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2 **Hydrocarbon degradation potential of heterotrophic bacteria isolated from oil polluted**
3 **sites in sakpenwa community in rivers state.**
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6 **ABSTRACT**

7 In this study, hydrocarbon degradation potentials of heterotrophic bacteria isolated from oil-
8 polluted soil were examined; Samples were collected from Sakpenwa, an oil producing
9 community in Tai LGA of Rivers State, and analyzed for physicochemical and
10 microbiological properties using standard techniques. Hydrocarbon utilizing bacteria (HUB)
11 were isolated by vapour phase transfer method using mineral salt medium. They were
12 subjected to constant shaking in a standard laboratory shaker for 30 days in Bushnell -Haas
13 agar supplemented with 5% of crude oil. Fifteen (15) bacterial isolates were screened for
14 hydrocarbon degradation potentials of which five bacterial isolates exhibited high
15 hydrocarbon degradability. The degradation study was determined by monitoring colour
16 change, Optical density (OD), pH, Total Petroleum Hydrocarbon (TPH), Total Cuturable
17 Heterotrophic Bacterial Counts (TCHBC) and Total Hydrocarbon Contents (THC)
18 respectively for each isolate. The mean total culturable heterotrophic bacterial counts ranged
19 from 1.65×10^7 to 2.27×10^8 cfu/ml while the mean total culturable hydrocarbon utilizing
20 bacterial counts ranged from 1.09×10^4 to 3.9×10^5 . The optical density varied from 0.09 ± 0.02
21 - 2.57 ± 0.00 and pH ranged from 2.98 ± 0.09 - 6.98 ± 0.09 . The elimination of oil spilled in the
22 environment can be achieved by microbial degradation when added up with some physical
23 and chemical methods.

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26 **KEY WORDS:** hydrocarbon, heterotrophic bacteria, polluted soil, degradation potentials
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30 **INTRODUCTION**

31 In Nigeria, 80% of the crude oil used is supplied from the South-South region of the country.
32 Therefore, as a result of high oil exploration activities going on in this part of the country
33 over years (Abu and Chikere, 2006), substances like gaseous emissions, oil spills, effluents
34 and solid waste are discharged into the environment, thus, polluting the environment (Nweke
35 and Okpokwasili, 2004). From statistics, the biotic component of the soil occupied not greater
36 than 5% soil space; living microbes including bacteria, archaea and fungi are responsible for
37 80-90% of soil processes and formation such as recycling of nutrients, transformation of
38 organic matter, and maintenances of soil structure in microbial decomposers (Nannipieri *et*
39 *al.* 2003). Since microorganisms in the soil are involved in various biogeochemical processes,
40 soil activities largely depend on them (Mikkonen, 2008).

41 The degradation of hydrocarbons is influenced by many factors (temperature, relative
42 humidity, soil structure, soil moisture, soil pH, soil biota, pollutant's structure, dose, toxicity,
43 and bioavailability). A study on the degradation of hydrocarbon by aerobic heterotrophic
44 bacteria by Ichor *et al.*, (2014), took cognisance of some of these factors.

45 **MATERIALS AND METHODS**

46 **2.1 Study area and sample collection.**

47 The study site was located at the oil polluted sites in Sakpenwa community in Ogoni land,
48 Tai Local Government Area, Rivers State. Soil samples were collected 500m and 1000m
49 away from the major spill sites. Fifty grams (50g) of the oil-polluted soil samples were
50 collected from each of the sampling points using a soil sampler. The collected soil samples

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51 were transported in plastic nylon bags from the polluted sites to the Department of
52 Microbiology, University of Port Harcourt laboratory for analysis within 24 hours (Iheanacho
53 *et al.*, 2014).

54 2.2 Samples preparation

55 The soil samples collected were passed through a mesh sieve (2mm pore size) to remove
56 large particles and were thoroughly mixed. Thereafter, 5g of each soil sample was suspended
57 in 45 ml of distilled water. The suspended samples were mixed properly in a rotary shaker at
58 100 rpm at room temperature ($28 \pm 2^{\circ}\text{C}$) for 1 hour, 30 minutes to liberate the organisms into
59 the liquid medium (Iheanacho *et al.*, 2014). The pH of the samples was also taken.

60 2.3 Isolation and enumeration of total heterotrophic bacteria

61 The total culturable heterotrophic bacterial count for each degradation set-up was enumerated
62 using the streak plate method (Odokuma and Okpokwasili, 1992). Serial dilutions of the
63 samples were made and 0.1ml aliquot of the 10^{-1} to 10^{-4} dilutions of each sample were
64 transferred onto well dried, sterile nutrient agar plates (in triplicate) and incubated at 37°C for
65 24. After incubation, the bacterial colonies that grew on the plates were counted and sub-
66 cultured onto fresh nutrient agar plates using the streak-plate method in order to obtain pure
67 cultures of each colony. Discrete colonies on the plates were then transferred into nutrient
68 agar slants, properly labelled and stored at 4°C as a stock culture for preservation and
69 identification (Odokuma and Ibor, 2002).

70 2.4 Enumeration of total culturable hydrocarbon utilizing bacteria (TCHUB)

71 The enumeration of Total Culturable Hydrocarbon Utilizing Bacteria (TCHUB) was done by
72 applying the vapour phase method described by Atuanya and Ibeh, (2004). Appropriate
73 diluent of 0.5ml of the samples collected from the two different set up (1000m and 500m
74 away from a particular polluted site) labelled A and B respectively were inoculated into
75 modified Mineral Salt Agar medium (MSA). The medium was made of 0.42g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$;
76 0.297g KCl; 0.85g KH_2PO_4 ; 0.424g NaNO_3 ; 1.27g K_2HPO_4 ; 20.12g NaCl; 250mg
77 Amphotericin B (sold as Fungizone) and 20g agar powder. These were weighed out and
78 hydrated in 1000mL of sterile distilled water in a conical flask. The media was sterilized by
79 autoclaving at 121°C , 15Psi for 15min, before dispensing into sterile Petri dishes. The gelled
80 Mineral Salt Agar (MSA) was inoculated with 0.5ml of serial dilutions of the polluted soil
81 sites A and B sample respectively. Filter paper (Whatman No 1) was saturated with bonny
82 light crude oil, and the crude oil impregnated papers were aseptically placed onto the covers
83 of Petri dishes and inverted. The hydrocarbon saturated filter papers supply hydrocarbon by
84 vapour-phase transfer to the inoculums (Chikere and Chijioko-Osuji, 2006). The plates were
85 incubated at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for seven (7) days. Colonies were counted from triplicates and mean
86 values were record and colonies formed were stored as stock cultures at a temperature of 4°C
87 for preservation and identification.

88 2.5 Gram staining

89 Isolates were subjected to Gram's reaction to check whether they are Gram-positive or Gram
90 negative. A thin smear was made on a clean grease-free glass slide. The smear was air dried
91 and heat-fixed by rapidly passing it over the flame of a Bunsen burner three times. The fixed
92 smear was covered with crystal violet stain for 30 to 60 seconds and rapidly washed off with
93 clean water and then flooded with Lugol's iodine for one minute. The iodine was washed off
94 with clean water and the smear was decolorized with alcohol and washed immediately with
95 clean water before counter staining with safranin for one minute. The stain was washed off
96 with clean water and the smear air-dried and observed under microscope using oil immersion
97 objective lens. Gram positive bacteria stained purple while gram negative bacteria stained red
98 or pink (Cheeshrough, 2005).

99 2.6 Motility test

100 Twenty-four hour (24hr) culture of the isolates in peptone broth was used for motility test.
101 Five drops of each isolate was placed on a cover slip and the concave shaped slide smeared
102 with Vaseline at the edge of the concavity, gently on the cover slip. This slide was carefully

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103 inverted and the drop on the cover slip observed under high power objective lens (40×)
104 (Cheeshrough, 2005).

105 **2.7 Biochemical tests**

106 The bacterial isolates obtained were characterized and identified based on their cultural,
107 morphological and biochemical characteristics using the scheme of Bergey's Manual of
108 Determinative Bacteriology (Chikere and Okpokwasili, 2003; Oboh *et al.*, 2006).

109 **2.7.1 Catalase test**

110 Three millilitres (3ml) of 3% hydrogen peroxide solution was poured into different test tubes
111 and sterile glass rod was used to introduce some colonies of the isolates into the test tubes
112 containing the hydrogen peroxide. The contents of the test tubes were observed for gas
113 bubbling. Catalase positive bacteria showed active bubbles while catalase negative did not
114 (Cheeshrough, 2005).

115 **2.7.2 Urease test**

116 Christensen's solid medium was prepared by dissolving 1g peptone; 5g NaCl; 2g K₂HPO₄
117 and 10g agar in 1000 ml of distilled water. Phenol red (6ml) was also added and the pH
118 adjusted to 7.0. This was sterilized at 121⁰C for 20 minutes at 15psi and allowed to cool to
119 50⁰C. 10 ml of 10% solution of glucose and 100 ml of 20% urea solution were sterilized by
120 filtration. The glucose and urea were mixed aseptically with the agar medium and dispensed
121 in 5ml amounts into bijou bottles and allowed to solidify in the slope position. Urease
122 positive bacteria indicated pink colour while urease negative bacteria did not show pink
123 colour (Cheeshrough, 2005).

124 **2.7.3 Sugar fermentation test**

125 The test was carried out to determine the ability of the isolates to ferment various sugars
126 which is indicated by the production of acids/gas. The following sugars were used: maltose,
127 glucose and lactose. From each sugar, 0.5g was dissolved in 50ml of peptone water and
128 sterilized by membrane filtration. A pinch of phenol red was added as indicator and 5ml
129 aliquots were aseptically dispensed into sterile test tube containing sterile Durham tubes
130 which were inverted in the sterile broth. The broth was inoculated with the isolates using
131 sterile wire loop and incubated at 30⁰C for 48 hours. The content was observed for change in
132 colour and/or the production of gas (Cheeshrough, 2005).

133 **2.7.4 Citrate utilization test**

134 This test was used to study the ability of organisms to utilize citrate present in Simon's
135 medium as a sole source of carbon for growth. Simon's citrate agar was prepared, dispensed
136 into test tubes, autoclaved and allowed to solidify in a slanting position. The isolates were
137 streaked from freshly prepared cultures and incubated at 37⁰C for 48 hours. The content of
138 the test tubes were observed for the development of growth with blue colour as opposed to
139 the original green colour of the medium which signifies citrate utilization (Cheeshrough,
140 2005).

141 **2.7.5 Voges –proskauer test**

142 The medium used for this test is glucose phosphate medium. After sterilization, the medium
143 was allowed to cool and the test organism was inoculated into the broth and incubated for
144 five days at 37⁰C. After incubation, 1.5ml of 5% alcoholic alpha naphthol and 0.5 ml of 40%
145 aqueous KOH were added. The test tubes were shaken vigorously and allowed to stand for 5
146 minutes. The content was observed for the development of pink or red colour (Cheeshrough,
147 2005).

148 **2.7.6 Methyl red test**

149 About 5 drops of methyl red solution was added to 2ml of a five- day old culture of the
150 isolates inoculated in glucose-phosphate broth. Red colouration indicated a positive test while
151 yellow colour indicated negative test (Cheeshrough, 2005).

152 **2.8 Statistical analysis**

153 Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS,
154 Version 20.0). Analysis of variance (ANOVA), P- values test of significance, was carried out

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155 at 95% level of confidence, P - values was use to determine the significance levels between
 156 various treatments and data obtained during the study.

157 **RESULTS AND DISCUSSION**

158 The prevailing soil bacteria present in the various study sites including the control were
 159 ascertained following standard microscopic, cultural and biochemical methods. The various
 160 identification techniques used for the characterization of the bacterial isolates were as shown
 161 in Table 1. The bacterial diversity present in the Control soil, Site A (500m) and Site B
 162 (1000m) of this study was as represented in Table 2 These isolated bacteria were subjected to
 163 hydrocarbon degradation test in order to ascertain those that possess the potential to degrade
 164 the hydrocarbon component of the crude oil Table 3.

166 Table 1: Characterization of bacteria isolated from the study sites.

167

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Strain	1	2	3	4	5	6
Gramstaining	-	-	+	-	-	-
Motility	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Glucose	+	-	-	+	-	+
Lactose	-	-	-	-	-	-
Butt stant	AB	BB	BB	AB	BB	AB
Gas	+	-	-	+	-	-
H₂S	-	-	+	-	-	+
Citrate	+	-	+	+	-	+
Urease	-	+	+	+	+	-
Methly red	+	+	-	-	-	-
V.P	-	-	+	+	+	+
Glucose	AG	A	A	A	A	A
Sucrose	-	-	-	-	-	-
Probable Organism	<i>Alcaligenes</i> sp.	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp.	<i>Acinetobacter</i> sp.	<i>Serratia</i> sp.	<i>Citrobacter</i> sp.

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169 AG =acid growth, AB =acid and base, BB=base base, A= acid.

171 Table 2: Culturable bacterial diversity presents in the various study sites

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Control	Polluted site (500m) away	Polluted site (1000m) away
<i>Acinetobacter</i> sp.	<i>Pseudomonas</i> sp.	<i>Alcaligenes</i> sp.
<i>Alcaligenes</i> sp.	<i>Bacillus</i> sp.	<i>Citrobacter</i> sp.
<i>Pseudomonas</i> sp.	<i>Acinetobacter</i> sp.	<i>Bacillus</i> sp.
<i>Serratia</i> sp.		<i>Acinetobacter</i> sp.
<i>Bacillus</i> sp.		

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177 Table 3 Culturable hydrocarbon utilizing bacterial isolates from the study sites.
178

Control	Polluted site (500m) away	Polluted site (1000m) away
<i>Acinetobacter</i> sp.	<i>Pseudomonas</i> sp.	<i>Alcaligenes</i> sp.
<i>Alcaligenes</i> sp.	<i>Bacillus</i> sp.	<i>Citrobacter</i> sp.
<i>Pseudomonas</i> sp.	<i>Acinetobacter</i> sp.	<i>Bacillus</i> sp.
<i>Bacillus</i> sp.		<i>Acinetobacter</i> sp.

179
180 In Table 4, the physical parameter for *Pseudomonas sp* was recorded for 30 days at 5 days
181 interval, the colour change mean ranged from 2536.67±234.79Pcu on day 0 to 6745.00
182 ±17.30Pcu for 30 days measured in Cobalt per Unit, while Optical Density ranged from
183 0.88±0.00 from day 0 to 1.77±0.00 for 30 days. The pH was drastically changing to acidic in
184 the experiment as it ranged from 6.18±0.02 to 5.26±0.05. The Total Culturable Heterotrophic
185 Bacterial Count (TCHBC) measured in Cfu/ml increases from 1646.67±55.08 on the previous
186 day to 42666.67±3055.05 for day 30. (TPH) decreased from 4.58±0.01 Mg/ml to
187 0.02±0.00Mg/ml, this show that *Pseudomonas sp.* is active in the degradation process. Then
188 the Total Hydrocarbon Content (THC) decrease in percentage to 68.33 ±2.01%.
189 Similarly the physical parameter for *Bacillus sp.* was recorded for 30 days at 5 days interval,
190 the colour change mean ranged from 2536.67±243.79Pcu on day 0 to 2416.67 ±35.12Pcu for
191 30 days measured in Cobalt per Unit, while Optical Density ranged from 0.88±0.00 from day
192 0 to for 30 days 2.39±0.01. There was a slight increase in pH as it ranged from 6.18±0.02 to
193 6.30±0.08. The Total Culturable Heterotrophic Bacterial Count (TCHBC) measured in
194 Cfu/ml increases from on the first day of 1180.00±20.00 to 34666.6±7577.35 for 30 days.
195 The Total Petroleum Hydrocarbon (TPH) decreased from 4.58±0.00 Mg/ml to 0.11±0.06
196 Mg/ml, this show that *Bacillus sp.* is really degrading the hydrocarbon but at a slow rate.
197 Then the Total Hydrocarbon Content (THC) decrease in percentage to 53.33± 4.04%.
198 Furthermore, the physical parameter results for *Acinetobacter sp.* were recorded for 30 days
199 at 5 days interval, the colour change mean ranged from 2536.67±243.79Pcu on day 0 to
200 5406.67±268.58 Pcu for 30 days measured in Cobalt per Unit, while Optical Density ranged
201 from 0.88±0.00 from day 0 to for 30 days 2.50±0.01. There was a drastically increase in pH
202 from 6.18±0.02 to 8.21±0.14. The Total Culturable Heterotrophic Bacterial Count (TCHBC)
203 measured in Cfu/ml increases from on the first day 1230.00±36.06 to 63333.33±28867.51 for
204 30 days. The Total Petroleum Hydrocarbon (TPH) decreased from 4.58±0.00 Mg/ml to
205 0.05±0.07 Mg/ml, this show that *Acinetobacter sp.* is really degrading the hydrocarbon but at
206 a slow rate. Then the Total Hydrocarbon Content (THC) decrease in percentage to
207 52.67±1.53 %.
208 Similarly, the physical parameter for *Alcaligenes sp.* was recorded for 30 days at 5 days
209 interval, the colour change mean ranged from 2536.67±243.79Pcu on day 0 to 4786.67
210 ±32.15Pcu for 30 days measured in Cobalt per Unit, while Optical Density ranged from
211 0.88±0.00 from day 0 to for 30 days 1.33±0.01. There was a slight decrease in pH as it
212 ranged from 6.18±0.02 to 5.30±0.08. The Total Culturable Heterotrophic Bacterial Count
213 (TCHBC) measured in Cfu/ml increases from on the first day 131.00±45.83 to 51000.00
214 0±165.15 for 30 days. The Total Petroleum Hydrocarbon (TPH) decreased from 4.58±0.00
215 Mg/ml to 0.04±0.00 Mg/ml, this show that *Alcaligenes sp.* is really degrading the
216 hydrocarbon. Then the Total Hydrocarbon Content (THC) decrease in percentage to 71.67±
217 0.58%.
218 However, the strain identify as *Pseudomonas sp.* possess a steady increase in optical density
219 (OD) from 0.88 to 1.29, which raises the pH from 4.59 to 5.26 and increase in colour change

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220 from 2536.67pcu to 6745.00pcu after 30 days of degradation monitoring As shown in Table
 221 4.5. OD increases slowly as seen in the second strain indentified as *Bacillus* sp. from 0.88 to
 222 2.39, pH of 5.57 – 6.30 and shows a decrease in colour change from 4393.67pcu to
 223 2416.67pcu during the degradation study. There was a steady increase in OD as seen in
 224 *Acinetobacter* sp. which increases from 0.88 to 2.50 for 30 days which raises the pH from
 225 5.46 to 8.21 and increase in colour change of 2536.67pcu to 5406.67pcu. Fourth Strain
 226 identified as *Alcaligenes* sp. showed an increase in OD from 0.88 to 1.33, while pH increases
 227 from 2.98 to 5.30 and increase in colour change shows a wide range of 2536.67pcu to
 228 4786.67pcu.

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 230 Table 4: Responses of selected hydrocarbon utilizing bacteria as used in biodegradation
 231 studies
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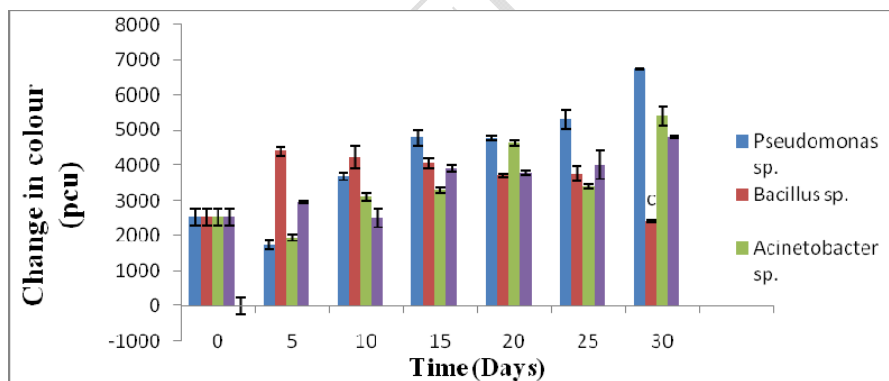
Day	Parameters	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp.	<i>Acinetobacter</i> sp	<i>Alcaligenes</i> sp
0	Colour (pcu)	2536.67±234.79	2536.67±234.76	2536.67±243.79	2536.67±243.79
	OD	0.88±0.00	0.88±0.00	0.88±0.00	0.88±0.00
	pH	6.18±0.02	6.18±0.02	6.18±0.02	6.18±0.02
	TCHBC(cfu/ml)	1646.67±55.08	1180.00±20.00	1230.00±36.06	1310.00±45.83
	TPH (mg/ml)	4.58±0.00	4.54±0.01	4.58±0.00	4.58±0.00
	THC (%)	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
5	Colour (pcu)	1746.67±122.20	4393.33±125.03	1946.67±83.27	2060.00±30.00
	OD	1.29±0.07	1.44±0.06	1.04±0.03	1.16±0.03
	pH	4.59±0.34	5.57±0.01	5.46±0.08	2.98±0.08
	TCHBC(cfu/ml)	1656.67±65.06	2190.00±101.49	1230.00±20.00	1773.00±61.10
	TPH (mg/ml)	2.84±0.00	2.80±0.02	2.93±0.00	2.86±0.00
	THC (%)	97.67±0.58	94.67±0.58	97.00±1.00	97.33±1.53
10	Colour (pcu)	3690.00±105.83	4240.67±320.78	3103.33±127.41	2496.67±271.54
	OD	1.15±0.00	1.22±0.09	0.91±0.02	1.05±0.03
	pH	4.61±0.45	5.64±0.03	5.51±0.02	4.20±0.02
	TCHBC(cfu/ml)	2030.00±170.00	2273.33±70.24	1600.00±163.71	2346.67±50.33
	TPH (mg/ml)	1.08±0.00	1.08±0.00	1.29±0.00	1.15±0.01
	THC (%)	93.00±4.36	87.33±0.58	94.33±0.59	94.00±1.00
15	Colour (pcu)	4780.00±233.02	4060.67±148.44	3293.33±66.58	3910.00±80.00
	OD	0.25±0.05	1.27±0.00	1.15±0.00	1.23±0.01
	pH	4.99±0.02	5.93±0.04	5.55±0.01	4.53±0.01
	TCHBC(cfu/ml)	35333.33±305.05	15266.67±305.12	18000.00±964.37	13866.67±583.15
	TPH (mg/ml)	0.62±0.00	0.62±0.00	0.68±0.00	0.65±0.01
	THC (%)	82.00±2.31	65.00±1.00	71.33±1.53	90.33±1.53
20	Colour (pcu)	4760.00±60.83	3715.00±37.75	4640.00±72.86	3790.00±69.28
	OD	1.45±0.00	1.32±0.00	1.38±0.00	1.24±0.03
	pH	4.83±0.29	5.95±0.02	5.83±0.05	4.73±0.10
	TCHBC(cfu/ml)	48000.00±458.58	82666.67±541.63	56666.67±527.25	19000.00±590.00
	TPH (mg/ml)	0.12±0.00	0.16±0.00	0.08±0.00	1.16±0.00
	THC (%)	76.33±0.58	63.33±1.15	59.00±1.00	72.33±1.53
25	Colour (pcu)	5300.00±270.14	3760.00±216.56	3396.67±56.86	4003.00±408.57
	OD	1.38±0.00	1.54±0.01	1.44±0.01	1.26±0.05

	pH	4.99±0.03	6.01±0.02	5.83±0.05	4.81±0.04
	TCHBC(cfu/ml)	46000.00±0.00	34666.67±305.05	17366.67±105.87	50000.00±480.89
	TPH (mg/ml)	0.07±0.00	0.14±0.00	0.05±0.00	0.05±0.00
	THC (%)	72.33±1.53	60.33±0.58	57.33±1.53	70.67±2.52
30	Colour (pcu)	6745.00±17.30	2416.67±35.12	5406.67±268.58	4786.67±32.15
	OD	1.77±0.00	2.39±0.01	2.50±0.01	1.33±0.01
	pH	5.26±0.04	6.30±0.08	8.21±0.14	5.30±0.08
	TCHBC(cfu/ml)	42666.67±305.05	34666.60±577.35	63333.33±287.51	51000.00±165.15
	TPH (mg/ml)	0.02±0.00	0.11±0.06	0.05±0.07	0.04±0.00
	THC (%)	68.33±2.01	53.33±4.04	52.67±1.53	70.10±0.58

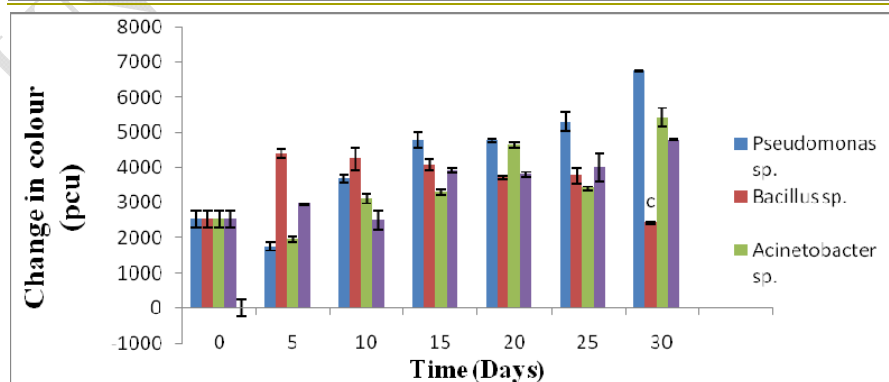
Mean ± standard deviation of triplicate determination

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The information from the degradation studies as shown in the table above (Table 4) were analysed and represented in bar charts. Changes in colour (pcu) against Time(Days) (Figure 1), Changes in total hydrocarbon content(%) against Time(Days) (Figure 2), Changes in Optical Density (OD) against Time(Days) (Figure 3), Changes in total petroleum hydrocarbon in (mg/ml) against Time (Days) (Figure 4) Changes in pH against Time(Days) (Figure 5), Changes in total culturable heterotrophic bacteria counts (cfu/ml) against Time(Days) caused by some of the isolates during the degradation studies were represented.



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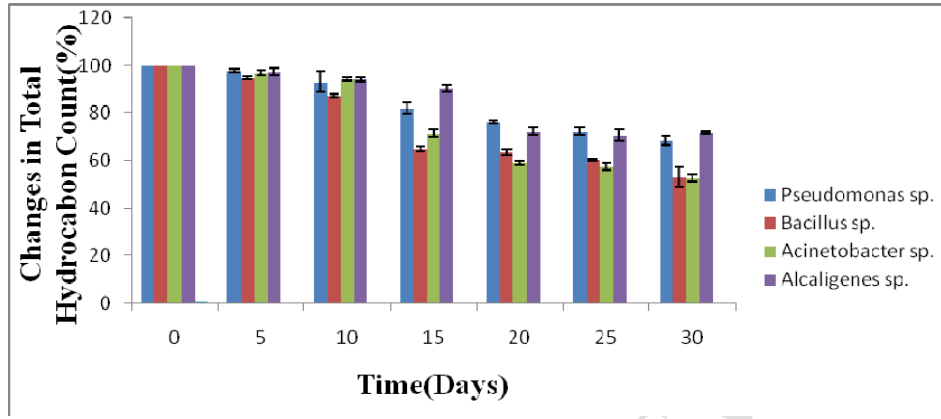


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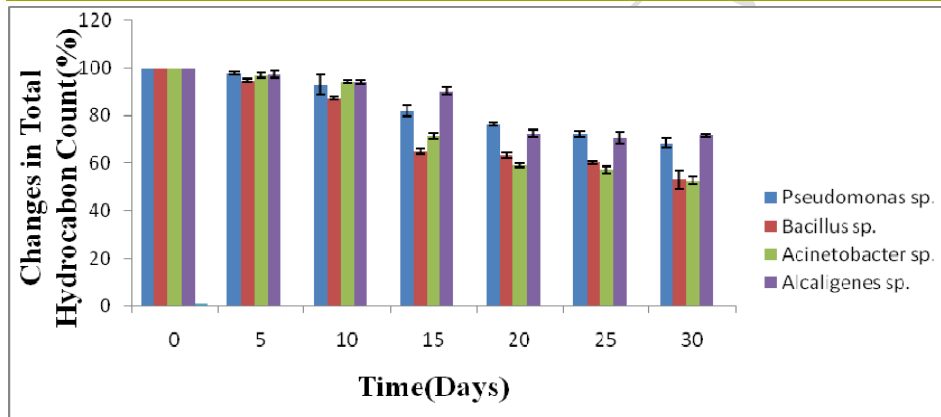
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Figure 1: Changes in colour (pcu) against Time(Days) caused by some of the isolates during the degradation studies (Data are mean \pm S.D of triplicate determinations)

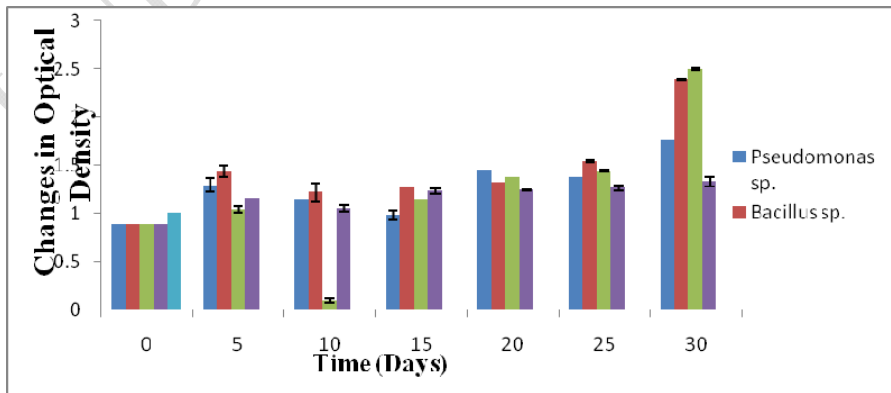


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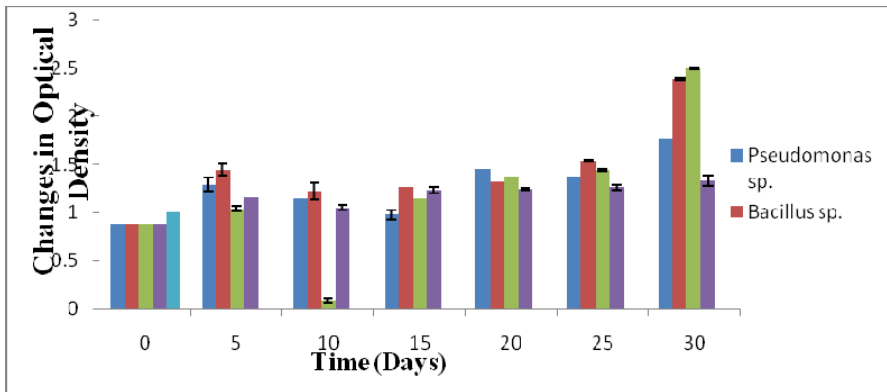


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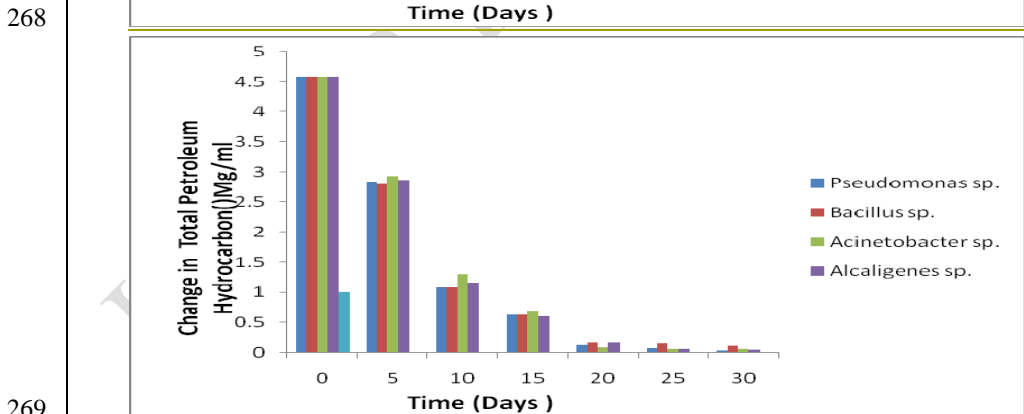
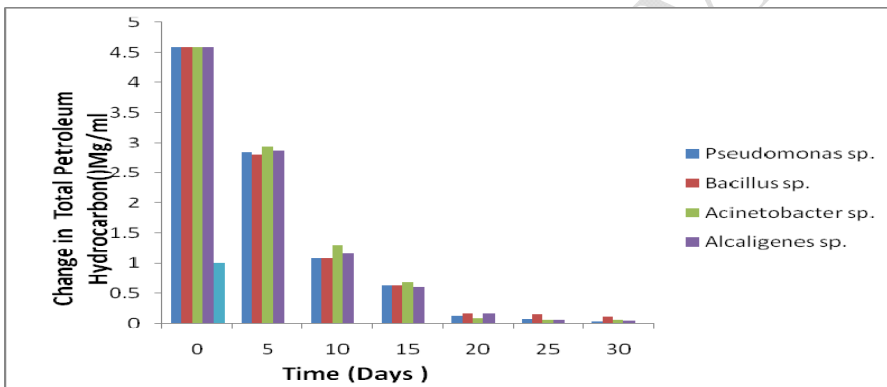
Figure 2: Changes in Total Hydrocarbon Content(%) against Time(Days) caused by some of the isolates during the degradation studies (Data are mean \pm S.D of triplicate determinations)



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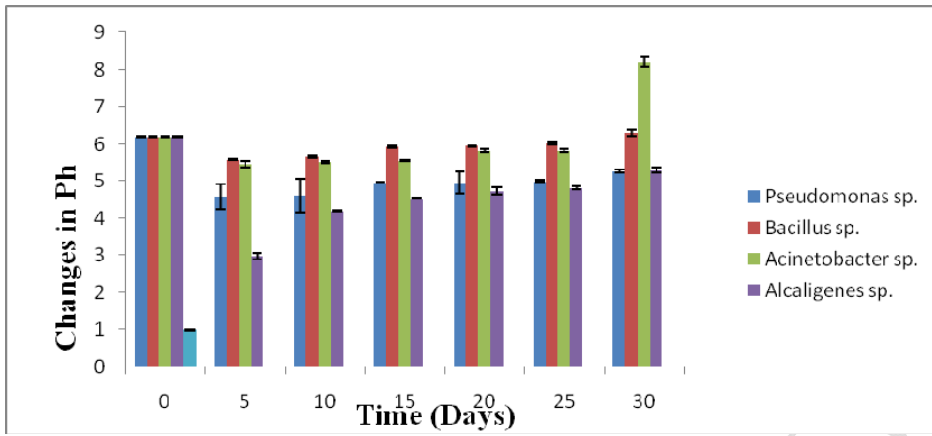


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263 Figure 3: Changes in Optical Density (OD) against Time in (Days) caused by some of the
264 isolates during the degradation studies (Data are mean \pm S.D of triplicate
265 determinations)
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270 Figure 4: Changes in total petroleum hydrocarbon in (mg/ml) against Time in (Days) caused
271 by some of the isolates during the degradation studies (Data are mean \pm S.D of
272 triplicate determinations)

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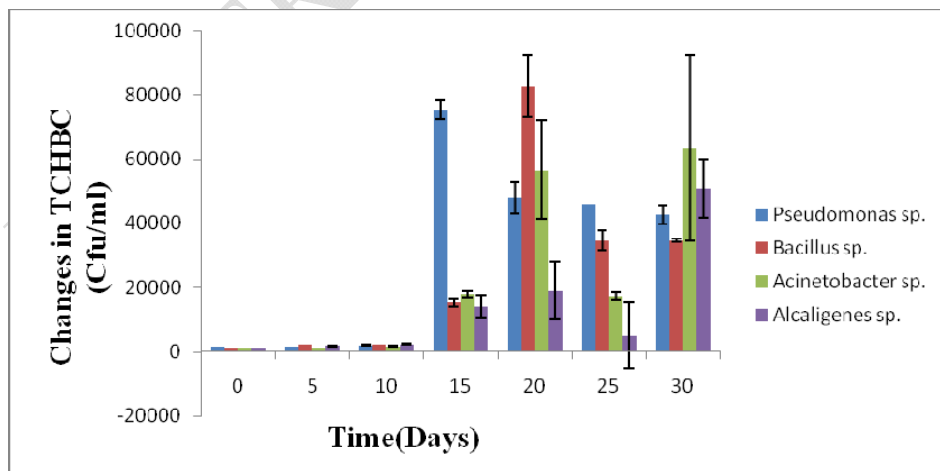
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Figure 5: Changes in pH against Time in (Days) caused by some of the isolates during the degradation studies (Data are mean \pm S.D of triplicate determinations).

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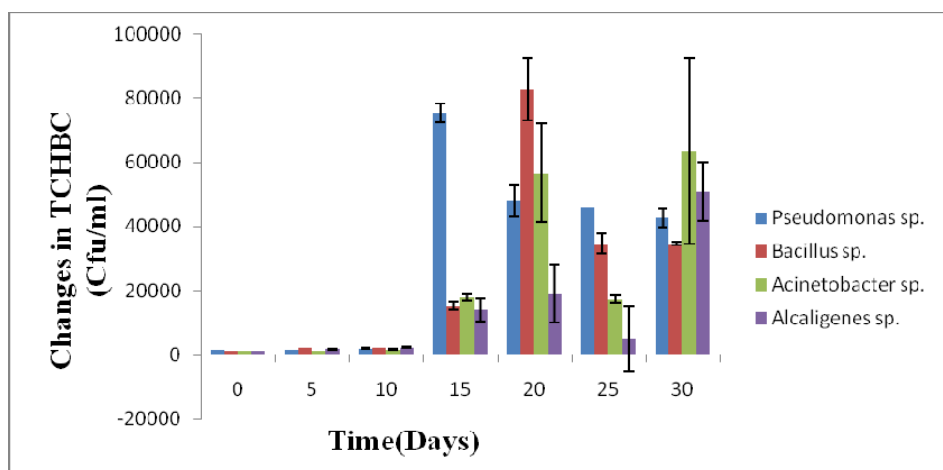


Figure 6: Changes in total cuturable heterotrophic bacteria counts (cfu/ml) against Time in (Days) caused by some of the isolates during the degradation studies (Data are mean \pm S.D of triplicate determinations)

The ability of these bacteria to be predominantly Gram-negative is contrary to Austin *et al.* (1997) who isolated predominantly gram-negative hydrocarbon utilizing bacteria from soil and aquatic environment. The isolation of *Bacillus* species is in agreement with the work of Okpokwasili and Okorie (1990), who found that *Bacillus* species could also be one of the predominant Gram positive organisms found in oil polluted areas

These findings showed that the biodegradation of complex hydrocarbons in nature usually required the cooperation of more than a single species. The microbial populations consisting of the strains belonging to various genera have been detected in petroleum-contaminated soil (Ilori *et al.*, 2006; Kim and Crowley 2007). This suggested that the strains from various genera have their roles in the hydrocarbon transformation processes (Ghazali *et al.*, 2004; Cunliffe and Kertesz 2006). Individual microorganisms can metabolize only a limited range of hydrocarbon substrates; hence assemblages of the mixed populations with overall broad enzymatic capacities would be required to achieve considerable biodegradation of petroleum hydrocarbons.

Statistical analysis of the result shows that there is significant difference between various heterotrophic bacteria and soil samples.

CONCLUSION

Environment pollution caused by released of a wide range of compound as a consequence of industrial progress has assumed serious proportions. To prevent development of hazardous waste the process of bioremediation has been followed. Our present study follows the isolation of hydrocarbon degrading bacteria from oil polluted sites. Sample was collected from contaminated sites of Sakpenwa Community, Tai L.G.A, Rivers State, These were brought then to the laboratory and isolation was done on the basis of gram staining. Biochemical tests were performed with isolates. The isolates were screened for their oil degrading capacity.

Having the knowledge of the processes and factors involves in the biodegradation experiment is of great ecological significance in the society. The elimination of oil spilled in the environment can be achieved by microbial degradation when added up with some physical and chemical methods

Comment [rev18]: ??????

Comment [rev19]: Contrary ? where is the contradiction?

Comment [rev20]: italicize

Comment [rev21]: see above

Comment [rev22]: which finding? Your findings doesn't show that

Comment [rev23]: this cannot be substantiated from the result section

Comment [rev24]: environmental

Comment [rev25]: release

Comment [rev26]: expunge

315 Further scale-up studies as applicable need to be carried out in increasing the degrading
316 ability and stability of the crude oil degrading isolate and its usage as a possible commercial
317 strain
318 We concluded that the use of consortium species makes biodegradation study more effective.
319

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