Impact Of Agrowastes on The Degradation of Crude Oil on Elem Sangama Wetland, Rivers State, Nigeria

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Abstract- ***This research investigated the possibility of using waste-derived compost to treat soils contaminated with crude oil. This research was aimed at assessing the effects of Palm Oil Mill Effluent (POME) and Cow dung on the soil physicochemical and biological indices in the wetland of Elem Sangama, Akuku Toru, Rivers State. Cow dung samples were collected from cattle abattoir in Rukpokwu, and the POME used in this research was collected from a local palm oil mill in Rukpokwu, Obio/Akpor Local Government Area of Rivers State. Dehydrogenase showed the highest activities in all the soil samples with a range of 15.32 to 30.73mg6^{-1} before and after bioremediation, while Alkaline phosphatase has the lowest soil reaction activities before bioremediation in the enzymatic reaction in impacted soil with cow dung. Oil impaction caused the decrease in the values of soil physicochemical properties including bacterial populations and enzyme activities. The treatment with Cow dung and POME caused significant increase (P=0.05) after bioremediation period. Ten litre (10L) of POME was used to amend the portion of land impacted with separate amounts of crude oil (10L, 15L, 20L, 25L, and 30L respectively). The most regular group of organisms were the Total Heterotrophic Bacterial Count (THBC) while the most advanced were the Total Petroleum Utilizing Bacterial Count (TPUBC) and Total Lipolytic Bacterial Count (TLBC). The populations of selected bacterial groups decreased significantly (P =0.05) after impaction and amendment with POME but increased above the control after the period of bioremediation. The result of waste material like cow dung and POME as binders can be considered as a sustainable approach to improve the physicochemical properties of the soil. 1kg, 2kg, 3Kg, 4kg and 5kg of cow dung was applied from the results obtained; the environment was remediated for use. From the results, it can be deduced that this research has proven the efficacy of POME and Cow dung in remediation of crude oil contaminated soil***

I. INTRODUCTION

Nigeria is a major player in the oil and gas exploration and exploitation operations. She gains and suffers from all activities relating to oil and gas production. This transportation of crude oil is often through pipelines and ocean liners. The transportation process is exposed to several forms of dangers including spills and theft. The spillage is due to several factors including pipeline cutting, sabotage, wrong sense of revenge, zeal to claim damages, willful destruction of properties/terrorism among others. Wetlands are ecologically sensitive and vulnerable to human disturbances. When a wetland is polluted, the ecosystem is altered, and agricultural activities are affected. Wetlands contaminated with heavy crude oil impaction can create un-conducive conditions in the soil. This is due to some inherent factors like poor aeration, immobilization of soil nutrients, loss of water-holding capacity, lowering of soil pH, and reduction in soil enzyme activities (Sathiya-Moorthi, 2008; Achuba and Peretiemo-Clarke, 2008) as well as inhibitory effect on the nitrate and phosphate reductase activities of plants (Odjegba and Atebe, 2007).

Crude oil is a natural product comprising of a complex mixture of various hydrocarbons created by the decomposition of plants remains from the carboniferous period under high temperature and pressure. Crude oil pollution is a threat to the environment and the remediation is a major challenge

to environmental research. Contamination of soil by crude oil could lead to a reduced microbial density and activities. Apart from its phytotoxicity, excess oil in soil may also limit the availability of nitrogen (John *et al*., 2014). In the case of relatively light crude oil contamination, it stimulates the soil biochemical processes such as organic matter decomposition, ammonification, nitrification, symbiotic and non-symbiotic nitrogen fixation and geochemical cycling of elements, which thereafter increases the number and activities of microorganisms.

Crude oil exploration and production (E&P) activities occur frequently in the natural wetlands of South-South Nigeria such as the Elem Sangama wetlands in Akuku Toru, Rivers State. Oil exploration and production (E&P) processes can contribute to the localized loadings of total petroleum hydrocarbons (TPH) in the environment through accidental spillage or oil leaks from producing wells, gathering lines, transportation lines and pits. Release of hydrocarbons into the environment is a major cause of soil pollution. Oil exploration and production (E&P) activities have multiple deleterious impacts on the wetland ecosystem. The adverse effect of crude oil on wetlands ranges from loss of vegetation to addition of toxic materials. Thus, wetland degradation in the South-South Nigeria resulting from oil exploration and production (E&P) activities has drawn national and regional attentions (Ike *et al*., 2014).

Elem Sangama wetlands in Akuku Toru are prone to crude oil and associated end-products contamination due to the exploration and production (E&P) activities in the area by major oil companies, leading to distortion in microbial dynamics and imbalance in soil health parameters. Accidental and deliberate crude oil spills have been and still continue to be, significant source of environmental pollution and poses a serious environmental problem, due to the possibility of air, water and soil contamination (Nwaugo *et al*., 2007).

Various approaches have been adopted in the cleanup of oil polluted areas. These include physical, chemical, mechanical and biological methods. Some of these methods are not environmentally friendly and may even add to the negative impact of the pollution. In addition, some of the cleanup methods are so specialized that they require special training to carry out. Some are also not practicable in certain terrains (Manish *et al*., 2019).
Bioremediation is an option that offers the possibilities to destroy or renders harmless various contaminants using natural biological activity (Vidali, 2001).

This work is designed to assess the effect of cow dung waste compost and POME on soil physicochemical and biological indices in the wetland of Elem Sangama, Akuku Toru Local Government Area of Rivers State.

II. MATERIALS AND METHODS

2.1 STUDY AREA

The study area is the Soibreibo River wetland of Elem Sangama, Rivers State. The Elem Sangama Community is 55.5km away from Port Harcourt City, Rivers State. The soil type is clay mixed with silt. The geographical coordinates are latitude 4.75- 4.78^{o} and longitude of 6.75 – 6.80^{o}. The area is of tropical climatic conditions with typical rain forest features. The portion of the wetland studied is the Elem Sangama section.

2.2 STERILIZATION PROCESSES

2.2.1 Petri-Sterilization and other Wares

The dishes are made of high-quality polystyrene material and are pre-sterilized by gamma irradiation. The bottom line of how this works is that the ionizing radiation produces disruptions in sub-atomic particles involved in the formation of the microorganism. Simply put, this radiation causes damage to the genetic material - the DNA or the RNA - of the organism's cell. If the DNA or RNA of a microorganism is damaged, the cell will die. In other words, radiation damages the hard drive of a bacterium, causing it to shut down for good. Gamma rays have a high penetration power so materials can be sterilized after filling them in the final container. Petri sterilized plastic were kept in Petri dish canister, while test tubes were wrapped in aluminum foil. Soil spatula was disinfected using 70 percent ethanol before use.

2.2.2 Sterilization of Media

All commercial media were prepared in accordance with the manufacturers specifications. The media were sterilized by autoclaving at 121^{o}C at 15 psi for 15 minutes. Each medium was reconstituted in distilled

water based on weight of the media and volume of water required, heated to dissolve before autoclaving to sterilize. These media include Nutrient Agar, Tributryn Agar (oxoid), modified mineral salt Agar, Petroleum modified mineral salt Agar and Pikovskaya Agar. They were then dispensed into pre-sterilized Petri dishes and allowed to gel before use.

2.3 SAMPLE COLLECTION

2.3.1 Cow dung composts

The composts were obtained from cattle abattoir in Rukpokwu, Obio/Akpor Local Government Area of Rivers State. The cow dung were collected from the abattoir and kept on experimental area to be remediated. This is enable quick access to the cow dung.

2.3.2 Palm Oil Mill Effluent (POME)

The POME used in this research was collected from a local palm oil mill in Rukpokwu, Obio/Akpor Local Government Area of Rivers State. This palm oil mill is privately owned and it produces palm oil in commercial quantities. The POME was collected at the point of discharge of the effluent after the oil has been extracted and it was collected in sterile plastic containers with screw covers.

2.3.3 Crude oil sample

The crude oil used was the Bonny Light Grade obtained from Nigerian Agip Oil Company Limited.The crude oil sample was collected in plastic container and stored till required for impaction.

2.3.4 Soil sample.

Using soil auger, soil samples were collected from three spots randomly selected from each of the plots at the depth of 0-20 cm. The samples were mixed to give a composite sample for each plot. Each composite soil sample was shared into two; one portion was sieved with 2mm mesh and stored at 4^0C until required for soil physicochemical analysis within a week of collection before and after remediation. The other portion was dried at room temperature and passed through a 2mm sieve after properly removing particulate matter. The soil so treated was used for enzymatic analysis. These analyses were done for each of the samples collected from each plot.

- POME effects on degradation of crude oil

The land was divided into 6 portions each measuring $3m^2$ and was two metres apart from each other. Four portions were intentionally impacted with 10L, 15L, 20L and 25L crude oil. The fifth and sixth wetland portions were left un-impacted. The fifth portion was left as negative control, while the sixth was amended with POME only as positive control (PC). After two weeks, all the crude oil impacted portions were amended with 10L of POME by spraying on the soil surface each before tilling manually and uniformly for two weeks intervals.

Cow dung wastes effects on degradation of crude oil

The land was divided into six plots of $3m^2$ each and $2m^2$ apart. This is to monitor the pace with which the bioremediation activities are taking. Five of the $3m^2$ soil plots were intentionally and evenly impacted with 15L of crude oil. The sixth plots were not impacted with cow dung, but left as positive control. Two of the plots impacted with oil were amended with 1kg, 2kg, 3kg, 4kg and 5kg of cow dung compost. The plots were tilled at two weeks to ensure turning and mixing. The remediation process lasted for 90 days.

III. RESULTS

EFFECT OF CRUDE OIL IMPACTION ON PHYSICOCHEMICAL PARAMETERS OF THE SOIL SAMPLE

Table 2: Physicochemical properties of soil samples before and after crude oil impaction and amendment with POME

	Before bioremediation						
		Soil	Treatment				
Parameter	C	PC	S+P+01	S+P+02	S+P+03	S+P+04	S+P+05
Temp^{o}C	28.1±0.21[a]	28.9±1.07[a]	29.3±0.62[b]	29.4±2.01[b]	29.5±2.04[b]	30.3±2.03[b]	30.1±3.02[b]

pH	6.90±0.03[a]	7.40±0.20[b]	7.3±0.21[b]	7.45±0.12[b]	7.10±0.20[b]	7.04±0.32[c]	6.6±0.22[a]
Org. Matter	21.24±0.23[a]	22.2±0.02[a]	26.7±0.23[b]	28.1±0.03[c]	30.8±0.20[c]	30.4±0.20[c]	20.4±0.2 [b]
NO_3	2.5±0.20[a]	2.4±0.20[b]	2.2±0.20[c]	2.2±0.02[c]	2.1±0.01[c]	2.1±0.01[c]	2.3±0.03[b]
PO_4	2.2±0.01[a]	2.5±0.01[b]	2.6± 0.03[b]	2.6±0.20[c]	2.2±0.01[d]	1.8±0.02[c]	2.1±0.01[a]
Mg	2.32±0.20[a]	2.6±0.20[a]	2.6±0.02[a]	2.6±0.03[a]	2.4±0.20[a]	2.3±0.03[a]	2.3±0.03 [a]
Ca $mgKg^{-1}$	1.30±0.03[a]	1.61±0.01[a]	1.77±0.01[a]	1.81±0.01[a]	1.83±0.20[a]	1.82±0.02[a]	1.2±0.01 [a]
Na $mgKg^{-}$	0.12±0.02[a]	0.19±0.03[a]	0.19±0.03[a]	0.20±0.01[a]	0.08±0.02[a]	0.08±0.02[a]	0.12±0.0 [a]
K $mgKg^{-1}$	0.76±0.03[a]	0.39±0.03[a]	0.38±0.02[a]	0.30±0.03[a]	0.25±0.01[a]	0.26±0.02[a]	0.76±0.0 [a]
			After bioremediation				
Temp^{o}C	28.2±1.20[a]	29.6±2.01[b]	29.4±2.02[b]	29.7±1.07[b]	29.7±2.01[c]	29.8±2.01[c]	28.5±1.1 [a]
pH	6.70±0.03[a]	7.0±0.0[b]	7.0±0.0[b]	6.4±0.02 [a]	6.9±0.03[b]	6.4±0.02 [a]	6.4±0.02 [a]
Org. matter	21.21±0.21[a]	23.0±0.01[b]	28.2±0.01[c]	28.22±2.1[c]	26.1±1.07[d]	26.2±2.20[d]	22.0±0.00[b]
NO_3	2.60±0.20[a]	2.80±0.20[a]	3.01±21[b]	3.01±0.01[b]	2.68±0.20[a]	2.14±0.02[c]	2.72±0.02[b]
PO_4	2.02±0.03[a]	2.43±0.02[b]	2.64±0.02[c]	2.70±0.01[d]	2.70±0.01[d]	2.32±0.03[b]	2.32±0.01[b]
Mg$mgKg^{-1}$	2.40±2.10[a]	2.66±0.20[a]	2.90±3.02[a]	2.71±0.01[a]	2.62±0.02[a]	2.52±0.02[a]	2.44±0.02[a]
Ca $mgKg^{-1}$	1.56±0.20[a]	1.76±0.03[a]	1.89±21[a]	1.89±0.01[a]	1.74±0.20[a]	1.55±0.01[a]	1.60±0,03[a]
Na $mgKg^{-1}$	0.21±0.01[a]	0.34±0.02[a]	0.38±0.02[b]	0.40±0.02[b]	0.32±0.01[a]	0.32±0.0[a]	0.23±0.01[a]
K $mgKg^{-1}$	0.54±0.01[a]	0.86±0.01[a]	0.88±0.02[a]	0.89±0.03[a]	0.75±0.01[a]	0.71±0.01[a]	0.59±0.03[a]

*Figures followed by the same alphabet are not significantly different but figures followed by different alphabets are significantly different ($P = 0.05$). ±sd= standard deviation

Table 3: Effect of volume of crude oil on bioloads of some bacterial groups in the soil before and after bioremediation ((cfu/g)

			Before bioremediation				
		Soil	Treatment				
	C	PC	S+P+O1	S+P+O2	S+P+O3	S+P+O4	S+P+O5
THBC	4.5×10^6	3.4×10^6	3.5×10^5	$2.2x\ 10^5$	3.5×10^4	3.1×10^4	$2.8\ x10^5$
NBC	2.2×10^4	1.1×10^4	4.5×10^3	3.6×10^3	1.4×10^3	1.2×10^3	$2.5\ x10^3$
PSBC	2.3×10^4	1.4×10^4	3.2×10^3	3.0×10^3	2.4×10^2	3.1×10^2	$1.2\ x10^3$
PUBC	2.3×10^4	3.2×10^4	$2.4x\ 10^4$	1.3×10^4	1.3×10^3	1.1×10^3	1.6×10^4
TLBC	2.8×10^4	1.3×10^4	1.0×10^4	1.0×10^4	1.2×10^3	1.1×10^3	1.2×10^4
			After bioremediation				
THBC	4.7×10^6	5.2×10^6	$6.4x\ 10^6$	6.7×10^6	3.5×10^6	3.5×10^4	$5.8\ x10^6$
NBC	$3.\ 3 \times 10^4$	2.4×10^4	5.3×10^4	4.6×10^4	2.5×10^4	2.1×10^3	$4.5\ x10^4$
PSBC	2.4×10^4	2.3×10^4	1.8×10^4	2.2×10^4	1.4×10^4	1.0×10^3	$1.3\ x10^4$
PUBC	2.3×10^4	3.2×10^4	3.9×10^4	2.1×10^4	$2.1x\ 10^4$	1.5×10^4	1.1×10^4
TLBC	1.2×10^4	2.3×10^4	2.8×10^4	1.8×10^4	1.2×10^4	9.3×10^3	1.8×10^4

* Values are average of triplicate experiments

Table 3: Effect of volume of crude oil on bioloads of some bacterial groups in the soil before and after bioremediation ((cfu/g)

			Before bioremediation				
		Soil	Treatment				
	C	PC	S+P+O1	S+P+O2	S+P+O3	S+P+O4	S+P+O5
THBC	4.5×10^6	3.4×10^6	3.5×10^5	$2.2x\ 10^5$	3.5×10^4	3.1×10^4	$2.8\ x10^5$
NBC	2.2×10^4	1.1×10^4	4.5×10^3	3.6×10^3	1.4×10^3	1.2×10^3	$2.5\ x10^3$
PSBC	2.3×10^4	1.4×10^4	3.2×10^3	3.0×10^3	2.4×10^2	3.1×10^2	$1.2\ x10^3$

PUBC	2.3 x 10^4	3.2 x 10^4	2.4x 10^4	1.3 x 10^4	1.3 x 10^3	1.1 x 10^3	1.6 x 10^4
TLBC	2.8 x 10^4	1.3 x 10^4	1.0 x 10^4	1.0 x 10^4	1.2 x 10^3	1.1 x 10^3	1.2 x 10^4
			After bioremediation				
THBC	4.7 x 10^6	5.2 x 10^6	6.4x 10^6	6.7 x 10^6	3.5 x 10^6	3.5 x 10^4	5.8 x10^6
NBC	3. 3 x 10^4	2.4 x 10^4	5.3 x 10^4	4.6 x 10^4	2.5 x 10^4	2.1 x 10^3	4.5 x10^4
PSBC	2.4 x 10^4	2.3 x 10^4	1.8 x 10^4	2.2 x 10^4	1.4 x 10^4	1.0 x 10^3	1.3 x10^4
PUBC	2.3 x 10^4	3.2 x 10^4	3.9 x 10^4	2.1 x 10^4	2.1x 10^4	1.5 x 10^4	1.1 x 10^4
TLBC	1.2 x 10^4	2.3 x 10^4	2.8 x 10^4	1.8 x 10^4	1.2 x 10^4	9.3 x 10^3	1.8 x 10^4

Table 4: Effect of oil impaction and bioremediation on some enzyme activities in the soil samples analyzed

	Soil Treatment				
	C	S+P+O1	S+P+O2	S+P+O3	S+P+O4
			Before bioremediation		
Dehy	26.73±1.8^a	20.2±0.4^b	15.3±0.3^c	12.7±0.2^d	10.52±0.2^e
Urease	3.43±0.3^a	2.23±0.2^b	2.01±0.1^b	1.62±0.2^d	1.04±0.2^e
Lipase	2.51±0.3^a	2.22±0.2^b	1.9±0.1^c	1.8±0.3^d	1.4±0.2^e
H^+ptase	3.4±0.1^a	3.0±0.0^b	3.63±0.3^a	2.2±0.1^c	1.8±0.2^d
OH ptase	3.3±0.2^a	2.8±0.2^b	2.6±0.2^b	2.4±0.2^b	1.3±0.1^d
Phenol oxide	2.5±0.1^a	2.3±0.1^b	2.1±0.1^c	1.9±0.1^d	1.1±0.1^e
			After bioremediation		
Dehy	26.73±2.3^a	38.71±2.1^b	32.35±0.4^c	25.72±0.4^d	18.57±0.1^d
Urease	3.43±0.3^a	3.90±0.3^b	3.5±0.1^c	3.4±0.2^a	2.4±0.2^c
Lipase	2.51±0.1^a	3.6±0.3^b	3.5±0.3^b	3.0±0.0^c	2.8±0.2^c
H^+ptase	3.4±0.2^a	3.6±0.1^b	3.8±0.2^b	3.0±0.0^d	2.3±0.3^e
OH ptase	3.3±0.1^a	3.2±0.2^a	3.0±0.0^b	2.9±0.3^b	2.4±0.1^c
Phenol oxide	2.57±0.3^a	3.3±0.1^b	3.0±0.0^c	2.8±0.2^c	2.5±0.1^a

Table 5: Effects of Cow dung on soil physicochemical properties before and after remediation

	Before remediation						
	Soil amendment						
Parameter	S±sd	S + 0 ±sd	S + C+01±sd	S + C + 02 ±sd	S + C+03±sd	S + C+04±sd	S+C+05±sd
Temp ^oC	28.1±2.1^a	29.2±2.3^b	29.4±1.4^b	29.41±2.1^b	29.7±2.3^b	30.1±2.2^b	30.4±2.2^c
Ph	6.8±1.2^a	7.2±0.2^a	7.2±0.2^a	7.2±0.2^a	7.3±0.3^a	7.3±0.3^a	7.4±0.2^a
Org matter	21.24±1.4^a	21.21±1.4^a	20.1±1.9^a	20.4±1.8^a	20.0±0.0^a	19.3±1.5^b	19.0±1.4^b
NO_3 gKg^{-1}	2.5±0.3^a	2.2±0.1^a	2.1±0.1^a	2.0±0.0^a	1.8±0.2^b	1.2±0.2^c	1.1±0.0^c
PO_4 gKg^{-1}	2.20±0.1^a	2.21±0.1^a	2.12±0.2^b	2.18±0.2^a	2.1±0.3^b	2.01±0.2^a	1.9±0.1^c
Mg mgKg^{-1}	2.32±0.1^a	2.13±0.3^a	2.12±0.3^a	2.10±0.1^c	2.10±0.1^c	2.0±0.0^a	1.8±0.2^b
Ca mgKg^{-1}	1.30±0.1^a	1.30±0.2a	1.25±0.1^b	1.10±0.1^a	1.00±0.1^b	1.00±0.1^c	0.98±0.1^c
K mgKg^{-1}	0.76±0.0^a	0.76±0.0^a	0.67±0.0^b	0.50±0.0^b	0.45±0.0^c	0.41±0.0^b	0.40±0.0^b
Mn mgKg^{-1}	1.12±0.2^a	1.12±0.2^a	1.00±0.0	0.87±0.0^c	0.67±0.0^c	0.40±0.0^c	0.38±0.0^c
Cr mgKg^{-1}	0.03±0.01^a	0.03±0.01^a	0.03±0.01^a	0.03±0.01^a	0.02±0.01^a	0.02±0.01^a	0.02±0.01^a
Cd mgKg^{-1}	0.03±0.01^a	0.03±0.01^a	0.02±0.01^a	0.02±0.01^a	0.02±0.0^a	0.02±0.0^a	0.02±0.0^a
Pb mgKg^{-1}	1.61±0.3^a	1.21±0.01^b	1.13±0.03^b	1.03±0.1^c	1.04±0.1^c	1.00±0.0^c	1.00±0.0^c
Zn MgKg^{-1}	13.1±0.1^a	14.1±0.1^a	14.5±0.02^b	14.5±0.02^b	14.7±0.3^b	14.8±0.3^b	15.2±0.2^c
C/N	15.3±0.2^a	14.6±1.3^b	10.4±0.8^c	10.0±0.8^c	10.3±0.4^c	10.5±0.3^c	11.2±0.2^c

				After remediation			
Temp ^{o}C	28.2±2.2^{a}	28.9±2.2^{b}	28.3±2.3^{a}	28.3±2.3^{a}	28.3±2.3^{a}	28.3±2.3^{a}	28.5±2.1^{b}
pH	6.9±0.3^{a}	6.6±0.2^{b}	6.3±0.1^{c}	6.3±0.1^{c}	6.4±0.2^{c}	6.7±0.2^{a}	6.9±0.3^{b}
Org.matter	21.21±0.1^{a}	24.66±0.3^{b}	28.71±0.3^{c}	27.6±0.3^{c}	23.2±-0.2^{c}	21.4±0.1^{c}	22.0±0.0^{b}
mgKg-1	2.6±0.2^{a}	3.2±0.0^{a}	5.8±0.2^{b}	5.6±0.1^{b}	5.4±0.1^{c}	5.4±0.2^{c}	5.4±0.1^{b}
NO_3 gKg^{-1}	2.02±0.1^{a}	3.56±0.1^{b}	4.22±0.2^{c}	5.34±0.2^{c}	3.11±0.1^{c}	2.21±0.1^{c}	2.20±0.2^{a}
PO_4 gKg^{-1}	2.40±0.3^{a}	3.76±0.1^{a}	5.63±0.1^{b}	7.53±0.1^{b}	3.61±0.3^{c}	2.88±0.1^{c}	2.80±0.2^{d}
Mg $mgKg^{-1}$	1.56±0.2^{a}	2.45±0.3^{b}	3.55±0.3^{c}	3.93±0.1^{c}	3.32±0.2^{d}	2.00±0.0^{e}	2.00±0.0^{b}
Ca $mgKg^{-1}$	0.54±0.1^{a}	1.19±0.3^{b}	1.30±0.2^{c}	1.37±0.1^{c}	1.00±0.0^{c}	0.88±0.1^{c}	0.80±0.1^{b}
K $mgKg^{-1}$	1.12±0.0^{a}	1.32±0.1^{a}	1.54±0.2^{b}	1.64±0.1^{b}	1.55±0.3^{b}	1.05±0.3^{b}	1.00±0.0^{c}
Mn $mgKg^{-1}$	0.03±0.0^{a}	0.03±0.0^{a}	0.03±0.0^{a}	0.03±0.0^{a}	0.03±0.1^{a}	0.03±0.1^{a}	0.03±0.1^{b}
Cr $mgKg^{-1}$	0.03±0.0^{a}	0.03±0.0.3^{a}	0.03±0.0^{a}	0.03±0.0^{a}	0.03±0.1^{a}	0.03±0.1^{a}	0.03±0.1^{b}
Cd $mgKg^{-1}$	1.60±0.2^{a}	1.31±0.1^{b}	1.32±0.1^{b}	1.32±0.2^{b}	1.22±0.2^{b}	1.12±0.2^{b}	1.10±0.3^{c}
Pb $mgKg^{-1}$	13.1±0.3^{a}	14.2±0.1^{a}	14.6±0.2^{b}	14.8±0.2^{b}	15.3±0.1^{c}	15.8±0.2^{b}	16.0±0.2^{c}
Zn $MgKg^{-1}$	15.3±0.1^{a}	13.1±0.3^{b}	10.0±0.1^{c}	9.8±0.3^{c}	9.7±0.3^{b}	9.5±0.3^{a}	9.3±0.3^{b}
C/N							

*Figures followed by the same alphabet are not significantly different but figures followed by different alphabets are significantly different (P = 0.05). ±sd= standard deviation

Table 7: Bioload of Specific Bacterial physicochemical groups in the soil before and after bioremediation (cfu/ml)

				Before remediation		
				Soil treatments		
Organisms	C S	S + C	S + C +0 1	S + C+ 02	S + C+ 03	S + C+04
THBC	4.5×10^{6}	3.7×10^{4}	3.9×10^{5}	3.3×10^{5}	2.5×10^{4}	3.5×10^{4}
TNBC	2.2×10^{4}	2.1×10^{2}	3.5×10^{2}	3.5×10^{2}	2.2×10^{2}	3.2×10^{2}
TPSBC	2.3×10^{4}	2.6×10^{2}	3.8×10^{2}	3.4×10^{2}	2.8×10^{2}	3.8×10^{2}
TPUBC	2.3×10^{4}	3.2×10^{3}	4.6×10^{3}	4.1×10^{3}	3.4×10^{3}	4.7×10^{3}
TLBC	2.8×10^{4}	1.9×10^{3}	3.3×10^{3}	3.2×10^{3}	2.5×10^{3}	$4.0x\ 10^{3}$

				After remediation		
				Soil treatment		
Organisms	CS	S + C	S + C+ 01	S + C+02	S + C+03	S+ C+04
THBC	4.7×10^{6}	6.0×10^{5}	6.8×10^{6}	7.2×10^{6}	7.0×10^{6}	7.4×10^{5}
TNBC	3.3×10^{4}	3.1×10^{4}	4.1×10^{4}	4.3×10^{4}	2.9×10^{4}	2.1×10^{4}
TPSBC	2.4×10^{4}	2.0×10^{4}	2.8×10^{4}	2.9×10^{4}	3.1×10^{4}	2.7×10^{4}
TPUBC	2.3×10^{4}	1.7×10^{4}	1.9×10^{4}	2.1×10^{4}	2.6×10^{4}	2.3×10^{4}
TLBC	1.2×10^{4}	1.2×10^{4}	2.1×10^{4}	2.4×10^{4}	2.5×10^{4}	2.2×10^{4}

* Figures followed by the same alphabets are not significantly different but figures followed by different alphabets are significantly different (P = 0.05).

Table 8: Enzyme activities in the impacted soil samples analyzed before and after bioremediation

			Before bioremediation			
			Soil treatments			
	CS	S+O	S+O+1	S+O+2	S+O+3	S+O+4
			Before bioremediation			

Dehy	24.73[a]	17.62[b]	15.32[c]	17.71[b]	17.80[b]	18.60[b]
Urease	3.43[a]	1.52[b]	1.56[b]	1.70[c]	1.73[c]	1.80[c]
Glucosidase	4.31[a]	3.07[b]	2.37[c]	2.41[c]	2.44[c]	2.80[c]
Lipase	2.51[a]	1.42[b]	1.62[b]	1.67[b]	1.69[b]	1.80[b]
H^+ptase	3.4[a]	1.49[b]	1.63[b]	1.67[b]	1.68[b]	1.74[b]
OH ptase	3.3[a]	1.11[b]	1.48[b]	1.40[b]	1.43[b]	1.82[b]
Phenol oxide	2.5[a]	1.38[b]	1.36[b]	1.36[b]	1.37[b]	1.44[b]
			After bioremediation			
Dehy	24.73[a]	18.67[b]	25.44[a]	29.74[c]	29.93[c]	30.73[c]
Urease	3.43[a]	2.11[b]	3.47[a]	3.61[a]	3.67[a]	3.73[a]
Glucosidase	4.31[a]	2.97[b]	4.46[a]	4.68[a]	4.73[a]	5.12[c]
Lipase	2.51[a]	1.93[b]	2.64[a]	2.78[a]	2.78[a]	2.95[a]
H^+ptase	3.47[a]	2.51[b]	3.02[a]	3.19[a]	3.19[a]	4.71[c]
OH ptase	3.37[a]	2.54[a]	3.41[a]	3.43[a]	3.47[b]	4.78[b]
Phenol oxide	2.57[a]	1.97[b]	2.66[a]	2.76[a]	2.93[c]	3.34[c]

*Figures followed by the same alphabet are not significantly different but figures followed by different alphabets are significantly different (P = 0.05). ±sd= standard deviation

IV. DISCUSSIONS

In the course of this research, there are abundant evidences that bioremediation process using cow dung and POME can revive the altered soil physicochemical properties affected by crude oil pollution. This bioremediation process occurred due to improvement in NO_3, PO_4 and Total Organic matter content of the impacted soil. This research agrees with the works of several authors including Liu *et al*., (2000); Abioye *et al*., (2012).

Positive correlation was observed between the level of soil degradation and the volume of oil involved. Oil has no NO_3 and PO_4 content, hence could dilute the concentration of the nutrients already present in soil (Nwaugo *et al*., 2007; 2008). The higher acidity of the soil impacted with higher oil volume agrees with the report of Nwaugo *et al.,* (2008) and Abioye *et al*., (2012). The petroleum impaction can hinder the gaseous exchange between soil and the atmosphere which aids heat retention. This could account for the initial slight increase in temperature before remediation. The no significant increase in metallic elements contents may be because the pollutant (oil) has no such metallic elements and cannot supply same to the soil but higher acidity caused solubilization of metallic ores.

After remediation, the values of organic matter, PO_4, and NO_3 increased significantly in all the soil samples, though at different rates according to the amount of the oil impaction on the soil. Values of 10L and 15 L oil impacted soil amended with POME showed highest increase while values of 20L oil impact on the soil was similar to the control. The POME also modified the effect of the oil and caused improved microbial activities as shown by the enzymatic reactions and physicochemical parameters that further improved the soil nutrients. However, the improvement was dependent on the volume of oil impaction on the soil. Results from POME amended soil without oil (positive control) agrees with the observations of improved values after amendment and bioremediation. Further observations in the bacterial spectrum analysis showed the adverse effect of crude oil on bacteria and consequent improvement after POME amendment and remediation. At the commencement, after impaction, the bacterial populations were low according to the concentrations of oil involved but their populations increased significantly after remediation. This indicated recovery from the pollution effect according to the oil content. At the end of the remediation process, 20L and 25L oil impacted soil had lower bacterial population compared to the other two (10L and 15L oil impacted). Obire *et al*., (2008); Abioye *et al*., (2012) reported that the level of oil impaction

(light, moderate or heavy) affected the rate of remediation as heavily polluted soil required more fertilizer and time to recover.

The Nitrifying and Phosphate Solubilizing Bacteria (NB and PSB) were mostly affected by the oil impaction. Nwaugo *et al*., (2008) findings agrees with the works of Pelczar *et al*., (2001) and Prescott *et al*., (2003), reported that the microorganisms involved in the mineralization of plant and microbial nutrients are highly affected by activities of the NB and PSB. Consequently, the improvement in soil physicochemical properties caused their increase after bioremediation as more nutrients for utilization became available and the environmental factor favoured their growth. On the other hand, Petroleum Utilizing Bacteria (PUB) and Total Lipolytic Bacteria (TLB), though equally affected before remediation, increased significantly after remediation. This could be attributed to the type of pollutant (crude oil). When the conditions are favourable, Petroleum utilizers will proliferate since it is their substrate. Similarly, the proliferation of lipolytic bacteria was expected because petroleum contains lipids (both saturated and unsaturated hydrocarbons). However, observations in the amount of oil impacting the soil showed that a high concentrations, even the PUB and LB are inhibited as observed in the 20L and 25L oil impacted soil.

Trends in the enzymatic activities are similar to those of the bacterial spectrum. Dehydrogenase activities are considered as the best indicator of soil microbial health because it occurs intracellularly in living bacterial cells (Masciandaro *et al*., 2001). Dehydrogenase which was significantly affected before remediation process improved significantly after the process, with the process with 10L and 15L going above the control. All the enzymes equally showed similar trend except phenol oxidase which showed a constant gradient with the concentration of oil impaction on the soil. The activities of all the enzymes were highest in the 10L and 15L oil impacted soil samples and lowest in the 25L oil impacted soil.

The findings in this research showed that soil amended with cow dung caused higher soil enzymatic activities than the control. This tallied with the research results Acosta-Martinez *et al*., (2003); Nwaugo *et al*., (2007); Chang *et al*., (2008). However, the problem with the 20L and 25L oil impacted soil could be referred to the volume of oil impacting the soil. Urease activities did not show much difference in the various soil samples and agrees with Lee *et al*., (2002) and Nwaugo *et al*., (2008) who found that low NO_3 content caused increased soil Urease activity. In addition, the results of the work of Chang *et al*., (2008) agreed with that of McCarty *et al*., (1994) that low pH caused increase in Urease activity. The soil pH could have resulted in the reduced effect on Urease activity observed in the work compared to dehyrogenase. Li *et al*., (2005) and Nwaugo *et al*., (2008) reports showed that urease activity is reduced in acidic soil, but higher in alkaline soil and the soil in this work was acidic. The high activity of Lipase at the end of the remediation process could easily be attributed to the presence of its substrate (lipids) in the soil. Both the pollutant (crude oil) and amendment agent (POME) contain some amounts of lipids. The activity of lipase has been reported to increase with the presence of lipids, hence tallies with the findings of this work. However, the remediation of all other environmental factors which affected bacterial proliferation also affected the lipase activity.

The findings of this work showed that the phenol oxidase activity correlated positively with the amount of crude oil impaction on the soil, as the higher the volume of oil impaction on the soil, the less the availability of oxygen due to the closure of soil pores. The phenol oxidase activity was reduced with high volume of oil impacting the soil because the organisms producing it were low. This tallied with the work of Nwaugo *et al*., (2007; 2008) showing that phenol oxidase is regulated by the amount of its substrate (the concentration of aromatic compounds available) and the amount of oxygen for the oxidative activity.

Results of acid and alkaline phosphatases indicated similar trends, though with acid phosphatases showing non-significant higher values, which could be attributed to the pH values observed. Both enzymes had higher values in the 10 L and 15 L oil impacted soil samples which tallies with findings in the PSBC. The PSB are the producers of the phosphatases, hence the enzymes (phosphatases) collaborated the PSBC. The 20L and 25L oil impacted soil samples had the lower values. Similar observations had been reported by Chang *et al*., (2008) who observed that organic

manure increased activities of both acid and alkaline phosphatases.

Results obtained in the characteristics of organic manure used (cow dung) showed that it was properly composted (matured). Kato *et al*., (2005) and Chang *et al*., (2008) reported that compost with C/N ratio of 9.5-12.5 and pH of 6.0-7.5 are mature. However, the observed higher amounts of NO_3, PO_4 EC in cow dung may be because of its high decomposition, solubilization and source. The crude oil impaction of the Orashi River Wetland in Egbema was observed to have caused nutrient depression and increased soil acidity and temperature. The increased acidity in oil impacted soil could be due to production of organic acids during its metabolism. The adverse effect of crude oil on the soil physicochemical properties agrees with the work of Nwaugo *et al*., (2007). Generally, the amended oil impacted soil samples showed higher NO_3, PO_4, organic matter and Electrical Conductivity than the negative control soil. This could easily be attributed to the effects of the organic fertilizers added. These fertilizers had considerable amount of NO_3, $PO_4$and other nutrients which enhanced their contents in the soil. The results obtained also showed that the higher the amount of compost added the greater the values of the nutrients available in remediating soil, indicating positive correlation. This assertion is further enhanced by the low heavy metallic elements in the soil as was also observed in the cow dung used. This is in consonance with the reports of Chang *et al*., (2008). Amendment of the impacted soil reduced the effect of the oil on soil properties because components of the compost acted as buffer or shield. In contrast to the above, after remediation, there was increase in the soil physicochemical properties, except the metallic elements that remained the same. Soil acidity also decreased as the pH values increased. While values in the negative control soil remained low, values in the organic manure amended soil samples increased statistically. The soil NO_3, PO_4, EC and organic matter all increased considerably. Soil Ca, K and Mg also increased which was attributed to the increase in mineralization activities in the soil which was enhanced by soil amendment.

The different physiological bacterial groups examined showed differences in their populations in the course of the research. There was a general decrease in all groups immediately after the oil impaction. However, there was slight improvement following the organic manure amendment. From the results, it is observed that the organic manure used, suppressed the effect of the crude oil impaction. This suppression positively correlated with the amount of manure used, i.e., the higher the manure applied, the higher the suppression of crude oil adverse effects. This agrees with the reports of Nwaugo *et al*., (2006) and Chang *et al*., (2008). The particles of the manure absorbed some of the effects of the oil impaction, causing some organisms to survive. This was because the higher the cow dung used, the lesser the effect of soil impaction on the bacterial groups.

Results of this research show that THB were the most abundant in the soil and were equally the most affected by the oil impaction. TPUB and TLB did not suffer much from the effects of the oil impaction. The effect of the oil impaction was less drastic on the TPUB and TLB. Crude oil had less effect on oil utilizing bacteria, which includes TPUB and TLB as both are degraders. The two bacterial groups easily used the crude oil as substrate for metabolism. However, observations from the results showed that oil impaction caused a significant decrease in their populations as well before the bioremediation exercise, showing the general toxicity of crude oil at the initial stage. The THB include oil degraders and non-oil degraders and it was these non-oil degraders that were extensively suppressed by the oil impaction.

After the remediation process, all the bacterial groups increased significantly in their populations, thereby indicating removal of the pollutant and availability of conditions favourable for bacterial proliferation. The organisms used the organic compost components as nutrients to enhance the metabolism of the crude oil. This is buttressed by the low bacterial proliferation in the negative control soil (oil impacted but unamended). The presence of NO_3, PO_4 and K in the compost served as initial nutrients for the bacterial groups in their lag growth phase before the metabolism of the crude oil. The metabolism of the Ca, N and P sources coupled with C from the crude oil ensured proliferation of all the organisms concerned. This research shows that metabolism of organic manure (POME and cow dung) caused significant

increase in all physiological groups. The remarkable increase in both TPUB and TLB could be attributed to the major substrate metabolized (crude oil and the organic manure). The proliferation of the phosphate solubilizing and nitrifying bacteria could be due to intermediate metabolic products from the primary degraders and removal of the oil. Before the remediation exercise, dehydrogenase showed the highest activities in all the soil samples with the range of 16.73 to 24.73 mg^{-1}, urease had 1.84-3.43 and glucosidase had between 2.07 to 4.31 mg^{-1}. In all cases, the lowest values were in the impacted soil while the highest were in the control. The lipase activity was the least affected though it also reduced like all the other enzymes. After the remediation process, all the enzymes increased significantly in activities, with enzymes in the cow dung amended showing higher values. Dehydrogenase is produced by heterotrophic organisms which is the most abundant in the soil, while lipase is attributed to mainly the TPUB and TLB. The increase in the population of THB, TPUB and TLB caused the consequent increase in the activities of both dehydrogenase and lipase. Observations in acid and alkaline phosphatases did not indicate much variation. This is could be attributed to the effect of pH as both enzymes activities correlated positively with pH of the medium involved. The pH amended soil did not differ significantly after remediation, therefore could not have affected the enzymes significantly. The acid and alkaline phosphatases were therefore within the same range in activities.

The phenol oxidase activity showed remarkable increase after the remediation process in the compost amended oil impacted soil samples. Like the other enzymes, its activity was significantly reduced by the oil impaction (before remediation). Nwaugo *et al*., (2008) reported that high concentration of aromatic hydrocarbons caused increase in phenol oxidase activity in petroleum produced (formation) POME impacted soil in Elem Sangama. The crude oil contained high concentration of aromatic compounds which could have caused the increased phenol oxidase activity observed after 90 days remediation exercise.

Finally, results of the bacterial groups and enzymatic activities in this work showed that the amendment with 3 cow dung gave higher values that those of 1 kg cow dung amendment this shows that more cow dung compost could be needed in the remediation of oil impacted soil.

CONCLUSION

Crude oil spillages in our environments have been a consistent challenge and as long as crude oil exploration takes place, spillage is bound to occur. This research provided a technique of bioremediation for the reclamation of oil contaminated soil. The overall aim of this research was to determine the feasibility of using POME and cow dung compost for the restoration of soils contaminated with crude oil. One of the purposes of this research includes returning the contaminated land to agricultural use. Bioremediation can be a viable and effective response to crude oil contaminated soil as seen from the result. There was visible positively enhanced remediation by the amendment of the crude oil contaminated soil with POME and Cow Dung. This study has demonstrated the potential of cow dung in enhancing the growth of microorganism for the remediation of crude oil contaminated soil. The bioremediation technique for contaminated soil with crude oil and/or other hydrocarbons is applicable in field because of its availability, low cost and its environment friendliness. Bioremediation for hydrocarbon polluted soil performed under aerobic conditions proved to be a potential method of remediation for most hydrocarbons' pollution soils. This study showed that optimizing cow dung was highly successful for cleanup of the artificially contaminated petrol-polluted soil and improved the fertility of the soil.

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