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Comparative Bioremediation Potentials of *Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium* KY085976 on Polluted Terrestrial Soil Treated with Oil Spill dispersant

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess the bioremediation potential of *Pseudomonas aeruginosa* and *Bacillus megaterium* on oil spill dispersant polluted terrestrial soil.
Study Design: The study employs experimental design, statistical analysis of data and interpretation.
Place and Duration of the Study: Polluted Terrestrial Soil (Ts) samples were collected from Kegbara-Dere community in Gokana Local Government Area of Rivers State with sterile shovel from three different spots at the same location and put in black polythene bags and transported to the microbiological laboratory within 24 hours for physicochemical and microbiological analyses. Oil spill dispersant (OSD/LT and OSD/Seacare) were obtained from Baker and Hughes Nigeria Limited in Rivers state, Nigeria.
Methodology: Standard microbiological procedures were used to enumerate, isolate and identify the bacterial isolates. *Pseudomonas aeruginosa* and *Bacillus megaterium* in oil spill dispersants

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contaminated soil were monitored over a period of 1, 7, 14, 21 and 28 days respectively for their bioremediation potentials.

Results: The presence of *Pseudomonas aeruginosa* (Pa) or *Bacillus megaterium*(Bm) in oil spill dispersant polluted soils enhanced decrease in Total Hydrocarbon Content (THC) of the soil. THC for control soil reduced from 18348.68 mg/kg to 9111.84 mg/kg; TS+OSD/LT+Bm, 18348.68 to 7092.11 mg/kg; TS+OSD/LT+Pa, 18348.68 to 6263.16(mg/kg); TS+OSD/LT+Bm+Pa, 18348.68 to 2473.68 mg/kg; TS+OSD/SC+Bm, 18348.68 to 6421.05 mg/kg; TS+OSD/SC+Pa, 18348.68 to 5618.42 mg/kg; TS+OSD/SC+Bm+Pa, 18348.68 to 5835.53 mg/kg, between the first (day 1) and last day (day 28). The percentage (%) bioremediation rate of polluted soil was as follows: control (TS(CRTL) 50.3%, TS+OSD/LT+Bm 61.3%, OSD/LT+Pa 65.9%, and OSD/LT+Bm+Pa 86.5% Whereas, TS+OSD/Seacare+Bm had 65.0%, OSD/Seacare+Pa 69.4%, OSD/Seacare+Bm+Pa 68.2% respectively. The highest percentages of THC in this study were from soil samples treated with oil spill dispersant and organisms while the least was observed in treatments without oil spill dispersant and organism. This suggests that microorganisms are more abundant in oil spill dispersant polluted soils than unpolluted soils.

Conclusion: From this study, it was observed that bioremediation of dispersant polluted environments could be achieved by stimulation of native microorganisms such as *Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium* KY085976 and this would be cost effective in the clean-up strategy for such pollutants.

Keywords: Bioremediation; oil spill; dispersants; OSD/LT; OSD/Seacare; Total Hydrocarbon Content (THC); terrestrial soil.

1. INTRODUCTION

Bioremediation is the use of microorganisms to remove or clean-up pollutants from the contaminated environment [1]. It is also a process, which depends on biological mechanisms to mineralized or transform concentration of pollutants to an innocuous state [2]. Bioremediation is among new technologies or approaches that derive its scientific justification from the emerging concept of environmentally friendly chemistry and engineering. This technology is a fast growing and promising remediation option that is increasingly studied and applied for pollutant clean up [3]. Bioremediation is effective, economic and ecofriendly; it leads to the complete mineralization of hydrocarbon (4). As such, the isolation of potentially applicable microorganisms to bioremediation of a variety of oily contaminations has received a lot of ink in the related literature [4,5]. However, the main drawback of bioremediation processes in a majority of cases is the slow biodegradation rate of hydrocarbons [6]. So many researchers have studied bioremediation, and their studies focused on hydrocarbons on account of frequent pollution of soil and ground water with this particular type of pollutant [7,8,9,10]. The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion and washing. Currently, accepted methods for removal of pollutants are incineration, land filling and safe disposal. Though, these technologies are expensive and can leads to incomplete decomposition of contaminants [11,12]. The conventional methods to clean-up oil spill from terrestrial and aquatic ecosystems are; mechanical, chemical and biological (microbial degradation) methods. The benefits in the use of biological agents is higher than those derived from the use of physical and chemical methods in the restoration of a contaminated environment [13,14,15,16,17]. Mechanical and chemical methods generally used to remove hydrocarbon from contaminated sites have limited effectiveness and can be expensive [18,19]. Physical and chemical methods to reduce hydrocarbon pollution are expensive [20]. In recent years microbial degradation of pollutants is a sustainable way to clean up the contaminated environment [21]. The success of oil spill bioremediation depends on one's ability to establish and maintain conditions that favour enhanced oil biodegradation rates in the contaminated environment, such as presence of microorganisms with appropriate metabolic abilities. Where there are frequent oil spills and difficult terrain for clean up exercise by mechanical means, bioremediation of oil spills become very important in that region [22]. Chemicals are used to change the characteristics feature of the oil [23]. Dispersants are chemical agents that reduce tension between oil and water interface thereby enhancing the natural process of dispersion by producing large amounts of

small droplets of oil that are drawn along into water column. Dispersants are most effective when applied immediately after a spill, before the lightest components in the oil evaporates. The use of dispersants in nearshore areas is expected to increase the exposure of aquatic organisms to petroleum [25]. Oil pollution, whether acute or chronic, has detrimental effects on agricultural lands and hence significant effects on plant growth [26]. Oil spill pollution has become a universal problem in industrialized and developing countries. It has cause a threat to our environment today by imposing a serious health hazard to human health, causes decrease in Agricultural productivity on terrestrial soil and economic loss [27,28,29]. On the terrestrial environment, oil spill cause extensive damages ranging from the destruction of terrestrial flora and fauna to biomagnification of the toxic components of the petroleum conversion of arable land to barren soils and the destruction of the aesthetic quality of the environment [30] the oil reduces the soil's fertility such that most of the essential nutrients are no longer available for plants and crop utilization [31]. the crude oil spillage in which hydrocarbons are found are large and complex molecules, and persistent in nature and may require a strong reagent to hinder their effect on terrestrial soil. oil spill pollution with dispersants can be degraded effectively by using microorganism activity. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbon as a source of carbon and energy [32,33,34]. Impact of the pollution is dependent on the volume of the hydrocarbons and the impact it has on the environment [35,36]. temperature, the availability of oxygen, ph, moisture content, the kind of microbe, the available nutrients and the type of soil are the conditions which control the breakdown of crude oil pollutants [37,38,39,40]. Hence, the essence for this study is to evaluate and compare the bioremediation potential of *Pseudomonas aeruginosa* and *Bacillus megaterium* on oil spill dispersant polluted terrestrial soil.

2. MATERIALS AND METHODS

2.1 Study Site and Sample Collection

The soil sampling was carried out at Kegbara-Dere community in Gokana Local Government Area of Rivers state, Nigeria. Kegbara-Dere is situated in the Niger Delta Area of Nigeria, between longitudes 7.01° and 7.07°E; and latitudes 4.08 and 4.2°N. Soil auger was used to collect soil sample from the polluted site and were put in sterile black polyethylene bag and labelled with masking tape, and then immediately taken to the microbiology laboratory, Rivers State University, for microbiological and some physicochemical analyses.

2.2 Source of Test Chemicals (Oil Dispersants)

The oil spill dispersants (OSD) used in the study work and their sources were; OSD/ LT and Seacare, all from Barker and Hughes Nig Ltd (formally mil park Nigeria limited) Port Harcourt.

2.3 Microbiological Analysis

2.3.1 Source of microorganisms (*Pseudomonas aeruginosa* and *Bacillus megaterium*)

the method described by [22] was adopted. pure cultures of these organisms were obtained from inoculation and incubation of soil samples using nutrient agar. Pure cultures were obtained by continuous subculturing [42]. isolates were inoculated into broth cultures [43].

2.3.2 Isolation of two test organisms

The test organisms (*Bacillus megaterium* and *Pseudomonas aeruginosa*) were selected because of their importance as active hydrocarbon degraders in crude oi polluted environments. They were isolated from the oil-polluted soil samples using spread plate method. soil suspensions were prepared by adopting ten-fold serial dilution. One gram of the soil sample was measured into a test tube and 9 ml of sterile distilled water was mixed with the sample. The suspension was properly shaken for 30 seconds to homogenize the solution and this served as the stock solution. Ten-fold serial dilution of all the homogenized mixture was carried out using prepared normal saline as diluents. Seven test tubes containing 9ml of normal saline was used for the serial dilution. Aliquots of 0.1 ml from 10^{-6} and 10^{-7} dilutions were introduced into duplicate sterile petri dishes using sterile pipettes and separately spread plated with flame sterilized bent glass spreader on well-dried Cetrimide agar plate (for *Pseudomonas aeruginosa*) and nutrient agar plates. The plates were incubated at 37 ^{o}C for 24 to 48 hours. After which bacterial colonies that formed during the incubation period were picked with sterile inoculating loop and were streaked on freshly prepared well-dried nutrient agar plates. The plates were incubated at 37°C

for 24 hr. discrete colonies on the plates were aseptically transferred into agar slants and bijoux bottles containing 10% (v//v) glycerol, properly labelled and stored as stock cultures for preservation and identification [42,41].

2.3.3 Confirmation of test organisms

The confirmation of the isolates was done according to the standard techniques in District laboratory practice in tropical countries [44] and was identified base on the Bergey's manual of Determinative Bacteriology after carrying out the morphological and various biochemical tests.

2.3.4 Soil preparation and application of organisms

Proper monitoring of the set up for each soil sample and Oil spill dispersants (OSD) in the laboratory was done. About 1500 g of soil sample collected from Kegbara-Dere Gokana L.G.A, was weighed into Seven plastic bowls. There were controls which were without organisms while the other set ups were augmented with organisms (*Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium* KY085976). About 20 ml of the Oil spill dispersant (OSD/LT and OSD/Seacare liquid detergent) was dispensed into each container containing the soil so as to contaminate them. They were mixed properly using sterile spatulas to enhance homogenization of the samples. About 30 ml of distilled water was also poured into each sample and properly stirred with spatula for oxygenation and to enable the organisms thrive successfully.

Bioaugmentation was the type of bioremediation carried out in which samples were augmented by adding 50 ml of broth culture organism (*pseudomonas aeruginosa* KX828570) to the first set up sample, 50ml broth culture organism (*Bacillus megaterium* KY085976) was added to the second set up sample and then 25 ml of each broth culture organism was added to the third set up sample containing 1500 g of pollute sample respectively, and they were kept at ambient temperature (28±2°C) for 28 days to determine the level of utilization by the organism *(Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium*KY085976). This method is referred to as ex situ bioremediation, whereby the polluted soil requires excavation and treatment can be carried out in the laboratory. This method of bioremediation can also be carried out on field or polluted sites.

2.3.5 Media used and its preparation

Nutrient Agar: It encourages the proliferation of organisms without segregating. 28 grams was dissolved in 1000ml of distilled water according to manufacturer's instruction. Oil Agar: The medium was prepared in the laboratory having the following composition of K_2HPO_4 (0.5 g), $MgSO_4$ (0.03 g), $NaCL_2$ (0.3 g), $MnSO_4$ H_2O (0.2 g), $FeSO_4$ H_2O (0.02g), $NaNO_3$ (0.03 g), ZnCl2 (0.3 g) and Agar agar (15 g) into 1 litre of distilled water [43]. Cetrimide Agar Medium: The preparation of this medium is by dissolving 45.3 gm in 1000ml distilled water, autoclaved at 15 psi (121°C) for 15 minutes. Cool to 45-50°C, prior to dispense. It is mainly for isolation of *Pseudomonas aeruginosa*. Nutrient Broth: This is a liquid medium used for the general cultivation of wide variety of microorganisms. The broth was prepared by dissolving 13g in 1000ml of distilled water.

Table 1. Experimental design (bioremediation set–up) for terrestrial soil sample

SET-UP	TREATMENT
SET UP 1	1500 g of soil+30 ml of Distilled
SET UP 2	1500 g of soil+20 ml of OSD/LT+30ml of Distilled H_2O+50 ml of *Bacillus megaterium*
SET UP 3	1500 g of soil+20 ml of OSD/LT+30ml of Distilled H_2O+50 ml of *Pseudomonas aeruginosa*
SET UP 4	1500 g of soil+20 ml of OSD/LT+30ml of Distilled H_2O+25 ml of *Bacillus megaterium*+25 ml *Pseudomonas aeruginosa*
SET UP 5	1500 g of soil+20 ml of OSD/SC+30ml of Distilled H_2O+50 ml of *Bacillus megaterium*
SET UP 6	1500 g of soil+20 ml of OSD/SC+30ml of Distilled H_2O+50 ml of *Pseudomonas aeruginosa*
SET UP 7	1500 g of soil+20 ml of OSD/SC+30 ml of Distilled H_2O+25 ml of *Bacillus megaterium*+25 ml *Pseudomonas aeruginosa*

2.3.6 Enumeration and isolation of bacterial population

The spread plate technique was used to inoculate the soil samples as described by [45]. The suspension was properly shaken for thirty seconds to homogenize the solution and this served as the stock solution. Ten-fold serial dilution of all the homogenized mixture was carried out using sterile distilled water as diluents. The plates were incubated at 37°C for 24 hours. Counting of colonies followed so as to estimate the microbial load.

Values were expressed as colony forming unit per g (Cfu/g). The colonial morphology such as shape, edge, colour, elevation, surface, opacity and their consistency were carried out. while biochemical assay was based on Gram staining reaction, Motility, Catalase, Oxidase, Coagulase, Indole, Methyl red, Citrate, Sugar fermentation tests [43]. And sub culturing of bacterial isolates was done to obtain pure culture. bacterial colonies were picked with sterile inoculating loop and were streaked on freshly prepared well-dried na plates [24].

2.3.7 Enumeration and isolation of hydrocarbon utilizing bacteria

Hydrocarbon utilizing bacteria (HUB) were enumerated as adopted from [46,47,17] using the vapour phase method (mineral salts medium with crude oil which served as the sole source of carbon). Isolated colonies were further purified by sub-culturing and identified using biochemical tests and microscopy [48]. It was done using Oil Agar (Mineral salt agar). Aliquots of 0.1ml from dilutions of 10^{-4} and 10^{-5} were also plated in duplicates on Mineral Salt Agar and Fungosol was added to the Mineral Salt Agar to suppress fungal growth. Spread plate method was used and filter paper (Whatman No 1) saturated with bonny light crude oil was aseptically placed onto the covers of the Petri dishes and inverted. The culture plates were incubated for 5 to 7 days at 37°C. Plates yielding colonies were afterwards enumerated, counted and were later sub-cultured into another plate to obtain pure cultures to be used for biochemical tests. The colonies counted were expressed as the colony forming unit (CFU) per gram of the soil, after applying the appropriate correction factor. The cultural, morphological and biochemical characteristics of the discrete bacterial isolates were compared with the recommendation in Bergey's manual of determinative bacteriology.

2.3.8 Enumeration and isolation of Oil Spill Dispersant (OSD) utilizing bacteria

Enumeration of Oil spill dispersant (OSD) utilizing bacteria was done by inoculating 0.1 ml aliquot of the dilutions unto mineral salt agar plates containing the OSD [49] Colonies were counted after 48 to 72 hours incubation at ambient temperature. The bacterial colonies on the plates after incubation were counted and sub-cultured onto fresh mineral salt agar plate to obtain pure cultures to be used for biochemical tests.

2.3.9 Stock solution or culture

Ten percent glycerol solution was prepared, dispensed in McCartney bottles and autoclaved at 121°C for l5minutes, it was allowed to cool, then the pure cultures were inoculated into each McCartney bottle, until the clear colourless solution turns turbid and were stored in the refrigerator. This served as storage medium for pure cultures for subsequent characterization [42].

2.3.10 Identification of test bacterial isolates

Identification of the bacterial isolates was done based on the method of [44].

The cultural, morphological and biochemical characteristics of the discrete bacterial isolates were compared with the recommendation in Bergey's manual of determinative bacteriology [48]. The morphological and biochemical test include; gram staining, motility, catalase, coagulase, oxidase, citrate utilization, sugar fermentations, indole production and methyl red tests [24]. Morphological identification observed was colour, shape, elevation, opacity, margin, size and texture. Microscopy was done under light-microscope to check for the bacterial Grams' reaction, shapes and arrangements. The biochemical test carried out includes motility, catalase, oxidase, citrate utilization, coagulase, indole production and fermentation of the following sugars: Glucose, lactose, mannitol, sucrose, and fructose.

2.4 Physicochemical Analysis

Moisture Content analysis: It was determined using moisture analyser. 10 g of polluted soil from each of the set up was weighed and was put inside of washed glass petri dish and was placed inside a hot air oven for drying. After

which the soils were immediately transferred into the desiccators for cooling. After cooling the soils were then reweighed and the grams gotten was subtracted from the initial 10g of the soil to get the moisture contents [29]. The soil pH was obtained with a pH meter (Hannah 8314) stabilized for 15 minutes and calibrated between pH 4 and 7 with standard buffer solution, according to [50]. This was determined by weighing 10 g of soil sample into the beaker and addition of 10 ml of distilled water. This was allowed to stand for 30 minutes and was stir occasionally with a glass rod. After which Insert the pH meter (previously calibrated) into the partly settled suspension and take the pH reading [51,52]. Soil Temperature: The temperature of the soil was measured ex situ with a mercury thermometer. This was done by inserting the thermometer in each of the set-up rubber. Constant temperature was recorded by allowing the thermometer to remain in the soil samples [53]. Total Hydrocarbon Content Analysis (THC): This was done using spectrophotometer. The procedure was undertaken 5 times (interval of 5 days) to form five replicates. During the setup process for spectrophotometric analysis, 10 g of soil sample were weighed from each of the setup rubbers containing 1500 g of soil sample into sterile beaker and 20 ml of xylene was added and shaken properly to extract the oil from the soil and this was allowed to digest for 30 minutes and the extracted oil were sieved with whatman No 1 filter paper into test tube that was transferred into colorimeter curvette and placed in a chamber known as infrared spectrophotometer analyser. The Total Hydrocarbon Content (THC) value was determined by comparison to a calibration curve obtained from dilution of a stock solution of a 1:1 bonny light crude and oil spill dispersant. The Ultraviolet light spectrophotometric measurement was at 420 nm and Total Hydrocarbon Content (THC) Oil Spill Dispersant (OSD) was at 560 nm [54,55].

2.5 Statistical Analysis of Data

Analysis of variance (ANOVA) and Duncan test was adopted using the IBM SPSS 20 software to analyze the significant differences. Data obtained were subjected to statistical analysis to determine the significant difference among the data obtained using one-way analysis of variance (ANOVA). A value of $p<0.05$ was considered significant while $p>0.05$ was considered not significant.

3. RESULTS AND DISCUSSION

Bacillus megaterium and *Pseudomonas aeruginosa* aided in remediating the oil dispersant-polluted terrestrial soil. Total Hydrocarbon Content (THC) results of soil samples augmented with bacterial species (*Bacillus megaterium* and *Pseudomonas aeruginosa*) for 28 days are shown on Fig 1. The result showed that the total hydrocarbon content decreased with increase in time, between the first (1) and last (28) days of the study. The THC results for days 1 and 28 are as follows: control, 18348.68 kg/kg and 9111.84 mg/kg, TS+OSD/LT+Bm, 18348.68 mg/kg and 7092.11 mg/kg, TS+OSD/LT+Pa, 18348.68 and 6263.16 mg/kg, TS+OSD/LT+Bm+Pa, 18348.68 mg/kg and 2473.68 mg/kg, TS+OSD/Seacare+Bm, 18348.68 mg/kg and 6421.05 mg/kg, TS+OSD/ Seacare+Pa, 18348.68 and 5618.42 mg/kg, ts+osd/seacare+bm+pa, 18348.68 mg/kg and 5835.53 mg/kg respectively. On the first day (day 1), the was relatively constant because proper augmentation had not taken place at that time. this indicates that the effect of time on petroleum hydrocarbon bioremediation rate is of great significance. Moreover, from the results obtained, it was observed that oil spill dispersant (osd/seacare) with *pseudomonas aeruginosa* was more effective than with *bacillus megaterium*, as well as (osd/lt) with *pseudomonas aeruginosa*. though osd/lt with mixed consortium showed the highest remediation rate compared to osd/seacare with mixed consortium. this observation is in line with the findings of [56], who reported that the use of microbial consortium mineralized pollutants more efficiently than individual isolates. [38] states that combination of bacteria is best in the degradation process than the use of a bacterium. the percentage (%) bioremediation rate of polluted soils is shown on fig 2 and they were as follows: control (ts(crtl) 50.3%, ts+osd/lt+bm 61.3%, osd/lt+pa 65.9%, and osd/lt+bm+pa 86.5% whereas, ts+osd/seacare+bm had 65.0%, osd/seacare+pa 69.4%, osd/seacare+bm+pa 68.2% respectively. the highest percentages of thc in this study were from soil samples treated with oil spill dispersant and organisms while the least was observed in treatments without oil spill dispersant and organism. this suggests that microorganisms are more abundant in oil spill dispersant polluted soils than in unpolluted soils. these results indicated that the added mixed bacterial culture enhanced the rate and extent of crude oil and oil spill dispersants biodegradation in the soil. other researchers [57,58] have

reported enhanced crude oil biodegradation in soil caused by inoculation of microbial slurry. moreover, in nature, biodegradation of crude oil typically involves a succession of species of microorganisms within the present consortia. consequently, study of bioremediation using bacterial consortia is encouraged because such mixed cultures display metabolic ability and superiority to pure cultures [59,60].

3.1 Molecular Characterization of the Two Organisms (*Pseudomonas aeruginosa* and *Bacillus megaterium*)

Molecular characterization was done on two bacterial isolates with the best degradative abilities on oil spill polluted marshland and terrestrial soil. The obtained 16s rDNA sequence showed the presence of *Bacillus megaterium*, and *Pseudomonas aeruginosa* (Phylogeny of the isolates) (Fig. 3). The obtained 16S rDNA sequence from the isolate produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database. The 16s rDNA of the isolates showed percentage similarity to other species at 99-100%. The evolutionary distances computed using the Juke-Cantor method were in agreement with the phylogenetic placement of the 16s rDNA of the isolates within the *Pseudomonas sp* and revealed a closely relatedness to *Pseudomonas sp* (KX828570) than other *Pseudomonas sp.*

3.2 Physicochemical Analysis

It is a known fact that oil spill on polluted soil affects the physical, chemical and biological characteristics of the soil [60]. The pH of soil samples shown in Fig. 4, ranged from 5.66 to 6.37 across the various set up. The highest soil pH (6.37) was recorded in the treated Terrestrial soil (TS+OSD/LT+Pa) while the lowest soil PH (5.84 to 6.16) was recorded in the Terrestrial soil control (TS(CTRL). There was no significant difference between the soil sample in soil pH. The soil pH of the soil sample (polluted terrestrial) sites were within the same range, and they were tending from slightly acidic towards neutrality. This result concord with the observation of [61,62], who indicated that a pH between 5 and 7.8 is favourable for the biodegradation activity of bacteria in the soil. [63] reported similar results on pH of crude oil polluted soils of Niger Delta. Plant grows in soil of pH between the ranges of 3 to 9. The non-significance difference between the soil pH in the experimental set up showed that the bioremediation of the polluted soil did not have any significant effect on soil pH [49]. The reduction in pH to slight acidic range in oil polluted soil inoculated with OSD could be attributed to acidic metabolites resulting from oil biodegradation. However, the pH range observed in the present study of terrestrial soil still fall within the pH range suitable for microbial growth indicating that these isolates exhibited optimal growth at pH range of 5.84 to 6.37. [64] reported that the growth of most microorganisms is usually greatest within a pH range of 6 to 8. Soil moisture content ranged from 0.03 to 0.4 across the soil samples. The highest soil moisture (0.4) was recorded in the terrestrial soil (TS+OSD/LT+Bm+Pa and TS+OSD/SC+Bm+Pa) while the lowest soil moisture (0.03) was recorded in the Terrestrial soil (TS(CRTL)). The moisture content result of soil samples in Fig. 5, sssssssshows the differences in the moisture content of the different experimental set-up, indicating the mixed consortium with different test chemical; OSD Polluted soil + *Bacillus megaterium*+ *Pseudomonas aeruginosa* (OSD+Bm+Pa) (0.4 g/10 g and 0.4/10 g) having the highest moisture content, followed by single organism application; Polluted soil + *Pseudomonas aeruginosa* (OSD+Pa)(0.2 g/10g and 0.2 g/10 g) is equal to Polluted soil + *Bacillus megaterium* (OSD+Bm)(0.2g/10g and 0.2 g/10 g), while Control (soil sample without added organism CTRL)(0.03 g/10 g) has the lowest. [28] report similar observation on the effect of moisture content on bioremediation potential of bio-stimulating and bio-augmenting agents. Alternatively, this study revealed the effects of different types of augmenting organisms, dispersants and crude oil on the moisture content of the affected soil. The high moisture content observed in the mixed consortium (Polluted soil + *Bacillus megaterium* + *Pseudomonas aeruginosa*) (OSD +Bm+Pa) could be due to its intrinsic moisture retention ability of the augmenting organisms while the control devoid of added organisms has least moisture content. These attributes (high moisture content) enhances the growth of microorganisms up to day 28 which was evident in their higher percentage bioremediation. The temperature reading for the soil sample in different set up ranged from 29°C to 30°C for control, 30°C to 33°C for oil spill dispersant (OSD/LT), while that OSD/SC range from 29°C to 34°C respectively. The highest temperature in day 28 was observed in (TS+OSD/SC+Bm+Pa), followed by

(TS+OSD/LT+Bm+Pa), and this was shown in Table 2. The temperature values obtained for the different oil spill dispersant polluted soil during the investigation study fall within the mesophilic range. This indicates that the temperature of the different oil spill dispersant polluted soils supported mesophilic bacteria throughout the investigation period. Similar result was reported by [52].

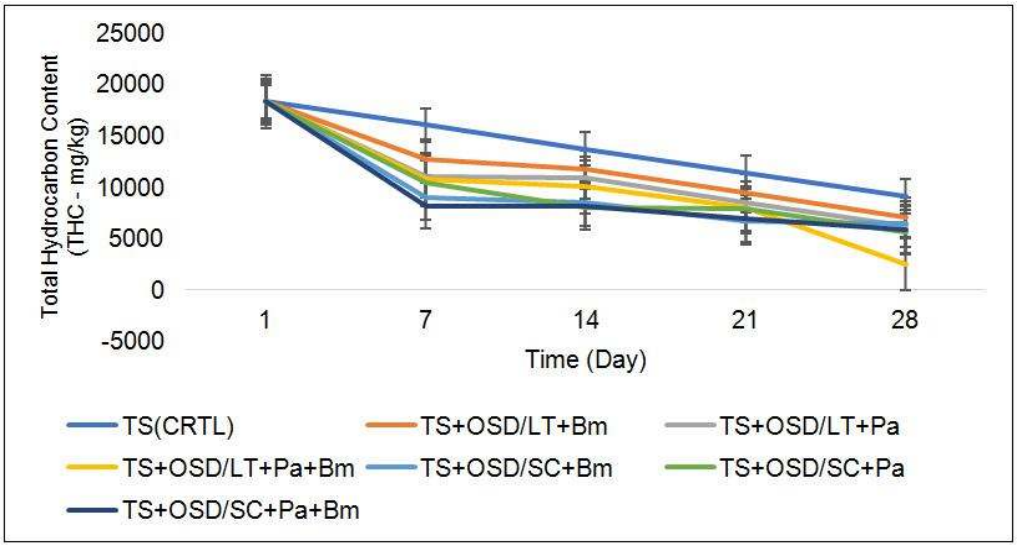

Fig. 1. Total hydrocarbon content (THC-mg/kg) during bioremediation of oil spill dispersant (OSD/LT and OSD/SC) polluted terrestrial soil using bio-augmenting organisms *Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium* KX085976

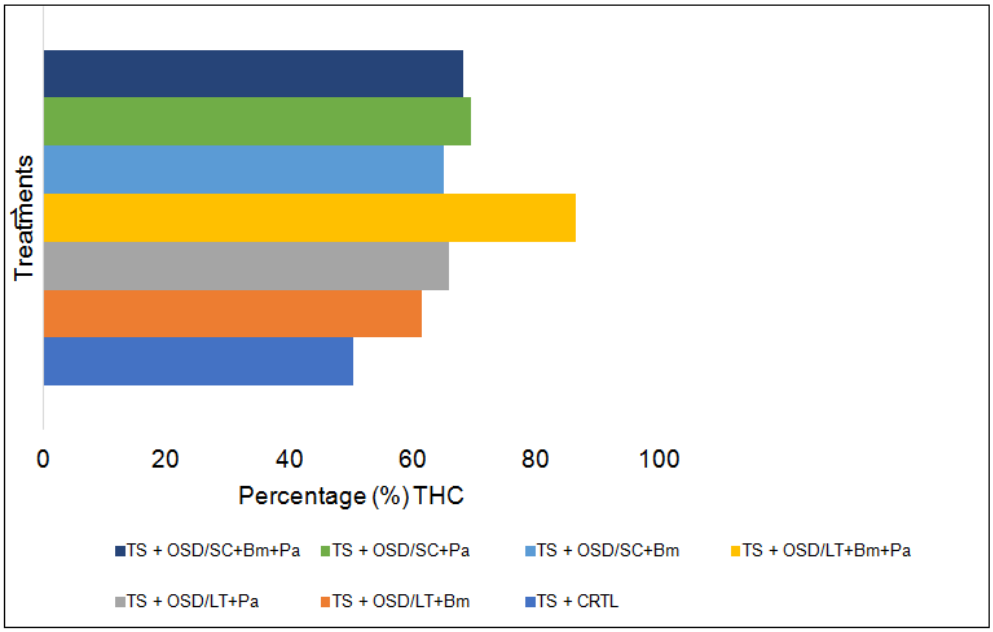

Fig. 2. % Bioremediation of total hydrocarbon content in different remediation treatment during bioremediation of oil spill dispersant (OSD/LT and OSD/SC) polluted terrestrial soil using bio-augmenting organisms *Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium* KX085976

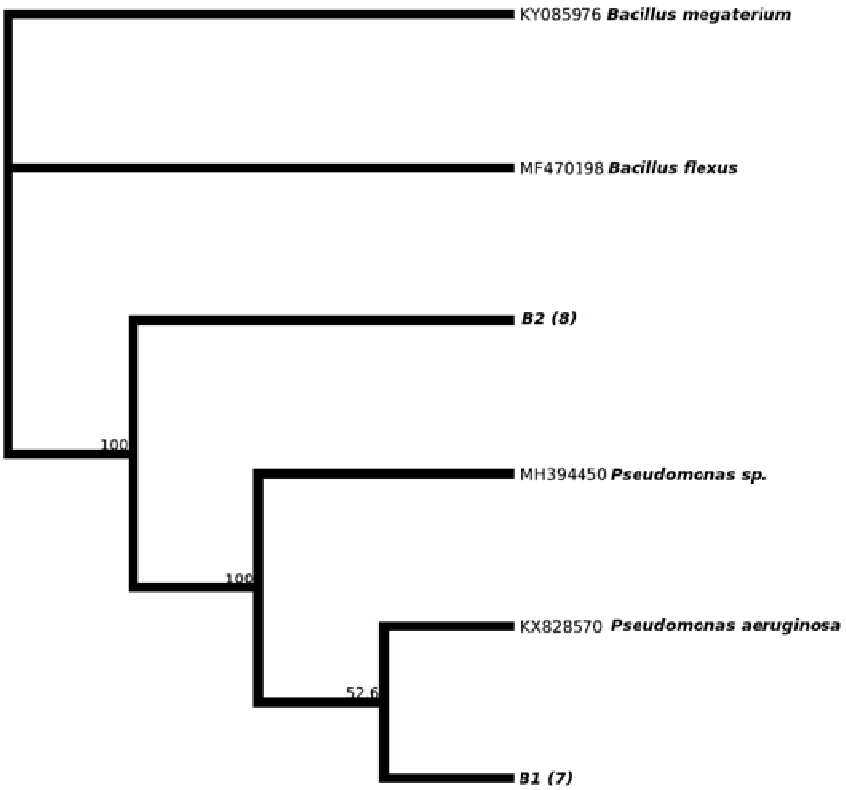

Fig. 3. Phylogenetic tree showing the evolutionary distance between the bacterial isolates

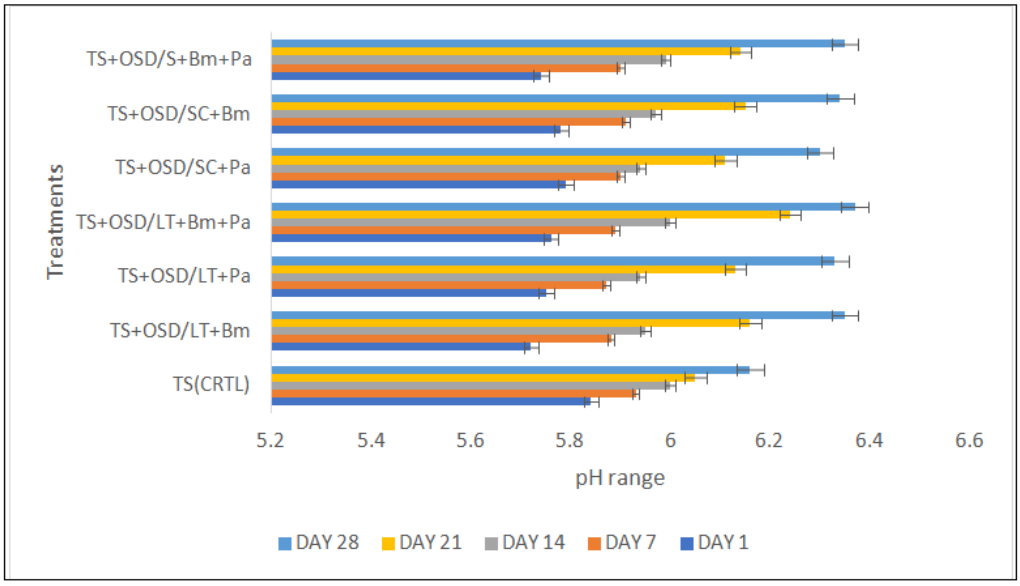

Fig. 4. Soil pH of different treatments during bioremediation of oil spill dispersant (OSD/LT and OSD/SC) polluted terrestrial soil using bio-augmenting organisms *Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium* KX085976

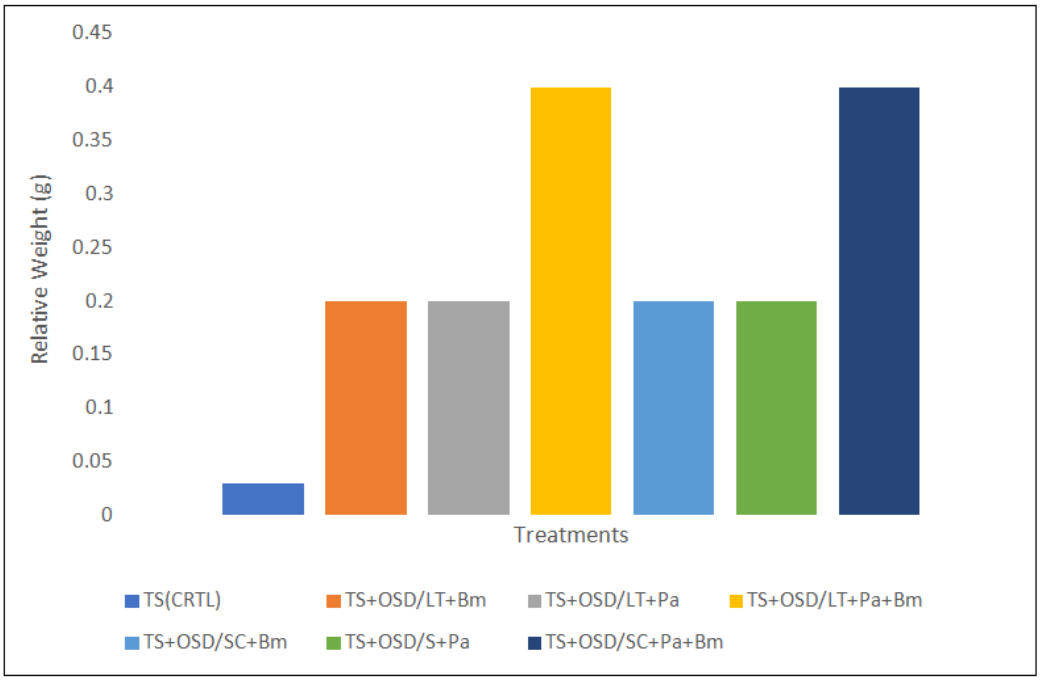

Fig. 5. Soil moisture of different treatments

Table 2. Physicochemical parameters of terrestrial soil

Terrestrial physicochemistry	Temp	pH	Moisture	THC
TS(CRTL)	29.8±0.45[a]	5.996±0.12[a]	.034±0.01[a]	13730.26±0.37[a]
TS+OSD/LT+Bac	31.8±1.09[b]	6.012±0.25[a]	.260±0.09[b]	11868.516±423[a]
TS+OSD/LT+Pse	31.2±0.84[ab]	6.004±0.23[a]	.240±0.05[b]	11017.260±454[a]
TS+OSD/LT+Bac+Pse	31.8±1.09[b]	6.052±0.25[a]	.360±0.05[b]	9932.820±572[a]
TS+OSD/SC+Bac	30.8±1.30[ab]	6.030±0.22[a]	.240±0.05[b]	9780.260±492[a]
TS+OSD/SC+Pse	30.8±1.30[ab]	6.008±0.20[a]	.240±0.05[b]	10069.820±493[a]
TS+OSD/SC+Bac+Pse	31.6±1.95[b]	6.024±0.23[a]	.380±0.04[c]	9489.480±505[a]
Total	31.11±1.28	5.909±0.37	.215±0.12	11302.521±444

Mean with the same alphabet across Row shows no significant different (p> 0.05)

Table 3. Microbial count during bioremediation process

Treatments	THB	PUB	DUB
TS(CRTL)	9.27±0.43[a]	7.02±0.46[a]	6.95±0.51[a]
TS+OSD/LT+Bm	9.40±0.46[a]	7.30±0.46[a]	7.08±0.52[a]
TS+OSD/LT+Pa	9.31±0.46[a]	7.28±0.45[a]	7.04±0.53[a]
TS+OSD/LT+Bm+Pa	9.31±0.41[a]	7.29±0.44[a]	7.05±0.52[a]
TS+OSD/SC+Bm	9.43±0.46[a]	7.29±0.43[a]	7.00±0.51[a]
TS+OSD/SC+Pa	9.36±0.46[a]	7.28±0.45[a]	6.99±0.54[a]
TS+OSD/SC+Bm+Pa	9.36±0.42[a]	7.30±0.45[a]	7.02±0.53[a]

Mean with the same alphabet across Row shows no significant different (p> 0.05)
KEYS: OSD/LT= Oil spill dispersant /LT, OSD/SC= Oil spill dispersant /Seacare, TS=Terrestrial soil, Bm= Bacillus megaterium. Pa= Pseudomonas aeruginosa, THC= Total hydrocarbon content, THB= Total heterotrophic bacteria, PUB= Petroleum utilizing bacterial, DUB= Dispersant utilizing bacterial, TBC= Total Bacillus count, TPC= Total Pseudomonas count

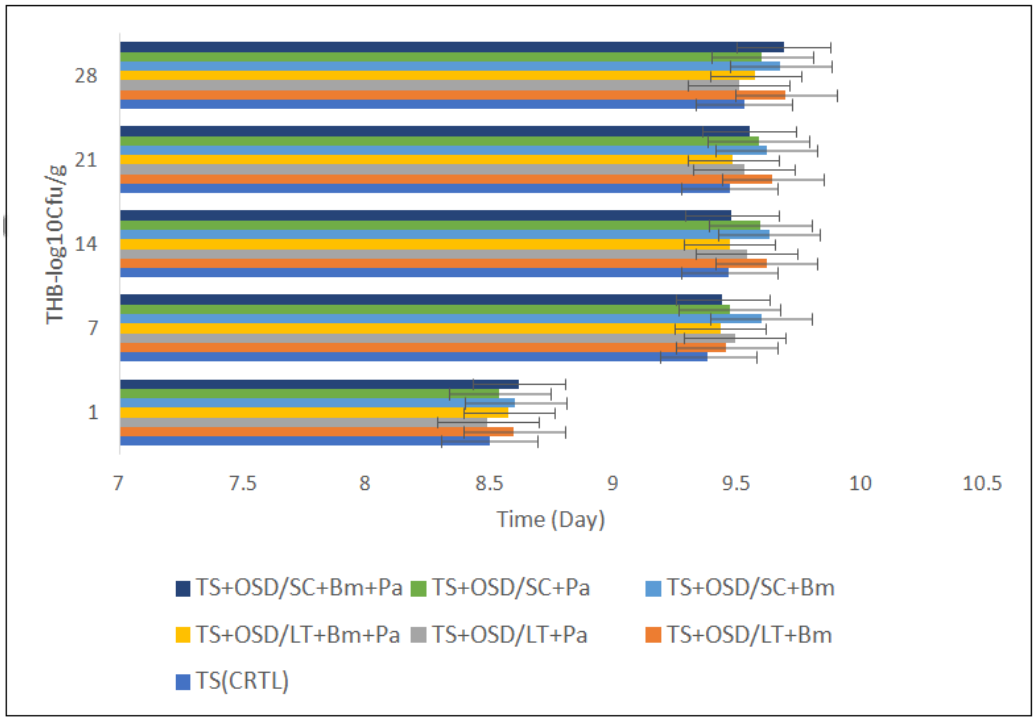

Fig. 6. Total heterotrophic bacterial count (THB) during bioremediation of oil spill dispersant (OSD/LT and OSD/SC) polluted terrestrial soil using bio-augmenting organisms *Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium* KX085976

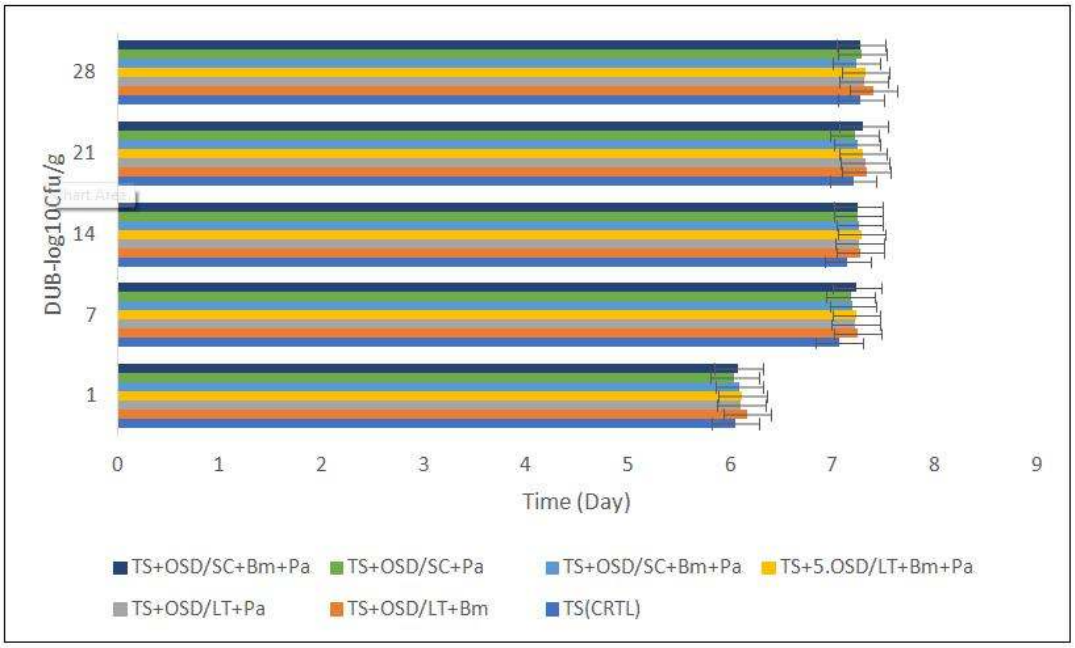

Fig. 7. Dispersant utilizing bacterial count (DUB (Log_{10}Cfu/g) during bioremediation of oil spill dispersant (OSD/LT and OSD/SC) polluted terrestrial soil using bio-augmenting organisms *Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium* KX085976

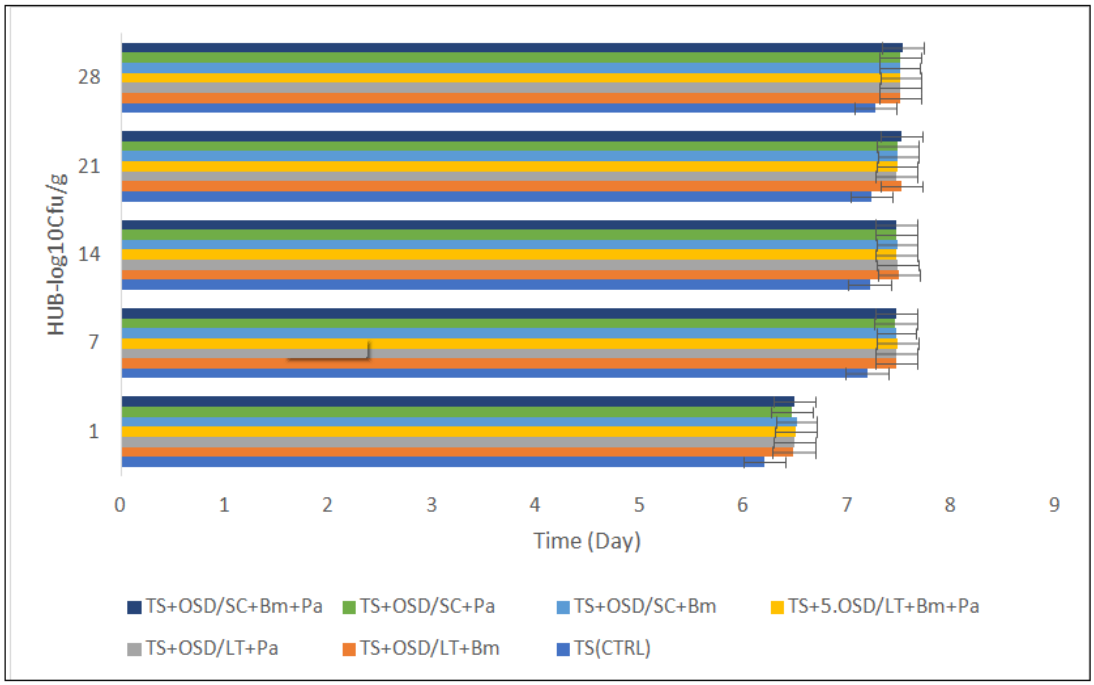

Fig. 8. Hydrocarbon utilizing bacterial (HUB) count during bioremediation of oil spill dispersant (OSD/LT and OSD/SC) polluted terrestrial soil using bio-augmenting organisms *Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium* KX085976

3.3 Bacteriological Analysis

It was observed that there was an increase in all the counts from the soil polluted with oil spill dispersants when compared with control. Table 3, shows the counts obtained from the study. Total heterotrophic bacterial population ranged from 8.498 log_{10}cfu/g to 9.528 log_{10}cfu/g. The highest count, 9.528log_{10}cfu/g was observed in soil sample polluted with oil spill dispersant (OSD/SC+Bm+Pa) while the lowest THB count, 8.498 log_{10}cfu/g was recorded in control (TS(CTRL), Fig. 6. The highest THB count was due to increase in hydrocarbon content and it is in agreement with [64,65]. The total Hydrocarbon utilizing bacterial count for terrestrial soil in Fig. 8, ranged from 6.201 Log_{10}Cfu/g to 7.528 Log_{10}Cfu/g. This increase is due the presence of hydrocarbon availability in the soil. This concurs with the findings of [52], who reported gradual increase in microbial population in contaminated soil. The inoculation of the soil samples on a Mineral salt agar (MSA) supplemented with the test chemical revealed that only 5 out of the 9 bacterial genera were able to degrade and utilize the two test chemicals as their sole carbon and energy source. This is likely to be as a result of the metabolic ability possessed by these oil-degrading bacteria (*Bacillus spp* and *Pseudomonas spp*.) which enables the utilization of hydrocarbon present in the crude oil as source of carbon and energy [66]. Similarly, the result obtained in this study is in line with the report of [67] who demonstrated that *Bacillus spp* and *Pseudomonas spp* are of high predominance in hydrocarbon polluted soil as a result of their ability to utilize it. There was an increase (day 21) and decreased (day 28) in total dispersant utilizing bacteria in soil samples used as inoculum. The increase could be due to the presence and activities of dispersants utilizers in the soil because, as they utilize the dispersants, there was multiplication in number. The counts ranged from 6.045 log_{10}cfu/g to 7.391 log_{10}cfu/g. The highest counts,7.391log_{10}cfu/g was observed in the soil sample polluted with oil spill dispersant (OSD/LT+Bm) while lowest DUB count 6.045log_{10}cfu/g was observed in control (TS(CRTL), Fig. 7. This suggests that at the introduction of the treatments (day 1), the concentrated dispersants were still very high and this inhibited bacterial growth since it is lethal. This observation concurs with the findings of [68, 69, 23,70] that oil spill dispersants support mild increase (stimulation) and decrease (inhibition) in the growth of dispersant degraders than

hydrocarbon-degraders and supported the growth of indigenous seawater bacteria confirming that the bacteria could utilize the nutrients available within the dispersants even at low concentrations. A consortium of micro-and macro-organisms known as biomass is able to use the dispersant as food. *Pseudomonas aeruginosa* KX828570*, Bacillus megaterium*KY085976*, Micrococcus sp, Serratia sp,* and *Proteus sp* were the most predominant dispersant utilizing bacteria found in this study. And this is in line with the findings of [70]. Other organisms present in the crude oil polluted soil such as *Streptococcus spp., Klebsiella spp., Listeria spp*., and *Escherichia coli* were not able to utilize dispersants products, their presence may be due poor hygienic practices in the community. With these, it shows that some of these isolates were not able to cope nutritionally. Similarity in test chemicals utilization by isolates could be as a result of adequate level of carbon source and phosphate components which has been reported by other investigators to play a role in the test chemical degradation [71,72]. Most of the THB isolated and identified across the two samples belonged to genera; *Pseudomonas aeruginosa* KX828570*, Bacillus megaterium*KY085976*, Micrococcus spp, Serratia spp, Klebsiella spp, Corynebacteria spp, Listeria spp, Bacillus spp*, and *Proteus spp*. This study reported that *Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium* KY085976 exhibited the best growth and had the highest levels of petroleum degradation. It was observed from the study that these organisms helped in remediating the pollutant caused by oil spill dispersants in the soil with time.

4. CONCLUSION AND RECOMMENDATION

The selection of proper microbial strains is the key step to a successful bioaugmentation. For a pollutant to be eliminated, it is very important to select microbial inoculant isolated from contaminated sites. Bioremediation using *Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium* KY085976 on oil spill dispersants pollutioncan improve the soil status. Therefore, based on the present research, it can be concluded and recommended that bioremediation of *Pseudomonas aeruginosa* KX828570 and *Bacillus megaterium* KY085976 should be regarded as a key component in the clean-up strategy for oil spill dispersants pollution. Since OSD/LT with mixed consortium are more degradable than OSD/Seacare, its use is more preferable during clean-up of oil spill polluted terrestrial soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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