International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Bioremediation Effects of *Bacillus subtilis* and *Pseudomonas aeruginosa* in Crude Oil Spill Affected Soils in Owaza Community, Abia State, Nigeria

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Abstract: ***The study analyzed the bioremediation effects of Bacillus subtilis and Pseudomonas aeruginosa in crude oil spill affected soils in Owaza community in Abia State, Nigeria. The point sampling method whereby soil samples were collected at random locations within the crude oil spill affected soils was employed for the study. The biodegradation experiments were carried out for 25 days in a nursery shed under natural environmental conditions. The experiments were carried out in four sets. Each of the sets had a control which was not with Cow dung or bacterial inoculation. The entire experimental setup were done in duplicates so that the collection of the samples will not disturb the degradation. The laboratory experiments involved well-structured series of analysis on the different soil samples inoculated with Bacillus subtilis and Pseudomonas aeruginosa. Descriptive statistics in form of Tables was employed for data presentation while Excel work sheet 2010 was employed for data analysis. Findings of the study revealed that at the end of the 25 days of the experiment, the mean population densities of Bacillus subtilis increased and recorded a total hydrocarbon content reduction of 54.4%. Similarly, the mean population densities of Pseudomonas aeruginosa increased at the end of the 25 days and recorded a total hydrocarbon content reduction of 54.71%. Findings further revealed that the combination of Bacillus subtilis and Pseudomonas aeruginosa significantly increased the population densities of bacteria isolates and recorded a total hydrocarbon contents reduction rates of 69.75%. The study concluded that Bacillus subtilis and Pseudomonas aeruginosa can lead to significant reduction in hydrocarbon contents in crude oil spill affected soils overtime, especially when more days can be deployed for the experiment. The study recommends that research expansion is therefore needed in this area for effective bioremediation techniques in cases of crude oil spill effects on soil quality.***

Keywords: Crude oil spill affected soils; Soil bioremediation; *Bacillus subtilis; Pseudomonas aeruginosa;* hydrocarbon content; Owaza community

1. Introduction

Oil and gas together constitute over 90% of Nigerian foreign-exchange earnings. The Niger Delta is the main seat of oil and gas production in Nigeria. It is a fact that all aspects of oil and gas exploration and exploitation have deleterious effects on the local ecosystem and biodiversity. Oil exploration by seismic companies involves surveying, clearing of seismic lines, and massive dynamiting for geological excavations. The explosion of dynamite in aquatic environments leads to narcotic effects and mortality of fish and other faunal organisms (Zabbey, 2014). Oil spillages routinely occur in the Niger Delta. Sources of oil entering the environment are variable, including pipeline leakage and rupturing, accidental discharges (e.g. tank accidents), discharges from refineries and urban centres, etc. There are also biogenic sources of hydrocarbons in the environment.

Bioremediation involves the processes where chemical substances are degraded by bacteria and other micro-organisms. The use of these microorganisms has been successfully applied for the treatment of waste and wastewater in controlled systems. The technique has been found to have a potential for broad applications in terrestrial and freshwater environments for treating soils and sediments contaminated with oil and other substances, as well as for coastal environments impacted by oil spills (Namkoong *et al*., 2002). Oil spills have been known to cause acute and long term damage to salt marshes and mangrove swamps. These impacts include; reduction in population and growth rate of the mangrove trees, acute and long term damage to salt marshes and mangroves, wide spread animal mortality as a result of the toxic effects of the oil and the disruption of the entire ecosystem (Xu & Obbard, 2004). For these reasons, microorganisms can be effective, economical, and non-disruptive tools for eliminating hazardous chemicals. Certain enzymes produced by microbes attack hydrocarbon molecules, causing degradation. The degradation of oil relies on having sufficient microbes to degrade the oil through the microbes' metabolic pathways (series of steps by which degradation occurs). Fortunately, nature has evolved many microbes to do this job. Throughout the world there are over 70 genera of microbes that are known to degrade hydrocarbons (Gray *et al*., 2000). These microbes usually account for less than 1% of natural population of microbes, but can account for more than 10% of the population in polluted ecosystems (Adebusoye *et al*., 2007).

Many microorganisms possess the enzymatic capability to degrade petroleum hydrocarbons. Some microorganisms degrade alkanes, others aromatics, and others both paraffinic and aromatic hydrocarbons. Often the normal alkanes in the range C_{10} to C_{26} are viewed as the most readily degraded, but low-molecular-weight aromatics, such as benzene, toluene and xylene, which are among the toxic compounds found in petroleum, are also very readily biodegraded by

many marine microorganisms. There are several different bioremediation techniques. The underlying idea is to accelerate the rates of *natural* hydrocarbon biodegradation by overcoming the rate-limiting factors. Several techniques can lead to the results striven for. Indigenous populations of microbial bacteria can be stimulated through the addition of nutrients or other materials. Exogenous microbial populations can be introduced in the contaminated environment. The addition of extra bacteria is known as bio augmentation. If necessary, genetically altered bacteria can be used. Once the bacteria are chosen, the engineer must carefully meet their nutritional needs by choosing the correct mix of fertilizer (Head, 1998). Furthermore, the contaminated media can be manipulated by, for example, aeration or temperature control. Two of these techniques, seeding with microbial cultures and environmental modification, commonly used are briefly explained.

Similarly, oil interferes with the functioning of various organ systems of plants and animals. It creates environmental conditions unfavorable for life; for example, oil on a water surface forms a layer which prevents oxygen penetration into water bodies, and this in turn leads to suffocation of certain aquatic organisms. Crude oil contains toxic components, which cause outright mortality of plants and animals as well as other sub-lethal damage. Generally, toxicity is dependent on the nature and type of crude oil, the level of oil contamination, the type of environment, and the selective degree of sensitivity of individual organisms.

Consequently, Owaza community under Ukwa West LGA being the oil producing area of Abia State has suffered many hazards as a result of oil spill and little or no effort has been made the responsible organisations to clean up and restore the marine and agricultural viability of the affected communities. Therefore in a bid to ameliorate the effects of these hazards on the residents of this area, this work was motivated to carry out this study and in essence recommend to the relevant authorities the efficiency of this technique in reducing the harmful effects of oil spill hazards on the residents of Ukwa West Local Government Area. This paper is meant to apply the techniques involved in the bioremediation of hydrocarbon polluted area. The outcome of the study will be of paramount importance to prove the efficiency in the use of bioremediation to clean up hydrocarbon pollution in the environment. This empirical study will be of immense benefit to the people as well as the appropriate authorities (National Oil Spill Detection and Response Agency, National Emergency Management Agency, National Environmental Standards and Regulations Enforcement Agency) charged with the responsibility of cleaning and restoring the polluted environment, also to the State and Federal government, various health agencies, oil companies working on various exploration sites by equipping them with some important facts which they need to incorporate in the clean-up and remediation of oil spill areas to preserve the environment and help host communities in resolving their soil pollution issues. It is based on this background that the current paper analyzed the bioremediation effects of *Bacillus subtilis* and *Pseudomonas aeruginosa* in crude oil spill affected soils in Uwaza community, Ukwa West LGA, Abia State, Nigeria. Thus, the study focused on ascertaining the effectiveness of *Bacillus subtilis* and *Pseudomonas aeruginosa* in cleaning oil pollution effects in affected soils in the study area.

2. Materials and Methods

1) Description of the Study Area

Owaza community is under Ukwa West local government area in Abia State (Figure 1). The study area is located geographically between latitude 4º09' N to 4º62' N and longitude 7º23' E to 7º´55' E (Figure 2). It has an area of 271 km² and a population of 88,555 at the 2006 census. The local government is the only crude oil producing area in Abia state. Its oil producing communities include: Owaza, Uzuaku, Umuokwor and Umuorie amongst others. The local government is divided into three main political units; Ipu unit, Asa unit and Ogwe unit. It practices rotational leadership system. Asa North is one of the political wards in it. The rainfall of the area occurs between March and October while the dry season prevails between November and February. The area is underlain by Benin formation, consisting of coarse sands interrupted by clay lenses of quaternary age. The study area experiences intense rainfall of an annual total of between 2000mm and 2300mm. This is induced by excessive evapotranspiration in the urban area due to prevalent high temperature. The average daily temperature experienced in the study area is about 28^0C in the dry season and 24^0C during the wet season (Nkwocha and Diene, 2010). The vegetation of the area is found within the rainforest zone. The vegetation types are characterized by tall trees about 30m (close to each other) and are interlaced with broad canopies which prevent direct temperature from impacting the soil. The examples of such trees are Iroko, Obeche, Mahogany, Oil palm amongst others. The soil types in the area are predominantly sandy and loamy soil which is a characteristics of coastal areas. The soil have relatively high porosity, infiltration capacity and low water retention capacity. Unprecedented loss of agriculture land use space has intensified agriculture in this area. Continuous cropping is the predominant agricultural practice in the area. Crops cultivated include; yam, cassava, maize, cocoa, oil palm etc.

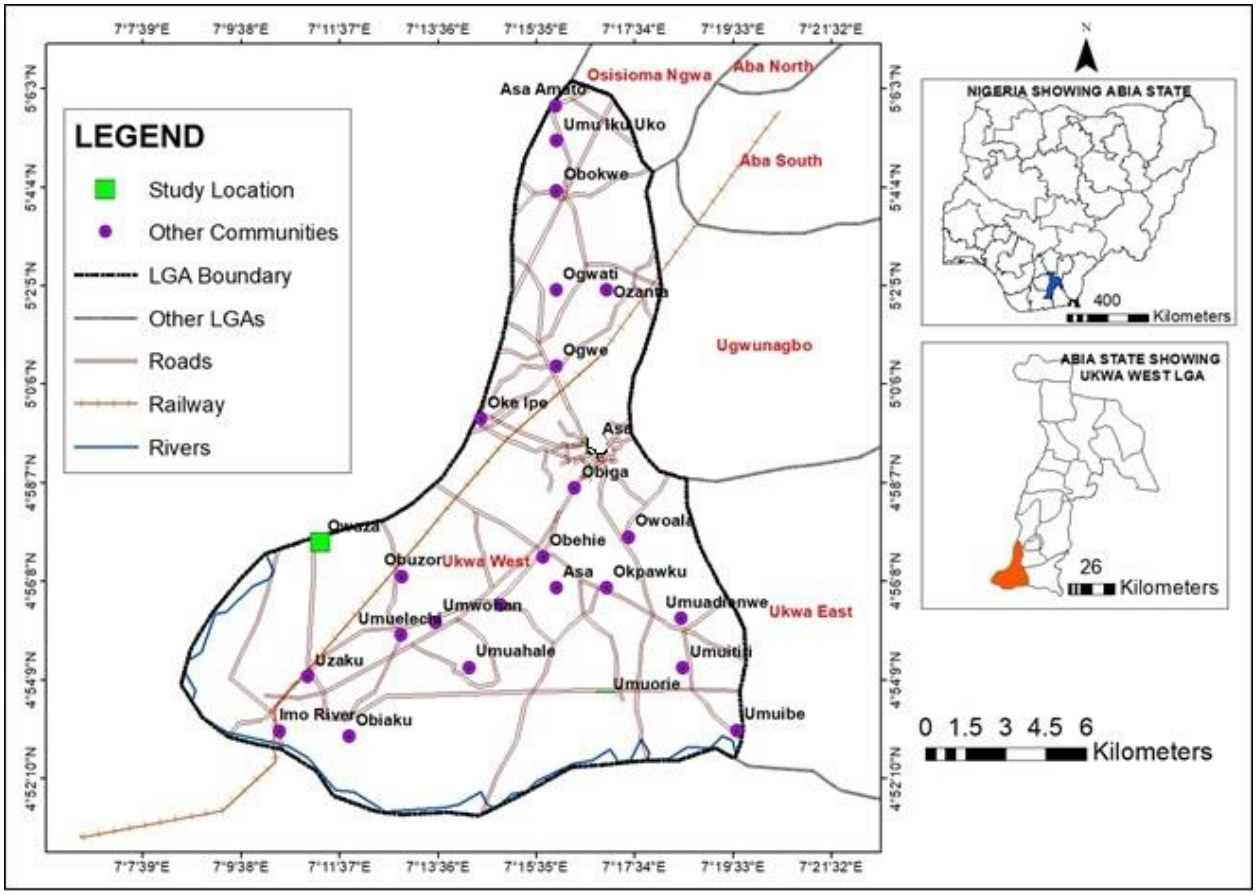

Figure 1: Ukwa West LGA (Source: Google Earth, 2024)

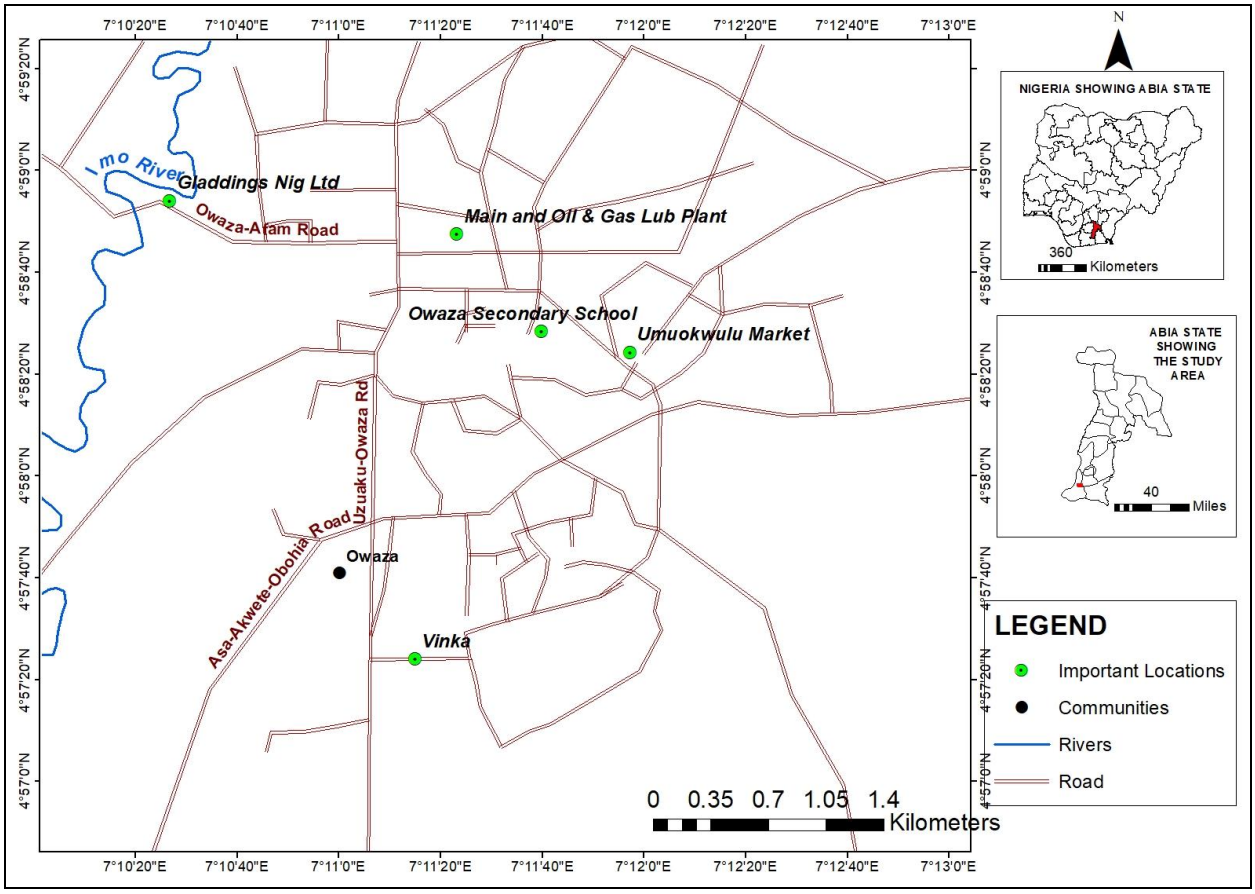

Figure 2: Owaza (Source: Google Earth, 2024)

2) Data Acquisition

The primary data sources were employed for this study. The primary data sources included utilizing the point samples whereby soil samples were collected at random locations within the crude oil spill affected soils in Owaza community. The biodegradation experiments were carried out for 24 days in a nursery shed under natural environmental conditions. The experiments were carried out in four sets. Each of the sets had a control which was not with Cow dung or bacterial inoculation. The entire experimental setup were done in duplicates so that the collection of the samples will not disturb the degradation. The samples were labelled using a combination of alphabets and numeric figures to differentiate each sample from the other. The samples were carefully transported to the laboratory for further laboratory analysis. The laboratory experiments involved well-structured series of analysis on the different soil samples inoculated with *Bacillus subtilis* and *Pseudomonas aeruginosa*.

3) Data Collection Techniques

The instruments for collection of the soil samples included a clean and sterilized hand trowel, a clean plastic which was used to collect 500gramms of the soil samples at each point of collection, and hand gloves for protecting the hands. Soil samples of 500 g each were collected aseptically with a clean hand trowel at three different points (at a depth of 0-15cm). The modified method of Odokuma and Dickson, (2003) was used. Each of the bacterial isolates were first cultured in 100ml Erlenmeyer flasks containing nutrient broth medium at 30^0C and 100rpm in a shaker incubator for 48 hours to increase the inoculum size. 1ml of each isolate was used to count in a Neubauer Haemocytometer and an inoculum size of 10^6 per ml was obtained. 100mls of normal saline solution containing 10^6 cells per ml was subsequently used to inoculate each soil sample. The inoculated soils were then mixed thoroughly using a sterile spatula to ensure uniform distribution of the bacterial cells. The samples were labelled using a combination of alphabets and numeric figures to differentiate each sample from the other. The samples were carefully transported to the laboratory for further laboratory analysis. The experimental design for the bioremediation process of crude oil affected soils are displayed on Table 1.

Table 1: Experimental design for the bioremediation process

Sterile	Non-sterile
BA + Cow dung + Polluted soil	BA + Cow dung + Polluted soil
500g + 500g	500g + 500g
PS + Cow dung + Polluted soil	PS + Cow dung + Polluted soil
500g + 500g	500g + 500g
BA+ PS + Cow dung + Polluted soil	BA + PS + Cow dung + Polluted soil
500g + 500g + 500g + 500g	500g + 500g + 500g + 500g
Control soil	Control soil
500g	500g

BA = *Bacillus subtilis;* PS = *Pseudomonas aeruginosa*

Appropriate grammes of the polluted and unpolluted soil samples were sterilised at 121^0 for 15 minutes and this was done for 3 consecutive times so as to exclude all viable micro-organisms present. This was done to ensure that only the inoculated bacterial isolates will degrade the oil contaminant present in the soil in order to determine their bio degradative ability. 20ml of sterile water was added to the soil at an interval of 4 days and mixed thoroughly so as to prevent drying of the soil and this was done throughout the period of the experiment. 50g of the soil samples were collected every eight days to extract residual oil and this was done by thoroughly mixing the soil and then using a sterile metal spoon to take the sample so as to have true representation of the treated soil sample. The bioremediation experiment results from the introduction of the two bacterial isolates into the different soil samples. The bacterial cell counts of the microorganisms were recorded as well as the total petroleum hydrocarbon results which were recorded alongside at the respective intervals of 5, 10, 15, 20 and 25 days for the study.

4) Data Analysis

The analysis was carried out in the microbiology laboratory of Nnamdi Azikiwe University Awka. The descriptive statistics using means, densities and Tables were employed for data presentation and analysis.

3. Results of the Analysis

Effects of *Bacillus subtilis* on oil spill affected soil

The information on Table 2 showed the mean population densities of *Bacillus subtilis* during bioremediation experiment. The information on Table 3 revealed that TPH on the fifth day reduced from 135.78 mg/kg to 119.28 mg/kg which represents a 12.2% decrease, on the tenth day the TPH reduced to 105.36 mg/kg representing a 10.2% decrease from the fifth day, on the fifteenth day the TPH reduced to 84.24 mg/kg which represents a 38.0% decrease from the start date, on the twentieth day the TPH reduced to 72.51 mg/kg representing a 46.6% decrease from the start date and a 8.6% decrease from the fifteenth day. Lastly, on the twenty fifth day the TPH reduced from 72.51 mg/kg to 61.93 mg/kg representing a 7.8% decrease from the twentieth day. However, from the start date to the twenty fifth day, a total of 54.4% reduction was experienced. The highest rate of reduction was found to have occurred on the fifteenth day whereas the total rate of reduction from the first day to the end of the experiment was 54.4%.

Table 2: Mean population density of *Bacillus subtilis* during bioremediation experiment (Cfu/g X 10^6)

Set up	Sterile						Non-sterile					
	500:500						500:500					
	Period (Days)						Period (Days)					
	0	5	10	15	20	25	0	5	10	15	20	25
BA	0.16	0.14	0.18	0.24	0.25	0.29	1	1.3	1.4	2.1	2.4	2.8
Control	0.09	0.11	0.12	0.13	0.14	0.17	0.86	0.89	0.92	0.95	0.99	1.2

BA- *Bacillus subtilis*

Table 3: Concentration of Residual Total Petroleum Hydrocarbon (TPH)

Experimental soil samples						
Period (days)	0	5	10	15	20	25
TPH (mg/kg)	135.78	119.28	105.36	84.24	72.51	61.93

Effects of *Pseudomonas aeruginosa* on oil spill affected soil

The information on Table 4 showed the mean population densities of *Pseudomonas aeruginosa* during the experiment. The information on Table 5 showed the results from the start date to the end date. The results therefore revealed that on the fifth day TPH reduced from 135.78 to 125.72 which represents a 7.4% decrease, on the tenth day the TPH reduced to 110.02 representing a 12.4% decrease from the fifth day, on the fifteenth day the TPH reduced to 83.46 which represents a 24.1% decrease from the tenth day, on the twentieth day the TPH reduced to 72.95 representing a 12.6% decrease from the fifteenth day, on the twenty fifth day the TPH reduced from 72.95 to 61.49 representing a 15.7% decrease from the twentieth day. The highest rate of reduction was found to have occurred on the fifteenth day whereas the total rate of reduction from the first day to the end of the experiment was 54.71%.

Table 4: Mean population density of *Pseudomonas aeruginosa* (PS) during bioremediation experiment (Cfu/g X 10^6)

Set up	Sterile						Non-sterile					
	500:500						500:500					
	Period (Days)						Period (Days)					
	0	5	10	15	20	25	0	5	10	15	20	25
PS	0.2	0.22	0.25	0.27	0.28	0.3	1.2	1.6	2.2	2.3	2.5	2.7
Control	0.09	0.11	0.12	0.13	0.14	0.17	0.86	0.89	0.92	0.95	0.99	1.2

Table 5: Concentration of Residual Total Petroleum Hydrocarbon (TPH)

Experimental soil samples						
Period (days)	0	5	10	15	20	25
TPH (mg/kg)	135.78	125.72	110.02	83.46	72.95	61.49

Effects of *Bacillus subtilis* and *Pseudomonas aeruginosa* on oil spill affected soil

The information presented on Table 6 showed the population densities of *Bacillus subtilis* and *Pseudomonas aeruginosa* during the experiment. The information presented on Table 7 the level of degradation of TPH in the polluted soils. For the Owaza soil sample, the TPH On the fifth day reduced from 135.78 to 106.19 which represents a 21.79% decrease, on the tenth day the TPH reduced to 98.47 representing a 7.27% decrease from the fifth day, on the fifteenth day the TPH reduced to 76.51 which represents a 22.40% decrease from the tenth day, on the twentieth day the TPH reduced to 54.22 representing a 29.13% decrease from the fifteenth day, on the twenty fifth day the TPH reduced from 54.22 to 41.08 representing a 24.23% decrease from the twentieth day. The highest rate of reduction was found to have occurred on the twentieth day whereas the total rate of reduction from the first day to the end of the experiment was 69.75%.

Table 6: Mean population density of *Bacillus subtilis* (BA) and *Pseudomonas aeruginosa* (PS) during bioremediation experiment (Cfu/g X 10^6)

Set up	Sterile						Non-sterile					
	500:500						500:500					
	Period (Days)						Period (Days)					
	0	5	10	15	20	25	0	5	10	15	20	25
BA+PS	0.21	0.24	0.27	0.33	0.32	0.35	1.4	1.9	2.4	2.8	2.9	3.2
Control	0.09	0.11	0.12	0.13	0.14	0.17	0.86	0.89	0.92	0.95	0.99	1.2

Table 7: Concentration of Residual Total Petroleum Hydrocarbon (TPH)

Experimental soil samples						
Period (days)	0	5	10	15	20	25
TPH (mg/kg)	135.78	106.19	98.47	76.51	54.22	41.08

4. Discussion

Findings of the study revealed that the population densities of *Bacillus subtilis* (BA) and *Pseudomonas aeruginosa* (PS) and when the two are combined during the experiment (Cfu/g X 10^6) increased between the start date and end date (25 days). The rate of degradation of total petroleum hydrocarbon varied. Thus, *Bacillus subtilis* and *Pseudomonas aeruginosa* can lead to significant reduction in hydrocarbon contents in crude oil polluted soils overtime, especially when more of days can be deployed for the experiment. The variation in results as regards percentage reduction rates in total petroleum hydrocarbon indicated 54.4% for *Bacillus subtilis*; and 54.7% for *Pseudomonas*

aeruginosa; and for both (*Bacillus subtilis* and *Pseudomonas aeruginosa*) it was 69.8%. Thus, more hydrocarbon contents were reduced from the crude oil spill affected soils in the study area. However, the experiment further revealed that when both bacteria isolates (*Bacillus subtilis* and *Pseudomonas aeruginosa*) were introduced into the bioremediation experiment higher hydrocarbon contents was reduced from the crude oil affected soils. These findings aligns with Mukherjee et al., (2017) and Song et al., (2017) who discovered that the diversity and population of soil bacteria as well as their physiological activities will to a large extent determine the level of degradation of hydrocarbons content in a polluted soil. Similarly, Pirnik *et al*., (1974) and Adegbite *et al*., (2020) have earlier reported that bacterial isolates feeds on the hydrocarbon contents in the crude oil polluted soils and biodegrades it at an efficiency rate of between 0.13% and 50%.

5. Conclusion and Recommendation

The focus of the study which was based on bioremediation of crude oil spill affected soils in Owaza community. The study made some findings which were: the study discovered that the addition of bacteria isolates into the crude oil spill affected soils increased their population densities over the experimentation period. Thus, higher percentage of petroleum hydrocarbon was liberated from the crude oil spill affected soils after 25 days of observation. Therefore, the addition of bacteria isolates of *bacillus subtilis* and *Pseudomonas aeruginosa* was discovered to be effective in the bioremediation of the crude oil spill affected soils Owaza community under Ukwa West LGA in Abia state. The study recommends that: research expansion is therefore needed in this area for effective bioremediation techniques in cases of crude oil pollution; research of this nature is very expensive and time consuming; thus, the government can support through adequate funding that will help to reduce cost and promote more accurate findings; the activities of the oil multinationals should be monitored at all levels in order to reduce their environmental impacts especially as it has to do with occasional crude oil spills in the study area.

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