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The Potential Use of NPK and Bacterial Isolates to Degrade Heglig crude Oil in Sudan

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A B S T R A C T

A study was carried out to examine the capabilities of three bacterial isolates to degrade crude oil in NPK- supplemented soil to which crude oil was added at 1, 3,5 and 10 %.750 mg NPK /100g soil at a ratio of 2:1:1was added to the polluted soil and mixed thoroughly. The tested bacterial isolates revealed great potentialities for the degradation of crude oil. The highest oil degradation percent 90% and 77.9% were recorded for Isolate HAC in 1% and 3% oil polluted soil after 12 weeks of incubation, respectively. The biodegradation percentages were upgraded to 98.61% and 93.11% in 1% and 3% oil polluted and NPK supplemented soil, respectively. Gas chromatographic analysis performed that Hegligcrude oil consisted of n-alkanes with chain length of C12 to C35and all the tested isolates could totally degrade chain hydrocarbons with C12, C14, C33 and C35 fractions.

Introduction

Soil contamination by petroleum hydrocarbons is one of the world's most common environmental problems (US EPM, 2000). Mechanical and chemical methods for remediation of hydrocarbon- polluted environment are often expensive, technologicaly complex and lack public acceptance (Vidali, 2001).Therefore, bioremediation processes become the most effective and acceptable method for removal of petroleum hydrocarbons.

However, the success of bioremediation technologies depends on biodegradation potentiality of the native populations. Usually, microbial isolates from the soil that are historically exposed to hydrocarbon pollution exhibit a higher potential of biodegradation than others with no history of such exposure (Leahy and Colwell, 1990). In addition to microorganism type, other factors that limit biodegradation include available nutrients, pH, temperature, moisture, oxygen, soil properties, and

contaminant concentration (Walter *et al*., 2005; Atlas and Bartha, 2006).

The activity of microorganisms could be greatly enhanced by biostimulation process which may involve the addition of electron acceptors, electron donors or nutrients (Nysönenetal., 2009). The effects of nutrients mainly nitrogen, phosphorus and potassium (NPK), aeration,biostimulation of indigenous soil microorganisms and inoculation of extraneous microbial consortia on the bioremediation of oil contaminated soil have been investigated (Ayotamuno *et al*., 2006; Sang-Hwan *et al*., 2007 and Yan *et al*., 2016)

Some reports on the influence of fertilizer application on degradation of hydrocarbons are available. For example, Lin *et al.* (1999) reported that fertilizer application in crude oil-contaminated soil enhanced marsh plant growth, soil microbial populations, microbial respiration rates and oil biodegradation rates. They concluded that bioremediation with fertilizer application significantly reduced total targeted linear hydrocarbon and aromatic hydrocarbon remaining in the soil, by 81% and 17%, respectively, compared to those of the non-fertilized controls.

The effectiveness of fertilizers application on the bioremediation of the crude oil in subarctic intertidal sediments was also reported by Pelletier *et al*. (2004). However, several authors have reported the negative effects of high NPK levels on the biodegradation of hydrocarbons (Chaˆıneau *et al*., 2005) especially on aromatics (Carmichae and Pfaender, 1997).

In this work the effect of NPK on the efficiency of the bacterial isolates to degrade the crude oil in soil contaminated with Heglig crude oil was examined.

Materials and Methods

An uncontaminated soil sample was obtained from oil production site of Heglig field in southern Kordofan, Sudan. Some physical and chemical soil properties were determined according to the methods described by Ryan *et al*. (2001). Four sets of bottles, each containing 100 g soil were prepared and the humidity of the soil was adjusted to approximately 60% of the field capacity. Crude oil was added to each set to make a concentration of 1%, 3%, 5% and 10 %.NPK was then added to each bottle at a rate of 375 mg, 167.5 mg and 187.5 mg per bottle, respectively.

Three bacterial isolates(HAC, HP and HWH) of known oil-degrading potential were obtained from Environment, Natural Resources and Desertification Research Institute, National Centre for Research, Sudan. The bacterial isolates were cultured in flasks containing Mineral Salt Medium (MSM) (Kennedy *et al*., 1993).The flasks were incubated on a reciprocal shaker at room temperature for 10-15 days. Ten ml of each culture were added to each soil set. An uninoculated set of NPK supplemented oil contaminated soil was also prepared as a control. Bottles were sealed with plastic lids and fixed with rubbers. Some pores were made on the top of the covers .The bottles were then, incubated at room temperature in the dark for 12 weeks and crude oil residues were determined at two weeks intervals.

Determination of the crude oil residues

The amount of crude oil residues in the soil was determined by the weight loss method of Bossert and Bartha (1984) in which 3g of soil were suspended in 10 ml of diethyl ether in a universal bottle for oil extraction. The solvent-oil mixture was evaporated at room temperature overnight. The percentage of oil

residues was then calculated according to Ijah and Ukpe (1992).

Gas Chromatographic analysis of residual crude oil

The analysis of crude oil residues was performed by Varina 3800 gas chromatography for saturate (alkanes) component on a FID detector under the following condition : column Sil POWA CB (50m x 0.21mm); initial temperature 40ºC; final temperature 290° C; ramp rate 3°C per min; carrier gas was nitrogen (64 ml/min); injection temperature 280° C.

Results and Discussion

Soil and crude oil analysis

The physico-chemical characteristics of Heglih soil were presented in table 1. The laboratory analysis revealed that the soil was clay, alkaline with high electrical conductivity. The nutrient contents were 0.0224% N, 1.5% P and 0.87ppm K. Analysis of Heglig crude oilis carried out by Petroleum Central Laboratory and data was presented in table 2.

The API value of Heglig crude oil is 31.03 (Table 2). Therefore, it was classified as a medium according to the American Petroleum Institute gravity (API, 1986).

Biodegradation of total petroleum hydrocarbon by the bacterial isolates

Figures 1, 2 and 3 display the estimation of crude oil degradation by the weight loss method for the three isolates at four different concentrations and when were supplemented with NPK. All isolates were capable of degrading crude oil. As expected, the biodegradation percentages have gradually decreased with increasing concentration of

crude oil. At 1% concentration, a degradation percentage of 90%, 70.9 % and 67% have been recorded for isolate HAC, HP and HWH, respectively. At 3% oil concentration, degradation percentages of 77.9,56 and 55% were recorded for HAC, HP and HWH, respectively while at 5% oil concentration 51%, 38.7% and 37.9% degradation were recorded for HAC, HP and HWH, respectively.

Biodegradation of total petroleum hydrocarbon in soil supplemented with NPK

All isolates were highly efficient in degrading the crude oil in soil supplemented with NPK. The percentage of degradation ranged from 97.85% to 98.61%, 93.11% to 94.21%, 71.33% to 88.13% and from39.50% to 40.12 % for1%, 3%,5% and 10% crude oil respectively.

Biodegradation of crude oil components

Residual oil components in 3% oil contaminated soil was determined before and after the incubation period(12 weeks) using GC. The result revealed 24 types of n-Alkanes hydrocarbons with chain lengths of C12 to C35of the crude oil before the soil was incubated. During the 12 weeks incubation period, the tested isolates showed different ability to degrade different crude oil components. Isolate HAC had highest degradation percentage of the component fractions compared with isolates HP and HWH which had approximately the same degradation ability. However, the lowest degradation of the component fractions was recorded for the control (Fig.3 and Table 3). The result also showed that the HAC and HWH isolates degraded the C33 completely while HP recorded 12.6%. In soils contaminated with 3% crude oil and supplemented with NPK fertilizer, all

isolates exhibited highest level of the component fractions degradation (Fig.4 and Table 4) and they could degrade C12, C13, C14, C33 and C35fractions completely.

Some previous studies have shown that most potential bacteria for petroleum hydrocarbon degradation have been isolated from areas contaminated with oil (Chaerun *et al*., 2004, Abu Shanab *et al*., 2015). The present results showed clear relationships between the rate of biodegradation and concentration of oil in the contaminated soil. Higher biodegradation rates were recorded in soil contaminated with 1 and 3% oil. This could be attributed to an increase in the activity of a particular soil microbe in this oil pollution level (Zajic and Supplisson, 1972). Ijah and Antai (2003a) reported high degradation of hydrocarbons in soil contaminated with 10% and 20% crude oil compared to those contaminated with 30 and 40% crude oil within a period of 12 months. The reason for the low efficiency of oil degradation by the microbes at 5%and 10% oil concentrations might be attributed to the toxicity of the oil to the microbial flora of the soil at these high concentrations of oil (5% and 10%). This trend of low biodegradation due to high

oil concentration has been reported by different authors (Rahman *et al*., 2000; Ijah and Antai, 2003b and Udgire*et al.*, 2015).

The bacterial community of oil contaminated soil showed greater changes by the addition of inorganic compounds such as P, K, Ca, and Mg (Evans *et al.,* 2004). Hollender *et al*. (2003) and Walworth *et al.* (2007) reported that, the addition of inorganic or organic nitrogen is an effective approach (biostimulation) to enhance the bioremediation process. Positive effects of nitrogen amendment on microbial activity and/or petroleum hydrocarbon degradation have been widely demonstrated by various authors (Chaˆıneau *et al*., 2005; Margesin, *et al*., 2003). Microorganisms are known to attack specific compounds present in crude oil which is a complex mixture of saturates, aromatics and polar compounds (Barathi and Vasudevan, 2001). Ainon *et al*. (2010) reported that gas chromatography analysis showed that the South Angsi crude oil components of C_{12} to C_{25} were more extensively degraded by *Actinomycits lwoffii* after 24 h of incubation compared to other bacteria over the same period.

Table.1 Physical and chemical characteristics of Heglig soil

Characters	Heglig soil
pH	7.62
EC (ds/m)	0.288
N (%)	0.0224
P (ppm)	1.5
O.C. (%)	0.56
O.M. (%)	0.96
Na (ppm)	16.05
K (ppm)	0.87
Ca(ppm)	0.80
Mg (ppm)	1.20
Moisture content (%)	5.589
Clay (%)	75.62
Silt (%)	12.50
Sand (%)	11.88

Table.2 Characterization of Hegligcrude oil

| characters	Heglig crude oil
Density (g/cm^3)	0.869
API (American Petroleum Institute) gravity	31.03
S.G. (Significant Gravity)	0.870
K (Characterization factor)	12.4
Crude oil %	3.6

Table.3 Degradation % by soil microorganisms of 3% crude oil component fractions after 12 weeks of incubation

| Component Fraction	Degradation (%)			
	Control	HAC	HWH	HP
C_{12}	22.76	53.19	32.87	58.65
C_{13}	12.17	73.43	48.81	54.00
C_{14}	1.521	77.72	57.09	61.24
C_{15}	15.96	82.66	66.85	72.55
C_{16}	5.98	79.35	57.27	65.65
C_{17}	7.31	80.75	58.62	64.66
Pr	10.73	71.60	42.61	44.47
C_{18}	24.76	83.16	64.01	65.28
Ph	20.42	77.39	38.26	50.76
C_{19}	34.48	88.18	72.40	74.08
C_{20}	32.72	83.32	63.07	62.68
C_{21}	33.07	85.60	68.06	62.91
C_{22}	28.74	84.70	62.75	59.29
C_{23}	15.43	82.76	53.58	49.05
C_{24}	11.43	77.28	46.31	43.61
C_{25}	6.34	78.23	40.21	38.76
C_{26}	5.78	77.38	39.62	36.08
C_{27}	5.56	74.44	35.84	29.33
C_{28}	4.61	69.81	29.17	22.20
C_{29}	7.28	64.98	25.05	15.24
C_{30}	4.76	58.80	13.00	6.22
C_{31}	23.76	60.17	24.18	24.18
C_{32}	14.24	60.46	24.68	11.28
C_{33}	8.79	60.86	39.25	16.14
C_{34}	5.52	100	100	12.60
C_{35}	19.20	72.83	65.17	47.77

Table.4 Degradation (%) by soil microorganismsof crude oil component fractions in the presence NPK after 12 weeks

| Component Fraction	Degradation (%)			
	Control	HAC+NPK	HWH+NPK	HP+NPK
C_{12}	22.76	100	100	100
C_{13}	12.17	100	100	100
C_{14}	1.521	100	100	100
C_{15}	15.96	97.77	96.63	97.65
C_{16}	5.98	95.53	93.84	95.43
C_{17}	7.31	95.26	92.72	93.78
Pr	10.73	93.42	91.83	93.37
C_{18}	24.76	95.33	94.19	95.71
Ph	20.42	94.30	94.07	95.38
C_{19}	34.48	97.70	97.56	97.31
C_{20}	32.72	96.91	96.73	97.37
C_{21}	33.07	96.38	96.63	97.32
C_{22}	28.74	96.20	96.65	97.27
C_{23}	15.43	95.86	96.41	96.90
C_{24}	11.43	95.77	96.43	96.89
C_{25}	6.347	92.67	93.56	94.00
C_{26}	5.787	90.78	91.25	93.33
C_{27}	5.56	90.71	91.11	91.35
C_{28}	4.61	89.91	90.58	90.71
C_{29}	7.28	89.82	89.73	90.05
C_{30}	4.76	82.34	82.43	83.62
C_{31}	23.76	77.43	77.70	78.64
C_{32}	14.24	88.14	85.25	87.40
C_{33}	8.79	100	100	100
C_{34}	5.52	71.34	53.33	52.943
C_{35}	19.20	100	100	100

Fig.1 Degradation %of crude oilby HAC isolate and HAC+NPK during 12 weeks period
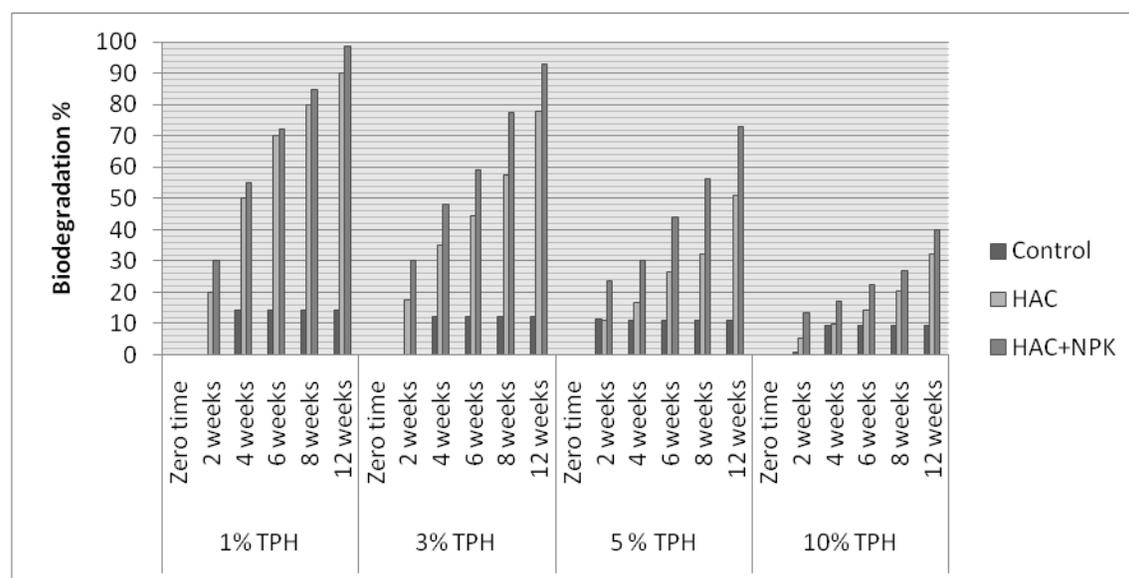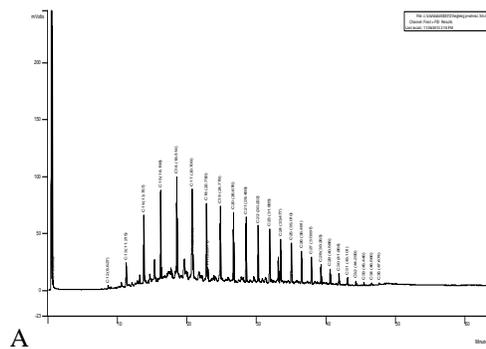
Fig.2 Degradation % of crude oil by HP isolate and HP+NPK during 12 weeks period
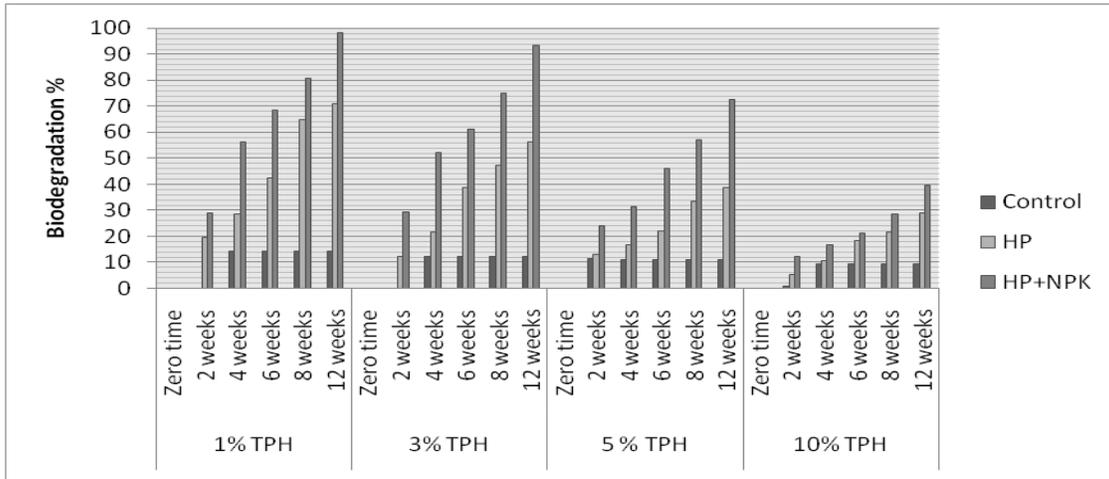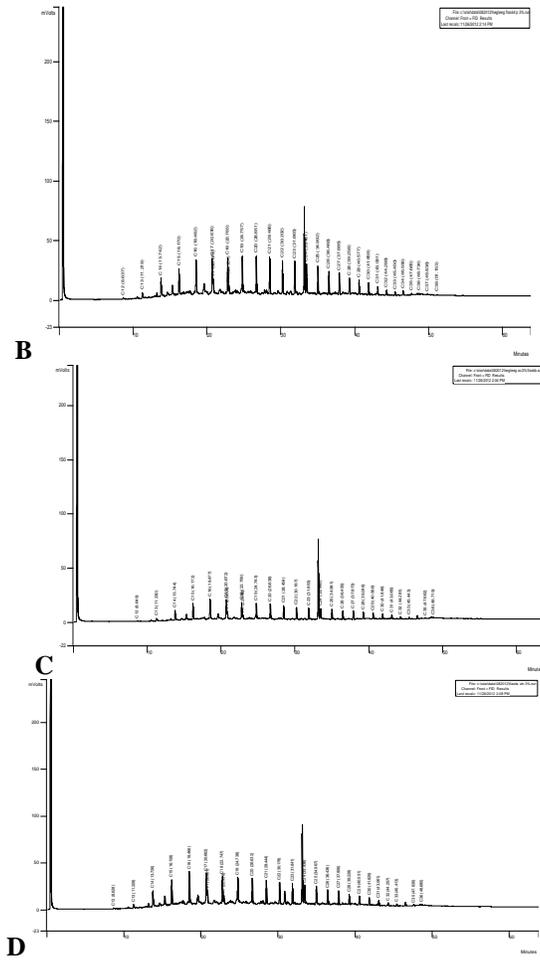

Fig.3 Degradation % of crude oil by HWH isolate and HWH+NPK during 12 weeks period
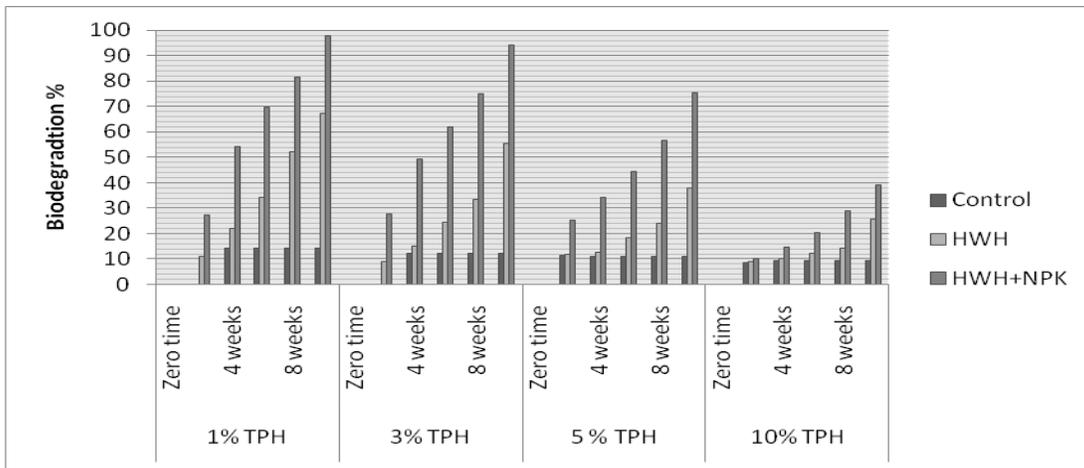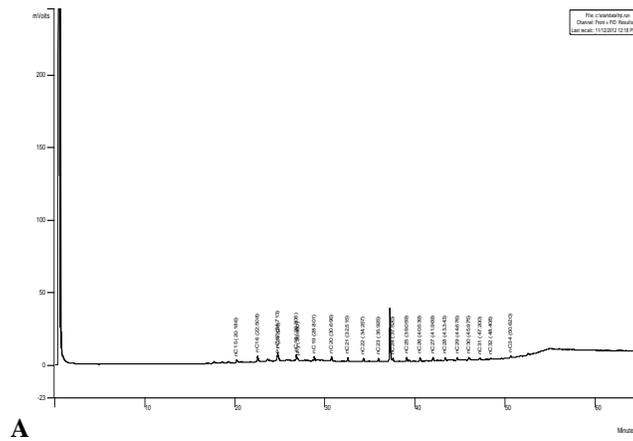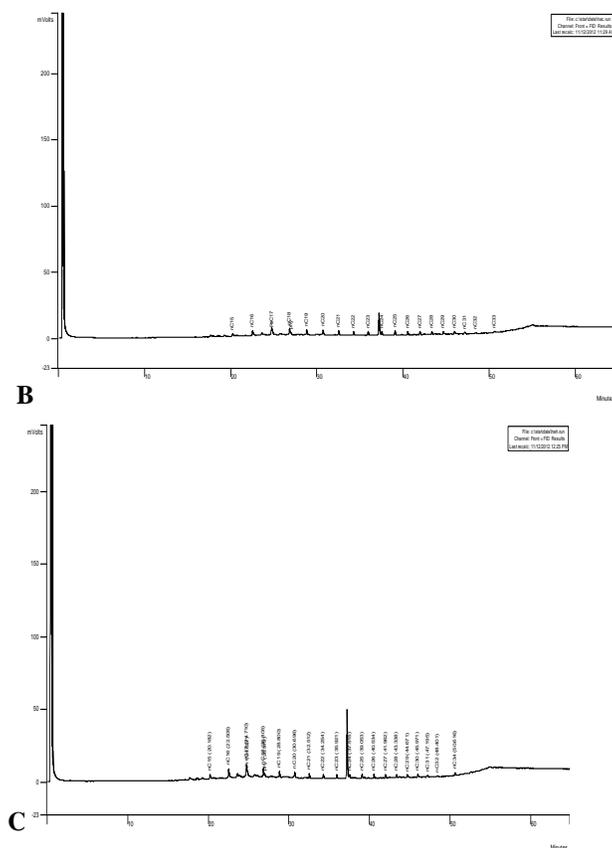

Fig.4 Chromatogram of TPH degradation in control (A), HP (B), HAC (C), and HWH (D) of Heglig crude oil after 3 month

Fig.5 Chromatogram of TPH degradation of Heglig crude oil by HP +NPK (A), HAC+NPK (B) and HWH +NPK (C) after 3 months

B

C

Malik and Ahmed (2012) reported that after 24 days of incubation the highest abiotic removal for lower alkanes (C13-C18) and higher alkanes (C21, C24 and C28) was 56 to 60%, respectively.

However, middle alkanes reported to have a lower level of degradation such as 51% for C16 and 40% forC17. The disappearance of significant quantities of short chain n-alkanes (C8-C11) during the experiment and the decrease of total hydrocarbons (aliphatic and aromatics) could also be related to abiotic factors, such as evaporation and dispersion as explained by Luzi*et al*. (2006).

In conclusion, all isolates were capable of degrading crude oil. The biodegradation percentages have gradually decreased with increasing concentration of crude oil. The study also presented that inorganic fertilizer containing nitrogen; phosphorus and potassium have great potentials in stimulating oil biodegradation in soil polluted with petroleum hydrocarbons.

Acknowledgments

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