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Implication of particle sizes on bioremediation of crude oil polluted sandy soil in Okolomade part of Niger Delta Basin, southern Nigeria

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ABSTRACT

Bioremediation is a cost-effective and environmentally friendly technology that exploits the capabilities of microorganisms to degrade organic pollutants leading to complete mineralization. It has become the most preferred technique for oil spill remediation on soil in Nigeria. The study aims to examine the implication of particle sizes on bioremediation of crude oil polluted sandy soil in Okolomade part of Niger Delta Basin, southern Nigeria. Once a week samples were collected for a total of 28 days and were analyzed for chemical and microbial content in an aerobic setting. The classification of the soil samples was done according to the U.S. Bureau of soil classification system, the soil samples were divided into X and Y, where X represented fine to coarse sand and Y represented very fine to coarse sand. The particle size distribution, total hydrocarbon content (THC), total heterotrophic bacteria count (THBC), total organic carbon, soil pH, and available nitrogen and phosphorus were the parameters investigated throughout the 28-day examination of the soil samples. The results shows that the total heterotrophic bacterial count and soil pH increased in all of the soil samples, with samples A for fine to coarse sand (X) and sample E for very fine to coarse sand showing the most significant increase with values of 120 Cfu × 105/g and 266 Cfu × 105/g, respectively. These samples also had the lowest coefficients of uniformity (Cu). The results further reveal that the total hydrocarbons content, available nitrogen and phosphorus, as well as total organic carbon, all decreased noticeably. In contrast to samples with higher coefficient of uniformity values, samples with lower coefficients of uniformity showed a higher decrease in hydrocarbon content, suggesting that particle size distribution affects bioremediation.

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0.0899 and 0.0942 were calculated to be the correlation coefficient of total hydrocarbon content vs coefficient of uniformity for fine to coarse sand (X) and very fine to coarse sand (Y). The contaminated soil samples are treated by combining pig manure, NPK 15:15:15, and the microorganism Pseudomonas aeruginosa, the total hydrocarbon content of sandy soil was reduced.

***Keywords*:** Bioremediation, Okolomade southern Nigeria, crude oil, pollution, particle sizes, Niger Delta Basin

1. INTRODUCTION

Crude oil spills are dangerous occurrences which often pose great threats to the environment, human health, food production and security (Omenna et al., 2023; Lu et al. 2014). Countries where exploration and exploitation of crude oil is taking place, crude oil pollution is a well-known environmental issue (Singh et al., 2020). Crude oils and its refined products are mainly hydrocarbons, and majority of them are bio-degradable (Prince 2002). However, accumulation of hydrocarbon contaminants in the environment constitutes a serious challenge to the ecosystem and human health (Omenna et al., 2023; Chen et al. 2015).

The remediation of oil-polluted soils in the Southern region of Nigeria has always been a serious problem confronting the region which requires urgent and effective solution (Erifeta 2017). Crude oil spills primarily affect terrestrial and aquatic ecosystems in Niger Delta Nigeria, where anthropogenic activities like increased upstream and downstream activity, vandalism of oil installations, and corrosion of overly old oil facilities can lead to large scale contamination of these environments (Michel and Finga 2016; Atlas, et al., 2011; Brkic' and Praks 2021; Das, et al., 2023).

Crude oil has caused environmental contamination despite its significance and extensive use on land (Choi, et al., 2002; Naveed et al., 2020; Chen, 2020). Oil spillage and discharges frequently happen as a consequence of explosion incidents during oilfield drilling; leakage from oil and gas pipelines and reservoirs, fuel tankers, and well waxing; and during overhauls of refineries and petrochemical manufacturing equipment (Brkic' and Praks 2021; Atlas, et al., 2011). It is also recognized as a serious threat to ecosystems and it takes several years or decades to recover from many environmental problems after the event of a spill (Hussain et al., 2015).

The presence of crude oil in the natural environment is a critical problem because it causes gradual soil degradation and occasionally leads to the permanent destruction of soil and loss of fertility (Balachandran 2012). This necessitates the utilization of methods that are environmentally friendly for cleaning up oil spills. One is the use of biological agents which, compared with physicochemical approaches, are better performing and more cost-effective (Wyszkowska et al., 2015).

Bioremediation is feasible, targeted, and capable of achieving high removal efficiency at a low cost. It is recognized as a cost-effective treatment technology for oil-contaminated soils (Cerqueira et al., 2014; Adetutu et al. 2015; Ali et al., 2020; Hazaimeh and Ahmed, 2021). It has been estimated that cleaning costs can be reduced by about 30 to 50% by bioremediation approaches (Lü et al., 2011). Bioremediation involves the removal, modification, immobilization, or detoxification of different chemicals and physical pollutants from the environment through the use of microbes and plants (Dvorak et al, 2017). The successful application of bioremediation techniques, such as bioaugmentation, biostimulation, and phytoremediation, for remediating oil spills was reported in numerous studies (Adams et al. 2015; Cai et al. 2016; Mrozik and Piotrowska-Seget 2010; Yavari et al. 2015). Field-scale bioremediation works were also conducted in some oil contaminated fields, and the obtained results were satisfactory. Most of them were ex situ methods, such as biopiles and prepared beds (Álvarez et al. 2017; Gomez and Sartaj 2013; Gomez and Sartaj 2014; Jørgensen et al. 2000), which are always time-consuming and expensive (Farhadian et al. 2008), and therefore unsuitable for mass soil.

Bioremediation is widely suitable and has been used for years (Han et al. 2008; Menendez Vega et al. 2007; Mishra et al. 2001; Pizarro-Tobías et al. 2015; Zhang et al. 2017). Whether bioremediation is successful mainly depends on the biodegrading microorganisms (Wu et al. 2016). Microorganisms use hazardous contaminants to produce energy and biomass, repairing the environment and halting more pollution in the process. The targeted contaminant is degraded by enzyme-powered bioprocess technology, which is made possible by the role of microorganisms as a catalyst. It is acknowledged that bioremediation can be used instead of conventional physico-chemical procedures to recover contaminated soil because it is more economical, less labor-intensive, safe, and environmentally benign (Drombrowski et al, 2016). In spite of its potential for environmental restoration, microbial bioremediation technology is currently less frequently used.

The physical, chemical, parent microbial population, environmental conditions, and accurate analysis are all factors that affect how effective bioremediation can be (Nedwell, 1999). Applying a particular fertilizer with nutrients of nitrogen, phosphorus, and potassium (NPK 15:15:15 fertilizer) to the polluted soil will accelerate the cleaning action that occurs naturally. Increasing nutrient levels in hydrocarbon polluted soils has been achieved through application of wastes of animal and plant origin (Adams et al., 2017; Agamuthu et al., 2013; El Mahdi & Aziz, 2019), and this has resulted in considerable reduction in the pollutant concentration through biodegradation. Wastes of animal and plant origin that have been investigated for use as nutrient source during bioremediation of petroleum hydrocarbon polluted soil include chicken manure, cow dung, goat manure, swine wastewater, brewery spent grain, food-waste compost, and wheat bran (Abioye et al., 2010; Adams et al., 2017; Agamuthu et al., 2013; Hara et al., 2013; Nwogu et al., 2015; Zhang et al., 2020).

Numerous laboratory investigations on the nutritional augmentation of naturally occurring microorganisms that degrade oil have found that this method holds potential for application in promoting oil decomposition (Zhang et al., 2019, Okoh et al, 2020; Arinze et al, 2022). Temperature, water runoff, substrate, and other environmental factors that are neither fully understood nor easily quantifiable affect how nutrients are applied in the field (Atlas, 1995).

In particle-size analysis, the mass fractions of clay, silt, sand, and gravel are measured. Understanding a material's particle size distribution might help one better comprehend its physical and chemical characteristics. It has an impact on the stability and load-bearing capabilities of soil and rocks. In many industrial items, including the production of printers, toners, cosmetics, and pharmaceutical products, it impacts the reactivity of solids participating in chemical reactions and needs to be strictly managed (Arinze et al, 2022). The surface area, which is a function of the soil particle size, is another aspect crucial to efficient restoration. At the water/oil contact in the soil, microorganisms that break down oil proliferate.

After 25 days, sand, laterite, topsoil, and clay soil, showed reductions in hydrocarbons of 70%, 69%, 49%, and 26% respectively, according to Arinze et al. (2022). Their findings demonstrate that the particle size distribution of soil influences bioremediation to some extent, as clay with a lower particle size proved more challenging to bioremediate, with sandy soil having the highest percentage decrease of hydrocarbon (70%) and clay being the lowest (26%).

Walter-Duru and Ekemube (2023). Used *Costus afer juice*, for the remediation of crude oil contaminated soil because of its rich nutrient composition of NPK which proved it as a biostimulant, their results showed an increased in the number of total heterotrophic bacteria count, consequently causing a reduction in total petroleum hydrocarbon and total polycyclic aromatic hydrocarbon.

According to Meghara et al. (2011), microbial proliferation and population growth are inversely correlated with oil surface area and lower the efficacy of soil remediation. According to a study by Adaba (2013), the particle size distribution parameter affects bioremediation. It was shown that the rate of hydrocarbon depletion increased with decreasing Cu and D50 levels. The correlation coefficient (R) of THC vs Cu for fine-to-coarse sand (X) is 0.867, while it is 0.923 for very fine-to-coarse sand, demonstrating that the soil's texture, colour, and sand content have an impact on the remediation strategy. Bearing in mind that each pollutant is unique in organic content and properties.

Emoyan (2020). Reported that there are differences between the types of dung employed in the remediation of soil that had been contaminated by crude oil. Cow dung proved to be more effective in bioremediation than pig dung and poultry droppings. Bacterias like Pseudomonas, achrombacter, arthrobacter, bacillus, flavobacter, nocardia, vibrio, connybacterium, alcaligeu and yeast and fungal species such as aspergilium, candida, cladspotum, penicillum, rhodomia, and trichodermia are some examples of naturally occurring microbes that are capable of decomposing petroleum hydrocarbons.

Abosede (2013). Evaluate the impacts of crude oil contamination on some soil characteristics using samples from contaminated site and control (unpolluted) at three different soil depths, recorded that crude oil has no significant effect on particle soil sizes (silt and clay) at different depths, however, the depth of sand particle were higher at 0.5cm depth than 10-15cm by 43.35%. The study reported that crude oil has no significant effect at different depths, but can be noted that the presence of the pollutant increases the bulk density while reducing the total porosity of the pore spaces attributed to clogging or blockage of pores spaces with crude oil, limiting drastically air and water circulation within the natural soil environment. His study further suggested that physical soil properties like saturated hydraulic conductivity, macro-porosity and total porosity and bulk density can be affected since these properties are controlled by pore spaces present in the soil

Hafiz et al. (2022) showed that the administration of bacteria isolates, *Bacillus subtilis* strain PM32Y, *Bacillus cereus* strain WZ3S1, *Bacillus* sp. strain SM73 and *Bacillus* sp. strain WZ3S3 in association with alfalfa significantly degraded petroleum hydrocarbons. Chimezie (2015) investigate the bioremediation of a polluted freshwater habitat, where he isolated, characterized, and assessed the hydrocarbon-utilizing fungi that may be used. His results revealed that Mucor species were more common in the liquid medium than Penicillium, which grew sparsely in the culture medium. The use of fungi in the bioremediation of oil available in the susceptible terrestrial environment has shown that they can be used to reduce the noxiousness associated with an oil spill in an arable land.

Savira and Ipung (2023) used Bacillus cereus for the remediation of kerosene contaminated soil, bacillus cereus believed to belong to the hydrocarbon clastic bacteria and also able to produce enzymes that hydrolyze proteins and complex polysaccharides and form endospores reduced the kerosene levels and the degradation rates by 26.58%.

Ojewumi et al, (2018) tested the effectiveness of Pseudomonas aeruginosa and aspergillus Niger for the bioremediation of treated crude petroleum and soil polluted with raw petroleum. They did the evaluation by monitoring the organic carbon content available in the soil for a period of 45 days and the influence of pH for a period of 25 days. They reported that the pH did not have effect on the level of bioremediation. With the application of P. aeruginosa and A. niger separately, they observed an enhancement level of bioremediation of raw crude polluted soil when compared to the treated crude polluted soil.

Suryatmana et al. (2018) reported that Azotobacter vinelandii and mushroom log waste were applied for the bioremediation of heavily polluted soil for petroleum degradation because Azobacter spp. accelerated the hydrocarbon degradation by shortening the remediation period and mushroom log waste increased the bacterial petrophylic growth rate and also in a shorter amount of time, their evaluation of the functional microbial available in the soil revealed that mushroom log waste could be used for the bioremediation of petroleum waste.

2. LOCATION AND GEOLOGY SETTING OF THE STUDY AREA

Niger Delta Basin is one of the world's hydrocarbon producing basins with its formation dated back to the Tertiary age (Alao et al 2013). It is situated in the southern part of Nigeria, which shares a border with the Atlantic Ocean. It is housed within the Gulf of Guinea and covers both the onshore and offshore of the Delta's province (Falebita et al 2015). The petroliferous Niger Delta Basin (NDB) lies between Longitudes 4.0° and 8.8°E of the Greenwich Meridian and Latitudes 3.0° and 6.5°N of the Equator (Figure 1). It is regarded as one of the world's largest regressive deltas and situated along the nose end of the northeast/southwest Benue Trough, an African Cratonic quasi-linear Cretaceous sedimentary depression (Doust and Omatsola 1990; Reyment 1965; Cratchley and Jones 1965; Mascle 1976).

The Niger Delta Basin is bounded by the Cameroon Volcanic Line to the east and the eastern-most West African transform-fault passive margin, Dahomey Basin to the west. It covers ~ 300,000 km^2 in area of land extending towards the Atlantic Ocean (Kulke 1995). Wu and Bally (2000) classified the Niger Delta Basin as a classical shale tectonic province due to the presence of over pressured shales and shale diapiric structures associated with the area. The overall sediment volume in the Niger Delta is ~ 500,000 km^3 (Hospers 1965) with ~ 10 km sedimentary thickness around the depocenters (Kaplan et al. 1994).

The Niger Delta Basin formed along a failed arm of the triple junction system (aulacogen) was initially developed in the late Jurassic following the breakup of the Gondwana into the African and South American plates (Burke 1972; Whiteman 1982). The southwestern coast of Nigeria and Cameroon which harbours two of the arms formed the West African passive continental margin, while the third failed arm developed to form the Benue Trough (Lehner and De Ruiter 1977). During Cretaceous to Tertiary time, synrift sediments were accumulated within the basin with the Albian age sediments being the oldest dated sediments.

Several episodes of transgression and regression led to the deposition of marine and marginal marine sediments and carbonates (Doust and Omatsola 1990). During the Early Santonian - Late Cretaceous, the occurrence of basin inversion marked the end of the synrift phase

Renewed subsidence resulting from the separation of the continents paved the way for the sea to transgress into the Benue Trough. Progradation of the Niger Delta clastic wedge continued during the Mid-Cretaceous into the depocenter located on top of the deformed continental margin at the spot where the triple junction was situated. During the Late Cretaceous, sediment progradation was interrupted by episodes of transgression (Whiteman 1982).

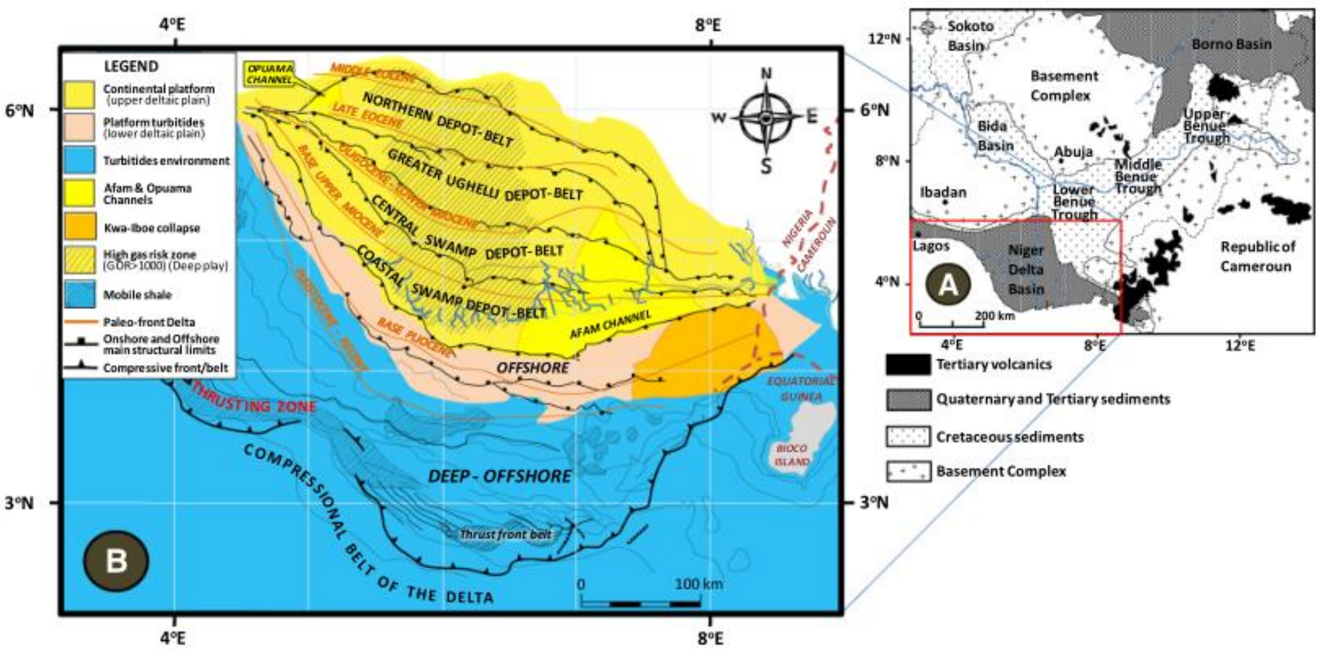

Figure 1. Geologic map of Nigeria showing the location of the Niger Delta Basin (**a**) (redrawn from Ebong et al. 2017) and sectional map of the Niger Delta depobelts and structural limits (**b**) (Redrawn from Doust and Omatsola 1990)

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The fault system in the Niger Delta is predominantly normal faults resulting from movements of ductile, over-pressured, deep-seated marine shale (Fig. 2). These processes over time have led to the deformation of the Niger Delta clastic wedge to a large extent (Doust and Omatsola 1990). Several of these faults which were due to slope instability along the continental margin were formed during delta progradation. These affected sediment dispersal, due to the syn- depositional episodes during the basin evolution. At depths, the faults flatten onto master detachment plane near the summit of the over-pressured marine shales around the base of the Niger Delta sedimentary succession. Pockets of complex structures in isolated areas are indicators of the density and style of faulting. Flank and crestal folds (i.e. simple structures) occur along individual faults. Hanging-wall rollover anticlines were built-up, due to listric-fault geometry and differential loading of the clastic wedge above over-pressured shales. Several complex structures, truncated by group of faults with varying amounts of throw, include collapsed crest structures with domal shape and strongly contrasting fault dips at depth.

The Niger Delta Basin is dominated by off lap cycles of fluvio-marine sedimentary fill in a stepwise pattern. This reflects sedimentary progradation towards the south, from the edge of the continent in the direction of the oceanic basement. The Tertiary Niger Delta stratigraphic evolution and underlying Cretaceous strata are well documented in Short and Stauble (1967).

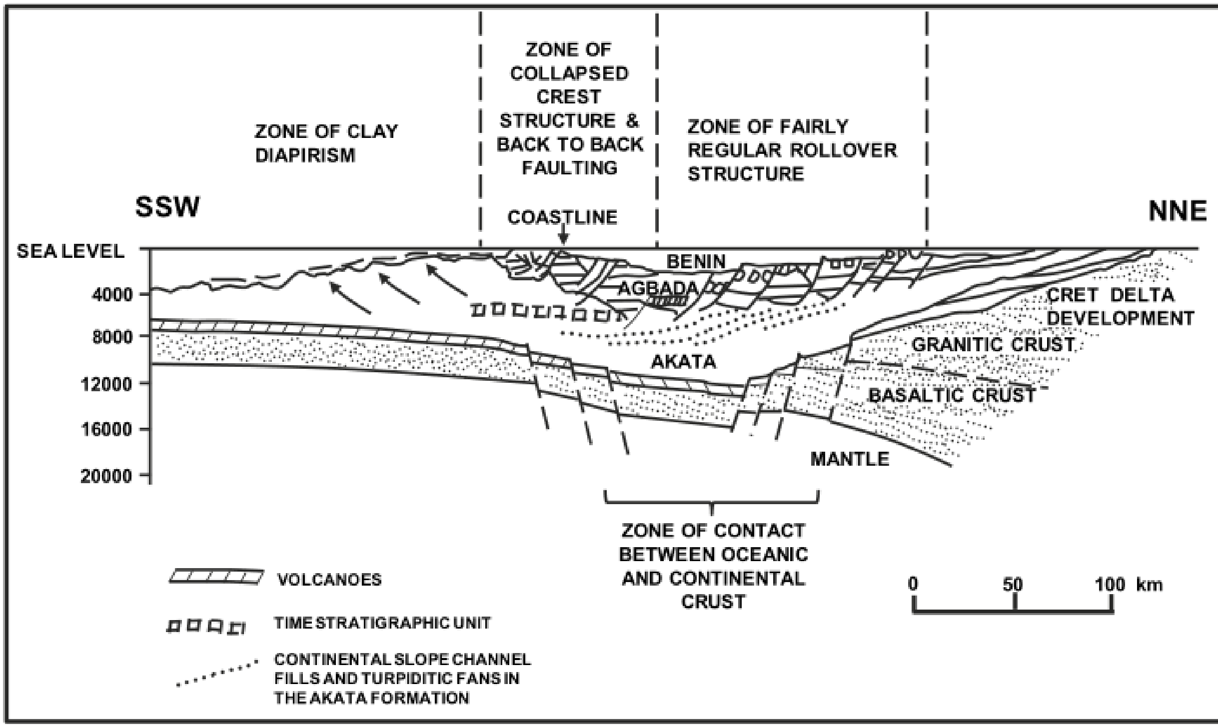

Figure 2. Schematic dip section of the Niger Delta Basin showing stratigraphic successions. (Redrawn from Whiteman 1982)

The three major subsurface lithostratigraphic units of the Niger Delta are the Akata, Agbada and Benin Formations. The sediment thickness of the Benin Formation is about 2100 m which was deposited in the continental phase of the Niger Delta (Weber and Daukoru 1976). Onyeagocha (1980) further acknowledge that the Benin Formation consist of mainly of sandstone, sands and gravel with clays occurring in lenses. The sands and sandstones are partly unconsolidated with varying thickness, and are of fine to coarse grained in texture.

3. MATERIALS AND METHOD

The soil samples utilized in this study were collected from Okolomade, a location that had been contaminated by oil, in the Abua/Odual Local Government of Rivers State, Nigeria. The soil sample was air dried and subjected to sieve analysis. By employing different sieve sizes (X sieve sizes: 10, 16, and Y sieve numbers: 8, 30, and 44), they were separated into classes X and Y. After being retained on each sieve, the soil samples were placed in plastic containers (2 for class X and 3 for class Y, for a total of 5 plastic containers for both classes). After proper gathering of information from the toxic soils, the remediation process commenced.

The contaminated soil samples are treated with 60g of NPK 15:15:15 fertilizer, 100g of pig manure, and 2 ml of pseudomonas aeruginosa inoculation in nutrient broth. After application, the five soil samples were turned (under an aerobic situation) to ensure a thorough mixture. Once a week samples were collected for a total of 28 days and were analyzed for chemical and microbial content in an aerobic setting. The following factors were identified: total heterotrophic bacteria count (THBC), soil pH, organic carbon content, available nitrogen, available phosphorus, and particle size distribution (Cu and D_{50}) respectively.

4. RESULTS AND DISCUSSION

The soil samples were categorized into two categories: X is fine to coarse sand and Y is extremely fine to coarse sand as shown in Table 1A and Table 1B. The samples were progressively passed through set of sieves and percentages of these samples were taken, as presented in Figure 3A and 3B below. The Cu and D_{50} values varied between the two classes (X and Y) as a result of the different sieve sizes.

Table 1A. Fine to coarse (X)

Parameters	Samples	
	A	**B**
D_{50}	0.4	0.5
Cu	3	3.8
D_{10}	0.18	0.17
THC mg/kg	15600	13800
TOC g/kg	7.048	7.457
Nitrogen g/kg	0.24	0.31
Phosphorous g/kg	4.27	4.30
Soil pH	2.13	1.57
THBC	295	38

Table 1B. Very fine to coarse sand (Y)

Parameters	Samples		
	C	**D**	**E**
D_{50}	23	25.95	19.15

Cu	4.3	8	2.1
D_{10}	0.07	0.2	0.19
THC mg/kg	15400	13000	18900
TOC g/kg	7.03	7.238	5.01
Nitrogen g/kg	0.243	0.3	0.14
Phosphorous g/kg	3.12	3.01	4.02
Soil pH	2.45	5.3	5.62
THBC	300	45	115

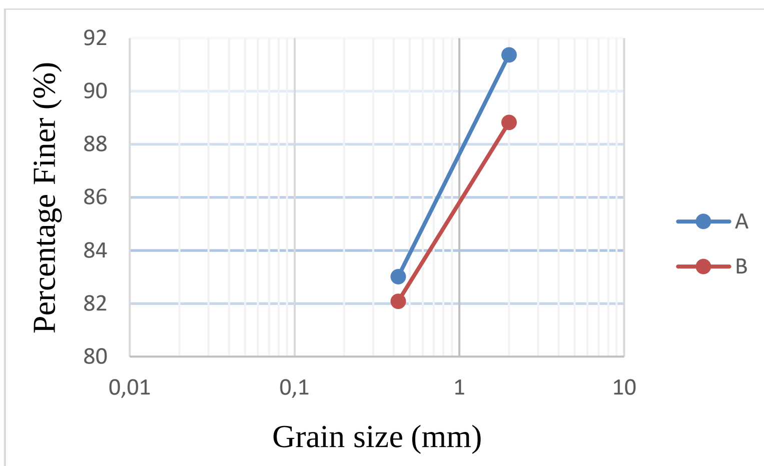

Figure 3A. Particle size distribution for fine to coarse sand (X)

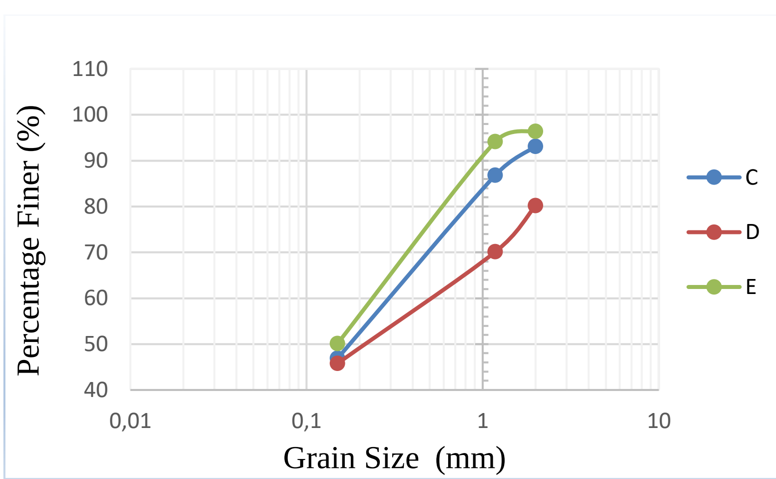

Figure 3B. Particle size distribution for very fine to coarse sand (X)

Total heterotrophic bacteria count, or THBC, increased from day 7 to day 28. However, sample A than sample B, showed a more noticeable rise in THBC, as indicated in figure 4A, with values of 120 Cfu × 105/g and 119 Cfu × 105/g, respectively for fine to coarse sand. By the end of remediation, it was found that Sample E's Total Heterotrophic Bacteria count for very fine to coarse sand recorded a noticeable increase of 266 Cfu × 105/g. The other samples (C and D) also showed an increase in THBC values of 172 Cfu × 105/g and 163 Cfu × 105/g, respectively as shown in figure 4B. This is in agreement with a report by Atlas (1984), Adaba et al., (2013) and Arinze et al., (2022).

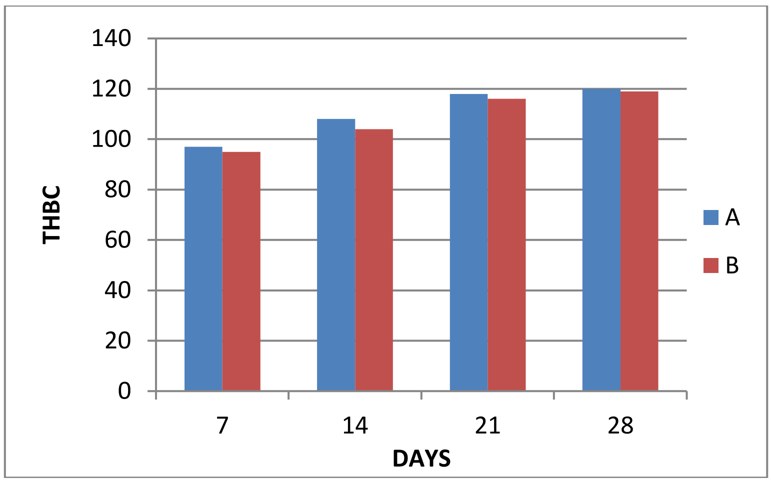

Figure 4A. Total Heterotrophic Bacteria Count Vs Days for fine to coarse sand (X).

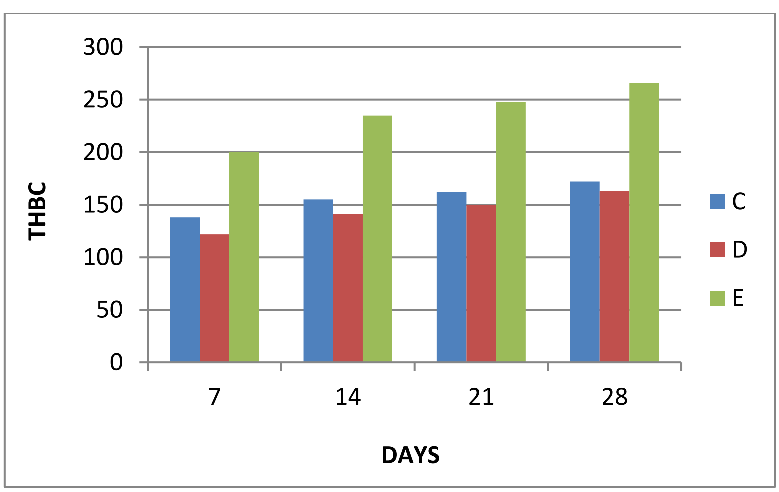

Figure 4B. Total Heterotrophic Bacteria Count Vs Days for very fine to coarse sand (Y)

As the THC levels dropped throughout the course of the days as a result of the bioaugmentation and biostimulation processes, the number of microorganisms in the soil increased. The rate at which crude oil is metabolized by soil organisms is accelerated, in accordance with Atlas (1984), by the addition of nutrients and oil-degrading bacteria.

Sample A had the lowest THC concentration (9000 mg/kg for fine to coarse sand) and Sample E had the lowest THC concentration (5000 mg/kg for very fine to coarse sand). As shown in figures 5A and 5B, Sample B had the highest concentration of THC at 9200 mg/kg for fine to coarse sand, Sample D had the highest value at 8600 mg/kg for very fine to coarse sand at the conclusion of the remediation period and Sample C recorded a value of 8400 mg/kg. Sample E had 1.26 g/kg for very fine to coarse sand and Sample A had the highest reduction in TOC (3.5 g/kg) for fine to coarse sand. The samples also had higher THBC values (120 Cfu × 105/g and 266 Cfu × 105/g, respectively), indicating that the bacteria were using the nutrients to grow in their numbers.

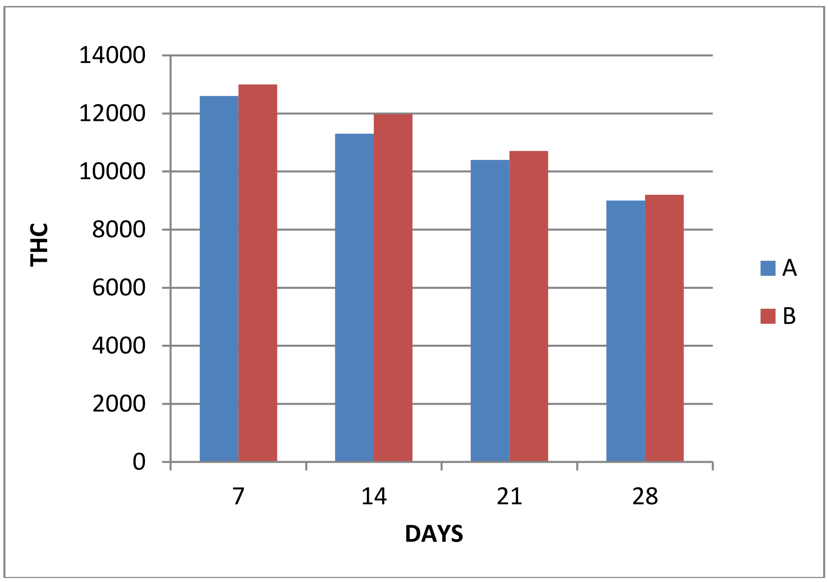

Figure 5A. Total Hydrocarbon Content Vs Days for fine to coarse sand (X)

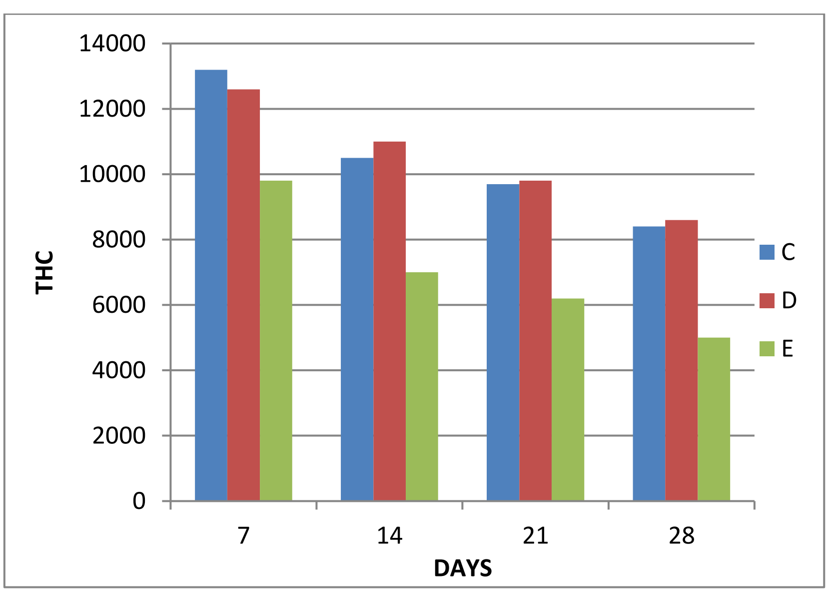

Figure 5B. Total Hydrocarbon Content Vs days for very fine to coarse sand (Y)

As shown in figures 6A and 6B, sample B had a reduction in Total Organic Carbon (TOC) of 3.74 g/kg for fine to coarse sand and sample D had a value of 3.98g/kg for very fine to coarse sand at the conclusion of remediation. After remediation, hydrocarbon loss was found to have increased significantly; samples A and E had the highest hydrocarbon loss at the end of remediation, at 42.31 and 73.54 for fine to coarse sand and very fine to coarse sand, respectively, as shown in figures 7A and 7B. Sample B recorded the least loss at 33.3 for fine to coarse sand, and sample D at 33.85 for very fine to coarse sand. The microorganisms present in the soil samples used the available nutrients, resulting in a significant reduction in the nutrients at the end of the remediation process. These microorganisms were more active in the samples with lower Cu values, namely sample A and sample E, which had nitrogen and phosphorus values of 0.170 g/kg and 0.102 g/kg and 1.09 g/kg and 1.57 g/kg, respectively.

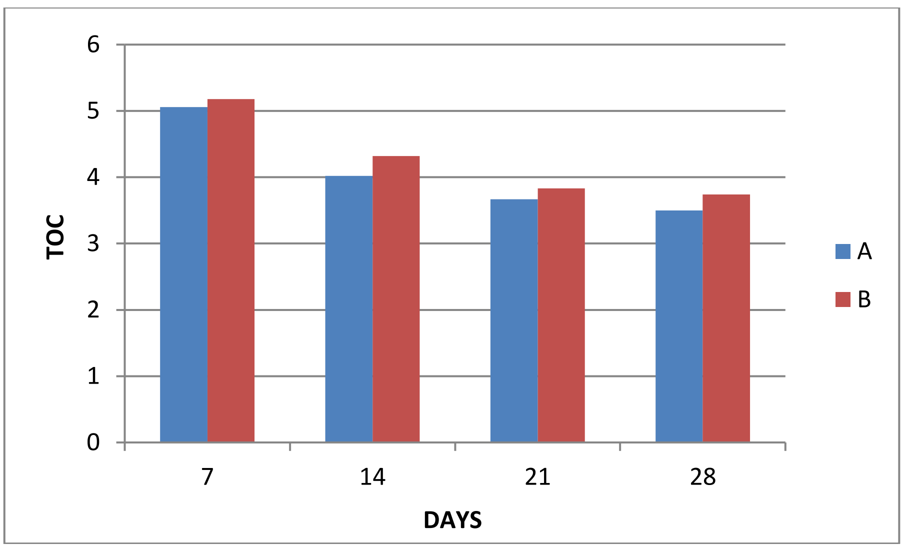

Figure 6A. Total Organic Carbon Vs days for fine to coarse sand (X)

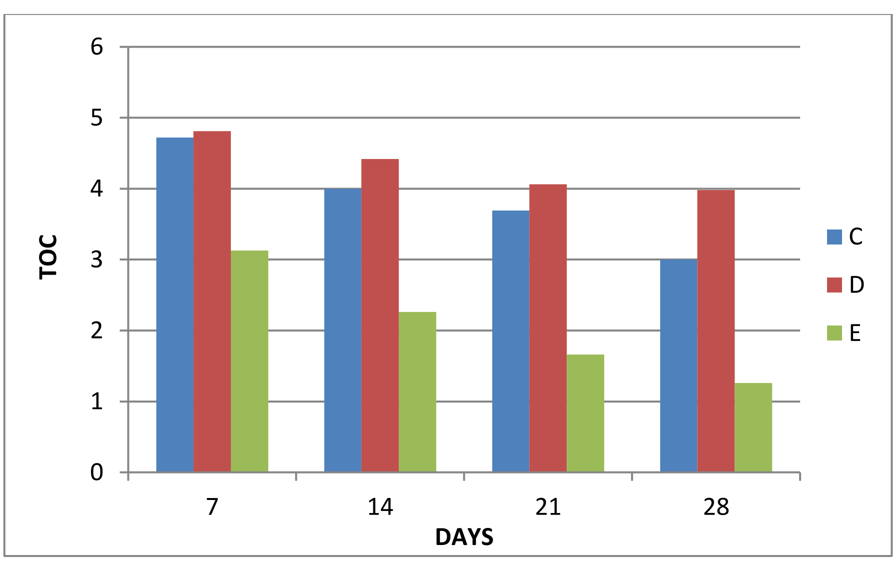

Figure 6B. Total Organic Carbon Vs days for very fine to coarse sand (Y)

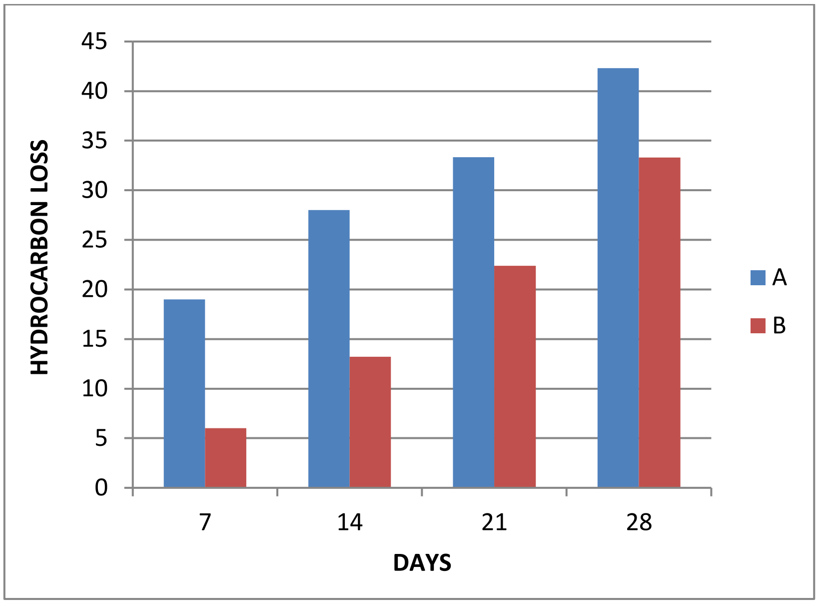

Figure 7A. Rate of Hydrocarbon Loss Vs for fine to coarse sand (X)

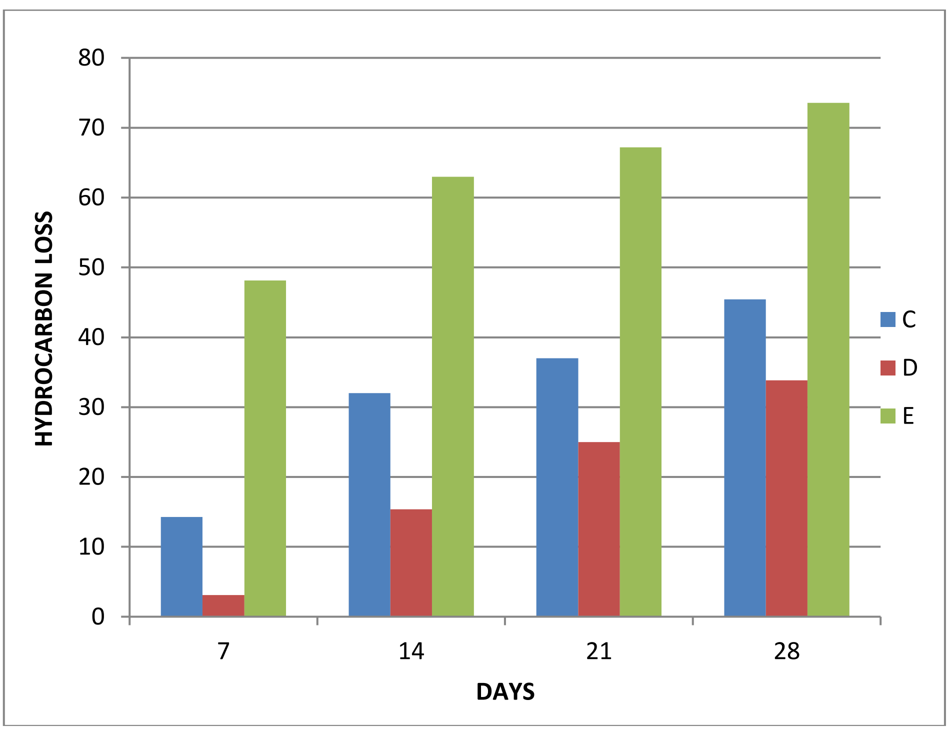

Figure 7B. Rate of Hydrocarbon Loss Vs days for very fine to coarse sand (Y)

For nitrogen, samples B, C, and D recorded values of 0.185 g/kg, 0.270 g/kg, and 0.299 g/kg, and for phosphorus recorded values of 2.02 g/kg, 1.76 g/kg, and 2.00 g/kg, respectively. The trend of nutrient reduction in soil samples over the remediation period is depicted in figures 8A, 8B, 9A, and 9B below.

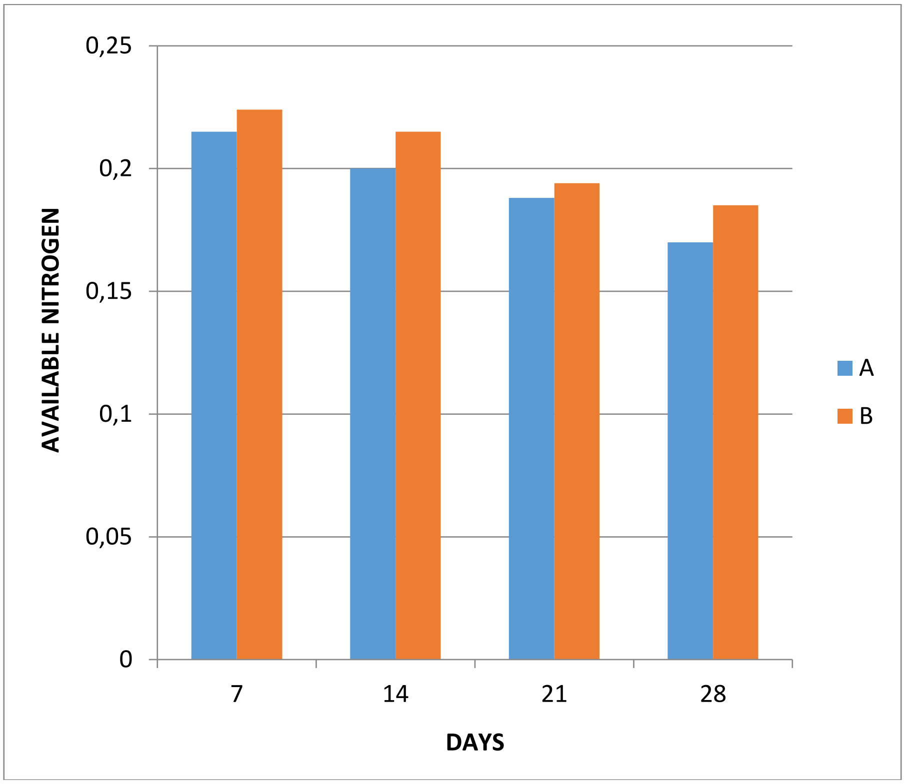

Figure 8A. Available Nitrogen vs. Days for fine to coarse sand (X)

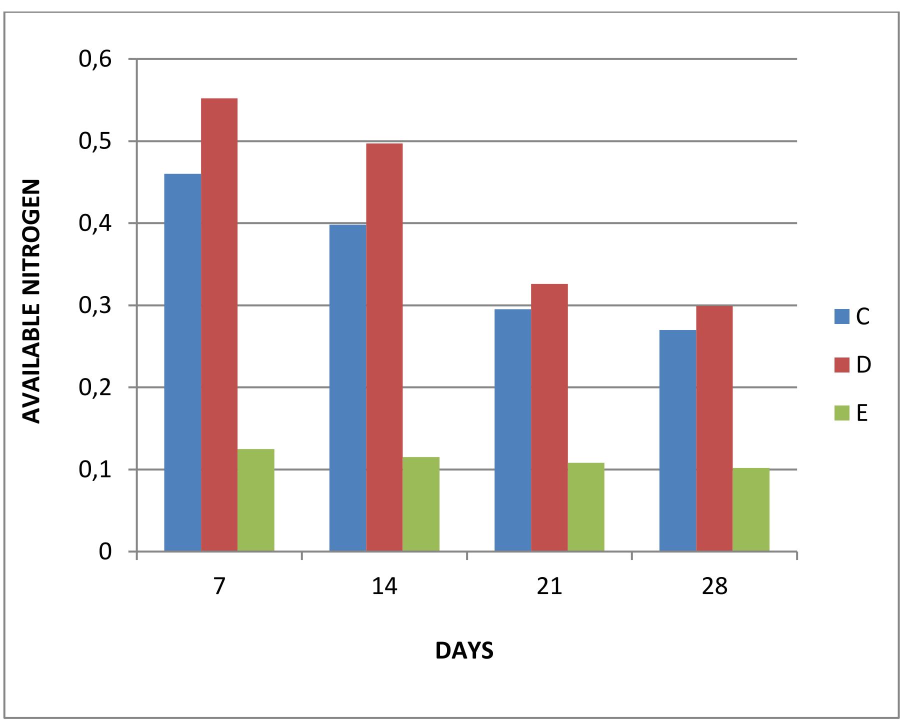

Figure 8B. Available Nitrogen vs. Days for very fine to coarse sand (Y)

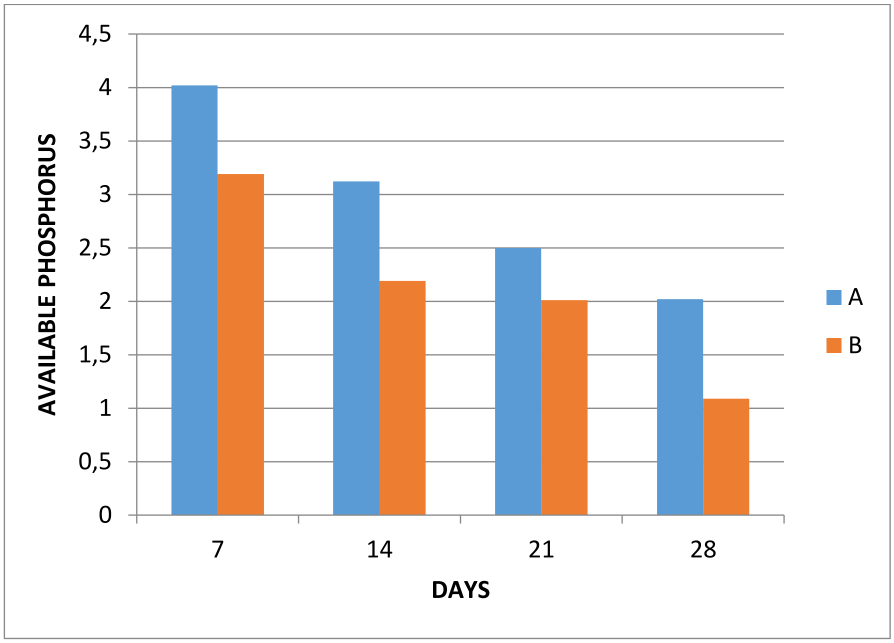

Figure 9A. Available Phosphorus vs. Days for fine to coarse sand (X)

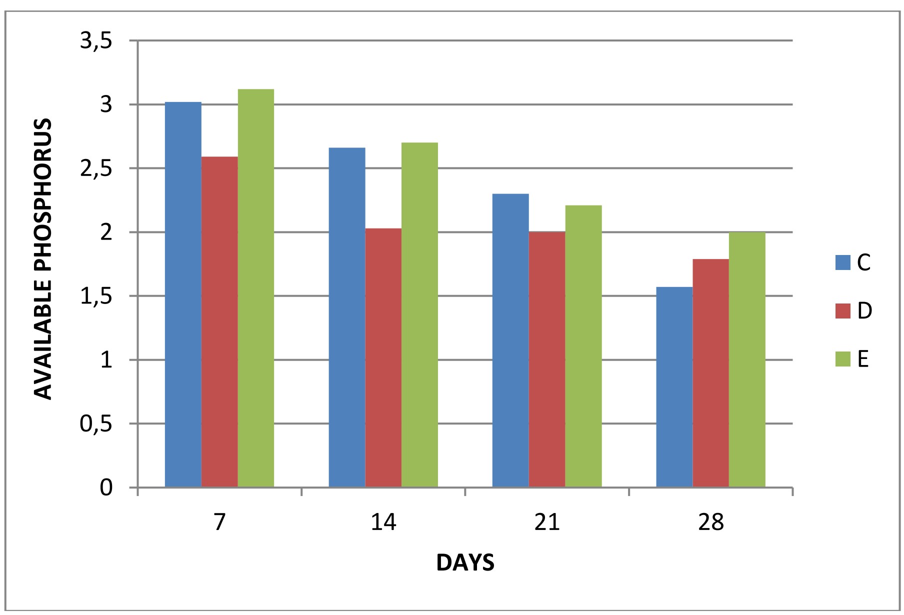

Figure 9B. Available Phosphorus vs. Days for fine to coarse sand (X)

Following the start of remediation, all soil samples showed a slight increase in pH, with values slightly moving towards the alkaline side in accordance with the Adaba (2013) report. However, sample A and sample E showed significant increases, with values for fine to coarse sand and very fine to coarse sand, rising to 4.31 and 6.6 respectively. As can be seen in figure 10A and 10B below, samples B, C, and D had pH readings of 4, 6, and 5.6, respectively.

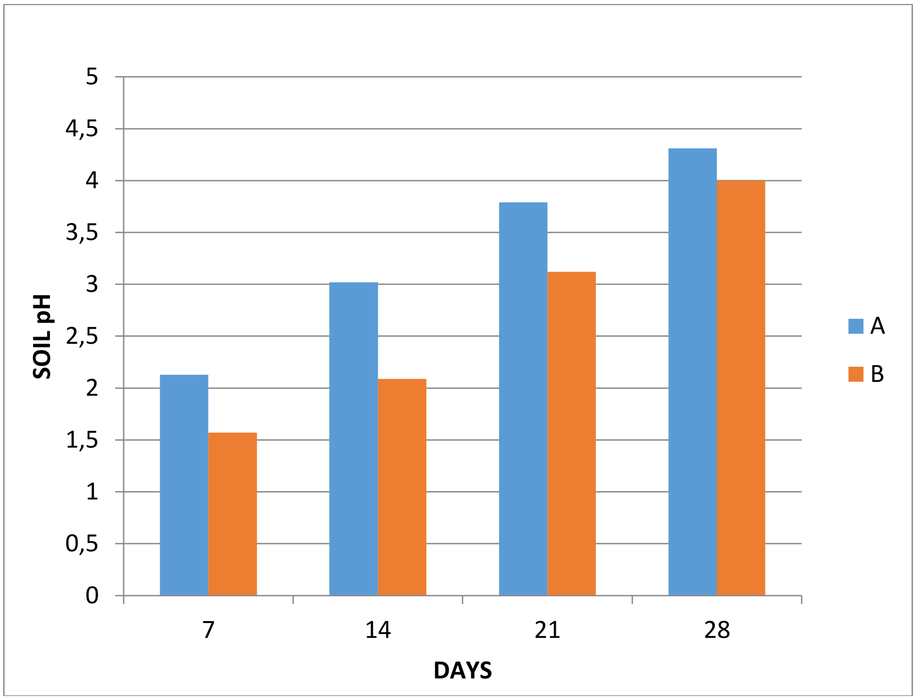

Figure 10A. Soil pH vs. Days for very fine to coarse sand (X)

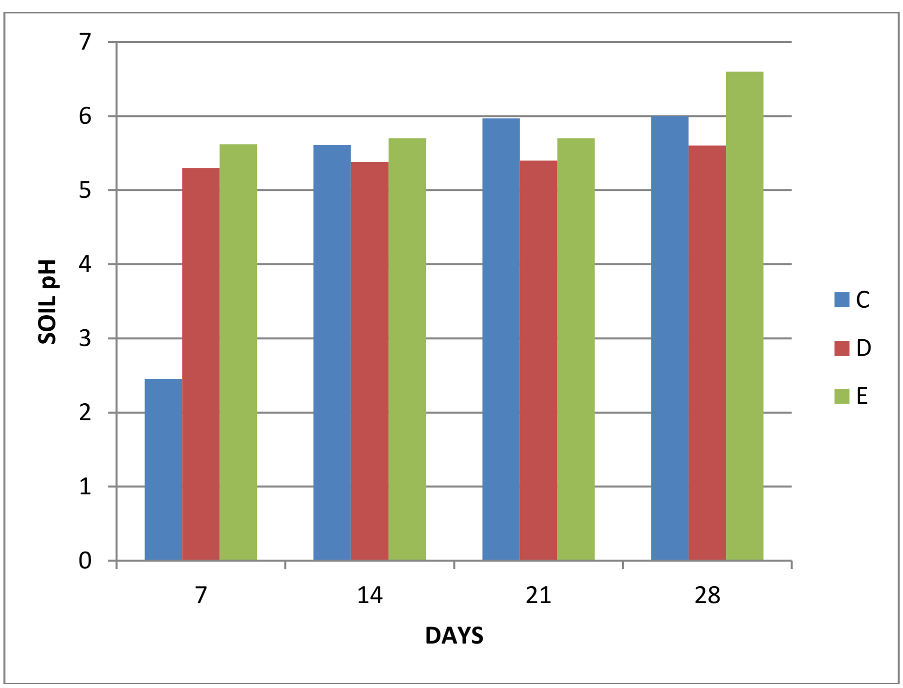

Figure 10B. Soil pH vs. Days for very fine to coarse sand (Y)

Studies by Adaba (2013) and Arinze et al. (2022) found that the faster the soil lost hydrocarbons, the lower the Cu and D50 values were, as shown in Table 2A and 2B, respectively. The lowest Cu value was in Sample A with a values of 3, which had a THC value of 9000 mg/kg for fine to coarse sand. Sample E also showed the lowest Cu value of 2.1 and a THC value of 5000 mg/kg for very fine to coarse sand. For fine to coarse sand, sample B had the highest Cu value (3.8), while for very fine to coarse sand, sample D had the highest Cu value 8. These findings imply that the properties of the particle size distribution have an impact on bioremediation. According to the results shown in the graph below, the higher rate of hydrocarbon loss is caused by larger voids in the soil sample, which is a condition that is more favourable for microorganisms to use nutrients than the class with higher coefficient of uniformity (Cu) values. With values of 0.0899 and 0.0942, respectively, the correlation coefficient (r) of THC Vs Cu for fine to coarse sand (X) is lower than that of very fine to coarse sand (Y).

Table 2A. Concentration of crude oil at last day vs Cu for (X)

	A	**B**
Coefficient of uniformity	3	3.8
Concentration of HC at last day	9000	9200

Table 2B. Concentration of crude oil at last day vs Cu for (Y)

	C	**D**	**E**
Coefficient of uniformity	4.3	8	2.1
Concentration of HC at last day	8400	8600	5000

5. CONCLUSION

Bioremediation methods are discrete and have proven successful in cleaning, maintaining, and reviving sites contaminated with crude oil, as evidenced by the appreciable biodegradation efficiencies recorded. The use of nutrients and microorganism on crude oil polluted soils hastens the rate of hydrocarbon loss, a process called bioremediation. The parameters determined includes: particle size distribution, soil pH, organic carbon content, available nitrogen, available phosphorus, total hydrocarbon content, total heterotrophic bacteria count (THBC). From the result of this study, it was observed that, the lower the Cu values, the faster the rate of hydrocarbon loss. This showed that, particle size distribution parameter has influence on bioremediation. The correlation coefficient(r) of THC vs Cu for fine to coarse sand (X) is 0.0899 while for very fine to coarse sand is 0.0942. Microbial biomass of both total heterotrophic bacteria increased on addition of nutrients of NPK 15:15:15 fertilizer, pig manure and microorganism (pseudomonas aeruginosa) on all the soil samples although, the nutrients were effectively utilized more by microorganisms present in soil samples with lower Cu values.

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