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Efficacy of enhanced natural attenuation (land farming) technique in the remediation of crude oil-polluted agricultural land

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Abstract

In this study, a bioremediation strategy that utilizes Remediation by Enhanced Natural Attenuation (RENA) technique otherwise termed (land farming) was adopted in the recovery of crude oil polluted farm lands. The levels of total petroleum hydrocarbon (TPH), polycyclic aromatic hydrocarbon (PAH) and selected heavy metals at three polluted sites were analyzed after 14 days of application of the RENA technique. TPH contents of polluted soils at sites 1, II and III decreased from 14, 569 to 45.7 mg/kg; 3,713 to 139 mg/kg; and 2,156 to 103 mg/kg, respectively. The reduction in the levels of PAH contents of the soils were as follows: 39.2 to <0.0001 mg/kg (site I); 12.0 to 2.38 mg/kg (site II) and 5.84 to <0.0001 mg/kg (site III). Similarly, most heavy metal contents of the polluted soils (sites 1, II and III) significantly decreased in their concentrations with the technique. Results obtained in this study suggest that the technique was efficient in the remediation of crude oil polluted agricultural lands.

Key words: Remediation, Total Petroleum hydrocarbon, polycyclic aromatic hydrocarbon, Heavy metals, Natural attenuation

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INTRODUCTION

The presence of inorganic ions, carcinogenic and growth inhibiting chemicals in crude oil, its effects on microorganisms and on human beings is well documented [1,2,3]. It is also evident that oil exploration and production activities have negative impact on the agricultural soils where such activities occur [4].

In the Niger Delta Area of Nigeria where oil spillage is a common feature [5], oil spills have destroyed a large part of the coastal vegetation, polluted potable water, damaged fertile agricultural lands (Fig 1) and led to ethnic and regional crisis.

Fig 1: Crude oil spillage site in the study area

It has been established that the main causes of oil pollution in Nigeria include; flow line/pipeline leaks, overpressure failures/overflow of process equipment components, hose failures on tanker loading systems, failures along pump discharge manifold (vibration effects) and sabotage [6,7]. Indeed, spill incidents have prompted research on cost effective and environmental benign clean-up strategies. According to Smith [8], thermal, physical, chemical, solidification and stabilization, and biological methods are among the remedial techniques that most workers have adopted in the clean-up of polluted environments. However, in this study, we investigated the efficacy of remediation by enhanced natural attenuation, RENA technique (also called land farming) in the recovery of polluted lands. This method involves the treatment of contaminated soil by spreading top soil on the contaminated soil, aerating the soil through tilling to enhance mixing with native soil, and increase available surface area for microbial activity.

MATERIALS AND METHODS

Description of study sites:

The study was performed on three crude oil-spill polluted sites located in the eastern Niger Delta of Nigeria (Fig. 2).

Fig 2: Map of eastern Niger Delta of Nigeria showing the study area

For each of the polluted sites, geographically similar areas unaffected by crude oil pollution were chosen as control. Throughout the period that preceded sampling, there were no reported cases of oil pollution at the control sites.

The study sites are located in the humid tropical rain forest in the Niger Delta area of Nigeria though the sites are on terrestrial portion of the zone devoid of swamps and surface water bodies. Mean daily temperature and rainfall of the area were 26^0C and 180mm respectively.

Field reconnaissance surveys were conducted to assess the extent of pollution of soil and vegetation, depth of impact, environmental sensitivity and site characteristics that may be required for the study. This visit influenced the design of the work.

Soil Sample Collection

At each of the polluted sites (sites 1, II, III), a sampling area of 100 x 100m^2 was delineated around the epicenter of spillage. Similarly, an area of 100 x 100m^2 was selected from the control.

The polluted sites were excavated using stainless steel shovels beyond the polluted depth of 20cm as established during site assessment exercise which was performed manually with the aid of soil auger. The excavated materials were heaped together. The rhizosphere of unpolluted or control soils (located 50m away from the perimeter of the polluted area) were also excavated manually using shovels and rakes (made of stainless steel) and transferred to the polluted sites which have been spiked and tilled. The excavated polluted soil and those of the un-polluted soil

were thoroughly mixed to homogeneity with shovels and rakes. The resultant soils mixture was windrowed to a height and width of 30cm and 50cm, respectively (Fig. 3).
The windrows which were constructed against the slope were turned three times per week for two weeks (14-days).

Fig 3: Windrows constructed at site II

The study areas had adequate supply of moisture as the study was carried out between the months of May and July, a period of peak rainfall in this region [9]

Sampling Technique
Soil samples for analyses were collected during site reconnaissance visits and after the remediation period of 14 days (for control and polluted sites).
Each sampling site (polluted and control) was divided into six (6) cells and from each cell composite soil samples were randomly collected at a depth of 0-15cm using a soil auger. The samples were bulked, placed in a well-labeled aluminum foil, sealed, transferred to the laboratory and stored in the refrigerator.

Extraction of crude oil and chromatographic analysis
Soil samples were crushed, air-dried for four (4) days and sieved through a 2mm mesh to obtain a uniform size. 5g of the homogenized soil sample was placed into clean and dry beaker. Crude oil contained in the sample was extracted using 10ml of 1:3 mixture of distilled hexane/dichloromethane. The extracts were charged to an alumina column. Hexane was used to

elute the non-polar fractions consisting of normal alkanes and isoprenoids while dichloromethane was used to elute the column for a fraction containing polycyclic aromatic hydrocarbons (PAHs). The resultant extracts were injected into a gas chromatograph (GC) (GC-SRI model 8640) while the chromatographic output was recorded on a computer. The chromatograms were quantified by the method of internal standards [Osuji *et. al*; 2006).

Estimation of selected heavy metals

The Food and Agriculture Organization of the United Nations method [10], which involves the digestion of sample in a beaker on a hot plate, was adopted for the breakdown of organic matter. 5.0g of sieved soil samples was placed into a long-form pyrex beaker and 25ml of a freshly prepared mixture of analytical-grade $HNO_3/H_2SO_4/H_2O_2$ 2:1:2 (V/V/V) was poured into the beaker. The beaker was covered with a watch glass, and set aside for 20 minutes to allow the initial reaction to subside. The beaker and its contents (with a few anti-bumping chips) were heated at reflux (maximum hot-plate temperature of 160C) for 3 hours until the initial volume was reduced to 3ml. The beaker was allowed to cool to room temperature. The contents were transferred into a 100ml volumetric flask and diluted with deionized H_2O. The concentrations of the selected heavy metals were estimated in an atomic absorption spectrophotometer (Buck, 200A) that operated with air-acetylene flame using the appropriate hollow-cathode lamps and coupled to a recorder.

Mean and standard deviation (SD) were calculated using conventional statistical formulae. Standard error (SE) was calculated as shown: $SE=SD/\sqrt[2]{N}$, where N is the number of replicates. SE was estimated at 95% confidence level by multiplying by 1.96.

RESULTS AND DISCUSSIONS

In the Niger Delta Area of Nigeria, most of the terrestrial ecosystems and shorelines in oil producing communities are important agricultural lands and are under continuous cultivation. Contacts with crude may result in damages to soil properties or plant communities. This was evidenced during field reconnaissance as localized quantities of crude oil were observed on the soil surface with impacted vegetation showing brownish to yellowish colorations (Fig 1). However, plants that were located 50m away from the impacted site appeared greenish and healthy.

Total Petroleum Hydrocarbons (TPH)

The GCs obtained in this study showed clear chromatographic fingerprint of crude oil. The measured concentrations of C_8 to C_{40} aliphatic and acyclic hydrocarbons in the polluted, remediated and control soil samples are shown in Table 1. As indicated in the Table 1, the GCs revealed the presence of pristane and phytane in all the polluted samples. The disappearance of light end n-alkanes of carbon numbers below C_{12} in most of the samples may indicate that the oil was slightly weathered after spill incident. It also suggest that the chemical constituents of the aliphatic components of the spilled oil may not have undergone significant alteration. In order words, the n-C_8 to n-C_{11} hydrocarbons of most of the polluted samples had been preferentially eliminated through evaporation [11, 12]. As is evidenced in Table 1, the hydrocarbon components of the spilled oil were higher in the polluted soils than those of the remediated soil samples. Indeed, the levels of hydrocarbon components of remediated soils did not vary

significantly from those of the control soil samples as the concentration in most of the remediated samples were below detection limit of ≤ 0.0001mg/kg.

The total hydrocarbon contents (mg/kg) of soils at sites 1 to III were; 14,569 (polluted), 139 (remediated), 0.37 (control) for site 1; 3,713 (polluted), 139 (remediated), 45.2 (control) for site II; 2,156 (polluted), 103 (remediated) and 2.20 (control) for site III. Investigations of Atlas and Bartha [13], holds that the most direct method of measuring the efficacy of a bioremediation process is by monitoring the rate of disappearance of hydrocarbons. The values of hydrocarbons observed in this study implied rapid disappearance of petroleum hydrocarbons when the polluted soils were subjected to RENA treatment.

Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs measured in this study have acenaphthene as the most abundant component with values ranging from 3.60 mg/kg to 17.2 mg/kg (Tables 2). The values of naphthalene and acenaphthylene in site III (Table 2) fell below detection limit of 0.0001mg/kg, indicating that the samples may have experienced mild evaporative weathering. With the exception of site II where fluorine, phenanthrene, anthracene and fluorathene were detected in minute quantities (Table 2), PAH levels for most samples were below detection limit of 0.0001mg/kg. In general, polycyclic aromatic hydrocarbons in the soils occurred in the following order of abundance: polluted >remediated ≥ control.

High-molecular mass PAHs are resistant to degradation and are source specific. Their presence in crude oil makes differentiation of sources of crude oil possible [12]. The marked reduction of the high molecular mass PAHs in the remediated samples would be attributed to stimulation of the rhizosphere to enhance microbial degradation of pollutants during the remediation process. This corroborates the earlier report of Cerniglia [14] who observed that microbial activities within the rhizosphere accounts for significant degradation of PAHs. Furthermore, it was reported by Lang and Wagner [15] that certain strains of bacteria have the capacity to produce biosurfactants that increases extractability of PAHs. Also, the marked reduction in the levels of PAHs for remediated samples may be attributed to the excavation and tilling of polluted and remediated soils. This is obvious as physical and chemical processes, including dilution, dispersion and volatilization that occurred during excavation and tilling of the soils may have influenced the observed decrease in PAH values.

Heavy metals in the samples

Results in Table 3 show significantly higher concentrations of heavy metals in the polluted soils when compared with those of the remediated and control soil samples. For instance, Pb levels in polluted, remediated and control soil samples (Tables 3) were as follows: 80.0mg/kg (polluted), 61.0mg/kg (remediated), 26.9mg/kg (control) for site I; 69.3 mg/kg (polluted), 65.4 mg/kg (remediated), 49.0mg/kg (control) for site II; and 86.6mg/kg (polluted), 62.5 mg/kg (remediated) and 9.62 mg/kg (control) for site III. Similar observations were recorded for As, Ba, Cd, Ni, Zn and Cr. There could be other contributory factors to heavy metal content of the polluted soils.

Flooding could lead to significant mobilization of heavy metals from soils particularly when readily oxidizable organic nutrients are available. Indeed, the levels of soluble heavy metals in the submerged soils may have been enhanced due to flooding. This is possible as records of

annual rainfall exceeded 1,200 mm in the study area, resulting in excess water accumulation in the season that preceded the sampling period. Such accumulation of water may have contributed to accumulation of metallic oxides, which probably might have increased mineralization by strains of microbial genera. It is common knowledge that certain strains of microbial genera increase in population on availability of excess hydrocarbons in soil. Excessive deposition of most heavy metals in the soil poses a lot of danger to agricultural soils. For instance, Cu ions have been demonstrated to inhibit root growth [16]. Also, presence of Ni in excess amounts is toxic to some soil fauna such as earthworms and low levels of Pb^{2+} ions are known to reduce heterotrophic activity of microflora [17].

CONCLUSION

Remarkable decrease in the levels of total petroleum hydrocarbons, polycyclic aromatic hydrocarbons and heavy metals in the remediated soil samples were observed when compared with those of the polluted soil samples. In fact, the values obtained in the remediated soils approached those of the control, for most samples. The implication of these findings is that remediation by enhanced natural attenuation or land farming promises to be one of the best options in the remediation of crude-oil polluted agricultural lands.

Table 1: Effects of RENA method on Total Petroleum Hydrocarbon (TPH) Contents of polluted soil (mg/kg)

Component	Site I			Site II			Site III		
	PS	RS	C	PS	RS	C	PS	RS	C
C_8	a	a	a	a	a	a	a	a	a
C_9	20.3±10.7	a	a	11.9±2.06	a	a	17.4±0.15	a	a
C_{10}	a	a	a	a	a	a	a	a	a
C_{11}	a	a	a	6.70±4.50	a	a	a	a	a
C_{12}	11.4±5.42	a	a	43.9±25.6	a	a	14.4±6.86	a	a
C_{13}	18.4±6.43	a	a	12.4±7.81	a	a	13.1±3.64	3.09±3.56	a
C_{14}	46.8±42.5	a	0.15±0.27	22.9±9.81	a	a	9.09±0.86	a	a
C_{15}	34.6±30.4	a	a	24.8±4.87	a	a	18.0±2.30	4.77±2.55	a
C_{16}	22.0±3.07	a	a	18.8±3.45	a	a	11.5±1.57	a	a
C_{17}	a	8.50±3.40	a	0.98±0.06	a	a	a	a	a
PRISTANE	1.05±1.01	a	a	409±23.8	20.3±10.7	a	198±8.65	a	a
C_{18}	1.57±1.31	a	a	537±27.6	25.8±13.7	a	203±8.67	a	a
PHYTANE	521±148	a	a	95.8±34.5	13.5±12.6	a	89.9±10.8	a	a
C_{19}	506±13.4	a	a	180±33.2	16.9±11.8	a	12.7±2.67	a	a
C_{20}	208±35.7	a	0.16±0.25	45.9±32.7	a	a	18.9±2.37	a	a
C_{21}	54.0±9.08	a	a	23.4±4.50	a	a	18.3±2.65	a	a
C_{22}	73.3±5.85	a	a	35.7±43.2	10.8±6.25	5.23±3.45	39.8±11.90	a	a
C_{23}	457±4.87	a	a	106±15.6	a	a	122±20.5	11.8±15.6	a

PS- Polluted soil; RS- Remediated soil; C- control.
Values are means ± S.E. for 4 replicates (n=4)
a = below detection limit (0.0001 mg/kg)

Table 1 contd.

Component	Site I			Site II			Site III		
	PS	RS	C	PS	RS	C	PS	RS	C
C_{24}	5.03±2.07	a	a	a	a	a	a	a	a
C_{25}	769±45.9	a	a	110±15.6	a	3.89±6.79	89.2±6.75	a	a
C_{26}	49.8±32.8	a	a	23.6±17.2	2.55±0.86	a	36.8±2.46	9.08±5.67	a
C_{27}	56.0±24.2	a	a	34.8±18.9	8.56±3.23	a	27.6±1.32	9.78±4.89	a
C_{28}	23.6±3.24	a	a	24.9±16.6	a	a	26.6±1.32	a	a
C_{29}	675±32.8	a	a	254±12.9	a	a	126±2.39	a	a
C_{30}	965±54.9	a	a	206±31.5	7.50±3.56	a	134±4.58	a	a
C_{31}	2376±896	a	a	907±54.3	a	a	201±2.85	9.67±4.98	a
C_{32}	1578±953	8.25±10.6	a	205±45.2	a	a	175±34.7	13.9±8.54	a
C_{33}	2098±767	5.25±9.73	a	136±23.5	a	a	108±38.2	a	a
C_{34}	785±467	a	a	86.0±56.2	a	a	87.5±30.7	6.89±4.98	a
C_{35}	362±34.6	a	a	110±65.8	18.7±8.80	4.78±2.78	116±34.4	12.7±5.76	1.33 ±0.56
C_{36}	77.8±46.9	a	a	67.3±12.8	a	a	55.4±23.8	8.75±3.23	a
C_{37}	60.7±32.9	12.8±3.30	a	13.7±11.3	a	a	24.8±8.89	6.77±1.86	a
C_{38}	35.8±4.98	a	a	12.5±16.7	a	a	18.9±23.7	6.75±1.87	0.98 ±0.35
C_{39}	57.5±34.0	a	a	38.4±22.5	11.6±5.71	a	23.6±18.6	a	a
C_{40}	a	a	a	9.31±0.21	2.94±0.72	a	3.98±0.87	a	a
TOTAL	**14,569±400**	**139±108**	**0.37±0.52**	**3,713**	**139**	**45.2**	**2,156±28.2**	**103±71.6**	**2.20 ±7.81**

PS- Polluted soil; RS- Remediated soil; C- control.
Values are means ± S.E. for 4 replicates (n=4)
a = below detection limit (0.0001 mg/kg)

Table 2: Effects of RENA Method on the Polycyclic Aromatic Hydrocarbon (PAH) (mg/kg) Contents of Soils

Component	Site I			Site II			Site III		
	PS	RS	C	PS	R S	C	PS	RS	C
Naphthalene	0.30±0.06	a	a	0.23±0.04	a	a	a	a	a
Acenaphthylene	4.53±2.72	a	a	0.29±0.19	a	a	a	a	a
Acenaphthene	17.2±8.22	a	a	4.67±3.90	a	a	3.60±2.37	a	a
Flourene	2.08±1.86	a	a	0.91±0.05	0.54±0.20	a	0.77±0.47	a	a
Phenanthrene	2.11±1.90	a	a	3.53±2.57	1.28±1.05	a	0.21±0.16	a	a
Anthracene	1.36±0.47	a	a	0.52±0.09	0.26±0.11	a	0.39±0.22	a	a
Flouranthene	0.87±0.45	a	a	0.66±0.14	0.30±0.15	a	a	a	a
Pyrene	0.22±0.16	a	a	0.66±0.42	a	a	0.35±0.60	a	a
Benzo(a)Anthracene	0.86±0.19	a	a	a	a	a	a	a	a
Chrysene	a	a	a	0.48±0.08	a	a	a	a	a
Benzo(b)flouranthene	a	a	a	a	a	a	a	a	a
Benzo (k) flouranthene	a	a	a	a	a	a	0.52±0.89	a	a
Benzo(a)pyrene	5.38±1.2	a	a	a	a	a	a	a	a

Indeno(1,2,3)pyrene	0 a	a	a	a	a	a	a	a	a
Dibenzo(a,h) Anthracene	a	a	a	a	a	a	a	a	a
Benzo (g,h,i) perylene	4.30±2.96	a	a	a	a	a	a	a	
Total	**39.2**	**a**	**a**	**12.0**	**2.38**	**a**	**5.84**	**a**	**a**

PS- Polluted soil; RS- Remediated soil; C- control.; Values are means ± S.E. for 4 replicates (n=4); a = below detection limit (0.0001 mg/kg)

Table 3 Mean Concentrations of Heavy Metals (mg/kg) in Polluted, Remediated and Control Soil Samples

Heavy Metals	Site I			Site II			Site III		
	PS	**RS**	**C**	**PS**	**RS**	**C**	**PS**	**RS**	**C**
Pb	80.0±21.3	61.0±59.9	26.9±14.4	69.3±25.7	65.4±26.9	49.0±16.6	86.6±13.5	62.5±6.31	9.62±3.10
As	a	a	a	a	a	a	a	a	a
Ba	15.1±3.13	14.6±3.39	12.5±3.32	17.2±4.88	8.75±5.32	4.50±3.07	13.0±3.83	12.7±3.12	6.09±4.44
Cd	7.88±4.53	3.28±5.24	2.47±3.45	15.4±22.9	9.80±5.24	5.70±3.10	17.6±2.38	12.4±2.43	1.63±2.38
Ni	87.7±103	85.7±66.7	56.7±33.0	116±94.8	77.0±10.3	20.6±12.7	96.5±9.40	10.2±88.5	22.9±4.72
Zn	65.3±5.00	54.3±40.5	31.5±13.1	90.4±24.1	59.7±18.6	39.2±16.7	25.0±14.9	7.60±33.5	4.70±3.16
Cr	1.66±3.71	2.08±3.59	a	12.5±13.8	6.25±3.67	4.18±1.23	12.5±13.8	2.08±3.59	a

PS- Polluted soil; RS- Remediated soil; C- control.
Values are means ± S.E. for 4 replicates (n=4)
a = below detection limit (0.0001 mg/kg)

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