing the area. An oil spill incident that occurred in June 2018 at Odhieje Community, Ahoada East Local Government Area in Rivers State of Nigeria, resulted in the discharge of large volumes of crude oil into several hectares of adjoining farmlands and forest. The environmental damages inflicted by this spill alone created environmental stress.

MATERIALS AND METHODS

Field Methods and Sampling Locations

The longitudes and latitudes of four sampling points, including a control where the study was conducted, are presented in Table 1. SP 2 was both the northernmost and easternmost sampling point (SP). Sampling was done according to the method of (Iwegbue 2007) at 0-15 and 15-30cm soil depths and samples were collected with a hand-held stainless auger. However, composite samples were collected for post-remediation assessment of the edaphic variables. Quality assurance procedures were strictly adhered to in sample collections and laboratory analyses.

Soil Remediation Exercise

Oil-impacted soil was excavated up to 0.5 m depth, spread, mixed with plants and animal dung, and plowed to promote

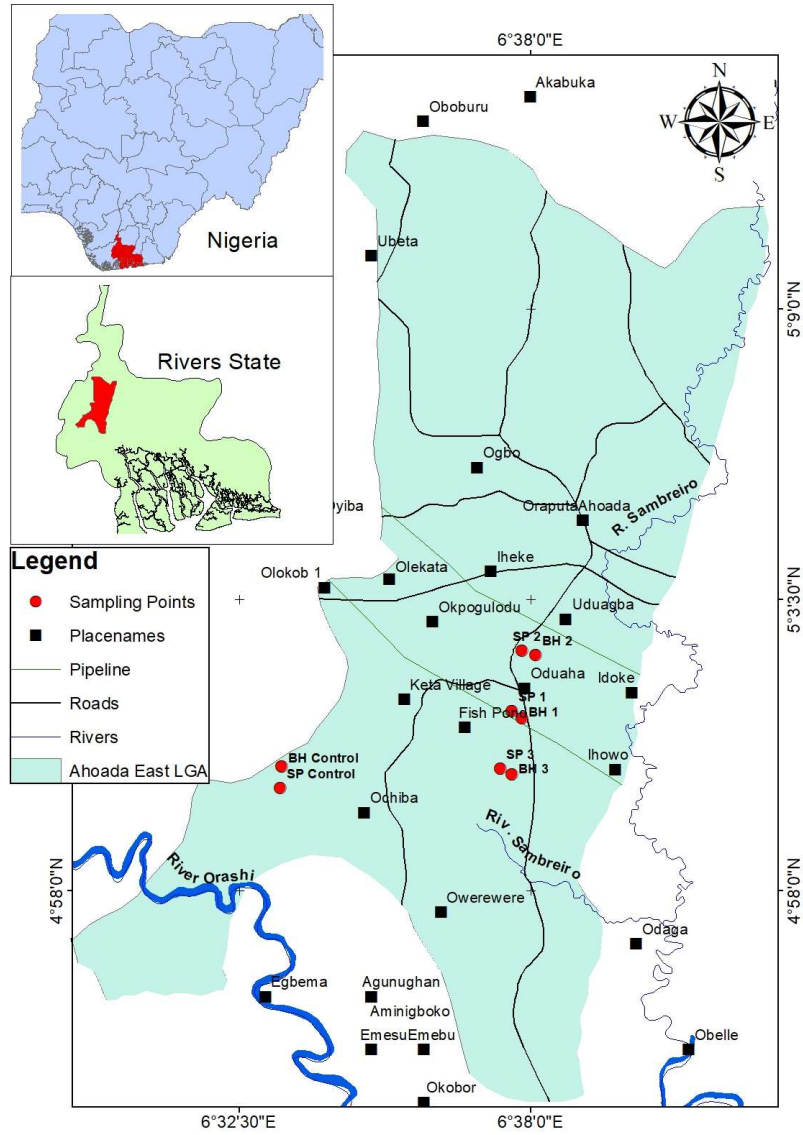

Fig. 1: Map of Odhiaje community and environs in Rivers State, Nigeria, showing the four sampling points (BH 1&SP 1, BH 2&SP 2, BH 3&SP 3, and BH Control/SP Control).

hydrocarbon degradation *in situ*. The procedures of plowing and tilling were done twice with a digger and spade and then homogenized. Composted plants and poultry dung, as well as nitrate-phosphate-potassium (NPK) fertilizer, were added during the homogenization procedure. Ridges (windrows) were constructed and then leveled severally at alternate times. Soil samples for petroleum hydrocarbons and other edaphic variables assessment were taken before and after a 17-week remediation period.

Table 1: Longitudes and latitudes of the sampling locations.

Location	Longitude	Latitude
SP 1	06°37'38.423"E	05°1'22.067"N
SP 2	06°37'50.022"E	05°2'30.607"N
SP 3	z06°37'26.824"E	05°0'16.69"N
SP Control	06°33'15.863"E	04°59'57.71"N

Laboratory Analyses

This was in keeping with the standard methods of (APHA 2002). THC content was obtained by shaking 10 g of a representative soil sample with 20 mL toluene, and oil was extracted. The extracted oil was determined with absorbance at 420nm wavelength in a Spectronic 21-D spectrophotometer. Concentration was then calculated with reference made to the standard curve that was prepared using

a known concentration of hydrocarbons in the extractant. Multiplication was made by the appropriate dilution factor.

To determine the soil pH, Pansu & Gautheyrou (2006) were used. The air-dried soil sample was sieved through a 2 mm sieve, and then 20 g of it was placed in a 50 mL beaker with 40 milliliters of distilled water. A glass rod was used to stir the mixture vigorously and made to stand for 30 min before reading off the pH value on a Corning pH meter (Model 7).

Pansu & Gautheyrou (2006) modified Bougoucous hydrometer method was used to determine textural classifications. A Solution containing sodium carbonate (8 $g.L^{-1}$) and hexametaphosphate (Calgon 44 $g.L^{-1}$) were used to disperse the soil samples. The pH of the solution stayed retained at approximately 8.3, and the textural triangular diagram was used to determine the textural classes.

For the exchangeable cations, 1 g sample of soil was put into a digesting tube, followed by an addition of 10 mL conc. HNO_3. At 96°C, the sample was put in the digester with intermittent stirring for 8 h. When the digestion process was completed, Whatman No. 42 filter papers were used to filter the sample into a 100 mL volumetric flask. The sample was prepared up to the 100 mL mark in the volumetric flask with distilled deionized water. The concentrations of K^+, Ca^{2+}, $Mg^{2+}$and Na^+ions in the supernatant solution were determined using a Varian Spectr-AA 600 Atomic Absorption Spectrophotometer (AAS), with air acetylene flame connected to it.

The sulfate ions presence in the soil sample was confirmed by the monocalcium phosphate extraction method. The soil sample is exposed to the air to become dried and then sieved. 10 g of the dried and sieved sample was put in a 50 mL Erlenmeyer flask. Monocalcium phosphate extracting solution is measured to just 25 mL and added the solution in the Erlenmeyer flask. The solution is shaken for 30 min at a frequency of 200 oscillations per minute. Charcoal measuring 0.25 g was added to each sample, and an extra 3 min of shaking was done. A Whatman No. 42, which is free from sulfate, was used to filter the solution. 10 mL of the filtrate from the extraction process was pipetted and transferred to a 50 mL Erlenmeyer flask. One milliliter of seed was added, and the solution was agitated. Addition of 0.5 g of $BaCl_2$. $2H_2O$ crystals were done, and the solution was put in a steady position for 1 min before a magnetic swirl was used to continuously swirl the flask until the crystals dissolved. HACH DR 2010 UV-visible spectrophotometer at a wavelength of 420 nm was used to read the transmittance at 3-8 min intervals. A linear graph to plot absorbance against the concentration and the absorbance reading was recorded. The concentration of sulfate in 10g of the soil sample was then calculated as:

$$MgSO_4\text{-}S \text{ / kg of soil} = \frac{\text{mg S/L} \times 0.025\text{L}}{0.010 \text{ kg soil}} =$$

$$= \text{mg S / L} \times 2.5$$

For available phosphorus, First, 15 mL of 1M ammonium fluoride and 25 mL of 0.5N HCl were added to 460 mL distilled water to prepare the extracting solution. Then 1g soil sample is air-dried and sieved through a 2mm mesh size. After weighing the dried-up soil sample, it was placed into a centrifuge tube, followed by the addition of extracting solution measuring 7 mL. This mixture stood stirred for 1 minute and then centrifuged. Two milliliters of the clear supernatant were transferred into a 20 mL test tube, and 5 mL of distilled water was added, followed by 2 mL of ammonium solution. 1 mL of chloride solution was added to the mixture. A spectrophotometer at 660 nm wavelength was used to measure the percentage transmittance in 20 min. A standard curve prepared with phosphate in soil standard solution was used in the determination of the amount of available PO_4^{3-} ion in the soil sample.[11]

The electrical conductivity (EC) of soil samples was determined on the filtrate obtained after filtering the suspension used for pH determination. The Lovibond conductivity meter (Model CM-21 bridge) was used in measuring conductivities in $\mu S.cm^{-1}$ (Pansu & Gautheyrou 2006).

Total nitrogen was determined by the Macro Kjeldahl method, as described by Pansu & Gautheyrou (2006). A typical soil sample weighing 5 g was shaken with 50 mL of 1N K_2SO_4. The phenol sulphonic Acid method was employed in determining the nitrogen content using the Aliquot of the resulting extract.

Organic carbon was determined by the wet combustion method of Pansu & Gautheyrou (2006). In duplicate, 2 g of soil sample was put into a 250 mL Erlenmeyer flask after it was weighed. 10 mL of 1N $K_2Cr_2O_7$ solution was added into the flask and swirled gently for the soil to be dispersed. An automatic pipette was used to make a rapid addition of 20 mL conc. H_2SO_4, directing the stream into the suspension. Instantaneously, the flask was shaken gently, make soil and reagents were mixed. After which it was shaken vigorously for 1 minute. The flask was then made to stand for 30 min on a sheet of asbestos. When thirty min had elapsed, 100 mL of distilled water was added. The addition of Four drops of o-phenanthroline-ferrous indicator and titration with 0.5N ferrous sulfate solution was carried out. The approach to the end-point of titration was marked by the solution changing from a greenish cast to a dark green color. At this point, the ferrous sulfate was added drop-wise until the color changed sharply from blue to red (maroon color) in reflected light against a white background. A blank titration was also made devoid

of soil samples to homogenize the dichromate. The result was computed and organic carbon was expressed in percentage.

Microbial Analysis

Soil samples were air-dried, ground, and sieved through a 2mm mesh size sieve. The oil-contaminated soil samples were serially diluted in ten folds, according to the methods of (Vallabhaneni 2012). Each sample of previously air-dried soil was vigorously shaken in 10 mL of sterile water to prepare a soil suspension. The soil suspension was put into the test tubes, and then, ten-fold serial dilution was done up to 10^{-5}.

In triplicate and from the dilution of 10^{-3} and 10^{-4} of each soil sample, 0.1mL aliquot was aseptically placed against Nutrient (NA) and Sabouraud Dextrose Agar (SDA) plates in clean form by pour plate methods of Brenner et al. (2005) and the spread plates methods of (Vallabhaneni 2012). Incubation of inoculated plates was done at 37°Centigrade for a duration of 18 to 24 h at ambient temperatures of 48 to 72 h for the details of total heterotrophic bacterial (THB) and fungal (THF) counts, respectively. Colonies that are distinct in incubated plates were counted and then expressed as colony-forming units per gram (cfu.g^{-1}) of the sampled soil.

The Hydrocarbon-Utilizing Bacteria (HUB) and Fungi (HUF) were cultured and enumerated on solid oil agar.

Statistical Analyses

The data analysis was carried out using SPSS v.23.0 and MS Excel 2020 software. Descriptive statistics and plots were employed to express variations in hydrocarbons and soil parameters. To determine variations in concentrations of hydrocarbons and other soil parameters across the locations at $p<0.05$, The one-way ANOVA test was employed, and mean separation was done with the Duncan Multiple Range test. To explore possible relationships between the Soil variables and the microbial community in the soil, the Pearson correlation coefficient (r) was utilized.

RESULTS AND DISCUSSION

Edaphic Variability and Oil Spill Remediation

Mean pH changed from 6.0±0.1, 5.4±0.2, 4.7±0.01 and 5.7±0.1 before remediation to 5.0±0.1, 5.5±0.1, 5.1±0.01 and 5.2±0.1 after remediation at SP 1, SP2, SP3 and the control (Table 2). Electrical conductivity generally increased from 53±2.5, 54.5±4.0, 28±2.0 and 33±4.0 at SP1, SP2, SP3 and control point, respectively, before remediation to 85±2.0, 90±3.5, 88±2.0 and 86±5.4 µS.cm-1 after remediation. Carbon-Nitrogen (C/N) ratios also varied from 16.5±1.6, 37.5±0.3, 66.0±2.5 and 9.0±3.0 at SP1, SP2, SP3 and control point respectively before remediation to 26.0±1.1, 37.5±0.3, 28.25±2.3 and 60.0±2.7 after remediation.

THC had decreased from 351.77±97.5 before remediation to 161.40±60.5 mg.kg^{-1} after remediation at SP 1; from 5035.30± 61.8 before remediation to 2587.30±55.1 mg.kg^{-1} after remediation at SP 2; from 2990.70±37.8 before remediation to 497.40±21.5 mg.kg^{-1} after remediation at SP 3 and from 65.96 ± 9.9 to 67.50±5.5 mg.kg^{-1} at the control point (Table 2). Available phosphorus contents decreased from 13.1±8.2 before remediation to 12.52±3.4 µg.g^{-1} after remediation at SP 1; from 33.95±1.35 before remediation to 10.70±1.1 µg.g^{-1} after remediation at SP 2; from 38.85 7.3 before remediation to 7.5±3.4 µg.g^{-1} after remediation at SP 3 and from 15.80± 1.7 before remediation to 11.25± 1.0 µg.g^{-1} after remediation at SP Control. Potassium concentration at SP 1, SP 2, SP 3, and the control point decreased from 64.1±1.3±71.8 ,7.7±75.6 ,14.1 and 1.3±59.0µg.g^{-1}, respectively, before remediation to 2.51±6.7, 1.79±4.5, 1.42±1.0 and 0.87±0.2 µg.g^{-1} respectively after remediation.

Organic carbon content decreased from 3.22±1.24 and 3.62±0.23% at SP 2 and SP 3, respectively, before

Table 2: Summary of physico-chemical characteristics of impacted soil before and after remediation.

Sample ID	Before Remediation							After Remediation				
	pH	EC [µS. cm^{-1}]	THC [mg.kg^{-1}]	Organic C [%]	C/N Ratio			pH	EC [µS. cm^{-1}]	THC [(mg.kg^{-1}]	Organic C [%]	C/N Ratio
SP1	5.95	53	351.8	0.97	16.5			5	85	161.4	1.56	26
SP2	5.4	54.5	5035.3	3.215	63.5			5.5	90	2587.3	1.5	37.5
SP3	4.7	28	2990.7	3.615	66			5.1	88	497.4	1.13	28.25
Control	5.7	33	66.0	0.545	9			5.2	86	67.5	1.2	60
Sample ID	**Before Remediation**							**After Remediation**				
	Av. P [µg.g^{-1}]	SO_4^{2-} [µg.g^{-1}]	K^+ [mg.kg^{-1}]	Na^+ [mg.kg^{-1}]	Ca^{2+} [mg.kg^{-1}]	Mg^{2+} [mg.kg^{-1}]	Av. P [µg.g] $^{-1}$	SO_4^{2-} [µg.g^{-1}]	K^+ [mg.kg^{-1}]	Na^+ [mg.kg^{-1}]	Ca^{2+} [mg.kg^{-1}]	Mg^{2+} [mg.kg^{-1}]
SP1	13.1	8.45	64.1	0.75	1.75	1.15	12.52	9	2.51	1.09	1.1	0.3
SP2	33.95	3.3	75.6	1.2	0.75	2.5	10.7	4	1.79	1.08	1.4	0.5
SP3	38.85	5	71.8	0.9	1.15	3.4	7.5	6	1.42	1.13	1.3	0.2
Control	15.8	6.2	59	0.85	0.9	2.05	11.25	5	0.87	1.17	0.8	0.5

remediation to 1.50±1.0 and 1.13±0.1% respectively after remediation**,** but increased from 0.97±0.22 and 0.55±0.2% at SP 1 and the control point respectively before remediation to 1.56 and 1.2±0.05% respectively after remediation. Sulfate ion concentrations increased from 8.45±0.4, 3.3±0.3 and 1.0±0.3 $\mu g.g^{-1}$ at SP 1, SP 2, and SP 3, respectively, before remediation to 9.0±0.1, 4.0±0.1, 6.0±0.1 $\mu g.g^{-1}$ after remediation. Still, they decreased from 6.2±0.2 before remediation to 5.0±0.1 after remediation at the control point. Magnesium ions decreased from 1.15±0.2, 2.5±0.1, 3.3±0.2 and 2.05±0.1 $mg.kg^{-1}$ at SP 1, SP 2, Sp 3, and the control point, respectively, before remediation to 0.3±0.1, 0.5±0.1, 0.2±0.1 and 0.5±0.1respectively after remediation. Total Nitrogen remained fairly unchanged at SP 1 (0.06±0.001%) but decreased from 0.05±0.001, 0.06±0.001, 0.06±0.001% at SP 2, SP 3 and the control point, respectively, before remediation to 0.04±0.001, 0.04±0.001 and 0.02±%0.001 respectively after remediation. Sodium concentration increased from 0.08±0.01, 1.2±0.01, 0.9±0.02 and 0.9±0.01 $mg.kg^{-1}$ at SP 1, SP 2, SP 3, and the control point, respectively, to 1.09, 1.08±0.03, 1.13±0.001 and 1.17±0.01 $mg.kg^{-1}$. Calcium ions had increased from 0.8±0.02 and 1.2±0.05 $mg.kg^{-1}$ at SP 2 and SP 3, respectively, before remediation to 1.4±0.001 and 1.3±0.02 $mg.kg^{-1}$ after remediation. Still, they decreased from 1.8±0.3 and 1.8±0.02 at SP 1 and the control point, respectively, before remediation to 1.1±0.1 and 0.8± 0.001 $mg.kg^{-1}$, respectively, after remediation (Fig. 2).

Of the textural classes, the composition of sand increased very slightly from 49±2 to 51±2 at SP 2. From 64±0.5 to 65±0.2% at SP Control before and after remediation (Fig. 3). However, it decreased from 60±1.5 to 47±1.0% at SP 1 and 74±1.3 to 63±1.0% at SP 3 before and after remediation respectively. Silt composition increased from 17±1.0 to 34±1.0 % at SP 1, 0.5±9 to 0.2± 26 at SP 3, and 14±0.4 to 28±0.1% at the control point before and after remediation. Clay composition decreased from 23±1.0 to 19±1.0 at SP 1, 25±1.2 to 23±1.0 at SP 2, 17±0.5 to 11±0.2 at SP 3, and 22±0.3 to 7±0.1% at the control point before and after remediation respectively.

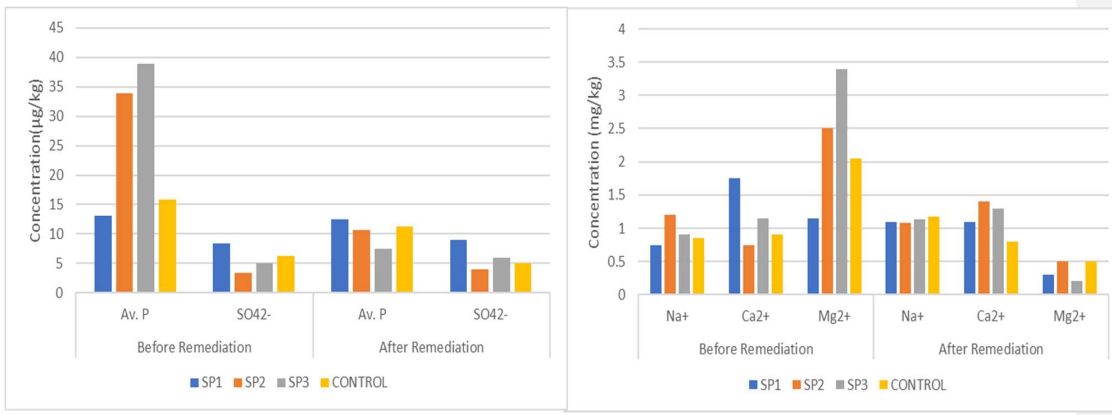

Fig. 2: Attenuation of physico-chemical parameters due to remediation.

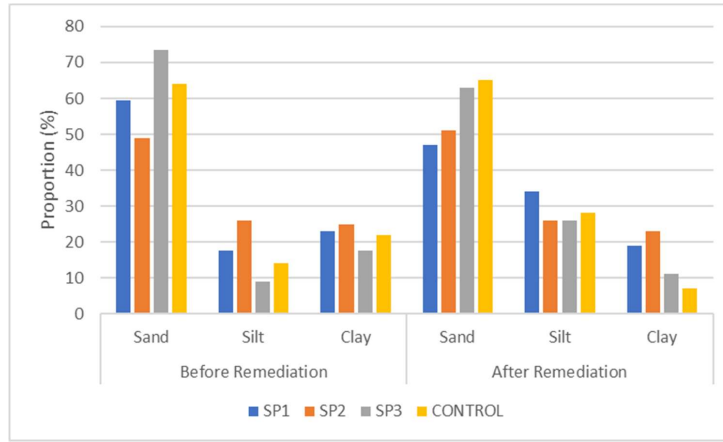

Fig. 3: Variations in soil characteristics before and after remediation.

The One-way ANOVA test revealed that the concentrations of THC ($Sig._{value}$ = 0.009), SO_4^{2-} ions ($Sig._{value}$ = 0.001), and sand compositions ($Sig._{value}$ = 0.045) all differed markedly across the sampling points at $p<0.05$. A post-hoc mean separation technique with the Duncan Multiple Range test (Table 2) revealed that the observed difference in THCs was between SP 1 = SP Control and SP 2; that in SO_4^{2-} ions was between SP 1 and the rest sampling points, while that in Sand composition was between SP 2 and SP 3. Using the Student's t-test to carry out a pair-wise comparison in concentrations of the soil variables showed that mean levels of EC (Sig.tvalue=0.039) and ΣN (Sig.tvalue = 0.058) & K^+ ions (Sig.tvalue = 0.004) differed significantly before and after the remediation exercise at the 95% confidence interval.

Variations in Microbial Population

The counts of microorganism groups in oil-impacted soil during the 17-week remediation exercise are shown in Tables 4 - 7. For the Total Heterotrophic Bacteria (THB) counts (Table 4), the population of the microorganisms increased in SP 1 from 5.3×10^5 at the commencement of the remediation exercise to 4.0×10^7 cfu.g^{-1} in week 9. Then, it decreased to 3.5×10^5 cfu.g^{-1} at the end of the exercise. At SP 2, microorganism counts increased from 6.8×10^6 at the commencement of the exercise to 3.1×10^8 cfu.g^{-1} in week 10 and then decreased to 4.4×10^6 cfu.g^{-1} at the end.

Table 3: Mean separation in edaphic variables impacted by the oil spill in Odhiaje community by Duncan Multiple Range (DMR) Test (p<0.05).

Sampling points				
Parameters	SP 1	SP 2	SP 3	SP Control
pH	5.633^a	5.433^a	4.833^a	5.533^a
EC	63.666^a	66.333^a	48.000^a	50.666^a
THC	288.310^a	4219.296^b	2159.600^{ab}	66.73^a
Organic C	1.166^a	2.643^a	2.786^a	0.763^a
∑N	0.060^a	0.046^a	0.050^a	0.046^a
C/N ratio	19.666^a	54.833^a	53.416^a	26.000^a
Av. P	12.906^a	26.200^a	28.400^a	14.283^a
SO_4^{2-}	8.633^c	3.533^a	5.333^b	5.800^b
K^+	43.570^a	50.996^a	48.340^a	39.623^a
Na^+	0.863^a	1.160^a	0.976^a	0.956^a
Ca^{2+}	1.533^a	0.966^a	1.200^a	0.866^a
Mg^{2+}	0.866^a	1.833^a	2.333^a	1.530^a
Sand	55.333^{ab}	49.666^a	70.000^b	64.333^{ab}
Silt	23.000^a	26.000^a	14.656^a	8.666^a
Clay	21.666^a	24.333^a	15.333^a	17.000^a

Values with the same superscript along the same row are not significantly different at
p<0.05; EC=Electrical conductivity; THC=Total Petroleum Hydrocarbons;
Av. P=Available phosphorus

At SP 3, THB increased from 4.4×10^6 to 2.5×10^8 cfu.g^{-1} in week 11 and then decreased to 4.1×10^6 cfu.g^{-1} at the end (Table 4). However, at the control location, microbial populations increased from 4.9×10^6 to 3.5×10^7 cfu.g^{-1} in week 8 and then decreased to 4.8×10^6 cfu.g^{-1} at the end of the exercise.

Table 5 shows that the Total Heterotrophic Fungi (THF) counts in the impacted site increased at SP 1 from 7.4×10^3 to 6.8×10^5 cfu.g^{-1} in week 10 and then decreased to 2.3×10^3 cfu.g^{-1} at the end of the exercise. THF counts also increased at SP 2 from 6.3×10^3 to 4.5×10^5 cfu.g^{-1} in week 12 and then decreased to 2.5×10^3 cfu.g^{-1} at the end. They increased at SP 3 from 6.4×10^3 to 5.5×10^5 cfu.g^{-1} in week 5 and then decreased to 2.5×10^3 cfu.g^{-1} at the end of the exercise. At the control location, THF counts increased from 4.5×10^3 to 3.7×10^4 in week 5 and then decreased to 4.2×10^3 at the end of the exercise.

The Hydrocarbon-Utilizing Bacteria (HUB) counts increased from 9.9×10^4 to 8.8×10^5 cfu.g^{-1} in week 10 and decreased to 9.3×10^3 cfu.g^{-1} at the end of the exercise in SP 1 (Table 6). At SP 2, it increased from 1.2×10^6 to 1.3×10^8 cfu.g^{-1} in week 11 and then decreased to 1.9×10^4 cfu.g^{-1} at the end. They increased from 9.9×10^5 to 7.6×10^7 cfu.g^{-1} in week 10 and then decreased to 7.4x 10^3 cfu.g^{-1} at the end of SP 3. HUB counts at the control sampling point also increased from 6.5×10^3 to 6.1×10^4 cfu.g^{-1} in week 6 and then decreased to 6.7×10^3 at the end of the remediation exercise.

Table 7 shows that the Hydrocarbon-Utilizing Fungi (HUF) counts increased from 9.1×10^2 to 8.4×10^4 cfu.g^{-1} in week 9 and then decreased to 2.1×10^2 cfu.g^{-1} at the end of the exercise at SP 1. At SP 2, HUF counts increased from 1.1×10^3 to 1.8×10^4 cfu.g^{-1} in week 11 and then decreased to 3.0×10^2 cfu.g^{-1} at the end. At SP 3, counts increased from 9.7×10^2 at the commencement of the experiment to 8.0×10^4 cfu.g^{-1} in week 11 and then decreased to 2.4×10^2 cfu.g^{-1} at the end. At the SP Control location, counts increased from 1.1×10^2 to 1.0×10^3 cfu.g^{-1} in week 10 and then decreased to 1.1×10^2 cfu.g^{-1} at the end of the remediation exercise.

Relationships Between Edaphic Variables and Microbial Community in Impacted Soils

Table 8 shows the Pearson's correlation coefficients (r) between the edaphic variables and microbial communities in impacted soils during the remediation period. At $p<0.05$, THB counts correlated positively with pH (r = 0.501) and sulfate ions (r = 0.500). At $p<0.01$, it correlated negatively with electrical conductivity (EC) (r = -0.701) and K^+ ions (r = -0.800). At $p<0.05$, THF counts correlated positively

Table 4: Total Heterotrophic Bacteria (THB) counts (cfu.g^{-1}) in impacted soils of Odhiaje community during the remediation period.

Sampling Points	Time (Weeks)																
	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 13	Wk 14	Wk 15	Wk 16	Wk 17
SP 1	5.3×10^5	5.1×10^5	5.3×10^5	4.5×10^6	4.1×10^6	4.4×10^6	3.1×10^7	3.7×10^7	4.0×10^7	4.8×10^6	3.2×10^7	3.1×10^7	4.5×10^6	4.7×10^6	4.5×10^6	4.8×10^5	3.5×10^5
SP 2	6.8×10^6	6.2×10^6	5.0×10^7	5.5×10^7	6.0×10^6	4.1×10^7	4.3×10^7	4.0×10^7	3.0×10^8	3.1×10^8	6.7×10^7	6.4×10^7	6.0×10^7	5.8×10^6	4.8×10^6	4.5×10^6	4.4×10^6
SP 3	4.4×10^6	4.4×10^6	4.8×10^6	3.5×10^7	3.0×10^7	4.0×10^6	3.1x 10^7	2.5×10^8	2.2×10^8	2.1×10^8	2.5×10^8	3.8×10^7	4.9×10^6	3.0×10^7	4.1×10^6	4.6×10^6	4.1×10^6
SP Control	4.9×10^6	4.8×10^6	4.1×10^6	5.1×10^6	4.8×10^6	4.3×10^6	3.1×10^7	3.5×10^7	5.0×10^6	5.1×10^6	3.0×10^7	4.1×10^6	3.1×10^7	4.4×10^6	4.4×10^6	4.1×10^6	4.8×10^6

Table 5: Total Heterotrophic Fungi (THF) counts (cfu.g^{-1}) in impacted soils of Odhiaje community during the remediation period.

Sampling Points	Time (Weeks)																
	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 13	Wk 14	Wk 15	Wk 16	Wk 17
SP 1	7.4×10^3	7.1×10^3	7.2×10^3	6.5×10^4	6.1×10^4	6.3×10^4	6.1×10^4	7.0×10^4	7.1×10^4	6.8×10^5	6.0×10^5	7.0×10^4	6.8×10^4	7.0×10^3	4.8×10^3	4.5×10^3	2.3×10^3
SP 2	6.3×10^3	6.4×10^3	7.9×10^2	7.0×10^2	6.8×10^3	6.5×10^3	6.4×10^3	6.1×10^3	5.5×10^4	5.2×10^4	5.5×10^4	4.5×10^5	4.1×10^5	5.1×10^4	6.8×10^3	4.0×10^3	2.5×10^3
SP 3	6.4×10^3	6.4×10^3	6.1×10^4	6.5×10^4	5.5×10^5	5.1×10^5	4.8×10^5	4.8×10^5	4.7×10^5	5.5×10^4	5.1×10^4	5.2×10^4	5.6×10^4	6.0×10^3	4.1×10^3	2.6×10^3	2.5×10^3
SP Control	4.5×10^3	4.1×10^3	4.0×10^3	4.5×10^3	3.7×10^4	4.1×10^3	4.1×10^3	4.0×10^3	4.7×10^3	4.5×10^3	4.1×10^3	4.1×10^3	3.5×10^4	4.4×10^3	4.1×10^3	4.0×10^3	4.2×10^3

Table 6: Hydrocarbon Utilizing Bacteria (HUB) counts (cfu.g^{-1}) in impacted soils of Odhiaje community during the remediation period.

Sampling Points	Time (Weeks)																
	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 13	Wk 14	Wk 15	Wk 16	Wk 17
SP 1	9.9×10^4	9.7×10^4	9.7×10^4	8.5×10^5	8.2×10^5	8.4×10^5	8.1×10^5	8.8×10^4	8.4×10^5	8.8×10^5	8.0×10^5	8.2×10^5	7.5×10^4	7.6×10^4	8.7×10^3	8.8×10^3	9.3×10^3
SP 2	1.2×10^6	1.3×10^6	1.7×10^6	1.1×10^7	1.3×10^7	1.4×10^7	1.1×10^7	1.0×10^8	1.1×10^8	1.0×10^8	1.3×10^8	1.1×10^8	1.7×10^7	1.9×10^6	1.8×10^5	1.5×10^4	1.9×10^4
SP 3	9.9×10^5	9.5×10^5	9.8×10^5	8.4×10^6	8.3×10^6	8.1×10^6	7.2×10^7	7.0×10^7	7.3×10^7	7.6×10^7	7.1×10^7	8.0×10^6	8.5×10^6	9.4×10^5	9.7×10^4	9.1×10^3	7.4×10^3
SP Control	6.5×10^3	6.6×10^3	6.7×10^3	7.1×10^3	7.0×10^3	6.1×10^4	6.0×10^4	7.8×10^3	7.8×10^3	7.4×10^3	7.3×10^3	7.1×10^3	7.5×10^3	7.0×10^3	6.8×10^3	6.7×10^3	6.7×10^3

Table 7: Hydrocarbon Utilizing Fungi (HUF) counts (cfu.g^{-1}) in impacted soils of Odhiaje community during the remediation period.

Sampling Points	Time (Weeks)																
	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 13	Wk 14	Wk 15	Wk 16	Wk 17
SP 1	9.1×10^2	9.3×10^2	9.1×10^2	9.5×10^2	8.7×10^3	8.5×10^3	8.1×10^3	8.5×10^3	8.4×10^4	8.1×10^4	8.0×10^4	8.1×10^3	8.5×10^3	8.7×10^2	5.5×10^2	3.7×10^2	2.1×10^2
SP 2	1.1×10^3	1.2×10^3	1.2×10^3	1.6×10^3	1.7×10^3	2.0×10^3	1.5×10^4	1.4×10^4	1.5×10^4	1.5×10^4	1.8×10^4	1.6×10^4	1.8×10^3	1.5×10^3	3.4×10^2	3.1×10^2	3.0×10^2
SP 3	9.7×10^2	9.5×10^2	8.8×10^3	$8.7 \times$	8.5×10^3	8.6×10^3	8.1×10^3	7.5×10^4	7.1×10^4	7.5×10^4	8.0×10^4	7.8×10^3	7.3×10^3	4.7×10^2	5.1×10^2	4.5×10^2	2.4×10^2
SP Control	1.1×10^2	1.0×10^2	1.7×10^2	1.2×10^2	1.4×10^2	1.2×10^2	1.8×10^2	1.5×10^2	1.8×10^2	1.0×10^3	1.0×10^3	1.5×10^2	1.2×10^2	1.7×10^2	1.4×10^2	1.7×10^2	1.1×10^2

Table 8: Correlation (r) matrix between the edaphic variables and Microbial groups in oil-impacted soils of the Odhiaje community.

Parameters	pH	EC	THC	Org.C	∑N	C/N	Av.P	SO_4^2	K^+	Na^+	Ca^{2+}	Mg^{2+}	Sand	Silt	Clay
THB	0.501*	-0.701**	-0.111	-0.174	0.200	-0.270	0.024	0.500*	-0.800**	0.123	0.027	0.151	0.072	-0.218	0.283
THF	0.520*	-0.280	-0.134	-0.259	-0.050	-0.267	-0.186	-0.173	0.067	-0.053	-0.032	0.820**	0.081	-0.157	0.133
HUB	-0.131	-0.707**	0.436	0.336	0.153	0.349	0.530*	-0.390	0.626**	-0.243	-0.429	0.733**	0.347	-0.504*	0.226
HUF	-0.193	-0.538*	0.233	0.254	0.137	0.232	0.456	-0.050	0.500*	-0.086	-0.249	0.506*	0.221	-0.324	0.150

*values are significant at p<0.05; **values are significant at p<0.01; THB=Total Heterotrophic Bacteria; THF=Total Heterotrophic Fungi; HUB=Hydrocarbon-Utilizing Bacteria; HUF=Hydrocarbon-Utilizing Fungi; EC=Electrical conductivity; THC=Total Petroleum Hydrocarbons; Org.C=Organic Carbon; C/N=Carbon/Nitrogen ratio; Av.P=Available

with pH (r=0.520), and at p<0.01, they correlated positively with Mg^{2+} ions (r = 0.820). At p<0.05, HUB counts correlated positively with available phosphorus (r = 0.530) and negatively with the composition of silt (r = -0.504). However, at p<0.01, it correlated positively with K^+ (r = 0.626) and Mg^{2+} ions (r = 0.733) and negatively with EC (r = -0.707). At p<0.05, HUF correlated positively with K^+ ions (r = 0.500) and Mg^{2+} ions (r = 0.506) and negatively with EC (r = -0.538).

Effect of Remediation on Soil Physicochemical Properties

The current work revealed high concentrations of petroleum hydrocarbons in soil and drastic decreases after remediation, similar to the work of Mafiana et al. (2021) and Chikere (2017) in the Niger Delta area of Nigeria. Soil-oil contaminant generally decreased by up to 50.41% at SP 2 after the 17-week remediation exercise. (Liu et al. 2010) also observed total petroleum hydrocarbon content reduction by 58.2% in treated plots after bioremediation for 360 days. The current work also revealed corresponding and appreciable improvement in the carbon-nitrogen ratio of soil, which got progressively lowered as the remediation proceeded and with the decay and release of more organic nitrogen in the soils. Essien and John (2010) also observed a similar trend in their work in Akwa Ibom State, Nigeria, carried out on the alluvial soils of the coastal plains of the Qua Iboe river wetlands.

By nature, organic carbon in soil is normally derived from flora and fauna, such as peat formation over time, plant fine roots yield, and microbial renewable organic materials from plants, animals, and others (Wang et al. 2013). However, the total organic carbon in the soil might be from crude oil contamination in the oilfield soils. The high concentration of THC in the oil-impacted soil might have resulted in the elevated total organic carbon content recorded. Wang et al. (2010) reported that a significant increase in the total organic carbon contents due to oil contamination is most likely because of the much higher THC concentration in spilled sites.

On a spatial basis, the current work reveals both slight increases and decreases in the pH of soil after the remediation exercise. Previous results from studies on oilfields in China also revealed an increase in soil pH as a result of oil pollution (Wang et al. 2010, Jia et al. 2009). The reason for the higher pH values in crude oil-contaminated soil in this study may be due to two factors: firstly, the hydrophobic nature of crude oil might encourage a potential scarcity in the shallow and underground layers of contaminated soil (Njoku et al. 2009), which could intensify the concentration of salt in the soil, and thereby raising the pH values when matched with the values in the control location. Secondly, the buildup of

exchangeable ions such as Calcium and sodium ions and a decrease in the exchangeable amount of acid and real cation exchange capacity have been revealed to be associated with oil-polluted soil (Osuji et al. 2006, Agbogidi et al. 2007). These mechanisms might also be responsible for the increases in pH values in oil-polluted soil. However, the mechanisms may not have operated in locations where decreases in pH were recorded in the current work.

The levels of petroleum hydrocarbons, SO_4^{2-} ions, and sand composition all differed significantly on a spatial basis. Edaphic properties that showed significant increases in their concentrations after remediation include electrical conductivity, total nitrogen, and K^+ ions. These all contributed to improved soil quality in the area. However, results of a previous study (Wang et al. 2010) showed that oil contamination could decrease available phosphorus concentration in soil by various degrees. A study carried out on the Momoge wetlands of China (Wang et al. 2010) showed a decrease in the concentration of available phosphorus as the time of oil exploration and production increased. Similar decreases were, however, observed in the current work only after remediation. In another experimental oil study, the available phosphorus in crude oil-polluted soil was reduced to as much as 66% in concentration in comparison with the control site as the crude oil content touched 30 $mg.kg^{-1}$ (Eneje et al. 2012). However, Liu et al. (2010) reported that available phosphorus concentrations are not considerably influenced by oil contamination.

From the extant study, lowered available phosphorus concentrations after remediation in all the impacted locations may have been enhanced for two reasons. First, THC in the soil could increase the carbon concentration. This may disturb the balance of soil nutrients. Soil microbes that utilize THC as a carbon source may well consume considerable amounts of available phosphorus when the hydrocarbons are degraded (Wang et al. 2013). Second, phosphorus solubility of phosphorus is exploited at pH near the neutral value, and higher pH values in some locations of the work may have also lowered the available phosphorus concentration when compared with that obtained in the control location. Phosphorus is an important macro-nutrient for plants and soil microorganisms. Decreased available phosphorus concentrations in impacted locations could alter the structural composition of vegetation and microorganisms in the soil, as well as reduce soil ecosystem services and values (Bello & Anobeme 2015).

Effects of Remediation on Microbial Populations

The endpoint and achievement of the oil spill and its bioremediation are dependent on the capability to start and preserve conditions that help enhance oil biodegradation rates in the contaminated environment. Scientific review that discussed various factors that affect the rate of oil biodegradation, including that by (Das & Chandran 2011), showed that the presence of microorganisms with suitable metabolic capabilities is an important requirement. Optimal rates of growth and biodegradation of hydrocarbon occur when these microorganisms exist. This process can be sustained if the pH is between 6 and 9 and the concentrations of nutrients and oxygen are sufficient. The physicochemical characteristics of the oil and the oil surface area are also vital factors of successful bioremediation. There are two basic approaches to oil spill bioremediation. The first, which was applied in the current study, is bioaugmentation, in which known oil-degrading bacteria are added to supplement the existing microbial population. The second is biostimulation, in which the addition of nutrients or other growth-limiting co-substrates stimulates the growth of indigenous oil degraders. Microbial counts in oil-impacted soils did not show appreciable change over weeks 1-3. Still, they did after week 3 when nutrients, consisting of composted plants and animal dung, as well as nitrate-phosphate-potassium (NPK) fertilizers, were introduced. Generally, counts peaked between weeks 6 and 14 and were least in week 17, marking the end of the remediation exercise in all the sampling points. The hydrocarbon-utilizing bacteria (HUB) counts peaked in week 10, and at week 17, all the microbial communities attained counts comparable to those of their respective control locations.

These exponential increases in microbial population are due to stimulatory effects by the composted plants and animal dungs, as well as NPK fertilizer introduced on the impacted soils. This effect has been explained to be due to the proliferation of microbes in soil, especially in the presence of growth nutrients (Semple et al. 2006, Hollender et al. 2003, Walworth et al. 2007). Findings by Ogbonna et al. (2007) revealed that there is a more rapid bioremediation of crude oil-contaminated soils with a combination of microorganisms, poultry manure, and fertilizers other than microorganisms or fertilizers alone. Selective enrichment of the soil with microbial species that have tolerance for extremely high oil concentrations could spontaneously be caused by the high oil content.

The soil became selectively enriched with very high oil concentration tolerant microbial species due to the excessive petroleum hydrocarbon content of the polluted area.

The general increases recorded in the various microbial community counts also confirm that hydrocarbon pollution does not only enrich the hydrocarbon utilizers but also enriches additional populations that utilize the by-products

that are not intact hydrocarbons, as observed by Walworth et al. (2007). According to (Das & Chandran 2011), bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in the environment. Microbial degradation is the major and ultimate natural mechanism by which one can clean up the petroleum hydrocarbon pollutants from the environment. Several bacteria are even known to feed exclusively on hydrocarbons. The hydrocarbon-utilizing bacteria and fungi also increased during the remediation period. These observations are consistent with the knowledge that inputs of hydrocarbon pollutants stimulate increases in microbial numbers (Ogbonna et al. 2007). These categories of microbes utilize hydrocarbons as their sole carbon sources of metabolism. Bello & Anobeme (2015) observed that hydrocarbonoclastic bacteria and fungi as oil degraders are ubiquitous in both the temperate and tropical environments of oil-polluted and unpolluted locations. This also explains the presence of hydrocarbonoclastic microbes even in the control sites. Ramdass & Rampersad (2021) have also reported the presence of a diverse microbial population, including novel oil-degrading filamentous fungi at eight oil-impacted sites in Trinidad.

Interactions of Edaphic variables and Microbial Groups

This study revealed that pH and Mg^{2+} ions had positive effects on the growth of the THF, while EC and K^+ ions appeared to inhibit the growth of the HUF. The recorded decrease in the pH of soil after remediation could be a result of the metabolic activities of the microorganisms, which produced pH-depressing metabolites. The utilization of crude oil by these organisms, which resulted in their population growth, also produced and accumulated acidic metabolites (Essien & John 2010). Electrical conductivity appeared to significantly inhibit the growth of HUF. This observation corresponded with decreases recorded in concentrations of K^+ ions, which usually contribute to the conductivity of soil and water media after the remediation exercise. Potassium and EC also showed inhibitory effects on growths of the THB, HUB, and HUF.

CONCLUSION

The current research set out to employ conventional enhanced natural attenuation techniques in the remediation of oil-impacted soil. This study reveals the effectiveness of bioaugmentation with simple locally-available manure, as well as synthetic fertilizer (NPK), in the restoration of the productivity of arable soil in the Odhiaje community in the Delta area of the River Niger, Nigeria. The addition of organic and inorganic nutrients was rapidly accompanied by microbial population growth in the soil, and this subsequently led to the consumption of the oil contaminant in the soil to comparable levels over a 17-week test period. There was a general decrease in the soil-oil contaminant by up to 50.41% after 17-week remediation, with a corresponding improvement in the carbon-nitrogen ratio of soil.

RECOMMENDATIONS

Enhanced Natural Attenuation (= Landfarming) technique should be recognized as the least expensive of the other bioremediation treatment methods in environmental management. The technique could be practiced with little or no expertise and in a natural environment.

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