Original Research Article

Bioremediation potential of *Pseudomonas aeruginosa* KX828570 on Crude oil spill Polluted Marshland and terrestrial soil treated with Oil Spill dispersant

ABSTRACT

Aim: To investigate bioremediation potentiality of *Pseudomonas aeruginosa* KX828570 on crude oil Polluted Marshland and Terrestrial Soil treated with oil spill dispersant

Study Design: 1500g of soil samples were weighed and transferred into sterile plastic rubbers labelled 1 to 4 for each of the soil. 50 ml of bio-augmenting agent and 20 ml of dispersant was respectively transferred into the rubbers accordingly except for the control. The setup was watered with 30 ml and tilled twice a week to provide moisture and more oxygen for the organisms to thrive.

Place and Duration of the Study: Soil samples were collected from K-Dere, Gokana L.G.A, and were transported to the Microbiology Laboratory of Rivers State University, Port Harcourt, Nigeria for analyses while Oil spill dispersant (OSD/LT and OSD/Seacare) were from Barker and Hughes Nig Ltd (formally mil park Nigeria limited), all in Rivers state, Nigeria. This investigation study lasted for 28 days and sampling was done every 7day period.

Methodology: Soil samples were inoculated and pure culture of *Pseudomonas aeruginosa* was obtained from the soil. Thereafter, 20ml of each of the Oil spill dispersant - OSD/LT and OSD/Seacare liquid detergent, was used to pollute 1500g soil sample, 50ml of (*Pseudomonas aeruginosa* KX828570), was used as augmentation alongside a control (without organism & treatment) and they were kept at ambient temperature (28±20C) for 28 days. Total hydrocarbon content and some physiochemical parameters was determined using standard method. Also, the standard plate count method was used for the enumeration of the total heterotrophic, dispersant utilizing and hydrocarbon utilizing bacteria. 1 g of soil sample was weighed and aseptically transferred into test tube containing 9ml sterile normal saline and was serially diluted to 10^{-7} and 10^{-5} dilutions were inoculated onto the mineral salt medium and nutrient agar medium respectively. Inoculated plates were spread using sterile bent glass rod and incubation at 37 0C followed. The duration of incubation were 24 hours and 5-7 days for the total heterotrophic bacteria, hydrocarbon utilizing, and dispersant utilizing bacteria respectively. This was done for all the soil samples.

Results: The pH of both soils ranged from 5.75 to 7.37 across the various set up. Temperature reading ranged from 27^0C to 34^0C. Soil moisture content ranged from 0.03 to 0.6 across the soil samples. Total Hydrocarbon Content (THC) for control, (without organism) of Oil spill dispersants (OSD/LT and OSD/Seacare) in terrestrial soil reduced from 18348.68(mg/kg) to 9111.84(mg/kg), Control with organism, (18348.68mg/kg) to (8065.79mg/kg), OSD/LT with *Pseudomonas aeruginosa*, (18348.68mg/kg) to 6263.16(mg/kg) and OSD/Seacare with *Pseudomonas aeruginosa*, (18348.68mg/kg) to 5618.42(mg/kg) respectively. While in marshland soil, control reduces from (68092.11mg/kg) to 42631.58(mg/kg), control with *Pseudomonas aeruginosa*, 68092.11(mg/kg) to (37434.21mg/kg), OSD/LT with *Pseudomonas aeruginosa*, (68092.11mg/kg to 35657.89mg/kg) and, OSD/Seacare with *Pseudomonas aeruginosa*, 68092.11(mg/kg) to 32302.63(mg/kg). The percentage (%) bioremediation rate of polluted soils, were as follows: controls (Marshland and Terrestrial) 37.4% and 50.3%, ML+Pseudo and TS+Pseudo 44.9% and 56.0%, OSD/LT+Pseudo 47.6% and 65.9%, OSD/Seacare+Pseudo 52.6% and 69.4% respectively. Oil spill dispersant(OSD-mg/k). In terrestrial soil, OSD/LT with *Pseudomonas aeruginosa*, reduced from 1776.32(mg/kg) to 598.65(mg/kg), OSD/Seacare with *Pseudomonas aeruginosa* 1776.32(mg/kg) to 513.16(mg/kg) while on marshland, the two test chemicals (OSD/LT and OSD/SC) have the same value 11513.16(mg/kg) to 5526.32(mg/kg). Total heterotrophic bacterial (THB) population ranged from 8.391to 9.760$\log_{10}$cfu/g across the marshland soil set up, terrestrial soil ranged from 8.498$\log_{10}$cfu/g to 9.720$\log_{10}$cfu/g. Dispersant utilizing bacterial count in marshland and terrestrial soil ranged from 6.013$\log_{10}$Cfu/g to 7.338$\log_{10}$Cfu/g and 6.045 $\log_{10}$Cfu/g to 7.301 $\log_{10}$Cfu/g respectively from Day 1 to the 28^{th} day. Hydrocarbon utilizing bacterial count ranged from 6.176 to 7.521$\log_{10}$Cfu/g.

Conclusion: From the investigation, remediation rate of *Pseudomonas aeruginosa* with Seacare was more degradable than *Pseudomonas aeruginosa* with LT. This shows that the organism, *Pseudomonas aeruginosa* have been found to be a potential bioremediation agent in oil spill dispersant polluted marshland and terrestrial soil.

Keyword: Bioremediation, Oil spill dispersant, OSD/LT, OSD/Seacare, Terrestrial soil, Marshland.

INTRODUCTION

The increase exploration and transportation of crude oil, through coastal communities has brought about the pollution of marshland (wetland) and terrestrial soil, which has become a serious environmental concern in Nigeria due to continuous change in the environment. The transportation method employed includes the use of pipelines overland and oceanic tankers. Most marshland are found in remote areas and were mostly polluted by oil spills (1). Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants (2). 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 soil and economic loss (3,4).

Dispersants are the main chemical used that reduced the interfacial tension between water and oil so that it breaks down the oil into droplets and quickly disperses into the water, its use is a topic of immense concern because of its potential ecological effects (5).The establishment of oil spill dispersant preparedness practices in marshland communities will be very crucial to reduce the impact from oil (6).Dispersants are mostly applied immediately after a spill before the lightest component in the evaporates (7,6).

The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion and washing. However, these technologies are expensive and can leads to incomplete decomposition of contaminants (8). Conventional methods to clean-up oil spill from terrestrial and aquatic ecosystems are; mechanical method, chemical method and microbial degradation. Mechanical and chemical methods generally used to remove hydrocarbon from contaminated sites have limited effectiveness (9,10). Mechanical cleaning of spilled oil and dispersant is nearly impossible in 'protected' ecosystems. Chemicals are used to change the characteristics feature of the oil (11).

In recent years microbial degradation of pollutants is a sustainable way to clean up the contaminated environment (12). Microbial degradation is the major and ultimate natural mechanism by which one can clean up the petroleum hydrocarbon pollutants and dispersants from the environment (13). This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbon as a source of carbon and energy (14, 9,10). The use of inexpensive equipment, environmentally friendly nature and simplicity of the process are some of the advantages over other remedial means such as chemical and mechanical treatments. This is the reason why the use of microorganisms capable of converting contaminants to harmless products by mineralization, generation of carbon(iv)oxide and water or by conversion into microbial biomass by exploiting its diverse metabolic abilities known as bioremediation has become an alternative technology (14, 15, 16). The most effective elimination of contaminates may be achieved by using microbial inoculants isolated from already polluted environments. Bioremediation involves the use of microorganisms to remove or neutralize pollutants from contaminated sites (17, 18, 4). The success of oil spill and residual dispersant bioremediation depends on one's ability to establish and maintain conditions that favour enhanced oil biodegradation rates in contaminated environment, such as presence of microorganisms with appropriate metabolic abilities. Several bacteria are even known to feed exclusively on hydrocarbons. The ability of this species to degrade crude oil in oil polluted soil site suggests that they could be used for the treatment of other oil wastes such as oil spill dispersant polluted terrestrial soil, marshland and water. Hence, the essence of this study; to investigate and compare the bioremediation potentiality of *Pseudomonas aeruginosa* on oil spill dispersant polluted marshland and terrestrial soil.

Materials and Methods

Study site and sample collection

The soil sampling was carried out at K-Dere community in Gokana Local Government Area of Rivers state, Nigeria. K-Dere is situated in the Niger Delta Area of Nigeria, between longitudes 7.010 and 7.07^0 E; and latitudes 4.08 and 4.2^0N. Sampling were done at two different sites, put in sterile black polyethylene bags and labelled with masking tape, and then immediately taken to the microbiology laboratory, Rivers State University, for microbiological and physicochemical analyses.

Source of Oil spill dispersants

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

Preparation of mineral salt medium (MSM)

The mineral salt medium was prepared in the laboratory having the following composition; $K_2HPO_4.7H_2O$ (0.5g); $MnSO_4$ H_2O (0.2g); $NaCl_2$ (0.3g); $ZnCl_2$ (0.03g); $MgSO_4$ (0.3g); $FeSO_4$ H_2O (0.02g) $NaNO_3$ (0.03g) and Agar Agar, (16g) in 200mls of sterile distilled water. Each salt was dissolved in distilled water before mixing. The pH of the solution was adjusted to 6.8. The medium was then sterilized by Autoclaving at 15 Ibs pressure (121 ^oC) for 15minutes (19).

Source of Microorganisms (*Pseudomonas aeruginosa*)

The method described by (20, 21) was adopted. Pure cultures of the organism were obtained from inoculation and incubation of soil samples using nutrient Agar. Pure cultures were obtained by continuous subculturing (22,23). Isolates was inoculated into broth culture (19).

Isolation of Test Organism

The test organism (*Pseudomonas aeruginosa*) was selected because of its importance as an active hydrocarbon degrader in crude oil polluted environment. It was isolated from the oil-polluted soil samples using the spread plate method (microbiological method). Soil suspensions were prepared by adopting Ten-fold serial dilution.1g of the soil sample was measured into a test tube and 9ml of sterile distilled water was mixed with the sample. 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 prepared normal saline as diluents. Seven test tubes containing 9ml of normal saline was used for the serial dilution. Aliquots of 0.1ml from 10^{-5} and 10^{-7} dilutions were introduced into duplicated sterile petri dishes using sterile pipette and separately spread plated with flame sterilized bent glass spreader on well-dried Cetrimide agar plate and nutrient agar plates. The plates were incubated at 37 ^oC for 24 to 48 hours. After which bacterial colonies that form during 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 ^oC for 24 hr. Discrete colonies on the plates were aseptically transferred into agar slants and bijou bottles containing 10% (v//v) glycerol, properly labelled and stored as stock cultures for preservation and identification (23,22).

Confirmation of Test Organism

The confirmation of the isolates was done according to the standard techniques in District laboratory practice in tropical countries (25), and was identified base on the Bergey's manual of Determinative Bacteriology after carrying out the morphological and various biochemical tests.

Bioremediation set–up (Experimental Design)

Table 1Experimental Design (Bioremediation set–up) for Both Soil

SET UP LABEL	SET UP CONSTITUENTS
1	1500g of Terrestrial soil+30ml of Distilled (Control)
2	1500g of Terrestrial soil +30ml of Distilled H_2O+50ml of *Pseudomonas aeruginosa*
3	1500g of Terrestrial soil+20ml of OSD/LT+30ml of Distilled H_2O+50ml of *Pseudomonas aeruginosa*
4	1500g of Terrestrial soil+20ml of OSD/SC+30ml of Distilled H_2O+50ml of *Pseudomonas aeruginosa*
5	1500g of Marshland soil+30ml of Distilled (Control)
6	1500g of Marshland soil +30ml of Distilled H_2O+50ml of *Pseudomonas aeruginosa*
7	1500g of Marshland soil+20ml of OSD/LT+30ml of Distilled H_2O+50ml of *Pseudomonas aeruginosa*
8	1500g of Marshland soil+20ml of OSD/SC+30ml of Distilled H_2O+50ml of *Pseudomonas aeruginosa*

Soil preparation and Application of organisms

Bioremediation set-up for a proper monitoring was set up for each soil sample and Oil spill dispersants (OSD), 1500g of the soil sample collected from K-Dere Gokana was weighed into Eight plastic rubbers. After that, there was a control which was without organisms while others were augmented with organisms (Pseudomonas aeruginosa). Twenty millilitre (20ml) of each of the Oil spill

dispersant - OSD/LT and OSD/Seacare was dispensed into each of the rubber containing the soil so as to pollute it and it was then mixed properly using a sterile spatula so as to enable the dispersants mix properly with the soil. Thereafter, 30ml of distilled water were used to watered the set samples and properly stirred with a spatula for the organisms to thrive successful and have more oxygen.
Bioaugmentation was the type of bioremediation carried out in which samples were augmented by adding 50ml of broth culture organism (*Pseudomonas aeruginosa*) to the first set up, and they were kept at ambient temperature (28 ± 2^0C) for 28 days. 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.

Total Hydrocarbon Content Analysis
Chemical Analyses
This was done using spectrophotometer.
During the setup process for spectrophotometric analysis, 10g of soil sample were weighed from each of the setup rubbers containing 1500g of soil sample into sterile beaker and 20ml 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 analyzer. The Total Hydrocarbon Content (THC) value was determined by comparing to a calibration curve constructed from dilution of a stock solution of a 1:1 bonny light crude and oil spill dispersant. The spectrophotometric measurement was at 420nm and Total Hydrocarbon Content (THC) Oil Spill Dispersant (OSD) was at 560nm (25, 26). For marshland soils, the extracted oil had high concentration so they were diluted with 10^{-1} dilution ratio before analyzing (4).

Sample Analysis
Moisture Content analysis
This was carried out by removing 10g of contaminated soil from each of the set up and weighing it inside of a wash glass, then they were placed inside a hot air oven for 1 hour at 110^0c for drying. After drying, the soil was immediately transferred into desiccators for cooling for 30 minutes. After which, the soil was then reweighed and the new weight in grams gotten were then subtracted (minus) from the initial 10 grams of the soil to get the moisture content value (27).
Moisture content was estimated as W_1-W_2/W_1× 100
Where W_1 = weight of the sample before drying
W_2 = weight of sample after drying.

Soil pH
This was determined by weighing 10g of soil sample into the beaker and 10ml of distilled water was added. Allowed to stand for 30 minutes and stir occasionally with a glass rod. Insert the pH meter (previously calibrated) into the partly settled suspension and take the pH reading (29)
Soil Temperature
The temperature of the soil was measure ex situ with a mercury thermometer. Constant temperature was recorded by allowing the thermometer to remain in the soil.

Media Preparation
Nutrient Agar
It is a general purpose medium supporting the growth of wide range of non-fastidious organisms. Nutrient agar was used for the isolation of total heterotrophic bacteria with the manufacturer's description of 28grams into 1000ml of distilled water.
Cetrimide Agar Medium
This is for the selective isolation of gram-negative bacteria, *Pseudomonas aeruginosa.*
The preparation of this medium is by dissolving 45.3gm in 1000ml distilled water, autoclaved at 15psi (121^0c) for 15minutes. Cool to 45-50^0C, prior to dispense.

Nutrient Broth

This broth is prepared for the multiplication of test organisms. The broth was prepared by dissolving 13g into 1000ml of distilled water, so we used the manufacture's specification to calculate depending on the quantity needed for experiment.

Stock Solution

Ten percent glycerol solution was prepared dispensed in McCartney bottles and autoclaved at 121°C for l5minutes, 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 (22, 24).

Isolation and enumeration of Hydrocarbon Utilizing Bacteria

Hydrocarbon utilizing bacteria (HUB) were enumerated as adopted from (4) using mineral salts medium with crude oil as the sole source of carbon. Isolated colonies were further purified by sub-culturing and identified using biochemical tests and microscopy (25). 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. Fungosol was added to the Mineral Salt Agar to suppress fungal growth. Spread plate method were used. A filter paper saturated with sterile crude oil was aseptically placed on the inside of the inverted Petri dishes and the culture plates were incubated for 5 to 7 days at 37 ^{o}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

Isolation and enumeration of Oil spill dispersant (OSD) utilizing bacteria

Enumeration of Oil spill dispersant (OSD) utilizing bacteria was done by inoculating 0.1ml aliquot of the dilution 10^{-5}into duplicated sterile petri dishes using sterile pipette and separately spread plated with flame sterilized bent glass spreader unto mineral salt agar plates containing the OSD (30, 31). The plates were incubated at 37 ^{o}C for 24 to 48 hours. Colonies were counted after 48 to 72 h incubation at ambient temperature. The bacterial colonies on the plates after incubation were counted and sub-cultured onto fresh mineral salt agar plate.

KEYS- OSD=oil spill dispersant; ML= Marshland soil; TS= Terrestrial soil; CRTL= Control; Pseudo= Pseudomonas aeruginosa; THC=Total hydrocarbon content; Temp=Temperature THB=Total heterotrophic bacterial; HUB=Hydrocarbon utilizing bacterial; DUB=Dispersant utilizing bacterial; SC=Seacare.

RESULTS AND DISCUSSION

Bioremediation of potential of *Pseudomonas aeruginosa* on oil spill dispersant polluted marshland and terrestrial was successful. *Pseudomonas aeruginosa* helped in remediating the polluted soils caused by oil spill dispersant by reducing pollutant in the soil. Total Hydrocarbon Content assay on soil samples (oil spill dispersant polluted marshland and terrestrial) augmented with bacterial specie (*Pseudomonas aeruginosa*) for 28 days was shown in Fig 1 and 3. The result showed that the total hydrocarbon content decreased with an increase in time, from the day 1 of the study, the control values for marshland and terrestrial soil reduced from 68092.11(mg/kg) to 43631.58(mg/kg) and 18348.68(mg/kg) to 9111.84(mg/kg) respectively. While the polluted soil samples of different treatments augmented with *Pseudomonas aeruginosa* were totally different from controls. The range of the two soil samples with different treatments from day 1 to day 28 are as follows: ML+Pseudo and TS+Pseudo ranged from 68092.11(mg/kg) to 37500(mg/kg) and 18348.68(mg/kg) to 8065.79(mg/kg), ML+OSD/LT+Pseudo and TS+OSD/LT+Pseudo ranged from 68092.11(mg/kg) to (35657.89mg/kg) and 18348.89(mg/kg) to 5618.42(mg/kg) respectively. This indicates that the effect of time on hydrocarbon bioremediation rate was significant. Furthermore, from the results gotten, it was observed that oil spill dispersant (OSD/Seacare) with *Pseudomonas aeruginosa* was more effective than oil spill dispersant (OSD/LT) with *Pseudomonas aeruginosa* in both polluted soils. The percentage (%) bioremediation rate of polluted soils, were as follows: controls (ML and TS) 37.4% and 50.3%, ML+Pseudo and TS+Pseudo 44.9% and 56.0%, OSD/LT+Pseudo 47.6% and 65.9%, OSD/Seacare+Pseudo 52.6% and 69.4% respectively. Generally, the highest percentages of THC in

this study were from soil samples treated with oil spill dispersant, while the least were persistently observed in treatments without oil spill dispersant and controls. This suggests that microorganisms are more abundant in oil spill dispersant polluted soils than unpolluted soils. In addition, the result obtained from mg/kg Dispersant OSD control for both soils (marshland and terrestrial), ranged from 11513.16(mg/kg) to 5986.84(mg/kg) and 1776.32(mg/kg) to 651.32(mg/kg) respectively. Whereas, the oil spill dispersant polluted marshland and terrestrial soil augmented with *pseudomonas aeruginosa,* showed higher bioremediation rate. In marshland soil, it was observed that the two treatments with *Pseudomonas aeruginosa* (OSD/LT and OSD/Seacare) has the same reduction values on day 28, but on day 21, OSD/Seacare degraded faster with the rate of 5842.11(mg/kg) than OSD/LT 7500(mg/kg). While in the terrestrial soil, OSD/Seacare+Pseudo has the highest degradation rate of 513.16(mg/kg). The OSD/LT + *Pseudomonas aeruginosa* degradation rate is said to be 598.65(mg/kg) and terrestrial soil +*Pseudomonas aeruginosa* without treatment (TS+Pseudo) degradation rate is said to be 625mg/kg. This means that oil spill dispersant (OSD/Seacare) is more degradable than (OSD/LT) using bio-augmenting organism *Pseudomonas aeruginosa*

The pH of both soils ranged from 5.75 to 7.37 across the various set up. The highest soil pH (7.37) and (6.37) was recorded in the treated Marshland soil (ML+OSD/Seacare+Pseudo) and treated Terrestrial soil (TS+OSD/LT+Pseudo) while the lowest soil PH (6.16 and 7.26) was recorded in the Terrestrial soil control (TS(CTRL) and Marshland control. There was no significant difference between the two soil samples in soil pH. The soil pH of the two samples (Polluted marshland and terrestrial) sites were within the same range, and they were tending from slightly acidic towards neutrality. This result concord with the observation of (34, 35), who indicated that a pH between 5 and 7.8 is favourable for the biodegradation activity of bacteria in the soil. (36) reported similar results on pH of crude oil polluted soils of Niger Delta. The non-significance difference between the soil pH in the two soils showed that the bioremediation of the polluted soil did not have any significant effect on soil pH (37). 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 marshland soil still fall within the pH range suitable for microbial growth indicating that these isolates exhibited optimal growth at pH range of 6.0 to 8.0. Reference (38) reported that the growth of most microorganisms is usually greatest within a pH range of 6 to 8.

Moisture Content

Soil moisture content ranged from 0.03 to 0.6 across the soil samples. The highest soil moisture (0.6) was recorded in the Marshland soil polluted with oil spill dispersants (ML+OSD+Pse) while the lowest soil moisture (0.03 and 0.07) was recorded in the Terrestrial and Marshland soil control(TS(CRTL and ML(CRTL).The moisture content result of both soil samples in Table 3, shows the differences in the moisture content of the different experimental set-up, indicating the treated soil ; OSD Polluted soil + *Pseudomonas aeruginosa* (OSD/LT +Pseudo and OSD/Seacare)(0.6g/10g and 0.2/10g) having the highest moisture content, followed by soil sample with organism application; ML+Pseudo and TS+Pseudo0.3g/10g and 0.1g/10g), while Control (soil sample without organism) CTRL)(0.03g/10g and 0.07g/10g) has the lowest. (4, 6) reported 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 oil spill dispersant with *Pseudomonas aeruginosa* (OSD+Pseudo) 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 bioremediation.

The temperature reading for the both soil samples ranged are as follows; for control, 29^0C to 30^0C and 27^0C to 30^0C, for oil spill dispersant (OSD/LT), 28^0C to 31^0C and 30^0C to 33^0C, while that OSD/SC range from 28^0C to 31^0C and 29^0C to 34^0C respectively. 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. Table 3-4 showed the mean and standard deviation of some physicochemical parameters carried out in the study.

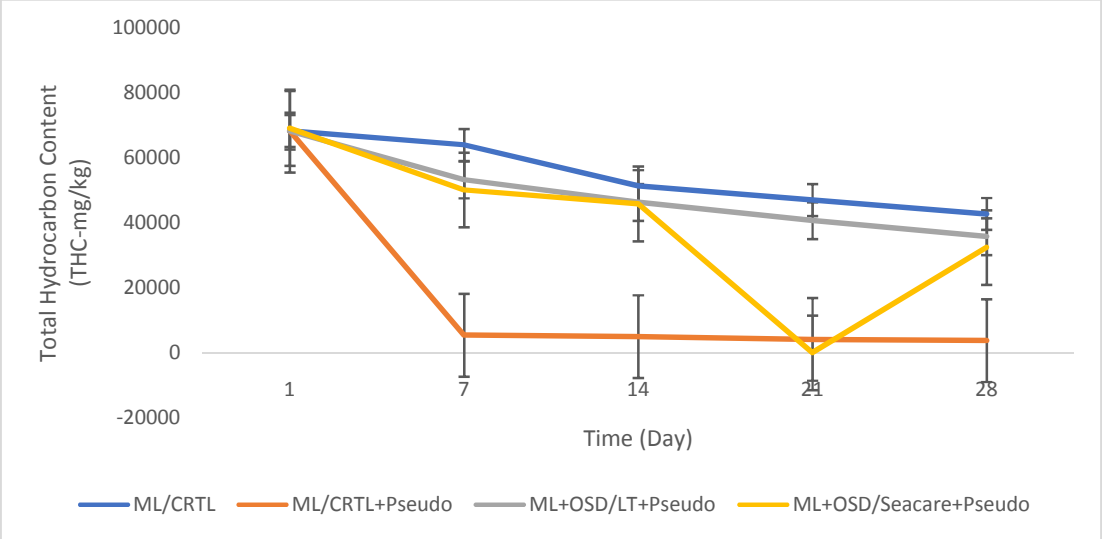

Fig 1: Total Hydrocarbon Content (THC-mg/kg) during bioremediation o oil spill dispersant (OSD/LT and OSD/SC) polluted marshland soil using bio-augmenting organism (*Pseudomonas aeruginosa* KX828570)

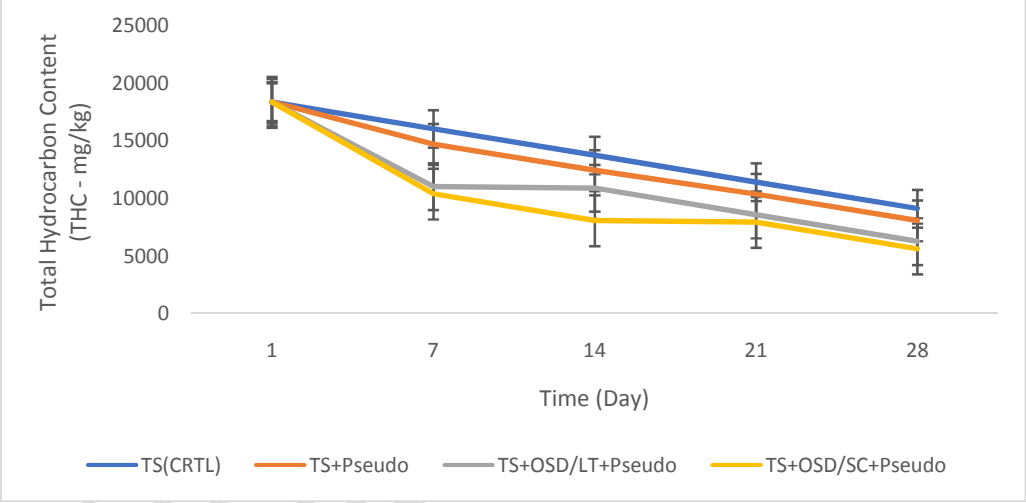

Fig 2: Total Hydrocarbon Content (THC-mg/kg) during bioremediation o oil spill dispersant (OSD/LT and OSD/SC) polluted terrestrial soil using bio-augmenting organism (*Pseudomonas aeruginosa* KX828570)

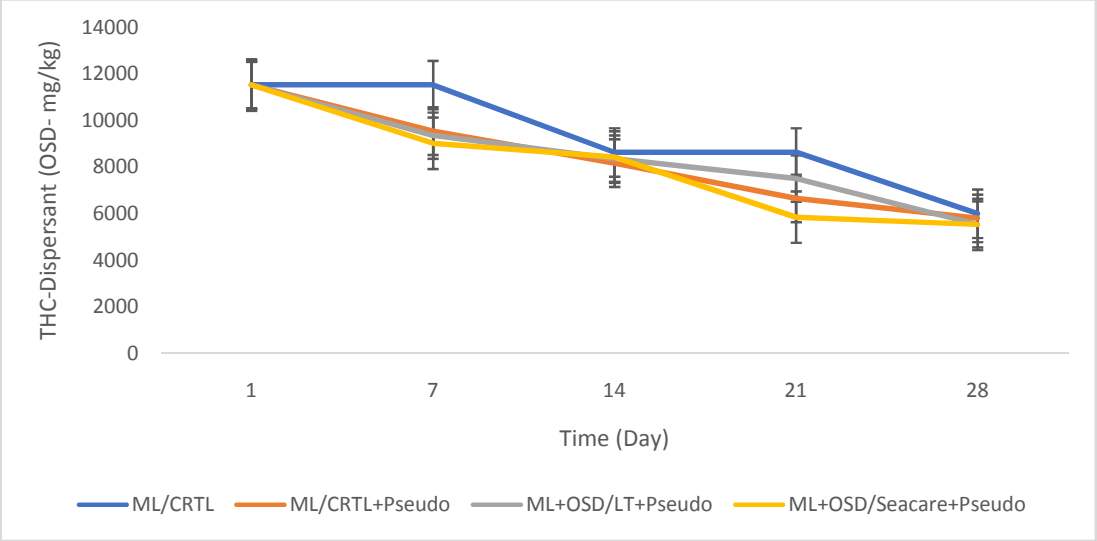

Fig 3: Total Hydrocarbon Content (THC- Dispersant OSD-mg/kg) during bioremediation o oil spill dispersant (OSD/LT and OSD/SC) polluted marshland soil using bio-augmenting organism (*Pseudomonas aeruginosa* KX828570)

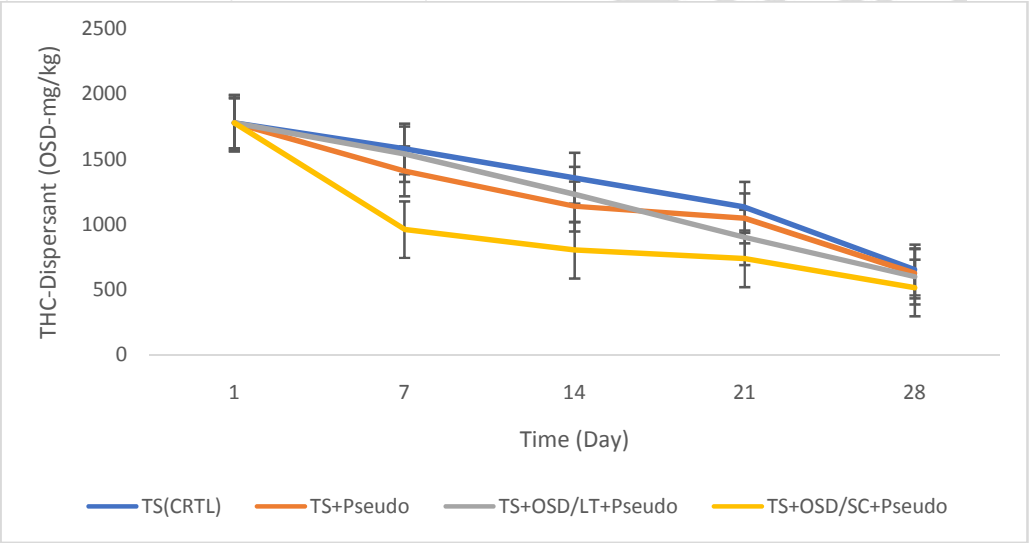

Fig 4: Total Hydrocarbon Content (THC- Dispersant OSD-mg/kg) during bioremediation o oil spill dispersant (OSD/LT and OSD/SC) polluted terrestrial soil using bio-augmenting organism (*Pseudomonas aeruginosa* KX828570)

Total Hydrocarbon Content carried on soil samples (oil spill dispersant polluted marshland) augmented with bacterial specie (*Pseudomonas aeruginosa*) was shown in Fig 1 and 3. It was observed in this study that the bioremediation rate of oil spill dispersant polluted marshland using *Pseudomonas aeruginosa* was successful. In the marshland soil sample, remediation value was high in the control while bioremediation potential of oil spill dispersant polluted marshland using *Pseudomonas aeruginosa* has the highest degradation rate

The reduction in THC value in the polluted soil samples might be attributed to microbial degradation as a result of the remediation process, in this case, the study shows that microorganisms utilized the hydrocarbon and dispersants as their energy source for their metabolic activities, while the high THC in polluted soil samples could be as a result of the toxicity of the oil to oil and OSD utilizers, making biodegradation to be slow or stopped. This agrees with of findings (43). Also, the increased in THC at 560nm Dispersant might be as a result of the toxicity of the oil spill dispersant (OSD). The petroleum degradation rate decreased with an increase in time. This suggested that the crude oil and oil spill

dispersant were not only slightly worn by atmospheric condition but biodegraded by hydrocarbon utilizing microbes, as supported by the number of peaks in the total hydrocarbon content.

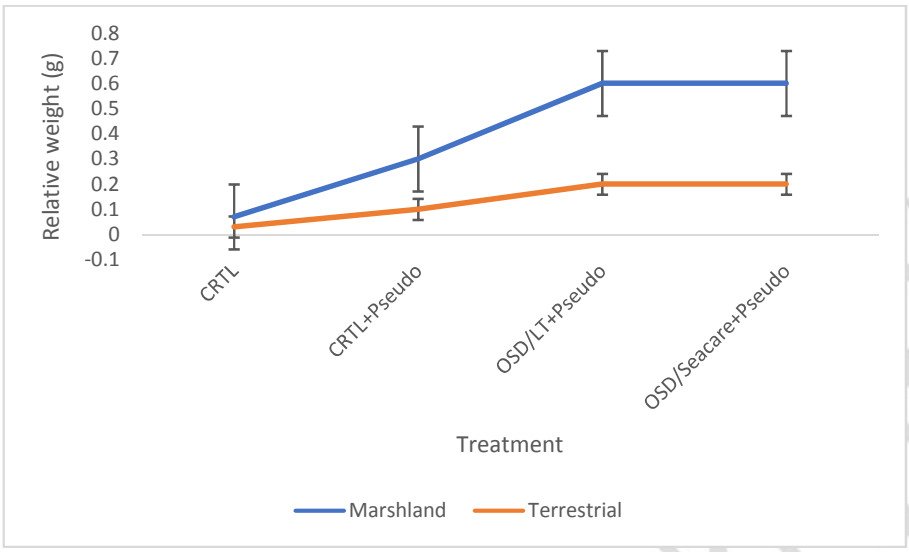

Fig.5: Soil Moisture Content (g) during bioremediation o oil spill dispersant (OSD/LT and OSD/SC) polluted terrestrial soil using bio-augmenting organism (*Pseudomonas aeruginosa* KX828570)

Table 2: Physicochemical Analysis of Terrestrial soil

Terrestrial Physicochemistry	**Temp**	**pH**	**Moisture**
TS (CRTL)	29.8±0.45^{a}	5.99±0.12^{a}	.034±0.01^{a}
TS + Pseudo	31.6±1.52^{b}	6.03±0.22^{b}	.120±0.03^{b}
TS +OSD/LT+Pseudo	31.2±0.84^{ab}	6.00±0.23^{a}	.240±0.05^{b}
TS +OSD/SC+Pseudo	30.8±1.30^{ab}	6.01±0.20^{a}	.240±0.05^{b}

Mean with the same alphabet across rows shows no significant difference (>0.05)

Table 3: Physicochemical parameters on marshland soil

Marshland Physicochemistry	**Temperature**	**pH**	**Moisture**
ML(CRTL)	29.2±1.30^{ab}	6.94±0.23^{a}	.066±0.01^{a}
ML+Pseudo	30.2±1.64^{ab}	6.95±0.29^{a}	.260±0.05^{b}
ML+OSD/LT+Pseudo	30.6±1.67^{ab}	6.99±327^{a}	.600±0.01^{b}
ML+OSD/SC+Pseudo	29.8±1.92^{ab}	6.76±0.34^{a}	.606±0.01^{b}

Mean with the same alphabet across rows shows no significant difference (>0.05)

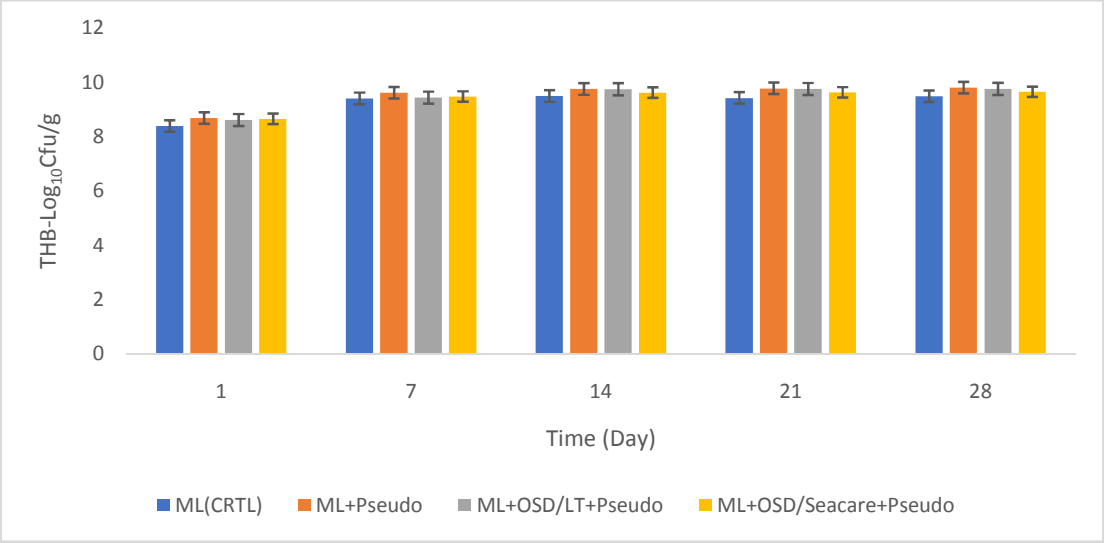

Fig 6: Total Heterotrophic Bacteria count (THB- log_{10} cfu/g) during bioremediation o oil spill dispersant (OSD/LT and OSD/SC) polluted marshland soil using bio-augmenting organism (Pseudomonas aeruginosa KX828570)

THB for terrestrial soil

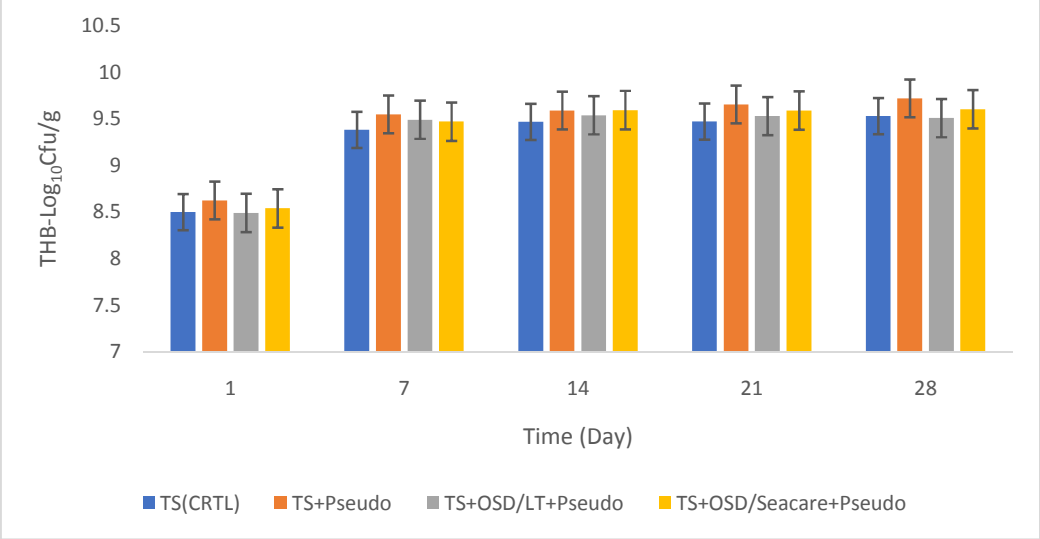

Fig 7: Total Heterotrophic Bacteria count (THB-log_{10} cfu/g) during bioremediation o oil spill dispersant (OSD/LT and OSD/SC) polluted terrestrial soil using bio-augmenting organism (*Pseudomonas aeruginosa* KX828570)

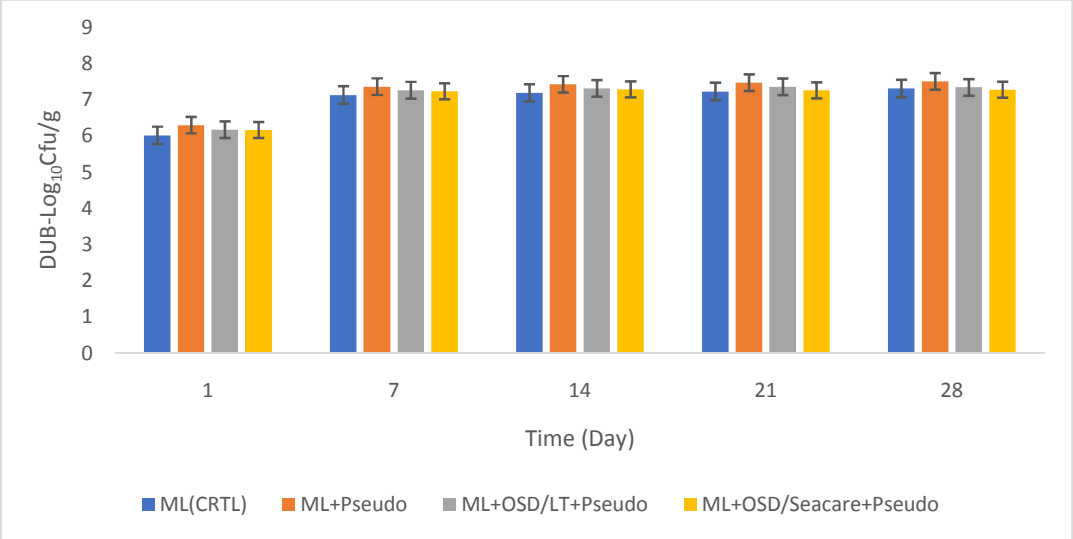

Fig 8: Dispersant Utilizing Bacteria count (DUB-log$_{10}$ Cfu/g) during bioremediation o oil spill dispersant (OSD/LT and OSD/SC) polluted marshland soil using bio-augmenting organism (Pseudomonas aeruginosa KX828570)

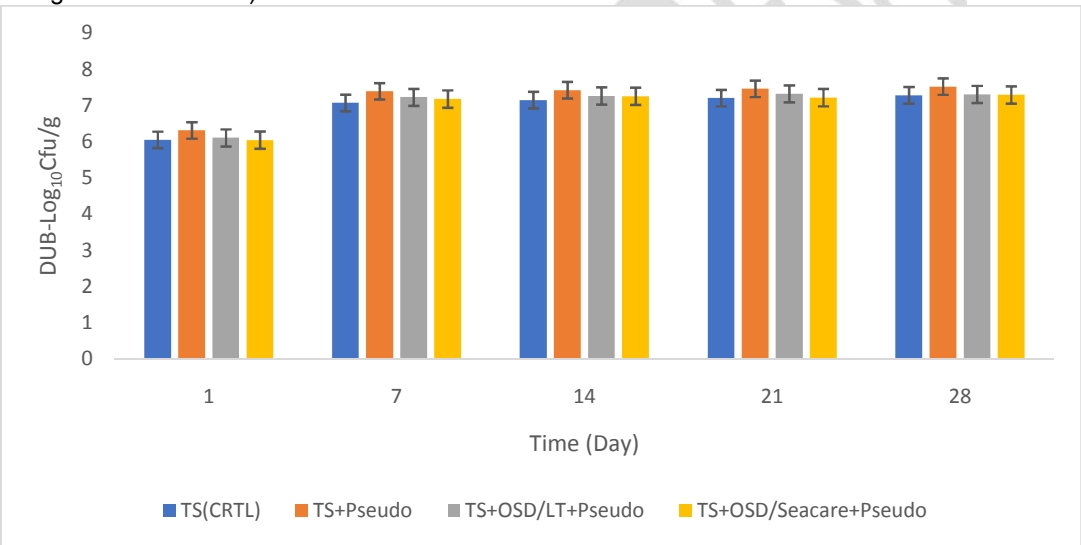

Fig 9: Dispersant Utilizing Bacteria count (DUB-log$_{10}$ Cfu/g) during bioremediation o oil spill dispersant (OSD/LT and OSD/SC) polluted terrestrial soil using bio-augmenting organism (Pseudomonas aeruginosa KX828570)

The result showed that there were higher population of bacterial counts in oil polluted soil with oil spill dispersant, at day 7, 14, 21 and day 28. Total heterotrophic bacterial (THB) population ranged from 8.391to 9.760log$_{10}$cfu/g across the marshland soil set up. The highest THB count 9.760log$_{10}$cfu/g was observed in the soil sample (marshland) polluted with oil spill dispersant (OSD/LT+Pseudo), while lowest THB count 8.391log$_{10}$cfu/g was observed in marshland control (ML(CRTL). While that of terrestrial soil ranged from 8.498log$_{10}$cfu/g to 9.720log$_{10}$cfu/g. The highest count 9.720log$_{10}$cfu/g was observed in soil sample (terrestrial) polluted with oil spill dispersant (TS+Pseudo), while the lowest THB count 8.498log$_{10}$cfu/g was recorded in terrestrial control (TS(CTRL). The highest THB count was due to increase in hydrocarbon content, which concord with the findings of (39,40). This shows that at the introduction of the test organism (*Pseudomonas aeruginosa*), the concentrated oil spill dispersant was still very high, which inhibited bacterial growth, as it is lethal to it.

There is an increased and slight decreased in total dispersant utilizing bacterial (DUB) count and Hydrocarbon utilizing bacterial count (HUB) in soil samples used as inoculum. The count for DUB in marshland and terrestrial soil increased from 6.013$\log_{10}$Cfu/g to 7.338$\log_{10}$Cfu/g and 6.045 $\log_{10}$Cfu/g to 7.301 $\log_{10}$Cfu/g respectively from Day 1 to the 28th day. The highest count 7.338$\log_{10}$Cfu/g was observed in the soil sample (marshland) polluted with oil spill dispersant (OSD/LT+Pseudo), while lowest DUB count 6.013$\log_{10}$Cfu/g was observed in marshland control (ML(CRTL). In terrestrial soil, the highest count 7.301$\log_{10}$Cfu/g was observed in the soil sample polluted with oil spill dispersant (OSD/LT+Pseudo), while lowest DUB count 6.045$\log_{10}$Cfu/g was observed in terrestrial control (TS(CRTL). The higher count may be attributed to the fact that the oil and dispersant served as a source of carbon and energy to the organisms and so encouraged their proliferation. The lower count observed on the Day 1 and 7 suggests the concentrated oil spill dispersants and crude oil were still very high, which inhibited bacterial growth, as it lethal to it. This observation is line with the reports of (41, 42,31) that oil spill dispersants support mild increases (stimulation) and decreases (inhibition) in the growth dispersants 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. Author (43) studied the biodegradability o three dispersants; Pars 1, Pars 2 and Gamlen OD4000. The study showed that, the highest growth of microorganisms was documented or either Pars 1 or Pars 2. Pars dispersants 1 showed more degradability in the first 24 h compared to others, and has more adaptability to the aquatic ecosystem. In HUB, polluted soil had higher bacterial count than the control. From Table 4 & 5, there was no significant difference between the various set up with different treatments ($p>0.05$). Bioremediation using *Pseudomonas aeruginosa* on oil spill dispersants pollution can improve the soil status.

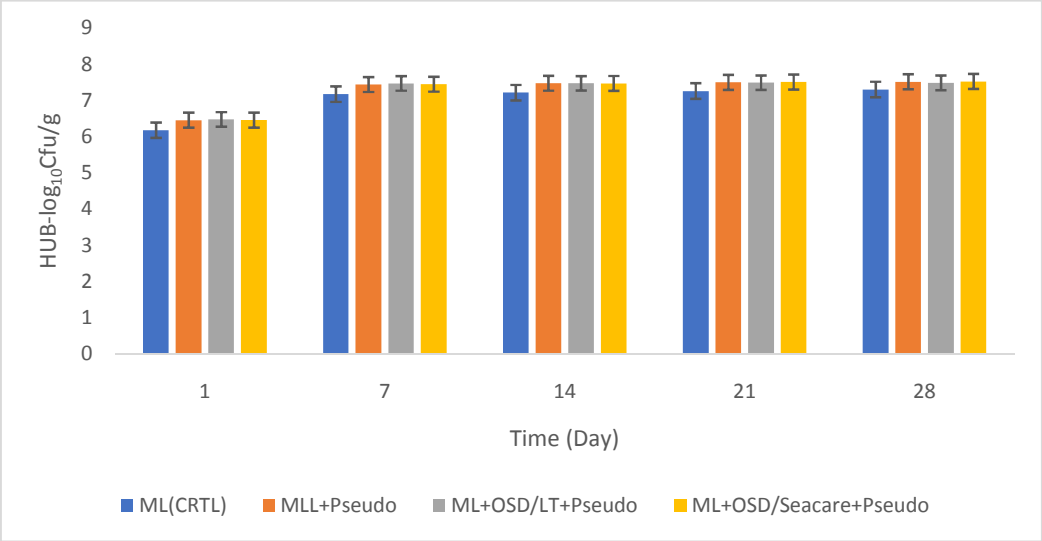

Fig 10: Total Hydrocarbon Utilizing Bacteria count (HUB-$\log_{10}$ cfu/g) during bioremediation o oil spill dispersant (OSD/LT and OSD/SC) polluted marshland soil using bio-augmenting organism (*Pseudomonas aeruginosa* KX828570)

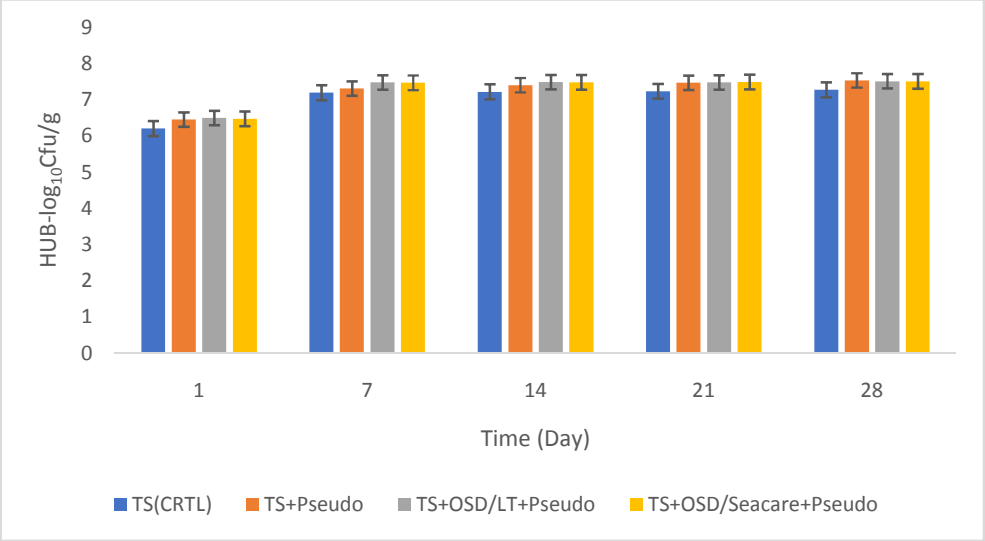

Fig 11: Total Hydrocarbon Utilizing Bacteria count (HUB-$\log_{10}$ cfu/g) during bioremediation o oil spill dispersant (OSD/LT and OSD/SC) polluted terrestrial soil using bio-augmenting organism (*Pseudomonas aeruginosa* KX828570)

Conclusion

The ability of this *Pseudomonas aeruginosa* KX828570 to degrade crude oil in oil polluted soil site suggests that they could be used for the treatment of other oil wastes such as oil spill dispersant polluted terrestrial soil, marshland and water. It is a welcome development in carry out bioremediation process using bio-augmenting organism *Pseudomonas aeruginosa* KX828570 in the oil spill dispersant polluted soil environment. From the investigation, remediation rate of *Pseudomonas aeruginosa* with Seacare was more degradable than *Pseudomonas aeruginosa* with LT.

Oil spill dispersant bioremediation on marshland and terrestrial soil can be promoted by augmenting the selected specie (s) isolated from polluted soil to the indigenous microorganisms in the soil

This result showed that this organism, *Pseudomonas aeruginosa* is potential bioremediation agents in oil spill dispersant polluted marshland and terrestrial soil.

It is recommended that oil companies and government parastatals carrying out remediation in the Niger Delta should be encouraged and mandated to use OSD/Seacare with *Pseudomonas aeruginosa* due to its high biodegradation potential over OSD/LT.

Since OSD/Seacare with *Pseudomonas aeruginosa* are more degradable in Marshland and Terrestrial than OSD/LT, its use should be preferred during cleaning of oil spill polluted Marshland and Terrestrial soil.

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