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Hydrocarbon degradation potentials of bacterial species isolated from leachate of Lemna waste dump site Calabar

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ABSTRACT

Hydrocarbon degradation potentials of bacteria species isolated from leachate samples of Lemna dumpsite, Calabar, Cross River State were investigated. Five (5) leachate samples were collected from the dumpsite with the use of PVC pipes and were analyzed using standard microbiological techniques. Results of microbial counts from the dumpsite leachates ranged from 10 x 10^4cfu/g to 2.0 x 10^5cfu/g. Bacteria isolates, from the leachate samples were subjected to hydrocarbon biodegradability screen test using kerosene, diesel and premium motor spirit (PMS) as substrate for carbon source. Bacteria genera isolated from the analyzed leachate samples were identified as *Pseudomonas, Bacillus, Citrobacter, Klebsiella, Aeromonas, Serratia* and *Shigella*. Among these isolates, *Pseudomonas* (CPS_1, CPS_2), *Bacillus* (CPS_2, CPS_{10}), *Aeromonas* (CPS_5), *Serratia* (CPS_{13}) and *Corynebacterium* (CPS_6; CPS_{16}) showed efficient kerosene biodegradability potentials compared to other of their bacteria counterparts. Also *Pseudomonas* (CPS_1, CPS_{12}), *Bacillus* (CPS_2, CPS_{10}), *Aeromonas* (CPS_5) and *Serratia* (CPS_{11}) were able to biodegrade diesel and premium motor spirit efficiently. However, in comparing hydrocarbon biodegradation potentials among the bacteria isolates from the analyzed leachate samples, *Pseudomonas* (CPS_1, CPS_{12}), *Bacillus* (CPS_2, CPS_{10}), *Aeromonas* (CPS_5) and *Serratia* (CPS_{11}) were efficient kerosene, diesel and premium motor spirit biodegraders, and this could be useful for bioremediation. This therefore calls for further research by microbiologist and other environmental agencies on the large scale applicability of these isolates for the bioremediation of hydrocarbon polluted environments, as this could serve as a more eco-friendly approach.

Keywords: Hydrocarbons, Dumpsite, Leachate, Degradation, Lemna.,

INTRODUCTION

Environmental pollution by hydrocarbons including petroleum and other petrochemical products have attracted much attention in recent decades (Youssef *et al.,* 2010), as the contamination of soil by hydrocarbons is

rapidly increasing due to global increase in crude oil exploration and exploitation, as well as increase in the usage of petroleum products (Okerentugba and Ezeronye, 2013).

Mishaps due to a number of causes such as corrosion of pipelines and tankers, sabotage, oil production operation (normal and operational failures), inadequate or non-functional production equipment, amongst others have resulted to the spillage of crude oil and contamination of our environment with hydrocarbons (Broomjiman *et al.,* 2009). Also, the presence of different types of automobiles and machinery have resulted in an increase in the use of lubricating oil and the spillage of these oils such as spent engine oil, used hydraulic oil, and used brake pad oil that contaminates our environment with hydrocarbon (Tiku *et al.,* 2016; Bassey *et al*., 2018). The presence of this hydrocarbon especially polycyclic aromatic hydrocarbon in the environment attracts public attention, as researches have shown that they are toxic, mutagenic and carcinogenic (Mandris & Lin, 2007). Prolonged exposure to high waste lubricating oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow and an increase rate of cancer (Husaini *et al.,* 2008; Bassey *et al*., 2016). In addition, polycyclic aromatic hydrocarbons have a wide spread occurrence in various ecosystems that contributes to the persistence of these compounds in the environment and the illegal dumping of these waste lubricating oils is an environmental hazard with global ramifications, they contain heavy metals and heavy polycyclic aromatic hydrocarbons that could also contribute to chronic hazards including mutagenicity and carcinogenicity (Clemente *et al.,* 2001; Van Hamme *et al.,* 2003; Bassey *et al*., 2016).

Microbial degradation has been described to be the most environmental friendly method for removal of hydrocarbons in the environment since the conventional physical and chemical method leads to introduction of toxic compounds and formation of sludge in the environment (Boonchan *et al.,* 2010). Researchers have reported that hydrocarbon degrading microorganisms are widely distributed in marine, fresh water and soil ecosystem (Oboh *et al.,* 2006). However, they do not occur alone but in mix consortia with heterotrophic microorganisms without degradation capabilities.

MATERIALS AND METHODS

The study area and sampling site

Leachate samples were collected from a dumpsite popularly called Lemna dumpsite which is an open dumpsite (latitude: $4^0$13' E and $5^0$15' E and longitude $8^0$15 S and $8^0$21'S) located in Ikot Effanga Mkpa ravine, in Calabar Municipality Local Government Area of Cross River State, South-South Nigeria. The sampling area is characterized by wet and dry seasons with high annual rainfall in the range of 350 – 400mm, mangrove vegetation and run-off estimated to reach 90%. The waste dumpsite is managed by Patson Environmental Services Limited (PESL) and receives wastes on daily basis. Most of these disposed wastes are mainly domestic and market wastes. At the time of sampling, the waste dumpsite covered an area of about 3,265m^2 and is less than 500m away from a small stream that serves as source of domestic and irrigation water for many households within the study site. The stream eventually empties into the Great Kwa river (see map), which is the only source of water intake to the state-owned water board company.

Leachate sample collection

The method described by Bassey *et al.* (2015) was slightly modified for effective sampling of leachate. Leachate samples were collected from 4 different locations using PVC pipes cut into four parts, each of 1m in length. The base end of each pipe was permanently sealed with a pipe cover and an adhesive while the top ends were just fitted with pipe covers (see figure 1). The pipes were perforated evenly at considerable distances from their base ends to allow for leachate percolation and collection. The whole pipe lengths were then buried into an already dug ground in each sampling point with small allowances at the top for easy access to the top ends (which were only temporarily sealed). The buried pipes at different depth were left for a period of three to four (3-4) weeks before sampling for the percolated leachates. Sterile Enema pumps were used for leachate collection into sterile bottles and labelled properly as LS1, LS2, LS3 and LS4. The samples were then transported immediately to the laboratory for physicochemical and metagenomics analysis.

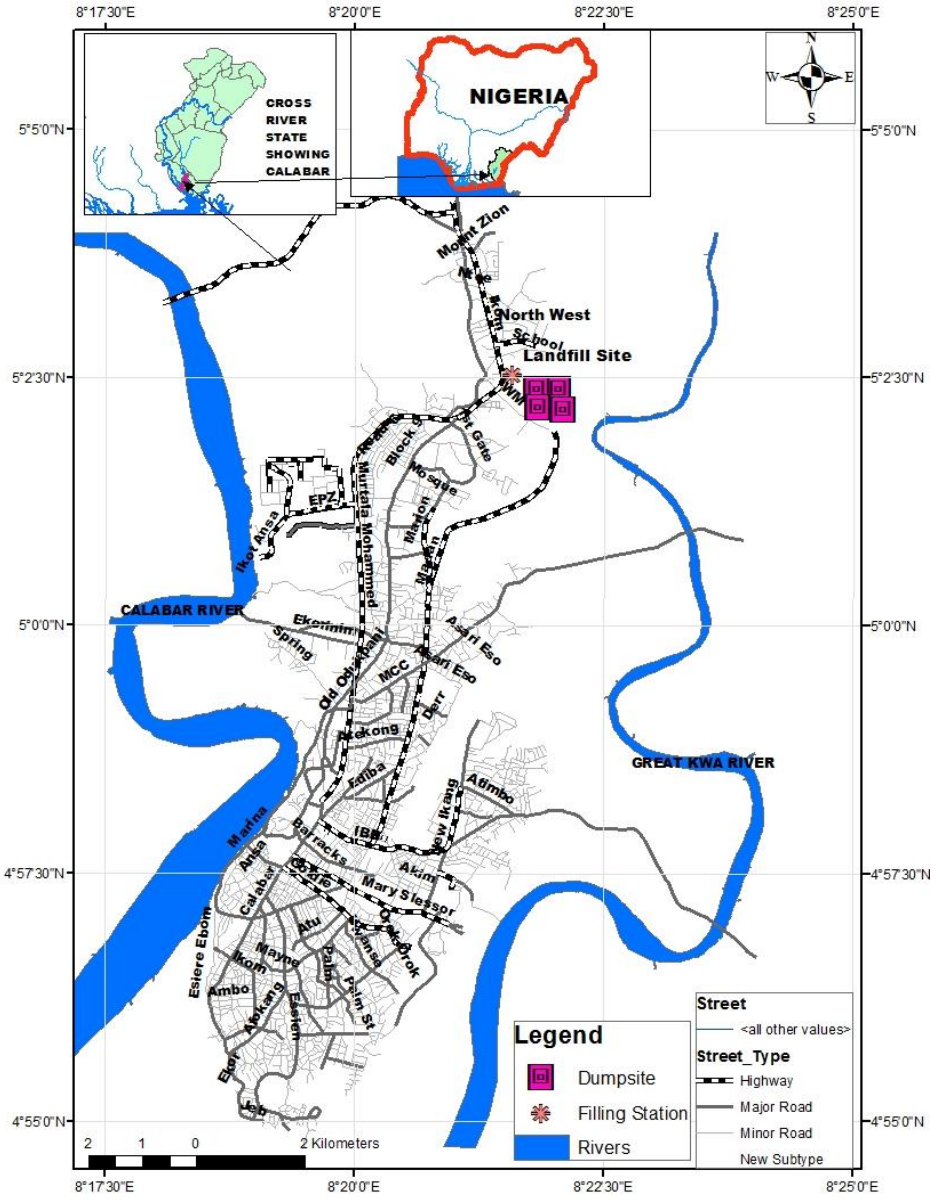

Figure 1: Map of study area

I **II**

Figure 1: Collection of leachate samples using perforated PVC. I = Perforated PVC buried at dumpsite; II = Percolated leachate sample after 4 weeks.

Physicochemical analysis of leachate sample

Turbidity was measured spectrophotometrically using standards according to HACH. Biochemical oxygen demand (BOD_5) was measured as the difference between initial oxygen concentration in sample and concentration after 5 days of incubation in DO at 20°C (APHA, 1991). Nitrite and nitrate were determined spectrophotometrically by turbidometry using barium chloride (APHA, 1991). Temperature was measured using a Celsius thermometer. Temperature, pH, dissolved oxygen (DO) and conductivity were measured were measured in-situ using appropriate meter (Jenway 970 DO_2) with sensitivity of ±0.1%. Electrical conductivity meter with sensitivity ±0.1% was used to measure conductivity. Salinity was determined using the argentometric method. Nitrite, nitrate, chloride, sulphate and total dissolved solids were done as previously reported (APHA, 1991). Atomic absorption spectrophotometer (AAS Model 2380) was used for the analysis of selected heavy metals (Cr, Cd, Zn, Ni, Pb, Co, Mn, Cu, and Fe) (Rorrer, 1999; Farstner and Wittman, 1979).

Kerosene, diesel and petrol samples

Kerosene, diesel and petrol samples were purchased at Udensco filling station located at yellow duke street, Calabar. They were placed into 1 litre plastic containers and transported to the laboratory for analysis.

Sample preparation

This was preformed according to the method described by Antai *et al.,* (2016) with slight modification. Ten milliliter (10ml) of the leachate samples was introduced into 90mls of sterile distilled water in a 100mls conical flask. The samples were vortexed to homogenize and allowed to stand for 10 minutes. From this initial dilution, 10-fold serial dilutions were carried out in clean sterile test tubes containing 9mls of sterile distilled water.

Purification of isolates

Bacterial colonies were picked at random and subcultured repeatedly into nutrient agar for purification. Purified isolates were stocked in nutrient agar slants for further studies.

Identification and characterization of isolates

Purified isolates were characterized by Gram morphology and biochemical test using schemes in Bergey's manual of determinative bacteriology (Holt *et al.,* 1994; Cheesbrough, 2000).

Hydrocarbon biodegradation potential of bacterial isolates

Modified micro-liter plate technique as described by Malatova (2005) was used in screening of the bacteria isolates for kerosene, petrol and diesel potentials. Pure bacterial isolates from the leachate samples were inoculated into 5mls of nutrient broth and incubated for 48 hours at room temperature. 0.1ml each of the pure bacteria isolates from the nutrient broth was then inoculated into 9.9mls of Bushnell Haas Broth contained in two different tubes one hundred (100µl) of sterilized kerosene, petrol and diesel oil were then pipette into the different test tubes. Controls containing only the kerosene, petrol and diesel were also set up.

All the test tubes were incubated for 16 days at room temperature without shaking. On the 16th day, 50µl of p-iodonitrotetrazolium violet (INT) indicator was added to each tube and the tube was further incubated for 24 hours. Kerosene, petrol and diesel biodegradation was indicated by pink to red precipitate, while the broth turbidity was also observed. At the end of the screen test, a high degree of precipitate and broth turbidity of both kerosene, petrol and diesel observed from the biodegradation activities of the bacteria isolates were assigned +++, while the formation of moderate and low degree of precipitate and broth turbidity were assigned ++ and + respectively. However, the absence of precipitate and turbidity during the biodegradation screen test by the bacteria isolates was assigned.

RESULTS

Morphological and biochemical characterization of bacteria isolates from the analyzed leachate sample

Table 1 presents the result of morphological and biochemical characterization of bacteria isolates from the analyzed leachate samples. It revealed that the bacteria genera were identified as *Pseudomonas, Bacillus, Citrobacter, Yersinia, Enterobacter, Serratia* and *Shigella*.

Table 1 : Morphological and biochemical characterization of bacteria isolates from leachate

Isolate code	Gram R x N	Shape	Oxidase	Catalase	Motility	Indole	Ornithine	Methyl Red	Voges proskauer	Urease	Citrate	Starch hydrotysis	Acid fast	Slope	Butt	H_2S	Gas	Probable organisms
CPS_1	-	Rod	+	+	+	-	-	-	-	-	+	-	-	Y	Y	-	-	*Pseudomonas sp*
CPS_2	+	Rod	+	+	+	-	-	-	+	+	-	-	-	Y	Y	-	-	*Bacillus sp*
CPS_3	-	Rod	-	-	-	*	-	+	-	-	*	*	-	Y	Y	+	AG	*Citrobacter sp*
CPS_4	-	Rod	-	*	-	-	+	+	-	-	-	*	-	Y	Y	-	AG	*Klebsiella sp*
CPS_5	-	Rod	+	-	-	*	-	-	-	-	+	-	-	R	Y	-	AG	*Aeromonas sp*
CPS_6	+	Rod	-	+	+	-	-	+	-	-	-	-	-	Y	Y	-	-	*Corynebacterium sp*
CPS_7	+	Rod	+	+	+	-	-	-	+	+	-	+	-	Y	Y	-	-	*Bacillus sp*
CPS_8	-	Rod	-	-	-	-	-	+	-	-	-	+	-	R	Y	-	AG	*Yersinia sp*
CPS_9	-	Rod	-	-	-	+	-	+	-	+	-	+	-	R	Y	+	AG	*Enterobacter sp*
CPS_{10}	+	Rod	+	+	+	-	-	-	+	+	-	+	-	Y	Y	-	-	*Bacillus sp*
CPS_{11}	-	Rod	-	-	+	-	-	+	-	-	-	-	-	R	Y	-	AG	*Pseudomonas sp*
CPS_{12}	-	Rod	+	+	+	-	-	-	-	+	+	-	-	Y	Y	-	-	*Pseudomonas sp*
CPS_{13}	-	Rod	-	-	+	-	-	+	-	-	-	-	-	R	Y	-	AG	*Serratia sp*
CPS_{15}	-	Rod	-	-	-	-	+	-	-	-	*	-	-	R	Y	-	-	*Shigella sp*
CPS_{16}	+	Rod	-	+	+	-	-	+	-	-	-	-	-	Y	Y	-	-	*Corynebacterium sp*

Legend; R = Red, Y = Yellow, AG= Acid and Gas, + = Positive, - =Negative * = Not determined,
CPS = Culture plate sample

Table 2 : Frequency of occurrence of isolated bacterial species samples of Lemna waste dumpsite

Isolated bacterial species	Frequency of occurrence percentage bacteria isolates			
	DSLS$_1$	**DSLS$_2$**	**DSLS$_3$**	**DSLS$_3$**
Pseudomonas sp	45(15.41)	43(15.30)	41(16.47)	40(18.51)
Bacillus sp	36(12.30)	43(11.03)	34(13.65)	32(14.81)
Citrobacter sp	28(9.59)	30(10.68)	22(8.84)	20(9.26)
Klebsiella sp	32(10.96)	34(12.09)	30(12.05)	27(12.5)
Aeromonas sp	35(11.99)	31(11.03)	32(12.85)	30(13.89)
Yersinia sp	21(7.20)	20(7.12)	15(6.02)	11(5.09)
Corynebacterium sp	18(6.16)	18(6.41)	12(4.82)	8(2.70)
Enterobacter sp	29(9.93)	30(10.68)	25(10.04)	21(9.72)
Serratia sp	15(5.14)	19(6.76)	10(4.01)	6(2.78)
Shigella sp	33(11.30)	29(10.32)	28(11.24)	21(9.72)
Total	292	281	249	216

DSLS = Dumpsite, Leachate sample

Frequency and percentage occurrence of bacteria isolates from the collected leachate samples

Table 2 presents the result of frequency of occurrence of bacterial isolates from the leachate samples of Lemna waste dump site. It showed that *Pseudomonas* had the highest frequency and percentage occurrence 45(15.4%) in Dumpsite Leachate-1; 43(15.30%) in Dumpsite Leachate-2; 14(16.47%) and 40(18.51%) in Dumpsite Leachate-3 while *Serratia* had the lowest frequency and percentage occurrence 15(5.14%) in Dumpsite Leachate-1; 14 (16.47%) in Dumpsite Leachate-2; 10(4.01%) in Dumpsite Leachate-3, 6(2.78%) as compared to other of their bacterial counterparts from the analyzed leachate samples.

Hydrocarbon degrading potential of selected isolates from dumpsite leachates

Figure 1 presents the result of hydrocarbon degradation potentials of selected isolates from dumpsite leachates. It showed that there was no significant difference in the hydrocarbon degradation potential of the selected isolates.

Figure 2 present the result of colony forming units of *Pseudomonas sp* grown on three petroleum fraction for 5 days. It showed that *Pseudomonas sp* had a significantly higher growth rate and degradation potentials on kerosene (on the 4th day) and diesel (on the 1st day).

Figure 3 present the result of colony forming units of *Bacillus sp* grown on three different petroleum fractions for 5 days. It showed that *Bacillus sp* had a significantly higher growth rate and degradation potentials on kerosene (on the 3rd day) compared; to other petroleum fractions they were incubated in.

Figure 4 present the result of colony forming units of *Aeromonas sp* grown in three petroleum fractions for 5 days. It showed that *Bacillus sp* had a higher growth rate and degradation potentials on kerosene (on the 3rd day) compared to other petroleum fractions they were incubated in.

Figures 5 present the result of colony forming units of *Klebsiella sp* grown in petroleum fractions in 5 days. It showed that *Klebsiella sp* had a significantly higher growth rate and hydrocarbon degradation potentials on kerosene (on the 3rd day) compared to other petroleum fractions they were incubated in.

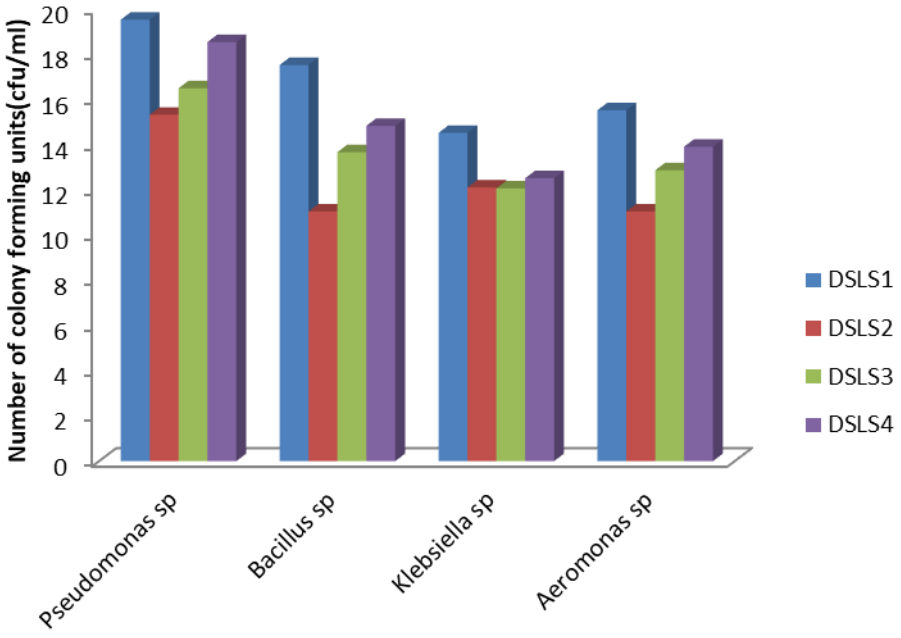

Figure 1: Hydrocarbon degrading potentials of selected isolates from dumpsite leachates

Table Analyzed	Data 2	
Two-way ANOVA		
Source of Variation	% of total variation	P value
Column Factor	52.60	< 0.0001
Row Factor	41.70	0.0002
Source of Variation	P value summary	Significant?
Column Factor	***	Yes
Row Factor	***	Yes

Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	3	52.26	17.42	27.67
Row Factor	3	41.43	13.81	21.94
Residual	9	5.667	0.6296	
Number of missing values	0			

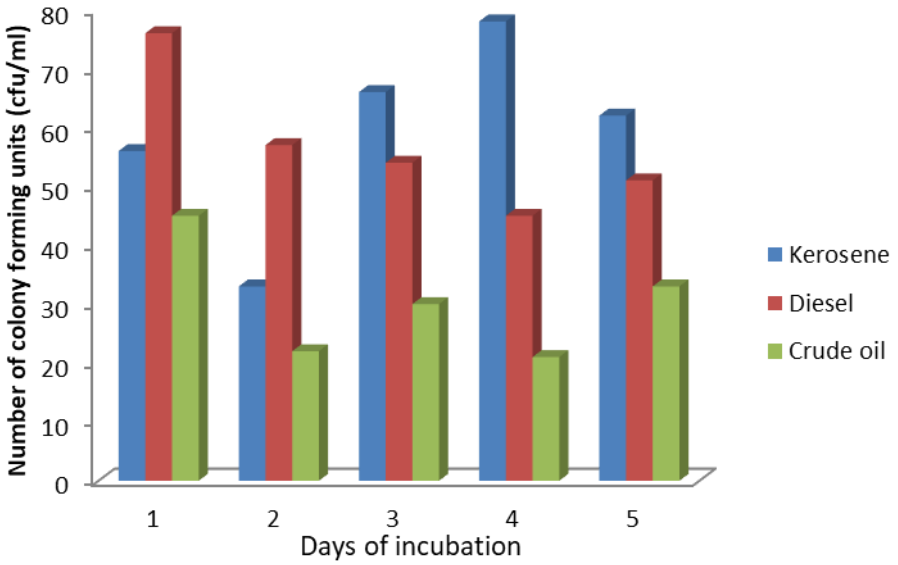

Figure 2: Bar chart showing colony forming units of *Pseudomonas* sp grown on three petroleum fractions after 5 days

Table Analyzed	Data 2	
Two-way ANOVA		
Source of Variation	% of total variation	P value
Column Factor	15.53	0.4274
Row Factor	55.69	0.0135

Source of Variation	P value summary	Significant?
Column Factor	ns	No
Row Factor	*	Yes

Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	4	712.3	178.1	1.079
Row Factor	2	2554	1277	7.740
Residual	8	1320	165.0	

Number of missing values	21

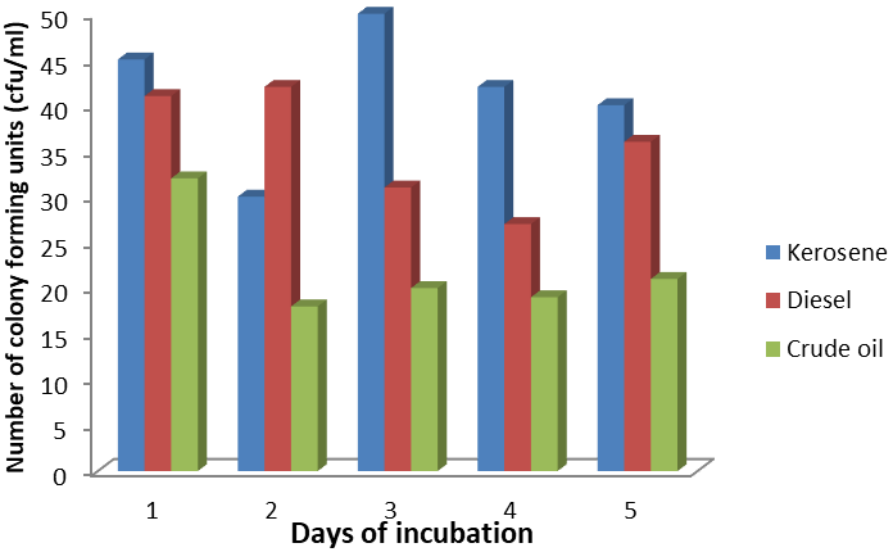

Figure 3: Bar chart showing colony forming units of Bacillus sp grown on three different petroleum fractions for 5 days.

Table Analyzed	Data 2			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Column Factor	12.68	0.3909		
Row Factor	65.73	0.0037		
Source of Variation	P value summary	Significant?		
Column Factor	ns	No		
Row Factor	**	Yes		
Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	4	190.3	47.57	1.174
Row Factor	2	986.5	493.3	12.17
Residual	8	324.1	40.52	
Number of missing values	21			

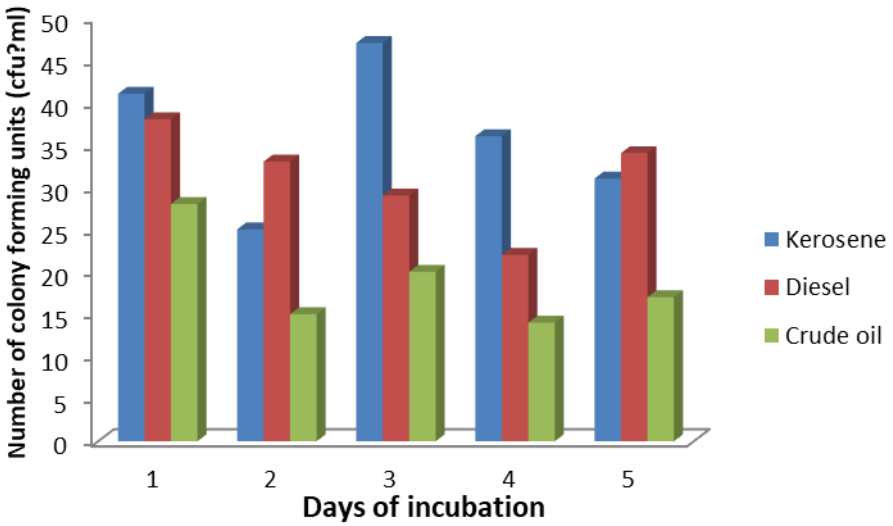

Figure 4: Bar chart showing colony forming units of Aeromonas sp grown in three petroleum fractions for 5 days

Table Analyzed	Data 2	
Two-way ANOVA		
Source of Variation	% of total variation	P value
Column Factor	22.71	0.1380
Row Factor	58.21	0.0037

Source of Variation	P value summary	Significant?
Column Factor	ns	No
Row Factor	**	Yes

Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	4	307.3	76.83	2.380
Row Factor	2	787.7	393.9	12.20
Residual	8	258.3	32.28	

Number of missing values	21

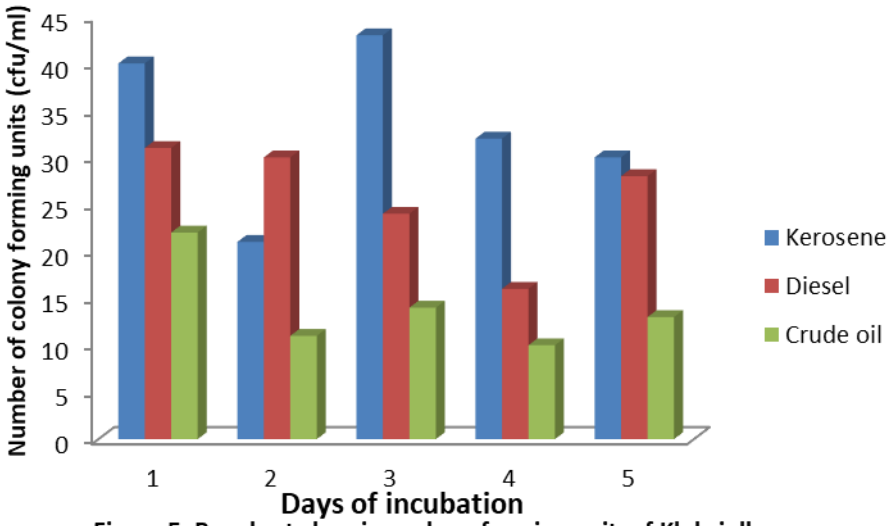

Figure 5: Bar chart showing colony forming unit of Klebsiella sp grown in petroleum fractions in 5 days

Table Analyzed	Data 2			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Column Factor	18.34	0.1863		
Row Factor	63.39	0.0025		
Source of Variation	P value summary	Significant?		
Column Factor	ns	No		
Row Factor	**	Yes		
Source of Variation	Df	Sum-of-squares	Mean square	F
Column Factor	4	271.3	67.83	2.008
Row Factor	2	937.7	468.9	13.88
Residual	8	270.3	33.78	
Number of missing values	21			

Screen test for hydrocarbon degradation by bacteria isolates from the analyzed leachtate samples

Table 3 presents the result of screen test for kerosene degradation by bacteria isolates from leachate samples. It showed that among the isolates tested, *Pseudomonas* (CPS_1, (PS_{12}), *Bacillus* (CPS_2, (PS_{10}), *Aeromonas* (CPS_5, (PS_{13}) and *Corynebacterium* (CPS_6, (PS_{16}) were efficient kerosene biodegraders compared to others.

Table 4 present the result of screen test for diesel biodegradation by bacteria isolates from the leachate sample. It showed that *Pseudomonas* (CPS_1, (PS_{12}) , *Bacillus* (CPS_2, (PS_{10}), *Aeromonas* (CPS_5), and *Serratia* (PS_{11}) were able to biodegrade diesel efficiently.

Table 5 present the result of premium motor spirit (PMS) biodegradation by bacteria isolates from the analyzed leachate samples. It showed that *Pseudomonas* (CSP_1, CPS_{12}), *Bacillus* (CSP_2, CPS_{10}), *Aeromonas* (CSP_5) and serratia (CPS_{13}) were able to biodegrade premium motor spirit (PMS).

Table 3 : Screen test for kerosene biodegradation by bacteria isolates from leachate samples

Isolate code	Colour of kerosene layers	Broth turbidity	Degree of precipitate	Colour of precipitate	Probable organism
CPS_1*	Pink	+++	+++	Pink	*Pseudomonas sp*
CPS_2*	Light pink	+++	+++	Light pink	*Bacillus sp*
CPS_3	Colourles	-	+	Colourles	*Citrobacter sp*
CPS_4	Colourles	-	-	Colourles	*Klebsiella sp*
CPS_5*	Light pink	+++	+++	Light pink	*Aeromonas sp*
CPS_6	Light pink	+	+	Light pink	*Corynebacterium sp*
CPS_7	Pink	+	+	Pink	*Bacillus sp*
CPS_8	Colourles	-	-	Colourles	*Yersinia sp*
CPS_9	Colourles	+	-	Colourles	*Enterobacter sp*
CPS_{10}	Black	+	-	Colourles	*Bacillus sp*
CPS_{11}*	Pink	+++	+++	Pink	*Serratia sp*
CPS_{12}*	Pink	+++	+++	Pink	*Pseudomonas sp*
CPS_{13}*	Pink	+++	+++	Pink	*Serratia sp*
CPS_{14}	Light pink	+	+	Light pink	*Serratia sp*
CPS_{15}	Colourles	-	+	Colourles	*Shigella sp*
CPS_{16}*	Light pink	+++	+++	Light pink	*Corynebacterium sp*
Control	Colourles	-	-	Colourles	-

Legend: +++ = High, ++ = Moderate, + = Low, -= Nill, * = Efficient kerosene biodegraders , CPS = Culture plate sample

Table 4 : Screen test for diesel biodegradation by bacteria isolates from leachate samples

Isolate code	Colour of diesel layers	Broth turbidity	Degree of precipitate	Colour of precipitate	Probable organism
CPS_1*	Pink	+++	+++	Pink	*Pseudomonas sp*
CPS_2*	Light pink	+++	+++	Light pink	*Bacillus sp*
CPS_3	Colourles	-	-	Colourles	*Citrobacter sp*
CPS_4	Colourles	-	+	Colourles	*Klebsiella sp*
CPS_5*	Light pink	+++	+++	Light pink	*Aeromonas sp*
CPS_6	Colourles	+	-	Colourles	*Corynebacterium sp*
CPS_7	Light pink	+	+	Light pink	*Bacillus sp*
CPS_8	Colourles	-	-	Colourles	*Yersinia sp*
CPS_9	Colourles	-	+	Colourles	*Enterobacter sp*
CPS_{10}*	Pink	+++	+++	Pink	*Bacillus sp*
CPS_{11}*	Light pink	+++	+++	Light pink	*Serratia sp*
CPS_{12}*	Pink	+++	+++	Pink	*Pseudomonas sp*
CPS_{13}*	Light pink	+	+	Light pink	*Serratia sp*
CPS_{14}	Light pink	+	+	Light pink	*Serratia sp*
CPS_{15}	Colourles	-	+	Colourles	*Shigella sp*
CPS_{16}*	Light pink	+	++	Light pink	*Corynebacterium sp*
Control	Colourles	-	-	Colourles	-

Legend: +++ = High, ++ = Moderate, + = Low, -= Nill, * = Efficient diesel biodegraders , CPS = Culture plate sample

Table 5 : Screen test for diesel biodegradation by bacteria isolates from leachate samples

Isolate code	Colour of PMS layers	Broth turbidity	Degree of precipitate	Colour of precipitate	Probable organism
CPS_1*	Pink	+++	+++	Pink	*Pseudomonas sp*
CPS_2*	Light pink	+++	+++	Light pink	*Bacillus sp*
CPS_3	Colourles	-	-	Colourles	*Citrobacter sp*
CPS_4	Colourles	-	+	Colourles	*Klebsiella sp*
CPS_5*	Light pink	+++	+++	Light pink	*Aeromonas sp*
CPS_6	Colourles	-	-	Colourles	*Corynebacterium sp*
CPS_7	Light pink	-	+	Light pink	*Bacillus sp*
CPS_8	Colourles	-	-	Colourles	*Yersinia sp*
CPS_9	Colourles	-	+	Colourles	*Enterobacter sp*
CPS_{10}*	Pink	+++	+++	Pink	*Bacillus sp*
CPS_{11}*	pink	+++	+++	pink	*Serratia sp*
CPS_{12}*	Light pink	+++	+++	pink	*Pseudomonas sp*
CPS_{13}*	Light pink	+	+	Light pink	*Serratia sp*
CPS_{14}	Colourless	+	-	Colourless	*Serratia sp*
CPS_{15}	Colourless	-	+	Colourles	*Shigella sp*
CPS_{16}*	Colourless	+	++	Colourless	*Corynebacterium sp*
Control	Colourles	-	-	Colourles	-

Legend: +++ = High, ++ = Moderate, + = Low, -= Nill, * = Efficient PMS biodegraders , CPS = Culture plate sample

TABLE 6 : Comparism of kerosene, diesel and biodegradation among bacteria isolates leachate samples

Isolate code	EKB	EDB	EPB	Probable organisms
CPS_1*	√	√	√	*Pseudomonas sp*
CPS_2*	√	√	√	*Bacillus sp*
CPS_3	x	X	x	*Citrobacter sp*
CPS_4	x	X	x	*Klebsiella sp*
CPS_5*	√	√	√	*Aeromonas sp*
CPS_6	x	X	x	*Corynebacterium sp*
CPS_7	x	X	x	*Bacillus sp*
CPS_8	x	X	x	*Yersinia sp*
CPS_9	x	X	x	*Enterobacter sp*
CPS_{10}*	√	√	√	*Bacillus sp*
CPS_{11}*	√	√	√	*Serratia sp*
CPS_{12}*	√	√	√	*Pseudomonas sp*
CPS_{13}*	x	√	√	*Serratia sp*
CPS_{14}	x	X	x	*Serratia sp*
CPS_{15}	x	X	x	*Shigella sp*
CPS_{16}*	x	√	x	*Corynebacterium sp*

Legend: EKB = Efficient kerosene biodegraders, EDB=Efficient Diesel biodegraders, EMPS = efficient premium motor spirit √ = yes, x = No., CPS = culture plate sample

Comparison of hydrocarbon biodegradation potentials among bacteria isolates from leachate sample

Table 6 presents the result of comparism of kerosene, diesel and premium motor spirit biodegradation by bacteria isolates from the analyzed leachate samples. It showed that in comparing the hydrocarbon biodegradation potentials among the bacteria isolates from the leachate samples *Pseudomonas* (CSP_1, CPS_{12}), *Bacillus* (CSP_2, CPS_{10}), *Aeromonas* (CSP_5) and *Serratia sp* (CSP_{11}) were efficient kerosene, diesel and premium motor spirit (PMS) biodegraders.

DISCUSSION

Hydrocarbon contamination resulting from the daily routines and activities of the petrochemical industry is one of the major problems of the oil rich Niger Delta region. Accidental releases of petroleum products are of particular concern in the environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants. Currently accepted disposal methods of incineration or burial insecure landfills can become prohibitively expensive when amounts of contaminants are large. Mechanical and chemical methods generally used to remove hydrocarbons from contaminated sites have limited effectiveness and can be expensive. Bioremediation is the promising technology for the treatment of these contaminated sites since it is cost-effective and will lead to complete mineralization. In this study, bacteria genera isolated from leachate samples in Lemna waste dumpsite were identified as *Pseudomonas, Bacillus, Citrobacter, Klebsiella, Chromobacterium violaceum, Micrococcus luteus, Aeromonas, Corynebacterium, Yersinia, Enterobacter, Serratia* and *Shigella.* The observation was not surprising, as similar study by Bassey *et al.,* (2015), who reported to have identified *Bacillus, Pseudomonas Staphylococcus aureus* and *Serratia* from Lemna dumpsite in Calabar, Cross River State.

The result of the screen test showed that among the isolates from leachate samples, *Pseudomonas* (CPS_1, CPS_{12}), *Bacillus* (CPS_2, CPS_{10}), *Aeromonas* (CPS_5), *Serratia* (CPS_{13}) and *Corynebacterium* (CPS_6, CPS_{16}) were efficient kerosene biodegraders, compared to other bacteria isolate counterparts, *Pseudomonas* (CPS_1, CPS_{12}), *Bacillus* (CPS_2, CPS_{10}), *Aeromonas* (CPS_5) and *Serratia* (CPS_{11}) were able to biodegrade diesel efficiently while Pseudomonas (CPS_1, CPS_{12}), *Bacillus* (CPS_2, CPS_{10}), *Aeromonas* (CPS_5) and *Serratia* (CPS_{13}) were able to biodegrade premium motor spirit (PMS). This observation is in agreement with of Ekhaise and Nkwelle (2011) who reported to have identified *Bacillus, Pseudomonas* and *Serratia* species that utilized spent motor oil. Also similar study by Tiku *et al.,* (2016) reported to have identified *Pseudomonas* and *Bacillus* species that biodegraded diesel. This observation agrees with findings of Mikessel *et al.,* (2013), who reported a higher abundance of crude oil utilizing bacterial consortiums at vary site of oil seepages. However, this observation confirms earlier findings that hydrocarbon provides a source of carbon and energy for the growth of hydrocarbonoclastic bacteria (Eze *et al.*, 2014).

Municipal solid waste dumpsites often produce high volume of leachates that are loaded with diverse microbial species, most of which have are hydrocarbonoclastic with high potentials for degradation of different fractions of hydrocarbons and other environmental contaminants (Bassey *et al*., 2018). The presence of these hydrocarbonoclastic bacteria in dumpsite leachates (DSW) and decomposing solid waste (DSW) of Lemna dumpsite could also be attributed to the disposed oil cans and used engine oils (Bassey *et al*., 2018), which is also an indication of the organisms persistence and utilization of these fractions of oil as their carbon source over time. However, *C. violaceum* isolated from an environment without a history of oil spill may not be an active hydrocarbon degrader unlike *Bacillus* sp and *Micrococcus* sp with much published literatures as efficient hydrocarbon degraders (Bassey *et al*., 2018; Bassey *et al.,*2016).

The ability of these microbial isolates to grow and proliferate in the medium could have been as a result of the direct or indirect use of this hydrocarbons as carbon and energy source for growth and this is in agreement with the findings of Yakimov *et al.,* (2012), Okerentugba and Ezeronye (2013). Also study by Husaini *et al.,* (2010) reported that hydrocarbons provide a source of carbon and energy for microbial growth, as they are readily assimilated as substrates and this explains the presence of microbial diversity in petroleum or waste oil contaminated soil samples. Margesin *et al.,* (2010), who carried out similar research, reported that the addition of hydrocarbon was the main driver for the increase of CFU and

diversity of heterotrophic hydrocarbonoclastic bacteria in crude oil polluted soil samples.

CONCLUSION

Cleaning up of hydrocarbons polluted environments is a crucial issue and of utmost concern in the Delta as its negative implications far outweighs its positive implications. A better understanding of the mechanism of biodegradation has a high ecological significance that depends on the indigenous microorganisms to transform or mineralize the organic contaminants. Hydrocarbon degradation potentials by some of the isolates could have been mediated by specific enzyme systems such as cytochrome P_{450} Alkane hydroxylases which have been implicated to play an important role in the microbial degradation of oil, chlorinated hydrocarbons, crude oil additives and many other compounds. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy. Based on the present study, it is pertinent to conclude that Lemna waste dumpsite harbors bacterial species with high potentials of hydrocarbon degradation.

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