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Microremediation of crude oil polluted soil using four individual and consortia of microorganisms

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ABSTRACT

This study was carried out to evaluate the potentials of Bacillus subtilis, Micrococcus luteus, Aspergillus niger and Candida spp to accelerate the remediation of soil polluted with crude oil. This was done for each microbe and as consortia of microbes after four weeks of incubation. The levels and the types of the petroleum hydrocarbon present in the soils samples at the beginning and after four weeks were determined using the gas chromatographic technique. The results obtained showed that the hydrocarbons were more reduced in soils inoculated with the microbes than in soils without any microbe. The highest reduction of the petroleum hydrocarbons were noticed in soils inoculated with B. subtilis alone followed by the soil inoculated with a consortium of the four microbes. Soil inoculated with Candida spp alone had the lowest reduction of petroleum hydrocarbon. The other soil parameters (soil pH, soil temperature and soil nutrient levels) measured were also affected by the growth of the microbes. There were statistical differences among the parameters tested in the different soil treatments ($P<0.05$; $P< 0.01$; $P< 0.001$). From the results obtained, it is suggested to use B. subtilis as single culture for cleaning up areas contaminated with crude oil and when the microbes are to be applied as a consortium, it will be better to combine the four microbes.

Keywords: Consortium, remediation, crude oil, pollution

INTRODUCTION

The presence of petroleum hydrocarbons in the environment is generally considered a public health and an ecological hazard. There are enormous economic and environmental impacts of oil pollution (Ezenne *et al.,* 2014). The impact of crude oil pollution tends to persist in soils until remediation measures are applied to such soils. To alleviate the problems faced with crude oil contamination, different techniques are being used to clean up sites contaminated with crude oil. Bioremediation through hydrocarbon biodegradation using selected microbial organisms has provided a favorable opportunity for cleaning crude oil contaminated soil because it is environmentally friendly and cost effective. The findings of Milic *et al.* (2009), showed that with the application of bioremediation techniques, the concentration of total petroleum hydrocarbons (TPH) can be reduced by 89% during a five-and-half month period.

Bioremediation of oil contaminated soil is a low cost technique and does not lead to secondary pollution. The stimulation of the biodegrading potential of indigenous microbial communities through bioaugmentation with mixed bacterial cultures (consortium) had been reported in some studies (Odokuma and Dickson, 2003, Onwurah, 2003 and Odokuma and Inor, 2002). Using consortia of microbes for bioremediation seems to be better than the use of individual microbes for bioremediation of petroleum hydrocarbon contaminated sites for some reasons. According to Thapa *et al.* (2007), in such consortia, biodegradation of different petroleum compounds occurs simultaneously but at different rates because different species of microbes preferentially attack different compounds. This leads to the successive disappearance of individual components of petroleum over time. Synergistic actions of the microbes increase the rate crude oil remediation.

The advantages of employing mixed cultures as opposed to pure cultures in bioremediation have been widely demonstrated (Ghazali *et al.,* 2004).The work of Hussein *et al.* (2012) showed that by using the mixed bacterial consortia which can efficiently degrade crude oil components, higher percentages of oil degradation can be achieved. A composition of different microorganisms having all possible enzymatic pathways can decompose more efficiently a complex mixture of hydrocarbons in soil, fresh and sea water than single microorganisms can. Thus complete mineralization of substrate can be achieved (Matvyeyeva *et al*., 2014). Various studies have examined the ability of consortia of hydrocarbon degrading microbes to degrade different petroleum products and the results have been encouraging, some species utilize the intermediates of degradation of original hydrocarbon produced by other species leading to complete degradation oil (Marquez-Rocha *et al.,* 2001; Atlas 1981). Omotayo *et al.* (2014) reported that re-inoculation of the contaminated soil with the rhizosphere bacteria consortia showed a higher degradation potential for use in the remediation of oil-polluted sites. Also, Malik and Ahmed (2012) studied the degradation of petroleum hydrocarbons by oil field isolated bacterial consortium, and found out that consortium gave a better ability of degrading long chain n-alkanes and crude oil at high concentration.

Although reports on the use of microorganisms to bioremediate crude oil contaminated soil are available, none to the best of our knowledge on the use of a consortium of *Bacillus subtilis, Micrococcus luteus, Aspergillus niger* and *Candida sp* is readily available. Therefore this study was carried out to evaluate the potentials of these microorganisms to enhance crude oil remediation as single and mixed cultures. Specific objectives of this study were to (i) examine the microbial response to crude oil contamination and assess their growth over a period of time and (ii) assess the ability of such microorganisms to remediate and impact on crude oil contaminated soil as single cultures and as mixed cultures.

MATERIALS AND METHODS

The soil used for the study was obtained from the Botanical garden of the University of Lagos, Akoka Lagos Nigeria while the crude oil used was Bonny light obtained from Shell petroleum Warri, Nigeria. The microorganisms used (*Bacillus subtilis, Micrococcus luteus, Aspergillus niger* and *Candida sp.*) were collected as pure isolates from the Microbiology Department of Nigerian Institute of Medical Research Yaba, Nigeria.

The microorganisms were cultured in nutrient broth (for the bacterial isolates) and

potato dextrose broth (for the fungi isolates) prepared as was described by Umar *et al.* (2013). The minimal salt medium used for the determining the microbial growth was prepared following the protocols described by Zuberer (1994). The soil was experimentally contaminated following the procedures outlined by Nwachukwu *et al.* (1999) by thoroughly mixing it with sterile bonny light crude oil to give 15% (wt/wt) pollution level. The soil was dispensed into beakers of 10cm x 10cm x 7cm (internal dimensions) with each beaker containing about 250g of soil. The contaminated soil was divided into seven groups and each group had three replicates. The replicates in each group represented a treatment and were inoculated each with 37.5ml of minimal salt broth containing either *B. subtilis, M. luteus, A. niger* or *Candida spp* as individual isolates, a combination of the bacterial isolates, a combination of the fungal isolates or a combination of all the four microbial isolates. The set ups were watered with 37.5mls of sterilized distilled water and were kept at room temperature for four weeks. A control experiment was set up in a similar manner but without any microorganism inoculated into it.

The microbial population densities were determined using standard plate count method (Nwachukwu and Ugoji, 1995). Soils samples were aseptically obtained from the set ups at beginning of study and after four weeks of study to determine the impact of the growth microbes on the hydrocarbon content, the pH and temperature and nutrient content of the soils. The pH of the soil samples were determined using the method described by Eckerts and Sims (1995). The sulphate, phosphate and nitrate levels of the soils were determined following the methods described by Ben Mussa *et al.* (2009)

The total petroleum hydrocarbon in the soil samples were extracted with dichloromethane. The level of residual TPH in each set up was determined using a gas chromatography- mass spectrophotometer (GC-MS) Hewlett Packard series 2 Model following the protocol outlined by La Dreu *et al.* (1997). Percentage degradation of the crude oil was determined as described by Marc *et al.* (2009) using the formula:

% degradation =

residual substrate in control - residual substrate in assay/Residual substrate in control X 100

Analysis of variance using one-way and two-way ANOVA was carried out and all statistical analyses were performed at $P \leqslant 0.05$ using Graphpad prism software for Windows v. 5™

RESULTS

Population Size of Microorganisms in Crude oil Contaminated soil at the beginning and the end of the Study

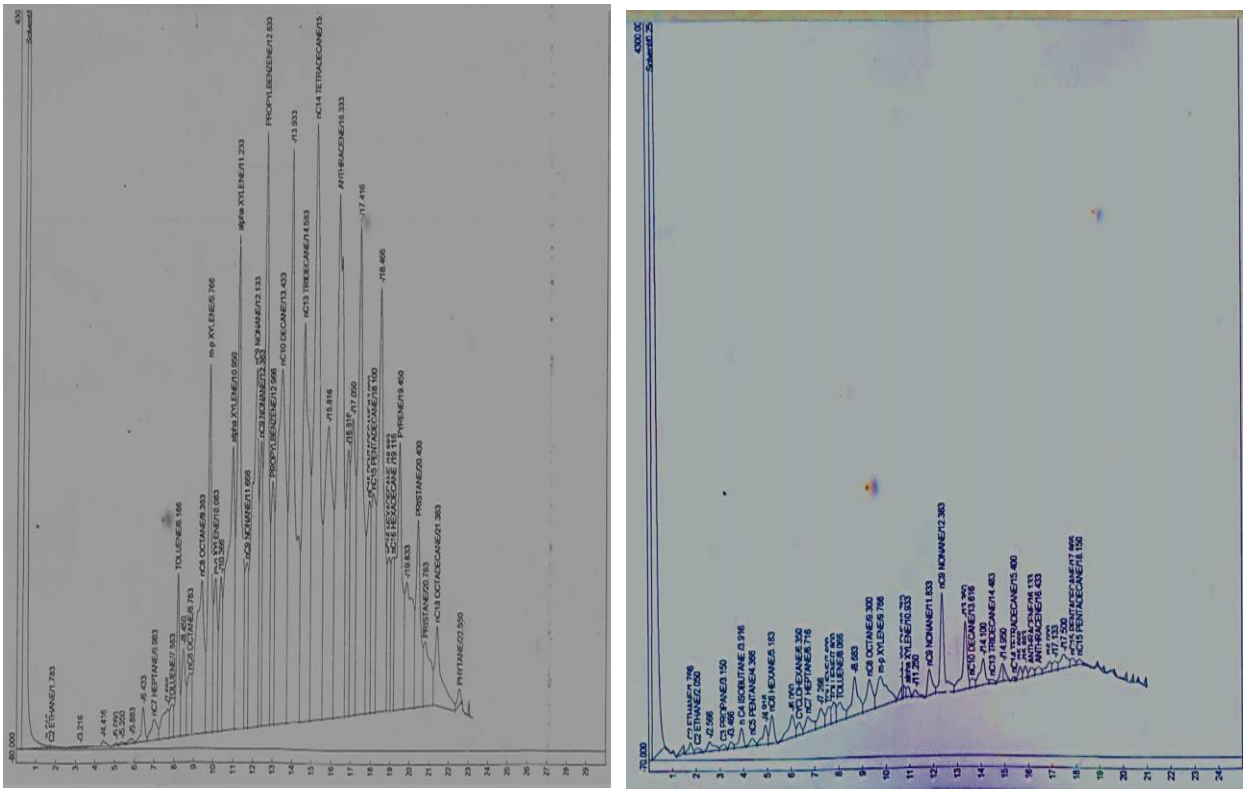
The population sizes of the microorganisms used in the study are shown in Table 1. The population sizes of the microorganisms were higher at the end of the study (week 4) than at the beginning of the study (week 0). The *B. subtilis* had the highest population size in both week 0 and week 4 followed by *M. luteus* and *A. niger* had the least population size in week 0 and week 4. The population sizes followed a general trend of *B. subtilis > M. luteus > Candida sp > A. niger*. The percentage growth of the microorganisms over the period of four weeks was in the trend of *B. subtilis > A. niger >M. luteus > Candida spp*.

Table 1: Population size of microorganisms in crude oil contaminated soil at the beginning and the end of the study ((cfu/gm) x 10^8)

Microorganism	Initial population size	Final population size	Percentage growth
B. subtilis	7.0	10.0	42.85
M. luteus	6.5	8.0	23.07
A. niger	4.8	6.2	29.17
Candida sp	6.0	7.0	16.67

Remediation of crude oil by the different microorganisms as single culture

The percentages of the hydrocarbons lost/degraded in the crude oil contaminated soil inoculated with different microorganisms are shown in Table 2. In the soils inoculated with individual microbes, there was 100% degradation of propyl benzene, octadecane and phytane by all the microbes. Tridecane and pristine were 100% degraded in the soils inoculated with *B. subtilis* and *M. luteus*. Also soils inoculated *B. subtilis, M. luteus* and *P. niger* had 100% degradation/loss of hexadecane and pyrene. In addition, 100% degradation of anthracene was noticed only in soil with *A. niger*. Various degrees of degradation of the other hydrocarbons were noticed in soils inoculated with the different microbes. Generally, soils inoculated with *B. subtilis* had the highest percentage degradation while the soil inoculated with *Candida sp* had the least percentage degradation of the hydrocarbons except for the case of heptane. Also, of all the hydrocarbons, heptane was the least degraded by the microbes. The percentage degradation of heptane and toluene was significantly higher ($P<0.01$) in soil inoculated with *B. subtilis* than in the soils inoculated with Candida sp and *A. niger*. Figure 1 shows the gas chromatogram of the initial and final petroleum hydrocarbons level in crude oil contaminated soil. Figure 2 shows the gas chromatogram of the soil crude oil contaminated soils inoculated with different microorganism at the end of study (after four week)

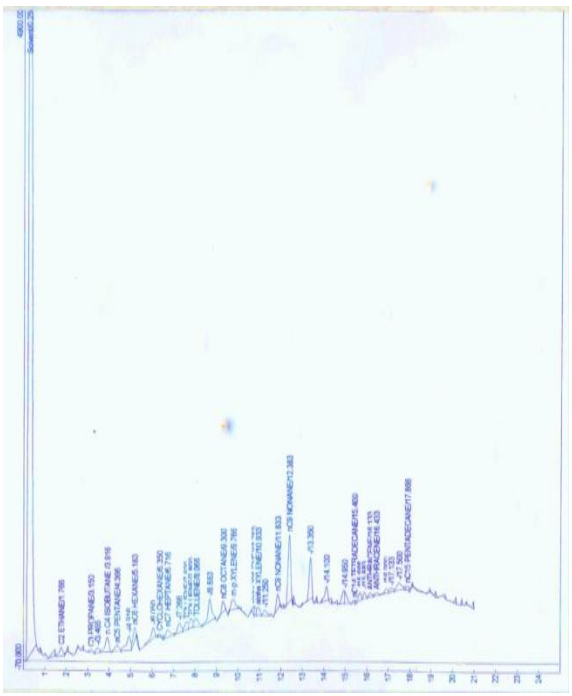


(a) (b)

Figure 1: Gas chromatogram of residual oil content for *Micrococcus luteus, Bacillus subtilis, Aspergillus niger* and *Candida spp* (treatment) at week 0 (a) and week 4 (b)

Table 2: The percentage of petroleum hydrocarbons lost/degraded in the soils contaminated with crude oil after four weeks

Hydrocarbon	**control**	***Bacillus subtilis***	***Micrococcus luteus***	***Aspergillus niger***	***Candida spp***
Heptane	2509.55	66.04	43.12	4.44	9.72
Toluene	2459.93	68.04	52.88	22.23	11.01
Octane	25943.66	97.30	91.80	81.35	77.53
Xylene	26123.49	96.12	89.34	67.88	59.89
Nonane	16306.33	74.05	68.48	47.62	30.67
Propyl benzene	16556.28	100	100	100	100
Decane	63202.99	100	99.78	98.43	96.80
Tridecane	54229.08	100	100	98.90	96.39
Tetradecane	75582.02	99.82	99.82	99.68	98.04
Anthracene	69146.99	99.64	99.69	100	96.59
Pentadecane	16792.82	98.60	98.43	96.14	86.26
Hexadecane	11022.32	100	100	100	81.07
Pyrene	31042.96	100	100	100	97.35
Pristane	9181.11	100	100	97.31	97.31
Octadecane	10931.45	100	100	100	100
Phytane	1090.32	100	100	100	100



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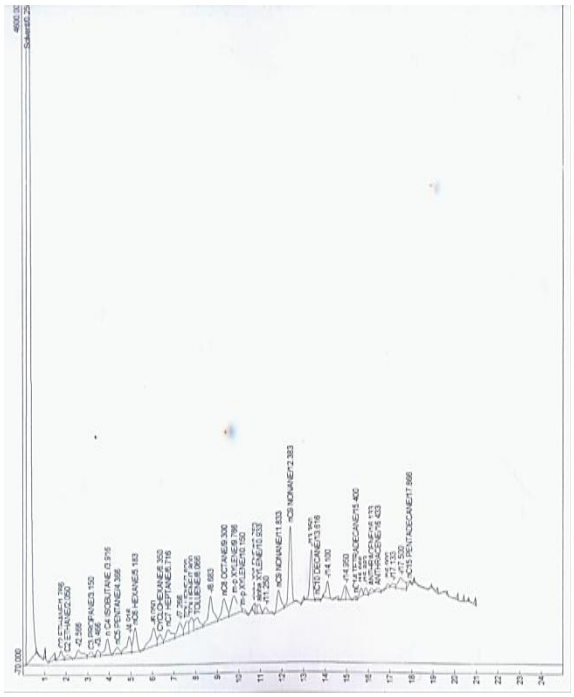

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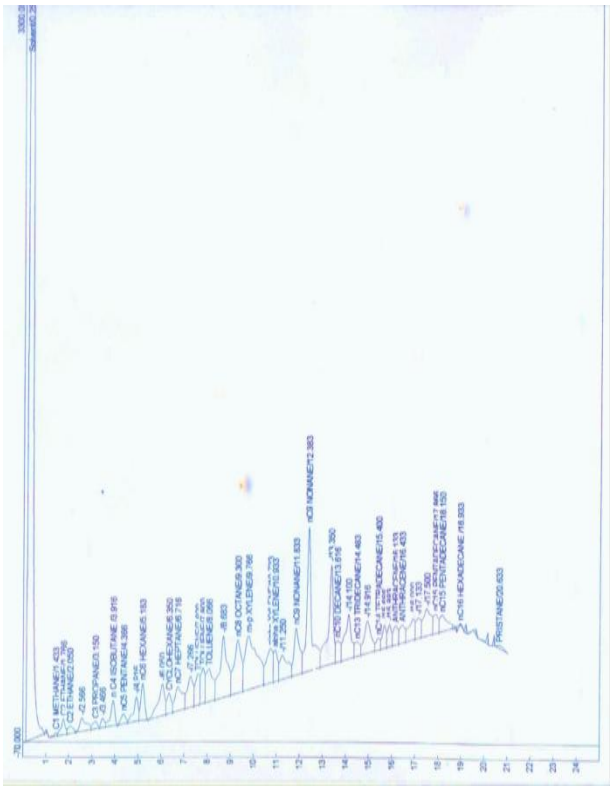
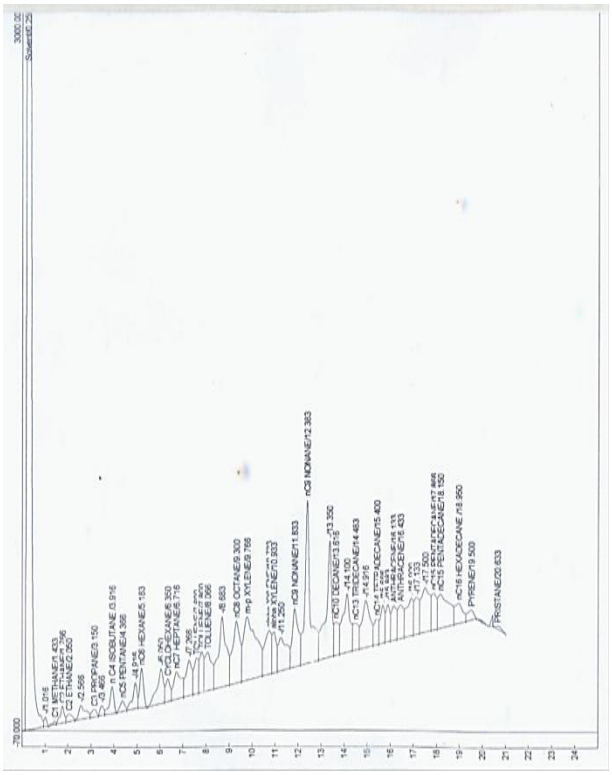


C



D

Figure 2: Gas Chromatogram of the hydrocarbons in the soil treated with different microogranisms at four weeks of growth (A = *B. subtilis,* B= *M. luteus,* C = *A. niger,* D= *Candida spp*)

Impact of Consortia of Microorganisms on the Petroleum Hydrocarbon Contents of Crude Oil Polluted Soil

The impact of different consortia of microorganisms on the petroleum hydrocarbon contents of crude oil polluted soil is shown in Table 3. The loss/degradation of the petroleum hydrocarbons was generally more in soil inoculated with the consortium of all the microbes than in the soils with either *B. subtilis* plus *M. luteus* or *A. niger* plus *Candida sp*. The degradation of the hydrocarbons was least in soil inoculated with *A. niger* plus *Candida sp*. Propyl benzene, pyrene, octdecane and phytane were totally degraded by all the microbial combinations. Hexadecane had 100% degradation in soils inoculated with *B. subtilis* plus *M. luteus* and a consortium of all the microbes. The combination of the bacterial strains (*B. subtilis* plus *M. luteus*) led to higher degradation or loss of the hydrocarbons in the soils than the combination of the fungal strains (*A. niger* plus *Candida sp*). The percentage of heptane and toluene lost from the soil inoculated with mixture of *A. niger* and *Candida sp* was significantly lower than that lost from the soil inoculated with mixture of all the four microbes ($P<0.05$)

Table 3: Percentage degradation of crude oil by consortium of isolates

Hydrocarbon	control	*B. subtilis + M. luteus*	*A. niger + Candida spp*	*B. subtilis + M. luteus + A. niger + Candida spp*
Heptane	2509.55	11.04	4.46	24.86
Toluene	2459.93	27.46	18.36	38.41
Octane	25943.66	83.14	80.04	86.87
Xylene	26123.49	71.61	65.13	79.41
Nonane	16306.33	55.65	41.77	56.99
Propyl benzene	16556.28	100	100	100
Decane	63202.99	99.19	97.87	98.92
Tridecane	54229.08	99.80	98.04	99.37
Tetradecane	75582.02	99.51	99.11	99.82
Anthracene	69146.99	99.65	98.15	99.06
Pentadecane	16792.82	98.95	92.73	96.69
Hexadecane	11022.32	100	98.93	100
Pyrene	31042.96	100	100	100
Pristane	9181.11	97.31	97.31	100
Octadecane	10931.45	100	100	100
Phytane	1090.32	100	100	100

Impact of Microbial growth on the pH of Crude Oil Contaminated Soil

The impact of the different microorganisms on the pH of crude oil contaminated soil is shown in Table 4. The pH of the soil without any of the microorganisms was higher at the beginning and at the end of the study than the pH of the soils inoculated with different microbes. Generally the pH of the soils increased at the end of the study. At the beginning of the study, the highest pH of 5.39 was recorded in the soil without microbial isolates and combination of the four isolates and the least pH of 5.26 was

recorded in the soil with *M. luteus*. However, at the end of the study, the highest pH of 6.53 was recorded in the soil with *M. luteus* and the least pH 6.40 was recorded in the soil with a combination of *B. subtilis* and *M. luteus*. Statistical analysis at 5% level of significance showed that the addition of crude oil to soil samples did not significantly affect the soil pH in this study as there was no significant difference in the absolute values of samples at ($P>0.05$). However, the pH of each treatment at the beginning of the study was significantly lower than the pH of the soil at the end of the study ($P<0.001$). combination of the four microbes had the least effect on the pH with 1.05 change.

Table 4: Impact of the different microorganisms on the pH of crude oil contaminated soil

SAMPLES	Initial Soil pH	Final Soil	Soil pH Change
Control	5.39	6.48	1.09
Bacillus subtilis	5.35	6.52	1.17
Micrococcus luteus	5.26	6.53	1.27
Aspergillus niger	5.34	6.45	1.11
Candida sp	5.28	6.52	1.24
M. luteus+B. subtilis	5.27	6.40	1.13
A. niger+ Candida sp	5.29	6.41	1.12
M.luteus+B.subtilis+A.niger+Candida sp	5.39	6.44	1.05

The Impact of Microorganisms on the temperature of crude oil contaminated soil

The temperature of the soil with the isolates varied at the different periods of study (table 5). The soil temperature was generally higher at the beginning of the study (week 0) than at the end of study (week 4).The temperature of the soils at the beginning of the study (week 0) was highest (35^oC) in soils inoculated with *B. subtilis* and mixture of the four microorganism but was lowest (34^oC) in the control soil, soils inoculated with *A. niger* and soil with a combination *A. niger* and *Candida sp*. Generally, at the end of the study (on week 4), the temperature of the soil varied between 32^oC (in the soil with no microbe, soil with *M. luteus* and soil with *Candida sp*) and 33.5^oC (in soil with mixture of *B. subtlis* and *M. luteus*). The soils inoculated with *B.subtilis* and *A.niger* had a temperature of 33^oC at the end of the study.

Impact of Microorganisms on the Nutrient content of Crude oil Contaminated Soil

The nutrient contents of crude oil contaminated with microorganisms at the end of week 4 are shown in Table 6. The total organic matter content, the percentage sulphate content, the percentage phosphate content and the percentage nitrate content of the soil after four weeks study are shown in table 5. The organic matter content of the soil in week 4 was highest (86.11%) in the soil without any microbe followed by the soil inoculated with a combination of *Candida* and *A. niger* and was lowest (33.11%) in the soil inoculated with *B. subtilis*. Also, the highest level of sulphate (82.14%) was recorded in the soil without any microorganism followed by the soil inoculated with a mixture of *Candida sp* and *A. niger* (66.99%) and was lowest (29.55%) in the soil inoculated with *B. subtilis*. The

phosphate and nitrate levels in the soil followed same trend. The nutrient levels in the soils with *B. substilis, M. luteus* and *A. niger* were lower than the nutrient levels in the soils without any microorganism (P<0.001). However, only the total organic matter and nitrate levels in the soil inoculated with *Candida spp* were significantly lower than the organic matter and nitrate content of the soils without microorganism (P<0.05). There was significant difference between the nutrient levels in the soils inoculated with different microorganisms (P<0.05, P<0.01 and P<0.001)

Table 5: The temperature of crude oil contaminated soil inoculated with different microorganisms at the beginning and at the end of the study (4 weeks)

SAMPLES	Initial soil temperature	Final Soil temperature	Soil temperature Change
Control	34	32	2.0
Bacillus subtilis	35	33	2.0
Micrococcus luteus	34.5	32	2.5
Aspergillus niger	34	33	1.0
Candida sp	34.6	32	2.6
M.luteus+B. subtilis	34.5	33.5	1.0
A.niger+Candida sp	34	32	2.0
M.luteus+B.subtilis+A.niger+Candida	35	33	2

Table 6: Nutrient content of Crude oil Contaminated Soil (the letters in superscript show the level of significance difference between the nutrients in the control and the soils inoculated with microorganisms. a = P<0.05; b = P<0.01; c = P<0.001)

Sample\Parameters	**Total Organic Matter (mg/ml)**	**Sulphate (SO_4^{2-}) (mg/ml)**	**Phosphate (PO_4^{2-}) (mg/ml)**	**Nitrate (NO_4^{2-}) (mg/ml)**
Control	86.11	82.14	48.47	70.78
Bacillus subtilis	33.11c	29.55c	12.39c	20.58c
Micrococcus luteus	39.78c	35.91c	16.71c	24.89c
Penicillum niger	60.77c	63.89b	32.01b	45.11c
Candida spp	73.05a	71.09	40.05	57.13a
M. luteus+ B.subtilis	51.09c	49.42c	27.11c	37.55c
A.niger+ Candida sp	67.78b	66.99a	35.12a	47.82c
M.luteus+B.subtilis+A.niger +Candida sp	45.15c	43.48c	22.03c	32.18c

DISCUSSION

Significant removal of petroleum hydrocarbons took place in the soil samples inoculated with petroleum-degrading active bacteria compared with the removal of petroleum hydrocarbon in the blank sample. The degradation of the petroleum hydrocarbon in the inoculated soil with the microbes is also similar to the findings of Milic *et al.* (2009) who reported that the

metabolically active microbial community play key role in the hydrocarbon biodegradation. The strains of microorganisms evaluated in this study showed differential oil degradation capacity and patterns. This conforms to the reports of some earlier studies which demonstrated that microbes have different capabilities of reducing /degrading hydrocarbons in soils (Ijah and Ukpe, 1992; Okoh, 2006). The higher degradation of the hydrocarbons in soils inoculated with either single culture or combined cultures of the bacterial strains than those in soils inoculated with fungal strains is in similarity with the reports of Umanu *et al.* (2013). The higher degradation of the hydrocarbons in the soils inoculated with bacterial strains compared to the degradation in the soils with fungal strains could be due to higher utilization of the hydrocarbons by the bacteria. As was noticed in this study, the growth of the individual microorganisms varied and *B. subtilis* had the highest growth over the period of study. Such can increase their activities and thus degradation of the higher carbons. Such is true for *B. subtilis* which increased better than the other microorganisms and coincidentally higher levels of degradation of the hydrocarbons were noticed in the soils inoculated with the same microorganism. This conforms to the reports of Ryan *et al.* (2007) who reported that fungi are usually slow growers and are less efficient in degradation than bacteria.

In the present work, the result of the degradation of the petroleum hydrocarbons by the consortium of the microorganisms is similar to that obtained by Nwadinigwe and Onyeidu (2012) who demonstrated that a consortium of *Pseudomonas* and *Bacillus* treatment has capabilities of bioremediating crude oil. According to Nwadinigwe and Onyeidu (2012), the toxic components of the crude oil might have been metabolized by these bacteria, which might have produced enzymes that could use petroleum as a substrate, thus degrading the crude oil. This agrees with the suggestion of Onwurah [2003], who reported that crude oil-degrading bacteria such as *Pseudomonas, Micrococcus*, and *Bacillus* could metabolize the toxic components of crude oil, leading to its degradation. Microorganisms produce enzymes in the presence of carbon sources which are responsible for attacking the hydrocarbon molecules. According to Thapa *et al.* (2007), in such consortia, biodegradation of different petroleum compounds occurs simultaneously but at different rates because different species of microbes preferentially attack different compounds. This can be attributed to the total loss or high percentage degradation of most hydrocarbons in soils with the combination of the four microorganisms as against the levels of degradation noticed in soils with other microbial combinations. Also, the improved degradation of the hydrocarbons in soils inoculated with mixed cultures of bacterial and fungal strains points to the fact the ability of fungi to biodegrade can be improved by mixing them with bacteria strains.

The results of higher degradation in the soils inoculated with the microorganisms suggest that the organisms used in this study could be responsible to the enhancement of degradation of the oil in the soil. This is similar to the observations of Omotayo *et al.* (2013) that the bacterial isolates were responsible for the oil degradation. Similar observations have also been reported by Adebusoye *et al.*. (2007), Mandri and Lin (2007) and Obayori *et al.*. (2009). The differences in the level that the different hydrocarbons were degraded in the various soils show that there are different capabilities of the microbes in degrading each hydrocarbon. This could be explained that consortium of hydrocarbon utilizing

microbes used in the study have different capability (Olanipekun, *et al.* 2015).

It is known that any factor that favours microbial growth can enhance their activities hence biodegradation. The growth and activity of soil microorganisms are very much dependent on the soil pH (Kalita and Devi, 2012). Soil pH is an important factor that can affect soil microorganism diversity (Killham, 2006). Alrumman *et al.* (2015) reported that relatively low soil pH values (6.0–6.5) soils are associated with higher heterotrophic bacteria CFU counts. The soil pH we observed at the end of the study for the soil treated with the different microorganisms are within this range. This could have accounted for the higher microbial load at the end of the study than at the beginning of the study. In biodegradation process, pH of the soil plays a predominant role (Njoku *et al..,* 2014). According to the findings of Kalitha and Devi (2012), significant degradation of petroleum hydrocarbons takes place at pH 4.5 and 7.5. According to the observations of Dibble and Bartha (1976), optimal mineralization of oily sludge takes place in a pH range of 5.0 to 7.8. Also Verstraete *et al.*. (1975) reported that a doubling rate of biodegradation of gasoline took place when the pH of soil was adjusted from 4.5 to 7.4. The pH of the soils inoculated with microbes is in the range of 5.39 and 6.52. These are within the range of optimal pH for biodegradation of the oil stated by the different researchers. The higher pH of soils inoculated with the microbes at end of the study compared to that of the control soil may be a pointer of the microbes favouring the change of soil from a more acidic nature to a less acidic or neutral one. Like Njoku *et al.* (2014) suggested, the differences in the pH of the soils with different microorganisms may infer that the various microbes used in the study have different abilities to affect soil pH.

Apart from pH, other factors like moisture, organic matter content and temperature influence biodegradation of crude oil. According to Ayotamuno *et al.*. (2006), appropriate soil moisture level is a good factor for bioremediation petroleum polluted soils. Ayotamuno *et al.* (2006) also showed that there is a negative relationship between soil nitrogen content and remediation period. Temperature plays very important biodegradation of petroleum hydrocarbon. Highest biodegradation rates generally occur in the range of 30-40^{o}C in soil environments (Jain *et al.* 2011). The reports of Chainuea *et al.* (2005) showed that addition of nutrients to soil stimulate microbial growth and degradation of petroleum hydrocarbon. Such microbes are known to use the nutrient for their growth and activities hence the reduction in the nutrient levels of the soils. These could be the cause of the reduced nutrient (sulphate, nitrate and phosphate) level noticed in the soils inoculated with the microbes when compared with level in the control soil. Also, a comparison of the nutrient levels in the soils with the different isolates showed an indication of lower nutrient contents in soils with higher hydrocarbon degradation. Manilal and Alexander (1991) reported that mineralization rates of contaminants are lower in soils a huge organic matter content which absorbs hydrophobic compounds. Such negative correlation between the nutrient content and the biodegradation of crude was also observed in this study and supports the opinion that use of the nutrients was highest in the media where the microbial activities (and biodegradation) were higher.

According to the findings of Paudyn *et al.* (2008), more degradation took place in soils with enough nutrient, moisture and oxygen. According to Rayner *et al.* (2007), natural attenuation of petroleum hydrocarbons in polar and subpolar soils is limited by low

nutrients, low temperatures, and water availability. This may imply the degradation of petroleum hydrocarbons is dependent on the level of nutrient, temperature and water availability. The extent of petroleum lost from the soils due to the microbial growth showed a relationship with the nutrient lost and microbial growth. *B. subtilis* showed the highest percentage growth within the study period and highest biodegradation of the petroleum hydrocarbons and the soil inoculated with the organized had the least nutrient level at the end of the study. This is a pointer to the fact that organisms that utilize the environment better can enhance remediation better that the others. This may also suggest that of the four microorganisms used in this study, *B. subtilis* utilizes and survives in crude oil contaminated soil more than the orders.

From the results obtained in this study, the four microorganisms have potentials to utilize petroleum hydrocarbon and enhance degradation of crude oil. Bacterial strains showed greater potential for hydrocarbon utilization and degradation as single and mixed cultures than the fungal strains. Using a combination of the four microorganisms for remediation of crude oil polluted soil is better than using either a mixture of the bacterial strains or a mixture of the fungal strains. We therefore recommend the use of a combination of microbial strains for remediation of crude oil polluted sites

REFERENCES

Adebusoye, S.A., Ilori, M.O., Amund, O.O., Teniola, O.D. and Olatope, S.O. (2007). Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. *World Journal. of Microbiology and Biotechnology*. 23, 1149-1159

Alrumman, S. A., Standing, D. B. and , Paton, G. I. (2015) Effects of hydrocarbon contamination on soil microbial community and enzyme activity. *Journal. of King Saud University – Science*, 27: 31–41

Atlas, R.M (1981). Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiology Review*, 45: 180-209.

Ayotamuno, M.J., Kogbara, R.B. and Taleat, M.O. (2006) Bioremediation of petroleum-hydrocarbon polluted agricultural soil at different levels of water application in Port Harcourt, Nigeria. *Journal. of Food, Agriculture and Environment*, 4 (3&4):214-217

Ben Mussa, S. A., Elferjani, H.S., Haroun, F. A. and Abdelnabi, F.F (2009) Determination of available nitrate, phosphate and sulphate in soil samples. *International. Journal. of PharmTech Research*. 1(3): 598-604

Chaıneau, C.H., Rougeux, G., Ye´pre´mian, C. and Oudot , J. (2005) Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil. *Soil Biology and Biochemistry* 37: 1490–1497

Ezenne, G.I., Nwoke, O.A. , Ezikpe, D.E., Obalum, S.E. and Ugwuishiwu, B.O. (2014) Use of poultry droppings for remediation of crude oil polluted soils: Effects of Application rate on total and polyaromatic hydrocarbon concentration. *International. Biodeterioration and Biodegradation*, 92:57-65

Ghazali, F.M., AbdulRahman, R.N.Z., Salleh, A.B. and Basri, M. (2004) Biodegradation of hydrocarbons in soils by microbial consortium. *International. Biodeterioration and Biodegradation*, 54: 61-67

Kalita, M. and Devi, A. (2012) Study on the effects of soil pH and addition of N-P-K fertilizer on degradation of petroleum hydrocarbon present in oil

contaminated soil. *International. Journal. of Chemical. and Petrochemical. Technology (IJCPT)*, 2 (3): 9-22

Le Dreau, Y., Jacquot, F., Doumenq, P., Guiliano, M., Bertrand, J.C. and Mille, G. (1997). Hydrocarbon balance of a site which had been highly and chronically contaminated by petroleum waste of a refinery from 1956-1992. *Marine Pollution Bulletin*. 34:456-468

Malik, Z.A. and Ahmed, S. (2012) Degradation of Petroleum Hydrocarbons by Oil Field Isolated Bacterial Consortium. *African Journal. of Biotechnology*, 11, 650-658.

Mandri, T. and Lin, J. (2007). Isolation and characterization of engine oil degrading indigenous microorganisms in Kwazulu-Natal, South Africa. *African Journal. of Biotechnology*. 6, 023-027.

Manilal, L.B. and Alexander, M. (1991) Factors affecting the microbial degradation of phenanthrene in soil. *Applied Microbiology and Biotechnology*, 35: 401-405

Marc, A., Diane, L., Gerald, T., Greer, C. and Fayolle-and Gulchad, F. (2009) Degradation of a mixture of hydrocarbons, gasoline and diesel oil additives by *Rhodococcus aetherivorans* and *Rhodococcus wratislaviensis*. *Applied and Environmental. Microbiology*. 75(24):7774-7782

Marquez-Rocha, F.J, Hernandez-Rodriquez, V. and Lamela, M.T. (2001). Biodegradation of diesel oil in soil by a microbial consortium. *Water Air and Soil Pollution*. 128: 313-320

Matvyeyeva, O. L., Vasylchenko, O.A. and Aliieva, O. R (2014). Microbial Biosurfactants Role in Oil Products Biodegradation. *International. Journal. of Environmental. Bioremediation and Biodegradation*, 2 (2), 69-74

Milić, J. S. Beškosk, V. P. Lići, M. V., Ali, S. A. M., Gojgić-Cvijović, G. Đ and Vrvić, M. M. (2009) Bioremediation of soil heavily contaminated with crude oil and its products: composition of the microbial consortium. *Journal. of Serbian Chemical. Society* 74 (4) 455–460

Njoku, K.L., Akinola, M.O., Nkemdilim, C.M., Ibrahim, P.M. and Olatunbosun, A.S. (2014) Evaluation of the Potentials of Three Grass Plants to Remediate Crude Oil Polluted Soil. *Current Advances in Environmental. Science* 2 (4): 131-137

Nwachukwu, S.U. and Ugoji, E.O (1995). Impacts of crude petroleum spills on microbial communities of tropical soils. *International. Journal. of Ecology and Environmental. Science*. 21:169-176

Nwachukwu, S.C.U; James, P and Gurney, T.R. (1999). Training of *Pseudomonas aeruginosa* as a polishing agent for cleaning environments polluted with hydrocarbons by step-up complex hydrocarbon utilization technique. *International. Journal. of Environmental. Education and Information*. 18(2): 53-66.

Nwadinigwe, A. O. and Onyeidu, E. G. (2012) Bioremediation of Crude Oil-Polluted Soil Using Bacteria and Poultry Manure Monitored through Soybean Productivity. *Polish Journal. of Environmental. Studies*. 21 (1): 171-176

Obayori, S.O., Ilori, M.O., Adebusoye, S.A., Oyetibo, G.O., Omotayo, A.E. and Amund, O.O. (2009). Degradation of ydrocarbons and biosurfactant production by Pseudomonas sp. strain

LP1. *World Journal. of Microbiology and Biotechnology*. 25, 1615-1623.

Odokuma L. O. and Inor M. N. (2002) Nitrogen fixing bacteria enhanced bioremediation of a crude oil polluted soil. *Global. Journal. Pure and Applied Science*. 8, (4), 455

Odokuma, L. O. and Dickson A. A. (2003) Bioremediation of a crude oil polluted tropical rainforest soil. *Global. Journal. of Environmental.* Science 2, (1), 29,

Okoh, A.I. (2006) Biodegradation alternative in the clean up of petroleum hydrocarbon pollutants. *Biotechnology and Molecular review*, 1 (20): 38-50

Olanipekun, O. O., Ogunbayo, A. O., Nwachukwu, S. C. U. and Bello, R. A. (2015) Comparative Study of Microbial Activities and Biodegradation-Abilities of Undefined Consortium in Some Hydrocarbon Contaminated Sites in the Niger Delta, Nigeria, *Journal. of Environmental. Protection*, 6, 138-145

Omotayo A. E., Egbomeade L.O., Taiwo O., Oyebamiji O.O. and Ilori M.O. (2013) Hydrocarbon Degradation by Free-Living Nitrogen-Fixing Bacteria. *Journal. of Scientific Research and Development*. 14, 75 - 84

Omotayo A.E., Shonubi O.O., Towuru E.G., Babalola S.E., Ilori, M.O. and Amund O.O (2014) Rhizoremediation of hydrocarbon-contaminated soil by *Paspal.um vaginatum* (Sw.) and its associated bacteria. *International. Research Journal. of Microbiology*. 5(1) 1-7

Onwurah, I. N. E. (2003) An integrated environmental biotechnology for enhanced bioremediation of crude oil contaminated agricultural land. *Bio-Research* 1, (2), 51,

Paudyn, K., Rutter, A., Rowe, R.K. and, Poland, J. S. (2008) Remediation of hydrocarbon contaminated soils in the Canadian Arctic by landfarming. *Cold Regions Science and Technology* 53: 102–114

Prescot, L. M., Klein, D. A. and Harley J.P. (2001) *Microbiology* 5Th Edition, McGraw Hill, Education, United States, PP. 32-60

Ryan, D., Leukes, W. and Burton, S. (2007) Improving the bioremediation of phenolic wastewater by *Tramates versicolor*. *Bioresource Technology* 98: 579-587

Thapa, B., Kumar, A., KC, and Ghimire, A. (2012) A Review on Bioremediation of Petroleum Hydrocarbon Contaminants in Soil. *Kathmandu University Journal. of Science, Engineering and Technology*. 8, (I), 164-170

Umar, H., Umar, A., Ujah, U.J., Hauwa, B., Sumayya, B.L and Shuaibu, M. (2013). Biodegradation of waste lubricating oil by Bacteria isolated from the soil. *Journal. of Environmental. Science, Toxicology and Food Technology*.3:62-67

Umanu, G., Akpe, A.R and Omoikhudu, A.R. (2013). Oil degradation assessment of bacteria isolated from used motor oil contaminated soils in Ota, Nigeria. *International. Journal. of Advanced Biological. Research*. 3(4): 506-513

Verstraete, W. Vanloocke, R. , DeBorger, R and Verlindne A. (1975) Modelling of breakdown and the mineralization of hydrocarbons in unsaturated soil layers. In: *Proceeding of 3rd International. Biodegradation Symposium*, Sharpley, J.M and A.M. Kalpan (Eds). Applied Science Publishers, London, pp 98-112.

Zuberer, D.A., (1994). Recovery and enumeration of viable bacteria. In: Methods in Soil Analysis. Part 2. Microbiological and Biochemical Properties. Pepper, I.L., 1995. *Environmental. Microbiology*. 2nd Edn., Academic Press, San Diego, pp: 232.