

PETROLEUM DEGRADING POTENTIALS OF BACTERIAL ISOLATES FROM RIVER ETHIOPE, DELTA STATE, NIGERIA

Abstract

Water samples were collected from twelve locations along the Ethiope River, Delta State, Nigeria to determine the petroleum degrading potentials of bacterial isolates and their x-ray mutants using standard methods. Bacteria isolated were *Bacillus* sp, *Klesiella* sp, *Escherichia* sp, *Micrococcus* sp, *Pseudomonas* sp, *Staphylococcus* sp and *Streptococcus* sp. All the isolates and their x-ray mutants possessed ability to degrade 1% (v/v) Forcados crude-oil with *Bacillus* sp, *Escherichia* sp and *Pseudomonas* sp isolated from a location, with reported crude-oil spillage, degrading 2.5% (v/v). X-ray mutation led to % kill that ranged from 3.58 (*Micrococcus* sp) to 11.59 (*Streptococcus* sp). There were reduction in crude-oil degradation in mutants from 0.33% (*Escherichia* sp) to 6.31% (*Pseudomonas* sp) with parent *Pseudomonas* sp (1.680ppm) and mutant *Streptococcus* sp (1.474ppm) being highest and least degraders respectively. Statistically significant differences ($t = 0.95$) in crude-oil degradation were observed for parents and mutants of *Bacillus* sp, *Escherichia* sp and *Pseudomonas* sp but not for *Streptococcus* sp, *Staphylococcus* sp, *klebsiella* sp and *Micrococcus* sp. Thus, irrespective of source of isolation on the Ethiope River, crude-oil degrading bacteria abound with increased ability on exposure to crude-oil pollution. The bacteria encountered in this study could be employed in bioremediation of crude-oil polluted environments.

Key words: Ethiope. River. Bacteria. Petroleum. Degradation. Parents. X-rays. Mutants. Bioremediation.

Introduction

River pollution occurs as a result of contamination of flowing surface water(s) that originate from a source. It occurs when waste and other different pollutants are discharged into a river without treatment and /or improper treatment. It has negative effects on aquatic ecosystem. The negative impacts on plants and animals often lead to decline in species and sometimes even to extinctions of entire species. Water is the best known and most abundant of all chemical components, which occur naturally on the surface of the earth or in aquifers under the ground. Most surface waters are almost always polluted because they are exposed to direct contamination from external sources or soil and basement rocks as it flow from the source downstream. Biologically, most natural waters contain a range of microorganisms, plants and animals that exist in a balanced ecological state. When this state is altered, it results in fatal consequences (Aluyi *et al.*, 2003).

River Ethiope, a fresh water river in Delta State, flows through several Local Governments Areas beginning from Umuaja in Ukwuani, through Ethiope East, Okpe, Ethiope

38 West, and Sapele Local Government Areas where it empties into River Jemison which
39 ultimately discharges into Benin River. At the Sapele end of the river, it is deep enough to
40 provide a harbor for ocean-going vessels. The river serves as a major source of water for
41 drinking, agriculture and transportation. Other purposes include fishing, recreation (beaches)
42 and other domestic purposes at various locations as there are different human settlements
43 along the bank of the river. The communities, through which the Ethiope River traverses, are
44 mainly peasant farmers whose food products include yams, corn, vegetables, cassava, plantain
45 and fruits. Agricultural activities in the area are also carried out along the bank of the Ethiope
46 River and agricultural wastes are discharged into the river as runoffs after rainfalls (Agbaire and
47 Obi, 2009). The river also re-distributes these wastes which may serve as plants/aquatic animal
48 nutrients. However, a host of these pollutants are inimical to the ecosystem which may also
49 have direct or indirect negative impacts on man as a result of bioaccumulation and/or
50 biomagnifications (Ekhaire and Anyasi, 2005).

51 Recently, the river has been reported to be polluted with crude oil believed to be
52 leaking from an oil pipeline belonging to NNPC which runs across the River at Okirighre near
53 Sapele (Addeh, 2012; Oghre, 2012). This and other pollutants may have altered the bacterial
54 qualities of the river (Aluyi *et al.*, 2006; Agbabiaka and Oyeyiola, 2012). It has been reported
55 that in the Niger-Delta region of Nigeria, bacterial isolates possessed the ability to degrade
56 crude oil irrespective of location of isolation apparently due to flooding of unpolluted plains with
57 contaminated surface waters with concomitant exposure of the bacteria therein to petroleum
58 pollutants during the wet season (Okoh, 2006). The aim of this study therefore was to
59 determine the potentials of some bacterial isolates of River Ethiope and their x-ray modified
60 mutants to degrade Forcados crude oil.

61

62

63 Materials and method

64 **Collection of samples:** Water samples were collected into sterilized 2L plastic containers from
 65 twelve (12) locations along the River Ethiope, Delta State, Nigeria for three months (April –
 66 June, 2012) in accordance with the procedures reported in APHA (1998). The stations were
 67 Umutu Bridge, Umutu-Agbor Road, Umutu; Obiaruku Bridge, Obiaruku; Uruoka Bridge, Abraka;
 68 Okotie River Resorts, Abraka; Abraka River Motel, Abraka; Swimming Pool at the Delta State
 69 University, Abraka; Mudi Beach, Abraka; Canoe anchor points at Sanubi, Eku, Igun and
 70 Okpara Waterside and Okirighre Bridge near Sapele (See Figure 1 below, map of study area).
 71 All collected samples were transported in ice-chest to the Microbiology laboratory at the Delta
 72 State University, Abraka for analyses within an hour of collection.

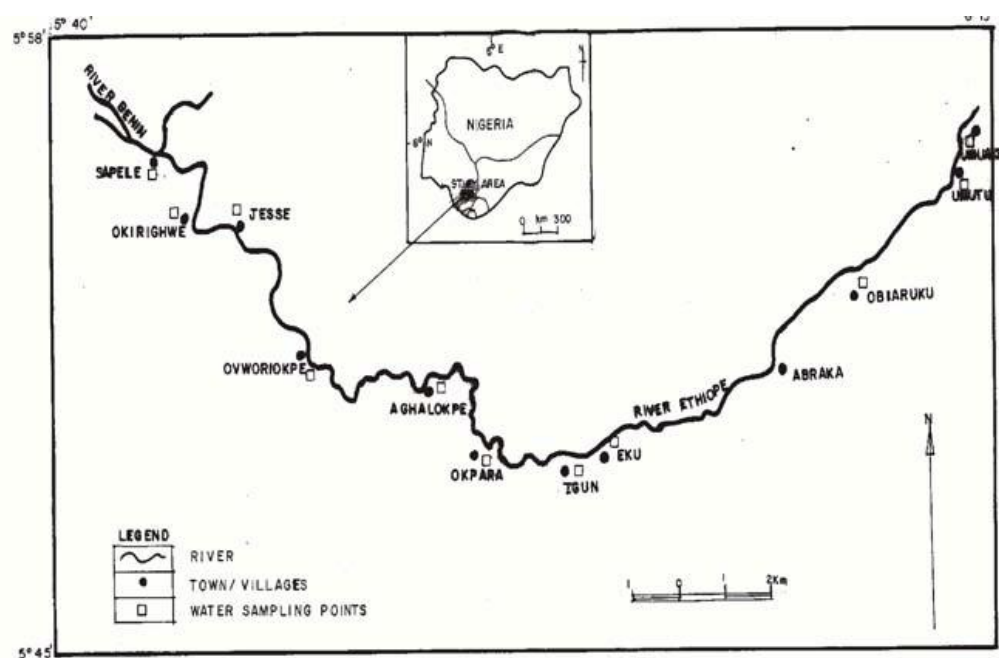


Figure 1: Map of the River Ethiope

77 **Isolation of bacteria:** The procedures of pour plate technique reported in Cheesebrough
 78 (2004) on Nutrient and MacConkey agar were used. Plates were incubated aerobically in an

incubator (Model TT-9082, Techmel and Techmel, U.S.A.) at 37°C for 24h. Pure cultures obtained were stored at +4°C on agar slants until required.

Identification of isolates: These were carried out in accordance with the procedures reported by Cowan and Steel (2004) in which isolates were biotyped for their: Gram reaction, growth under aerobic and anaerobic conditions, ability to form endospores, motility, possession of Oxidase, Catalase and Coagulase enzymes, ability to produce H₂S, ability to utilize citrate, ferment sucrose, lactose and glucose with production of gas. Results obtained were matched with the isolates described by Cowan and Steel (2004).

Determination of crude-oil degrading potentials of isolates: The procedures reported by Idise *et al.*, (2009) were adopted using 0.1, 0.5, 1.0, 1.5 and 2.5% (v/v) Forcados crude in minimum basal medium (MBM). The MBM, made up of Glucose 25g, NaNO₃ 15g, MgSO₄.7H₂O 1.3g, KCl 1.3g, KH₂PO₄ 3.8g, FeSO₄.7H₂O 10mg, ZnSO₄.7H₂O 1.0mg, Agar 37.5g and 2.5litres of distilled water, was prepared and the pH adjusted from 3.08 to 6.93 and sterilized. 0.1, 0.5, 1.0, 1.5 and 2.5mL of Forcados crude oil were respectively (aseptically) dispensed into 99.9, 99.5, 99.0, 98.5 and 97.5mL of molten MBM in five separate conical flasks. The flasks were swirled to homogenize the mixtures. Ten milliliters (10mL) of the mixtures of each flask was dispensed into sterile Petri dishes in duplicates and labeled for each isolate. Lawn cultures of isolates were prepared in accordance with procedure adopted by Sharma (2009). Inoculated plates were incubated aerobically at 37°C for 24h and observed for growth.

Mutagenesis of isolates with x-rays: A modification of the procedures reported by Idise and Ameh (2010) was used in which 10mL of 1.725Log₁₀cfu/mL 24h-cultures of isolates were exposed to x-ray (UX Universal mobile X-ray Machine, Made in England) flashes for five times. X-ray treated plates were, after each flash, wrapped in aluminium foil to avoid photo-oxidation and immediately incubated aerobically for 24h at 37°C to determine viable counts.

104 **Determination of crude-oil degrading potentials of parents and x-ray mutants:** These
 105 were carried out in accordance with the procedures reported by Idise and Ameh (2010). Using
 106 160mL1% (v/v) Forcados crude in MBM dispensed into each 250mL conical flask, 40mL of
 107 1.685×10^6 cfu/mL 24h-cultures of each isolate was added to the respective flasks and
 108 incubated aerobically at room temperature ($28 \pm 2^\circ\text{C}$). Separate flasks were used for parents
 109 and their x-ray mutants. Growth was determined at day zero (one hour after incubation) and at
 110 three-day intervals for 30days using a spectrophotometer (Model 721-2000vmcs, Made in
 111 China) at 560nm

112 **Determination of total petroleum hydrocarbons (TPH):** These were determined using ASTM
 113 D3921-96 in ASTM Vol. 11 (2002) thus: thirty milliliters of carbon tetrachloride (CCl_4) was
 114 added to one liter of the water sample to dissolve the hydrocarbons and shaken vigorously for
 115 two minutes. The mixture was poured into a separating funnel and shaken intermittently for
 116 three minutes and expelled the air by releasing the valves of the funnel. The funnel was then
 117 rinsed with twenty milliliters of carbon tetrachloride (CCl_4). The resulting oil was drained into a
 118 fifty milliliter volumetric flask through a funnel containing cotton wool to which had been added
 119 anhydrous sodium sulphate ($\text{Na}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$) to remove traces of water from the oil. The
 120 separating funnel was then rinsed with ten milliliters of CCl_4 to remove all the oils. The Infra-red
 121 spectrophotometer was calibrated at 420nm using dry Forcados Dry crude oil with
 122 concentrations between 100 and 500 mg/L to obtain a standard curve. To ten milliliters of the
 123 extracted oil was added 0.5grams of deactivated floriscil. The resultant supernatant contained
 124 the total petroleum hydrocarbons (TPH) while the sediment contained the biogenic
 125 hydrocarbons. The TPH was then determined using the supernatant on the Infra-red
 126 spectrophotometer (Model Cecil CE 7400, Made in England) at Optical Density of 420nm. The
 127 concentration of the sample was extrapolated from the standard curve. The values were
 128 obtained using the following relation:

129
$$\text{TPH (ppm)} = \frac{\text{Absorbance} \times \text{DF} \times 50}{\text{Volume of sample}}$$

131
132 Where: DF = Dilution factor
133 50 = initial extraction volume
134

135 **Statistical analyses:** Descriptive statistics and t-tests were carried out on the data obtained
136 using Microsoft excel 1998-2003 Package

137 **Results**

138 The results of this study (Table 1) show that seven bacterial genera were isolated from the
139 different locations along the Ethiope River, Delta State, Nigeria. These were *Bacillus* sp,
140 *Klesiella* sp, *Escherichia* sp, *Micrococcus* sp, *Pseudomonas* sp, *Staphylococcus* sp and
141 *Streptococcus* sp. Two to five genera were isolated from each sample location.

142
143 Table 1: Bacteria isolated from locations

S/N	Location	Bacteria	No. of genera
1.	Umutu bridge, Umutu	<i>Bacillus</i> sp, <i>Escherichia</i> sp,	2
2.	Obiaruku Bridge, Obiaruku	<i>Bacillus</i> sp, <i>Escherichia</i> sp	2
3.	Uruoka Bridge, Abraka	<i>Bacillus</i> sp, <i>Escherichia</i> sp	2
4.	Okotie River Resorts, Abraka	<i>Klebsiella</i> sp, <i>Micrococcus</i> sp, <i>Staphylococcus</i> sp, <i>Streptococcus</i> sp	4
5.	River Motel, Abraka	<i>Bacillus</i> sp, <i>Escherichia</i> sp, <i>Pseudomonas</i> sp, <i>Staphylococcus</i> sp, <i>Streptococcus</i> sp	5
6.	Delta State University Swimming Pool, Abraka	<i>Escherichia</i> sp <i>Klebsiella</i> sp, <i>Pseudomonas</i> sp, <i>Staphylococcus</i> sp, <i>Streptococcus</i> sp	5
7.	Mudi Beach, Abraka	<i>Escherichia</i> sp <i>Klebsiella</i> sp, <i>Pseudomonas</i> sp, <i>Staphylococcus</i> sp, <i>Streptococcus</i> sp	5
8.	Canoe Anchor point Sanubi-Eku	<i>Bacillus</i> sp, <i>Escherichia</i> sp	2
9.	Canoe Anchor point, Eku	<i>Bacillus</i> sp, <i>Escherichia</i> sp	2
10.	Canoe Anchor point, Igun	<i>Escherichia</i> sp <i>Klebsiella</i> sp	2
11.	Canoe Anchor point, Okpara Waterside	<i>Bacillus</i> sp, <i>Escherichia</i> sp	2
12.	Okirighre Bridge, Sapele.	<i>Bacillus</i> sp, <i>Escherichia</i> sp, <i>Pseudomonas</i> sp	3

144
145 The crude-oil degrading ability of the bacterial isolates is presented in Table 2. It was observed
146 that all the isolates from the sample locations possessed the ability to degrade 1% (v/v) crude

147 oil except *Bacillus* sp, *Escherichia* sp and *Pseudomonas* sp that were isolated from Okirighre

148 Bridge, Sapele that degraded 2.5% (v/v) crude oil.

149 Table 2: Crude-oil degrading ability of the bacterial isolates

Location	Isolates	0.1% (v/v) Crude	0.5% (v/v) Crude	1% (v/v) Crude	1.5% (v/v) Crude	2.5% (v/v) Crude
Umutu Bridge, Umutu	<i>Staphylococcus</i> sp	+	+	+	-	-
	<i>Escherichia coli</i>	+	+	+	-	-
	<i>Bacillus</i> sp	+	+	+	-	-
	<i>Streptococcus</i> sp	+	+	+	-	-
Obiaruku Bridge, Obiaruku	<i>Staphylococcus</i> sp,	+	+	+	-	-
	<i>Bacillus</i> sp	+	+	+	-	-
	<i>Streptococcus</i> sp	+	+	+	-	-
Uruoka Bridge, Abraka	<i>Staphylococcus</i> sp,	+	+	+	-	-
	<i>Bacillus</i> sp	+	+	+	-	-
	<i>Streptococcus</i> sp	+	+	+	-	-
Okotie River Resorts, Abraka	<i>Staphylococcus</i> sp,	+	+	+	-	-
	<i>Klebsiella</i> sp	+	+	+	-	-
	<i>Micrococcus</i> sp	+	+	+	-	-
River Motel, Abraka	<i>Staphylococcus</i> sp,	+	+	+	-	-
	<i>Escherichia coli</i> ,	+	+	+	-	-
	<i>Bacillus</i> sp	+	+	+	-	-
	<i>Streptococcus</i> sp	+	+	+	-	-
Delta State University Swimming Pool, Abraka	<i>Klebsiella</i> sp	+	+	+	-	-
	<i>Pseudomonas</i> sp	+	+	+	-	-
Mudi Beach, Abraka	<i>Klebsiella</i> sp,	+	+	+	-	-
	<i>Escherichia</i> sp	+	+	+	-	-
	<i>Streptococcus</i> sp	+	+	+	-	-
Canoe Anchor point Sanubi-Eku	<i>Staphylococcus</i> sp,	+	+	+	-	-
	<i>Escherichia coli</i> ,	+	+	+	-	-
Canoe Anchor point, Eku	<i>Bacillus</i> sp	+	+	+	-	-
	<i>Streptococcus</i> sp	+	+	+	-	-
Canoe Anchor point, Igun	<i>Staphylococcus</i> sp	+	+	+	-	-
	<i>Klebsiella</i> sp	+	+	+	-	-
Canoe Anchor point, Okpara Waterside	<i>Bacillus</i> sp	+	+	+	-	-
	<i>Streptococcus</i> sp	+	+	+	-	-
Okirighre Bridge, Sapele.	<i>Pseudomonas</i> sp	+	+	+	+	+
	<i>Bacillus</i> sp	+	+	+	+	+
	<i>Escherichia</i> sp	+	+	+	+	+

150

The effects of x-ray mutagenic treatment on bacterial isolates are presented in Table 3. It was observed that the % kill ranged from 1.14 (*Bacillus* sp) to 9.78 (*Pseudomonas* sp) for one x-ray flash; 0.65 (*Pseudomonas* sp) to 6.32 (*Klebsiella* sp) for two x-ray flashes; 1.21 (*Pseudomonas* sp) to 8.67 (*Klebsiella* sp) for three x-ray flashes; 2.50 (*Staphylococcus* sp) to 6.18 (*Streptococcus* sp) for four x-ray flashes and 0.00 (*Micrococcus* sp and *Pseudomonas* sp) to 13.07 (*Staphylococcus* sp) for five x-ray flashes.

157

Table 3: Effects of x-ray treatment on isolates

Isolate	Number of x-ray flashes					Isolate	Number of x-ray flashes				
	1	2	3	4	5		1	2	3	4	5
<i>Bacillus</i> sp						<i>Pseudomonas</i> sp					
Initial	1.76	1.77	1.77	1.78	1.77	Initial	1.84	1.55	1.65	1.54	1.70
Final	1.74	1.66	1.74	1.72	1.73	Final	1.66	1.54	1.63	1.50	1.70
% kill	1.14	6.22	1.70	3.37	2.26	% kill	9.78	0.65	1.21	2.60	0.00
<i>Klebsiella</i> sp						<i>Staphylococcus</i> sp					
Initial	1.75	1.74	1.73	1.74	1.73	Initial	1.80	1.80	1.60	1.60	1.50
Final	1.60	1.63	1.58	1.65	1.64	Final	1.70	1.70	1.75	1.56	1.76
% kill	8.57	6.32	8.67	5.17	5.20	% kill	5.56	5.56	3.13	2.50	2.67
<i>Escherichia</i> sp						<i>Streptococcus</i> sp					
Initial	1.79	1.80	1.80	1.78	1.81	Initial	1.73	1.82	1.80	1.78	1.76
Final	1.71	1.73	1.68	1.71	1.64	Final	1.72	1.75	1.70	1.67	1.53
% kill	4.47	3.89	6.67	3.93	9.39	% kill	0.58	3.85	5.56	6.18	13.07
<i>Micrococcus</i> sp											
Initial	1.76	1.78	1.77	1.79	1.76						
Final	1.73	1.70	1.73	1.74	1.76						
% kill	1.71	4.49	2.26	2.79	0.00						

159

The crude-oil degrading potentials of the x-ray modified bacterial isolates are presented in Table 4. It was observed that while *Escherichia* sp, *Bacillus* sp and *Klebsiella* sp degraded 2.5% (v/v) crude oil, other isolates degraded 1% (v/v) crude oil.

163

Table 4: Crude-oil degrading potentials of the x-ray modified bacterial isolates

Isolate	% crude oil degraded	Isolate	% crude oil degraded
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<i>Escherichia</i> sp	2.5	<i>Micrococcus</i> sp	1.0
<i>Bacillus</i> sp	2.5	<i>Klebsiella</i> sp	1.0
<i>Pseudomonas</i> sp	2.5	<i>Staphylococcus</i> sp	1.0
		<i>Streptococcus</i> sp	1.0

165

166 The mean values of crude-oil degraded by isolates is presented in Table 5 while the crude oil
 167 degradation by parents and mutants are presented in Figures 2a – 2g for *Bacillus* sp,
 168 *Pseudomonas* sp, *Escherichia* sp, *Streptococcus* sp, *Klebsiella* sp, *Micrococcus* sp and
 169 *Staphylococcus* sp respectively. It was observed that parents degraded more than their
 170 mutants with reductions that ranged from 0.14% in *Streptococcus* sp to 6.31% in *Pseudomonas*
 171 sp. Parent *Pseudomonas* sp (1.680ppm) was highest degrader while mutant *Streptococcus* sp
 172 (1.474ppm) was least degrader.

173

174 Table 5: Mean values of crude-oil (ppm) degraded by isolates

Isolate	Parent (ppm)	Mutant (ppm)	% (decrease) (% ppm)	Isolate	Parent (ppm)	Mutant (ppm)	% (decrease) (% ppm)
<i>Bacillus</i> sp	1.579	1.496	(5.26)	<i>Escherichia</i> sp	1.536	1.531	(0.33)
<i>Staphylococcus</i> sp	1.652	1.622	(1.82)	<i>Micrococcus</i> sp	1.590	1.538	(0.63)
<i>Pseudomonas</i> sp	1.680	1.574	(6.31)	<i>Klebsiella</i> sp	1.578	1.528	(3.17)
<i>Streptococcus</i> sp	1.476	1.474	(0.14)				

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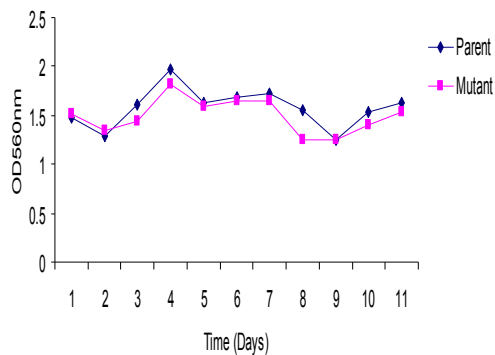


Figure 2a: Biodegradation of crude oil by parent and mutant *Bacillus* sp

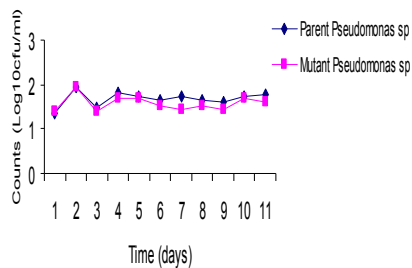


Figure 2b: Crude oil degradation by parent and mutant *Pseudomonas* sp

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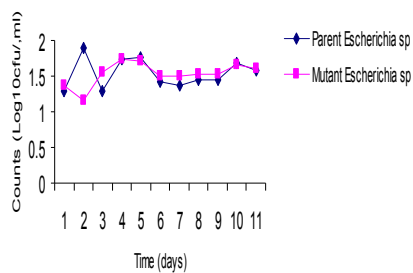


Figure 2c: Crude oil degradation by parent and mutant *Escherichia* sp

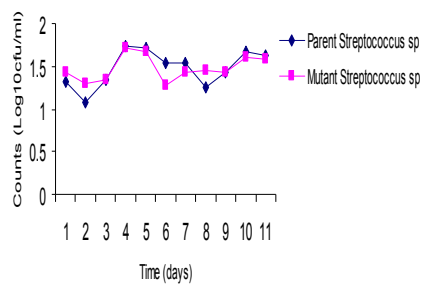


Figure 2d: Crude oil degradation by parent and mutant *Streptococcus* sp

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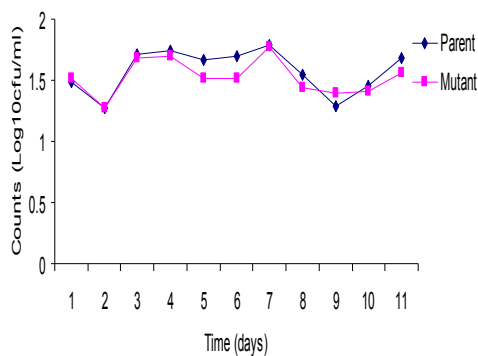


Figure 2e: Crude-oil degradation by parent and mutant *Klebsilla* sp

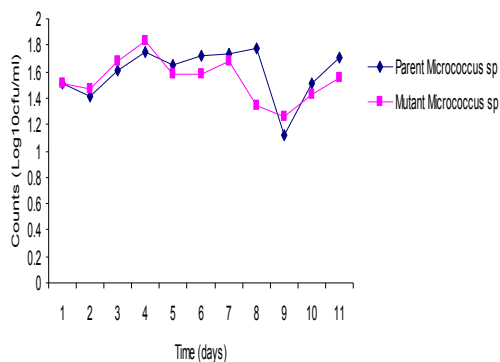


Figure 2f: Crude-oil degradation by parent and mutant *Micrococcus* sp

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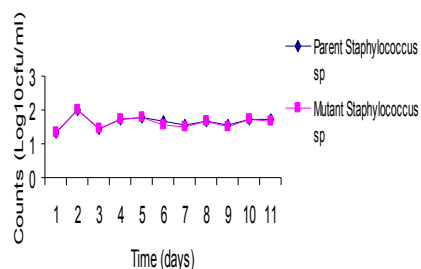


Figure 2g: Crude oil degradation by parent and mutant *Staphylococcus* sp

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183 The t-test for crude oil degradation by parents and mutants showed statistically significant
 184 differences at 95% confidence level in *Bacillus* sp, *Escherichia* sp and *Pseudomonas* sp but
 185 none in *Streptococcus* sp, *Staphylococcus* sp, *klebsiella* sp, and *Micrococcus* sp.

186

187 Discussion

188 River Ethiope, a clear fresh water body, which flows for over 100km to empty into the Benin
 189 River (Omo-Irabor and Olobaniyi (2007), is used extensively for recreational and occupational
 190 activities such as fishing, swimming, washing, bathing and for domestic purposes by the
 191 inhabitants of villages and towns along its bank. Aside these, River Ethiope overflow its bank
 192 annually carrying all the domestic wastes into the river in addition to those attributable to
 193 runoffs during rains. These wastes, in addition to accidental release of spent oil and crude oil
 194 from spills into the Ethiope River, probably accounts for the variation in the number of bacterial
 195 genera from the sampled locations (Table 1). The numbers of isolates (genera) were found to
 196 be higher in those locations where recreational activities were high i.e. in beaches and
 197 swimming pools established along the river bank. The bacterial isolates obtained in this study -
 198 *Bacillus* sp, *Klesiella* sp, *Escherichia* sp, *Micrococcus* sp, *Pseudomonas* sp, *Staphylococcus* sp
 199 and *Streptococcus* sp (Table 1), have previously been reported in fresh water bodies from
 200 Nigeria by Ekhaize and Anyasi (2005), Aluyi *et al.* (2003, 2006), Duru and Nwanekwu (2012)
 201 and Agbabiaka and Oyeyiola (2012).

202 The ability of all the isolates obtained to degrade 1% (v/v) crude-oil (Table 2) with isolates from
 203 Okirighre bridge, Sapele, where there was a report of oil spillage, were able to degrade 2.5%
 204 (v/v) crude-oil (Addeh, 2012), agree with the reports by Okoh (2006) of widespread bacterial
 205 species with the ability to degrade crude-oil, even in unpolluted environments. The results also
 206 agree with the observations of Benter-Coker and Ekundayo (1997), Idise and Ameh (2010),
 207 Idise *et al.* (2009, 2010, 2012) that microbial isolates from petroleum oil-polluted soils and
 208 water possessed the metabolic potentials to degrade oil.

209

210 The observed reduction in counts, with exposure to increasing number of x-ray flashes in Table
 211 3, as well as the % of kill of isolates agrees with reports of Idise and Ameh (2010) and Idise *et*
 212 *al.* (2010, 2012) of same trend with mutational treatments using x-rays apparently due to
 213 damages in the DNA of the isolates after mutation leading to an inverselt proportional
 214 relationship between the number of x-ray flashes and the bacterial counts..

215

216 The observation that the x-ray modified isolates (mutants) possessed the potentials to degrade
 217 crude-oil, with isolates from the oil-polluted location having increased potentials, corroborates
 218 the reports of other workers on increased abilities of isolates after mutational treatment, as well
 219 as increased ability of mutants, due to enzyme induction (Idise and Ameh, 2010; Idise *et al.*,
 220 2010, 2012). However, the observed reduction in ability of mutants to degrade 1% (v/v) crude-
 221 oil (Figures 2a – 2g) could be due to reversal of x- rays mutational effects in the isolates. These
 222 results agree with the findings of Idise and Ameh (2010) who reported reduced crude-oil
 223 degradation by x-ray mutants of *Bacillus* sp obtained from motor spent-oil contaminated soil in
 224 Abraka. The results also corroborates the findings of reduced crude-oil degradation from 0.14%
 225 in *Streptococcus* sp to 6.31% in *Pseudomonas* sp (Table 5) with *Pseudomonas* sp (1.680ppm)

226 and mutant *Streptococcus* sp (1.474ppm) being highest and least crude-oil degraders
227 respectively.

228

229 The statistical analyses further corroborated above findings of reduced ability of x-ray mutants
230 to degrade crude-oil (1% v/v).

231

232 **Conclusion:** Two to five of seven bacteria (*Bacillus* sp, *Klesiella* sp, *Escherichia* sp,
233 *Micrococcus* sp, *Pseudomonas* sp, *Staphylococcus* sp and *Streptococcus* sp) were isolated
234 from each of the twelve locations along the River Ethiope. All the isolates possessed the ability
235 to degrade 1% (v/v) crude-oil even after mutation using x-rays, with increased ability on
236 exposure to crude-oil pollution. However, there were reduced potentials to degrade crude-oil
237 after x-ray mutation. Thus, irrespective of source of isolates on the Ethiope River, Nigeria,
238 crude-oil degrading bacteria abound and the bacteria obtained in this study could be employed
239 in bioremediation of crude oil pollution on the Ethiope River and/or other environments.

240

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