

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

by

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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 *Streptococcus* sp, *Escherichia* sp and *klebsiella* sp but not for *Bacillus* sp, *Staphylococcus* sp, *Pseudomonas* 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 and reduced ability on x-ray mutation. The bacteria encountered in this study could be employed in bioremediation of crude-oil polluted environments.

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

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

determine the potentials of some bacterial isolates of River Ethiope and their x-ray modified mutants to degrade Forcados crude oil.

Materials and method

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

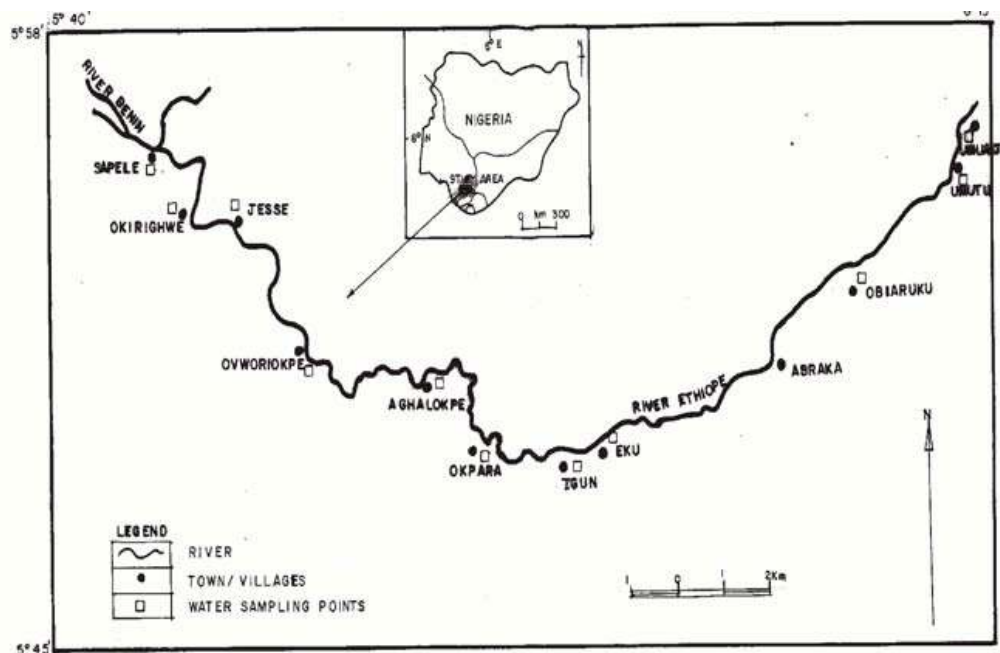


Figure 1: Map of the River Ethiope

Isolation of bacteria: The procedures of pour plate technique reported in Cheesebrough (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 24hours. 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 at 37°C for 24hours to determine viable counts.

Determination of crude-oil degrading potentials of parents and x-ray mutants: These were carried out in accordance with the procedures reported by Idise and Ameh (2010). Using one hundred and sixty milliliters of 1% (v/v) Forcados crude in MBM dispensed into each 250mL conical flask, 40mL of $1.685 \text{Log}_{10} \text{cfu/mL}$ 24h-cultures of each isolate was added to the respective flasks and incubated aerobically at room temperature ($28 \pm 2^\circ\text{C}$). Separate flasks were used for parents and their x-ray mutants. Growth was determined at day zero (one hour after incubation) and at three-day intervals for 30days using a spectrophotometer (Model 721-2000vmcs, Made in China) at 560nm.

Determination of total petroleum hydrocarbons (TPH): These were determined using ASTM D3921-96 in ASTM Vol. 11 (2002) thus: thirty milliliters of carbon tetrachloride (CCl_4) was added to one liter of the water sample to dissolve the hydrocarbons and shaken vigorously for two minutes. The mixture was poured into a separating funnel and shaken intermittently for three minutes and expelled the air by releasing the valves of the funnel. The funnel was then rinsed with twenty milliliters of carbon tetrachloride (CCl_4). The resulting oil was drained into a fifty milliliter volumetric flask through a funnel containing cotton wool to which had been added 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 separating funnel was then rinsed with ten milliliters of CCl_4 to remove all the oils. The Infra-red spectrophotometer was calibrated at 420nm using dry Forcados Dry crude oil with concentrations between 100 and 500 mg/L to obtain a standard curve. To ten milliliters of the extracted oil was added 0.5grams of deactivated floriscil. The resultant supernatant contained the total petroleum hydrocarbons (TPH) while the sediment contained the biogenic hydrocarbons. The TPH was then determined using the supernatant on the Infra-red spectrophotometer (Model Cecil CE 7400, Made in England) at Optical Density of 420nm. The

concentration of the sample was extrapolated from the standard curve. The values were obtained using the following relation:

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

Where: DF = Dilution factor
50 = initial extraction volume

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

Results

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

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

The crude-oil degrading ability of the bacterial isolates is presented in Table 2. It was observed that all the isolates from the sample locations possessed the ability to degrade 1% (v/v) crude oil except *Bacillus* sp, *Escherichia* sp and *Pseudomonas* sp that were isolated from Okirighre Bridge, Sapele that degraded 2.5% (v/v) crude oil.

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	+	+	+	+	+

Key: + = growth, - = no growth

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.

Table 3: Effects of x-ray treatment on isolates

Isolate	Number of x-ray flashes					SD	SE
	1	2	3	4	5		
<i>Bacillus</i> sp							
Initial	1.76	1.77	1.77	1.78	1.77	0.007	0.003
Final	1.74	1.66	1.74	1.72	1.73		
% kill	1.14	6.22	1.70	3.37	2.26		
<i>Klebsiella</i> sp							
Initial	1.75	1.74	1.73	1.74	1.73	0.008	0.004
Final	1.60	1.63	1.58	1.65	1.64		
% kill	8.57	6.32	8.67	5.17	5.20		
<i>Escherichia</i> sp							
Initial	1.79	1.80	1.80	1.78	1.81	0.19	0.009
Final	1.71	1.73	1.68	1.71	1.64		
% kill	4.47	3.89	6.67	3.93	9.39		
<i>Micrococcus</i> sp							
Initial	1.76	1.78	1.77	1.79	1.76	0.013	0.006
Final	1.73	1.70	1.73	1.74	1.76		
% kill	1.71	4.49	2.26	2.79	0.00		
<i>Pseudomonas</i> sp							
Initial	1.84	1.55	1.65	1.54	1.70	0.123	0.055
Final	1.66	1.54	1.63	1.50	1.70		
% kill	9.78	0.65	1.21	2.60	0.00		
<i>Staphylococcus</i> sp							
Initial	1.80	1.80	1.60	1.60	1.50	0.134	0.06
Final	1.70	1.70	1.75	1.56	1.76		
% kill	5.56	5.56	3.13	2.50	2.67		
<i>Streptococcus</i> sp							
Initial	1.73	1.82	1.80	1.78	1.76		

Final	1.72	1.75	1.70	1.67	1.53	0.035	0.016
% kill	0.58	3.85	5.56	6.18			
	13.07						

Key: SD = Standard deviation, SE = Standard error

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.

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

Isolate	% crude oil degraded	Isolate	% crude oil degraded
<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

SE = 0.00 and SD = 0.00

The mean values of crude-oil degraded by isolates are presented in Table 5 while the spectroscopic values are presented in Table 6. It was observed in Table 5 that parents degraded more than their mutants with reductions that ranged from 0.14% in *Streptococcus* sp to 6.31% in *Pseudomonas* sp. Parent *Pseudomonas* sp (1.680ppm) was highest degrader while mutant *Streptococcus* sp (1.474ppm) was least degrader. While the SD ranged from 0.001 to 0.075, the SE ranged from 0.001 to 0.15.

The values for TPH in Table 6 ranged from 8.378 in parent *Staphylococcus* sp to 8.526 in parent *Streptococcus* sp while it ranged from 8.320 in mutant *Pseudomonas* sp to 8.526 in mutant *Streptococcus* sp.

Table 5: Mean values of crude-oil (ppm) degraded by parents and mutants of isolates

Isolate	Parent (ppm)	Mutant (ppm)	% decrease	SD	SE
<i>Bacillus</i> sp	1.579	1.496	5.26	0.006	0.04
<i>Staphylococcus</i> sp	1.652	1.622	1.82	0.02	0.15
<i>Pseudomonas</i> sp	1.680	1.574	6.31	0.075	0.053
<i>Streptococcus</i> sp	1.476	1.474	0.14	0.001	0.001
<i>Escherichia</i> sp	1.536	1.531	0.33	0.004	0.003
<i>Micrococcus</i> sp	1.590	1.538	0.63	0.037	0.026
<i>Klebsiella</i> sp	1.578	1.528	3.17	0.035	0.025

Table 6: Spectroscopic values

Organism	Control	Sample	TPH
<i>Bacillus</i> sp Parent	0.00	16.842	8.504
<i>Bacillus</i> sp mutant	0.00	17.042	8.421
<i>Staphylococcus</i> sp Parent	0.00	16.696	8.378
<i>Staphylococcus</i> sp mutant	0.00	16.756	8.348
<i>Pseudomonas</i> sp Parent	0.00	16.640	8.426
<i>Pseudomonas</i> sp mutant	0.00	16.852	8.320
<i>Streptococcus</i> sp Parent	0.00	17.048	8.526
<i>Streptococcus</i> sp mutant	0.00	17.052	8.524
<i>Escherichia</i> sp Parent	0.00	16.928	8.469
<i>Escherichia</i> sp mutant	0.00	16.938	8.464
<i>Micrococcus</i> sp Parent	0.00	16.820	8.462
<i>Micrococcus</i> sp mutant	0.00	16.924	8.410

<i>Klebsiella</i> sp Parent	0.00	16.844	8.472
<i>Klebsiella</i> sp mutant	0.00	16.944	8.422

Key: TPH = Total petroleum hydrocarbons

The crude oil degradation by parents and mutants are presented in Table 7a while the values of t-test are presented in Table 7b. It was observed that mutant *Streptococcus* sp (1.47 ± 0.005) was the least degrader while parent *Pseudomonas* sp (1.68 ± 0.05) was the highest degrader. The statistical analyses in Table 7b showed that while parents and mutants of *Bacillus* sp, *Staphylococcus* sp, *Pseudomonas* sp and *Micrococcus* sp showed no statistically significant differences in crude oil degradation, there were in parents and mutants of *Streptococcus* sp, *Escherichia. coli* and *Klebsiella* sp.

Table 7a: Crude oil degradation by parents and mutants of isolates

	Parent Bacillus	Mutant Bacillus	Parent Staph	Mutant Staph	Parent Pseud	Mutant Pseud	Parent Strep	Mutant Strep	Parent Esch	Mutant Esch	Parent Micro	Mutant Micro	Parent Kleb	Mutant Kleb
Day														
1	1.486	1.523	1.343	1.309	1.339	1.379	1.329	1.419	1.279	1.36	1.516	1.516	1.481	1.515
4	1.28	1.342	1.976	1.997	1.956	1.934	1.087	1.287	1.884	1.158	1.417	1.462	1.275	1.267
7	1.618	1.437	1.432	1.439	1.465	1.382	1.338	1.333	1.282	1.544	1.61	1.682	1.719	1.679
11	1.972	1.826	1.743	1.749	1.803	1.706	1.726	1.704	1.734	1.739	1.745	1.826	1.749	1.702
14	1.633	1.596	1.753	1.77	1.752	1.707	1.716	1.678	1.753	1.712	1.651	1.586	1.664	1.516
17	1.677	1.651	1.69	1.55	1.643	1.51	1.539	1.281	1.426	1.503	1.723	1.583	1.701	1.518
21	1.722	1.647	1.558	1.488	1.74	1.446	1.529	1.439	1.367	1.488	1.735	1.672	1.794	1.772
24	1.557	1.252	1.692	1.649	1.644	1.507	1.25	1.446	1.446	1.533	1.771	1.348	1.539	1.444
27	1.253	1.25	1.53	1.507	1.621	1.448	1.436	1.433	1.46	1.516	1.121	1.255	1.285	1.401
30	1.533	1.401	1.747	1.7	1.743	1.692	1.678	1.608	1.688	1.669	1.504	1.433	1.462	1.403
33	1.638	1.527	1.705	1.683	1.772	1.604	1.626	1.59	1.574	1.615	1.701	1.55	1.689	1.562
N	11	11	11	11	11	11	11	11	11	11	11	11	11	11
Mean (X)	1.58	1.50	1.65	1.62	1.68	1.57	1.48	1.47	1.54	1.53	1.59	1.54	1.58	1.53
SD	0.20	0.18	0.18	0.19	0.17	0.17	0.21	0.15	0.20	0.17	0.19	0.16	0.18	0.15
X±SE	1.58±0.06	1.5±0.05	1.65±0.05	1.62±0.06	1.68±0.05	1.57±0.05	1.48±0.06	1.47±0.05	1.54±0.06	1.53±0.05	1.59±0.06	1.54±0.05	1.58±0.06	1.53±0.05

Key: Staph = Staphylococcus sp, Pseud = Pseudomonas sp, Strep = Streptococcus sp, Esch = Escherichia sp, Micro = Micrococcus sp,
Kleb = Klebsiella sp

Table 7b: Values of t-test for Crude oil degradation by parents and mutants of isolates

Isolate	t-cal	t-crit	Decision	Pearson correlation
<i>Bacillus</i> sp	2.5976	1.1825	H ₀ accepted	0.85
<i>Staphylococcus</i> sp	2.1199	1.1825	H ₀ accepted	0.97
<i>Pseudomonas</i> sp	3.8939	1.1825	H ₀ accepted	0.86
<i>Streptococcus</i> sp	0.0834	1.1825	H _A accepted	0.83
<i>E. coli</i>	0.0669	1.1825	H _A accepted	0.08
<i>Micrococcus</i> sp	1.1413	1.1825	H ₀ accepted	0.64
<i>Klebsiella</i> sp	2.0499	1.1825	H _A accepted	0.89

Discussion

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

Nigeria by Ekhaize and Anyasi (2005), Aluyi *et al.* (2003, 2006), Duru and Nwanekwu (2012) and Agbabiaka and Oyeyiola (2012).

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

The observed reduction in counts, with exposure to increasing number of x-ray flashes in Table 3, as well as the percentage of kill of isolates agrees with reports of Idise and Ameh (2010) and Idise *et al.* (2010, 2012) of same trend with mutational treatments using x-rays. This could apparently be due to damages in the DNA of the isolates after mutation leading to an inversely proportional relationship between the number of x-ray flashes and the bacterial counts. Statistical analyses in Table 3 showed that SD ranged from 0.007 (*Bacillus* sp) to 0.19 (*Escherichia coli*) while SE ranged from 0.003 (*Bacillus* sp) to 0.055 (*Pseudomonas* sp).

The observation that the x-ray modified isolates (mutants) in Table 4 possessed the potentials to degrade crude-oil, with isolates from the oil-polluted location having increased potentials, corroborates the reports of other workers on increased abilities of isolates after mutational treatment, as well as increased ability of mutants, due to enzyme induction (Idise and Ameh, 2010; Idise *et al.*, 2010, 2012). The observed reduction in ability of mutants to degrade 1% (v/v)

crude-oil in Table 5 could be due to reversal of x- rays mutational effects by the isolates apparently due to DNA repair mechanisms. The statistical analyses in Table 5 supports above results as while the SD ranged from 0.001 to 0.075, the SE ranged from 0.001 to 0.053 in *Streptococcus* sp and *Pseudomonas* sp respectively. The spectroscopic values in Table 6 also corroborate this reduction in crude-oil degradation by mutants. The crude-oil degradation by parents and mutants in Table 7a as well as their statistical analyses in Table 7b both also support the above findings. These results agree with the findings of Idise and Ameh (2010) who reported reduced crude-oil degradation by x-ray mutants of *Bacillus* sp obtained from motor spent-oil contaminated soil in Abraka. The results also support the findings of reduced crude-oil degradation from 0.14% in *Streptococcus* sp to 6.31% in *Pseudomonas* sp (Table 5) with *Pseudomonas* sp (1.680ppm) and mutant *Streptococcus* sp (1.474ppm) being highest and least crude-oil degraders respectively. However, while there were no statistically significant differences in crude-oil degradation by parents and mutants of *Bacillus* sp, *Staphylococcus* sp, *Pseudomonas* sp and *Micrococcus* sp, there were in parents and mutants of *Streptococcus* sp, *Escherichia coli* and *Klebsiella* sp.

Conclusion: Two to five of seven bacteria (*Bacillus* sp, *Klesiella* sp, *Escherichia* sp, *Micrococcus* sp, *Pseudomonas* sp, *Staphylococcus* sp and *Streptococcus* sp) were isolated from each of the twelve locations along the River Ethiope. All the isolates possessed the ability to degrade 1% (v/v) crude-oil even after mutation using x-rays, with increased ability on exposure to crude-oil pollution. However, there were reduced potentials to degrade crude-oil after x-ray mutation. There were observed statistically significant differences in crude-oil degradation by parents and mutants of *Bacillus* sp, *Staphylococcus* sp, *Pseudomonas* sp and *Micrococcus* sp, while no differences were observed in parents and mutants of *Streptococcus* sp, *Escherichia coli* and *Klebsiella* sp. Thus, irrespective of source of isolates on the Ethiope

River, Nigeria, crude-oil degrading bacteria abound and the bacteria obtained in this study could be employed in bioremediation of crude oil pollution on the Ethiope River and/or other environments.

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