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Research Article

Ecotoxicity Evaluation of Remediated Soils in Niger Delta Region Using Maize (*Zea mays*) and Okra (*Abelmoshus esculentus*) Plants

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ABSTRACT

The study was aimed at evaluating ecotoxicity of remediated crude oil contaminated soil in Rivers State using maize (*Zea mays*) and okra (*Abelmoshus esculentus*) plants in order to ascertain the bioremediation approaches adopted. Research was designed to evaluate the bioaugumentation efficiency of hydrocarbon degrading bacteria and the biostimulation efficiency of organic nutrients in bioremediation, thereafter, the strength of the remediated soil was evaluated using okra and maize plants in the Rivers State University agricultural farm, Port Harcourt, Nigeria. The experiment was conducted for a period of 98 days, from May to August 2022. Analyses were carried out after 2 weeks of planting and afterwards, at weekly intervals. Thirty-six (36) experimental pots were employed, each having about 1000g of farm soil which was prepared according to six different percentages of the remediated soil in triplicates each, for okra and maize plants. These experimental setups were left for acclimatization before planting viable okra and maize seeds. The grown plants were analyzed for their physical growth parameters and statistically compared using statistical package for service solutions (SPSS). Ecotoxicity assessment of the remediated soil revealed that the bioremediated soils supported plant growth as there were no significant differences ($P>0.05$) observed between the plants grown on the control soil, on the different percentages of remediated soil and on the remediated soil. Results showed that the bioremediation protocols employed in this study could restore the initial status of the soil for agricultural sustainability purposes, thereby, boosting the economy and local content of the States affected by oil spills. It is therefore, recommended that, soils and environments contaminated with crude oil hydrocarbons in the Niger Delta can be bioremediated and the soil quality restored for agricultural purposes to improve agricultural sustainability.

Keywords: Agricultural sustainability, Crude oil, Soils, Ecotoxicity, Bioremediation.

Introduction

The initial status of our natural resources is constantly deteriorating due to the various anthropogenic activities practiced either by individuals or by industries which leads to environmental pollution (Awari *et al*., 2020). Hydrocarbon spill poses a major threat to the natural resources including; soil, vegetations/farm lands, environments especially in oil producing states in Nigeria as a result of frequent crude oil spillage or accidental discharge during refining into the water bodies and farmlands (Ogbonna *et al*., 2012). These have over the years impacted negatively on affected areas and has also jeopardized the sustainability of our natural resources (Bento *et al*., 2005). Moreover, despite its negative effect on ecological diversity, it has affected agriculture, most crops often destroyed, and great land areas left infertile and polluted, fishes dying and other aquatic food sources, as well as the local economy of these affected areas (Awari *et al*., 2018). Furthermore, the health of

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crops, soil, environments and humans in this region is increasingly becoming unbearably affected due to the high effects of oil spills as these hydrocarbons pollutes natural resources ranging from the rural communities to the urban areas, affecting the communities at large (Menkith and Amechi, 2019).

The Niger Delta region is an area in south south and south eastern part of Nigeria, comprising of wet and dry lands which covers about 70,000 square kilometres. The region spans over 20,000 square kilometres, is located in the Atlantic coast of Southern Nigeria where the River Niger divides into numerous tributaries (UNEP, 2007). The region which consists of a number of distinct ecological zones, costal ridge barriers, mangrove swamps, fresh water swamps, forests, and low land rain forest is dominated by rural communities that depend solely on the natural environment for subsistence living (UNEP, 2007). UNEP (2007) reports stated that, more than 70% of the people depend on natural environment for their livelihood and the region is home to more than 10 million people. Niger Delta region is richly endowed with natural resources with oil and gas accounting for over 85% of the National Gross Domestic Product (GDP), over 95% of the National budget and over 80% of the national wealth (Bento *et al*., 2005). Nevertheless, the region remains the poorest, largely due to the ecologically unfriendly exploitation of oil and State's policies that expropriate the indigenous peoples of Niger Delta of their rights to these natural resources. It is believed that since the advent of oil exploration some decades ago, the region has become the breadwinner of the nation, accounting for over 90% of the nation's export earnings since 1975 (NNPC, 2015). The region cuts across nine states of the Federation as follows: Abia, Akwa Ibom, Bayelsa, Cross River, Delta, Edo, Imo, Ondo and Rivers as illustrated in Figure 1. (UNEP, 2007). The study was carried out in Rivers State in the Niger Delta region of Nigeria. This region falls within the tropical rain forest zone with high rainfall and thick vegetation cover (Ahmadu and Egbodion, 2013). The ecosystem of the area is highly diverse and supportive of numerous species of terrestrial and aquatic flora and fauna. Major occupation of the inhabitants of the area is agriculture of which some of the arable crops produced by the farmers include cassava, yam, cocoyam, okra, maize, rice etc. (Hamamura *et al*., 2006). With continuous discovery of more oil wells, the States experience deviations in its natural environment following the resultant effects of pollution due to spillage that occur during exploitation activities. Oil spillage has become great menace to the environment and poses great threat to economic development as it results to land, air and water pollution (Ekpebu and Ukpong, 2013). According to Ojanuga *et al.*, (2003), the rich alluvial soil of the Niger delta coupled with copious web of fish and salt water bodies provide the necessary incentives for the people who are predominantly farmers and fishers. UNEP, (2007) report shows that 60% of the population depends on the natural, living and non-living resources of the environment for livelihood. Ojanuga *et al.*, (2003) also observed that, the coastal swamps of Niger Delta are grossly under-utilized for agricultural purposes both in terms of the fraction of available land under cultivation and effectiveness of cropping and sustenance management. Furthermore, Awari *et al*., (2018), stated clearly that, the stressed environment can proffer solution to the production of bioactive substance like enzymes which are of great industrial purposes and contribute to the local content value of Nigeria economy. However, crude oil and their products has over the years turned out to be not only the major source of energy for industry and daily life but has successfully sustained the economy of many nations (Ogbonna *et al*., 2012). The discovery of oil in these areas and subsequent oil production activities has over the years resulted to untold diverse environmental hazards and general misnomer in the culture, economics and way of the people. Such hazards include; oil spillage, gas flaring, gas leakage, erosion, as well as water and air pollution with attendant health problems (Ekpebu and Ukpong, 2013). During an oil spill, massive quantities of liquid hydrocarbons are accidentally released into the environment. Due to this spill, there is wide spread and long term pollution which disrupts the local ecosystem (Ikuesan *et al*., 2017). The soil is then contaminated with a gross effect upon the terrestrial life. As leaking pipelines, running through villages, farms, creeks and rivers in the Niger Delta, are a major source of pollution, sickness and economic ruin for the people of the Niger Delta, farmland polluted by oil is rarely rehabilitated, destroying livelihoods (Orji, *et al*., 2012).

Hydrocarbon pollution of the environment resulting from oil spills and accidental discharge during crude oil processing occurs regularly and has proven to be a risk factor to reduction of ecological diversity. Over the years, oil has polluted the environment beyond sustainability (Antai *et al.*, 2014). Discharge of hydrocarbons into the environment accidentally or as a

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result of anthropological actions has proven to be the leading source of soil and environmental pollution (Wemedo *et al.,* 2018; Ogbonna *et al.,* 2012). Soil pollution by hydrocarbons results in huge deterioration of autochthonous system as build-up of toxic substances in plants and animals tissues may lead to death or mutagenesis (Antai *et al.,* 2014).

Oil pollution causes damage to human health, agricultural land and fish ponds. It can also result into long-standing ecological malfunctioning and poor environmental wellbeing in the Niger Delta (Ojanuga *et al*., 2003). The ecological devastation occasioned by oil exploration has rendered farming and fishing which are the main occupations of the rural people of this region, useless (Antai *et al.,* 2014). These have created health hazards and rendered fishing and farming activities almost impossible (IPIECA, 2000).

Hydrocarbon pollution of soil can occur in several ways; from natural seepage of hydrocarbons in areas where petroleum is found in shallow reservoirs, to accidental spillage of crude oil on the ground. Regardless of the source of contamination, once hydrocarbons come into contact with the soil, they alter its physical and chemical properties (Albert and Tanee, 2011), causing toxicity and lowering or destroying the quality of the soil. In such circumstances, the soil itself will become a source of pollution (IPIECA, 2000). Microbiological cultures, enzyme additives, or nutrient additives that significantly increase the rate of biodegradation to mitigate the effects of the discharge is defined as bioremediation agents (Baeck *et al.,* 2004). Bioremediation agents are classified as bioaugmentation agents and biostimulation agents, based on the two main approaches to oil spill bioremediation. Numerous bioremediation products have been proposed and promoted by their vendors, especially during early 1990s, when bioremediation was popularized as “the ultimate solution” to oil spills (UNEP, 2007).

Soil which also acts as reservoir of residual pollution, releases contaminants into groundwater or air over extended periods of time, often after the original source of pollution has been removed (IPIECA, 2000). Hydrocarbons can come into direct contact with vegetation through spillage onto roots, stems or leaves (Zeiger, 2011). Ahmadu and Egbodion (2010) also found out that farmers in the oil rich region have lost their lands and are consequently forced to emigrate to other communities in search of better livelihood, exerting additional pressures on natural resources in such areas. Hence, this study is designed to evaluate the efficacy of hydrocarbon degraders in combination with organic nutrients: goat manure and fish wastes to enhance bioremediation in the shortest possible period of time so as to evaluate the ecotoxicity of the remediated soil in order to restore the soil and the environment to its initial status and for sustainability, that can boost the economy of the oil producing states and regions previously affected by crude oil spills.

Materials and Methods

Study Area

The choice of Agricultural Teaching Farm of Rivers State University, Port Harcourt was premised on the following factors; the farm has recorded no history of crude oil spill over the years, availability of water, easy accessibility and sufficient space with relatively flat topography. The experimental plot was mapped out covering a total area of 27.5625m^2, measuring 5.25m x 5.25m. The farm land area used for the study is a pristine patch of land within coordinates 4.80474 Lat.N4^{0}48’1707804’’ and 6.97579 Lon.E6^{0}58’33.15828’’. The well-secured area serves as a centre for training, research, demonstration, production of crops including yam, cassava, cocoyam, sweet potato, maize, rice, beans, plantains, vegetables and fruits as well as development for sustainable agricultural practices.

Collection of Samples

Collection of soil samples

Top soil samples were collected using procedures stated in the Food and Agricultural Organization (FAO, 2007) after tilling with sterile manual soil auger from a depth of 0-15cm. The soil samples which were of sandy-loamy texture with specific gravity of 2.61 were bulked after collection to obtain composite soil sample, transferred into fresh, unused black polyethylene bags (Ogbonna *et al*., 2012; Ikuesan, 2017) and transported immediately to the Microbiology Laboratory of the Rivers State University, Port Harcourt, Rivers State.

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Collection of crude oil sample for bioremediation

Twenty litres of Bonny light crude oil was aseptically collected in large sterile plastic jerry cans from an oil company located at Nembe Creek, Bayelsa State.

Collection of seeds for bioassay

The improved varieties of these seeds were obtained from Agricultural Development Project (ADP), Rumodumaya, Port Harcourt, Rivers State.

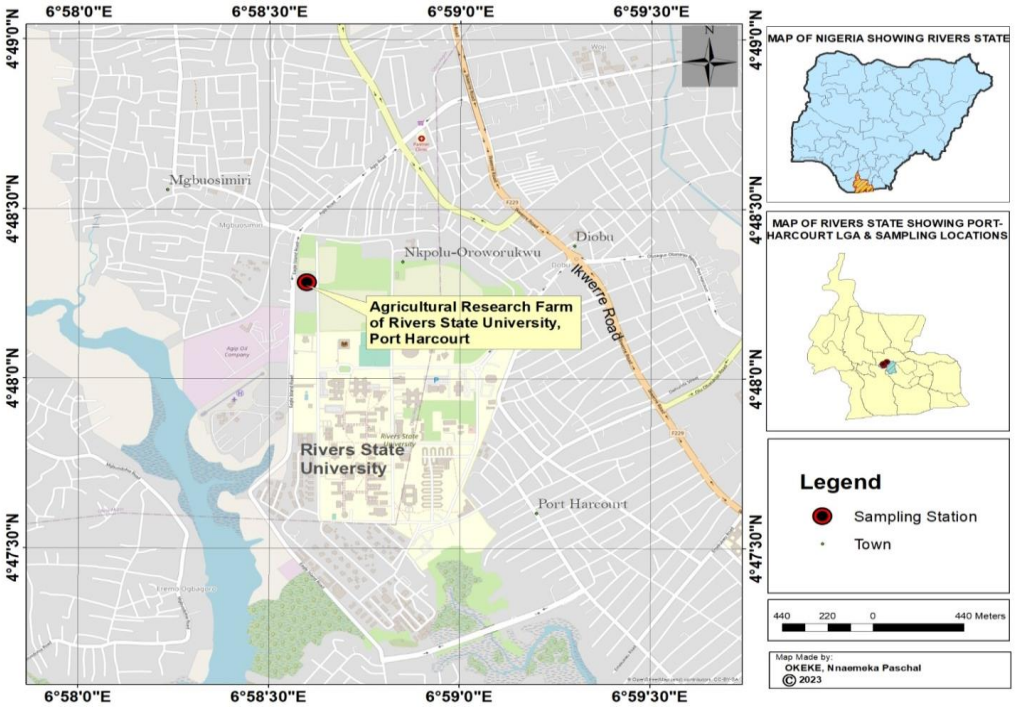

Fig.1. Map showing the Sampling Location of the Study Area in Port Harcourt, Rivers State

Determination of Microbial Counts of Uncontaminated and Contaminated Soils

The soil samples were processed according to method adopted by Prescott *et al* (2005). A portion (10g) of the homogenously mixed soil samples were aseptically transferred into 9 mls of 1% peptone water and properly mixed. Then, 1mls of the aliquots was further diluted up to 10^{-7} using tenfold serial dilution. Thereafter, bacteria and fungi were cultured and isolated using Nutrient Agar (NA) and Sabouroud Dextrose Agar (SDA) prepared according to manufacturer's instruction. Distinct representative bacterial and fungal colonies were purified by repeatedly sub-culturing onto freshly prepared respective media by streak-plate method and incubated for 24 hours and 72 hours respectively. Pure cultures of bacteria were aseptically transferred into 10% (v/v) glycerol suspension, while, pure cultures of fungi were aseptically transferred unto SDA slant in Bijou bottles, well labeled and stored as stock cultures for preservation of the bacterial and fungal isolates (Oyeleke and Manga, 2008). The microbial counts for the different concentrations of the crude oil contaminated soil samples which included: total heterotrophic bacterial count (THBC) and total fungal count (FC) (Obire *et al*., 2008), hydrocarbon utilizing bacterial count (HUBC) and hydrocarbon utilizing fungal count (HUFC) (Orji *et al*., 2012) were determined on the baseline of the uncontaminated and contaminated soil samples and on the soil samples after the application of the different treatments, at seven days interval. The means and percentages of the microbial counts obtained were then statistically analyzed using ANOVA.

Bioremediation Experiment Protocols

The experimental plot containing a total of thirty nine experimental units in which the bioremediation experimentation protocols was conducted in controlled conditions were prepared and used for bioremediation. In each experimental units, five thousand (5000g) of composite soil were weighed into fresh unused black polyethylene bags which were perforated with spatulas to allow for aeration and orderly laid out in the

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experimental plot were made up of thirteen units in three replicates and these represented the three batches of contamination levels, containing thirteen experimental units, with each batch having a representative control sample. These were left undisturbed for six days, thereafter, on the seventh day, two batches containing thirteen experimental units each, were contaminated with crude oil using 5% and 10% contamination levels accordingly (Ogbonna *et al*., 2012; Menkit and Amechi, 2019) while, the third batch also containing thirteen experimental units were left uncontaminated. All experimental units were homogenously mixed to obtain composite soil samples using different spatula. The experimental plot was again left for three weeks so as to allow acclimatization with the new environment (Ogbonna *et al*., 2012; Ikuesan, 2017; Menkit and Amechi, 2019). Applications of different treatments were carried out where the pure cultures of the bioaugumenting organisms were inoculated into the experimental plots accordingly. Similarly, the prepared nutrients for biostimulation obtained were introduced into the experimental plots accordingly (Bento *et al*., 2005). All experimental units were watered regularly and tilled to allow for sufficient aeration (Chaineau *et al*., 2002). Composite samples (10g portion) were then collected by homogenizing 10g portions of soil from each experimental unit for monitoring, after seven days and subsequently, at seven days interval by chromatographic analyses. The Total Petroleum Hydrocarbon (TPH) contents were determined and the percentage bioremediation (%BR) were thus, extrapolated. The differences in the amounts of TPH were derived (Bento *et al*., 2005; Nrior and Echezolom, 2016).

Ecotoxicity Evaluation of Remediated Soil

Bioassay for ecotoxicity evaluation of remediated soil was conducted using maize (*Zea mays*) and okra (*Abelmoshus esculentus*) plants by adopting the method of Zucconi *et al* (1981), Salanitro *et al* (1997) and USEPA (2004). The remediated soil sample (100%RS), normal soil sample (100%NS) and contaminated soil sample (100%CS) served as controls and three other test pots that had both the remediated soil sample and the normal soil sample, mixed in three different ratios; 3:1, 2:2 and 1:3 to obtain three other different percentages; (75%RS), (50%RS) and (25%RS) respectively, of bioremediated soil, making six test pots were suspended in transparent test pots to allow for visualization of roots. The experiment was conducted in triplicates, making, a total of eighteen test pots each for maize and for okra plants, and labelled accordingly. The soils were properly mixed, watered to allow for maximum aeration and left undisturbed for seven days before planting (Ogbonna *et al.*, 2012). Each of the eighteen test pots for maize planting was plated with four (4) viable seeds of maize and also, each of the eighteen test pots for okra planting was also plated with four (4) viable seeds of okra and left for fourteen (14) days (Salanitro *et al*., 1997). The test pots were watered every three days throughout the growing season, while, weeding was done by hand picking (Ogbonna *et al.*, 2012). At the end of the growing test period, test pots were analyzed scored as shoot was visible. Some vegetative growth parameters which included; the shoot lengths, leaf lengths and leaf width were measured in Centimeters using a meter rule two weeks after planting and at seven-day interval for up to eight weeks (Ogbonna *et al.*, 2012). The average measurements of the growth characteristics were considered and recorded in Centimeters.

Statistical Analyses of Data

All experiments were statistically analyzed using statistical package for service solutions (SPSS) where, one-way ANOVA followed by Duncan's multiple comparism test was used to compare the significant differences. An unpaired t-test in which a two-tailed *P*-value was calculated and results were presented as mean ±SD where necessary. Statistical significances were reported as a *P*-value of less than 0.05 at 95% confidence interval.

Results

Results of the means and percentages of microbial counts of the uncontaminated soil, 5% and 10% contaminated soil samples during bioremediation are as shown in Tables 1 to 3 below.

The means of the THBC ranged between 7.39 $\log_{10}$cfu/g (control sample) and 8.40 $\log_{10}$cfu/g (treated samples). The means of the FC ranged between 4.17 $\log_{10}$cfu/g (control sample) and 4.31 $\log_{10}$cfu/g (treated samples). The means of the HUBC ranged between 3.68 $\log_{10}$cfu/g (control sample) and 4.01 $\log_{10}$cfu/g (treated samples), while, the means of the HUFC ranged between 3.57 $\log_{10}$cfu/g (control sample) and 4.08 $\log_{10}$cfu/g (treated samples). The percentage hydrocarbon utilizing bacteria and fungi were calculated (Tables 1 to 3).

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Table 1: Mean and Percentage Microbial Count (log_{10}cfu/g) of Uncontaminated Soil during Bioremediation

Experimental units	Treatment	Total heterotrophic bacteria	Fungi	Hydrocarbon utilizing bacteria	Hydrocarbon utilizing fungi	Percentage (%) hydrocarbon utilizing bacteria	Percentage (%) hydrocarbon utilizing fungi
EU 1	US (control 1)	7.39 ±0.71	4.17 ±0.07	3.68 ±0.15	3.57 ±0.15	49.79	85.61
EU 2	US+GM	7.87 ±1.10	4.21 ±0.11	3.84 ±0.25	3.76 ±0.18	48.79	89.31
EU 3	US+FW	8.11 ±1.29	4.23 ±0.07	3.80 ±0.22	3.92 ±0.46	46.86	92.67
EU 4	US+BC	8.18 ±1.35	4.21 ±0.04	3.79 ±0.40	4.06 ±0.56	46.33	96.44
EU 5	US+CM	8.10 ±1.29	4.16 ±0.07	3.88 ±0.45	4.08 ±0.56	47.90	98.07
EU 6	US+GM+FW	8.34 ±1.48	4.26 ±0.10	3.91 ±0.31	3.79 ±0.18	46.88	88.97
EU 7	US+GM+CM	8.21 ±1.38	4.22 ±0.06	3.89 ±0.30	3.96 ±0.44	47.38	93.84
EU 8	US+GM+BC	8.36 ±1.50	4.31 ±0.26[a]	3.82 ±0.25	3.83 ±0.20	45.69	88.86
EU 9	US+FW+CM	8.40 ±1.53	4.22 ±0.06	3.86 ±0.26	3.97 ±0.45	45.96	94.08
EU 10	US+FW+BC	8.26 ±1.42	4.19 ±0.04	3.81 ±0.24	4.07 ±0.55	46.13	97.14
EU 11	US+CM+BC	8.34 ±1.49	4.21 ±0.06	3.86 ±0.26	3.82 ±0.19	46.28	90.74
EU 12	US+GM+FW+BC	8.29 ±1.44	4.19 ±0.05	3.92 ±0.31	3.87 ±0.23	47.29	92.36
EU 13	US+GM+FW+CM	8.38 ±1.52	4.22±0.06	4.01 ±0.26	3.91 ±0.25	47.85	92.65

Key:US:uncontaminated soil,GM:goatmanure,FW:fishwastes,BC:*Bacillus amiloliquefaciens*,CM:*Comamonas testosteroni* Not significantly different (p>0.05)

Table 2: Mean and Percentage Microbial Counts of 5% Crude Oil Contaminated Soil during Bioremediation

Experimental units	Treatment	Total heterotrophic bacteria	Fungi	Hydrocarbon utilizing bacteria	Hydrocarbon utilizing fungi	Percentage (%) hydrocarbon utilizing bacteria	Percentage (%) hydrocarbon utilizing fungi
EU 1	US(control1)	7.39 ±0.71	4.17 ±0.07	3.68 ±0.15	3.57 ±0.15	49.79	85.61
EU 14	5%CS (control 2)	7.47±1.01	4.17 ±0.11	4.04 ±0.10	3.95 ±0.13	54.08	94.72
EU 15	5%CS+GM	7.76 ±1.25	4.22 ±0.11	4.09 ±0.13[a]	4.19 ±0.29[a]	52.71	99.29
EU 16	5%CS+FW	8.02 ±1.46	4.16 ±0.16	4.07 ±0.12[a]	4.15 ±0.33[a]	50.75	99.76
EU 17	5%CS+BC	8.06 ±1.50	4.17 ±0.25	3.91 ±0.14[a]	3.99 ±0.33[a]	48.51	95.68
EU 18	5%CS+CM	7.98±1.44	4.17 ±0.41	4.00 ±0.08[a]	4.01 ±0.33[a]	50.13	96.16
EU 19	5%CS+GM+FW	8.33 ±1.73	4.19 ±0.11	4.13 ±0.13[a]	4.03 ±0.06[a]	49.58	96.18
EU 20	5%CS+GM+CM	8.20 ±1.61	4.14 ±0.13	4.10 ±0.13[a]	4.08 ±0.11[a]	50.00	95.55

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Table 2 Continued:-

EU 21	5%CS+GM+BC	8.30 ±1.70	4.17 ±0.14	4.04 ±0.08[a]	4.14 ±0.32[a]	48.67	99.28
EU 22	5%CS+FW+CM	8.36 ±1.75	4.12 ±0.16	4.09 ±0.10[a]	4.09 ±0.09[a]	48.92	99.27
EU 23	5%CS+FW+BC	8.00 ±1.45	4.14 ±0.21	4.02 ±0.08[a]	4.02 ±0.35[a]	50.25	97.10
EU 24	5%CS+CM+BC	8.30 ±1.70	4.26 ±0.26	4.07 ±0.10[a]	4.17 ±0.30[a]	49.04	97.88
EU 25	5%CS+GM+FW+BC	8.22 ±1.64	4.21 ±0.08	4.16 ±0.15[a]	4.15 ±0.13[a]	50.61	98.57
EU 26	5%CS+GM+FW+CM	8.30 ±1.70	4.15 ±0.15	4.12 ±0.11[a]	4.13 ±0.11[a]	49.64	99.52

Key:US:uncontaminated soil,GM:goatmanure,FW:fishwastes,BC:*Bacillus amiloliquefaciens*,CM:*Comamonas testosteroni* Not significantly different (p>0.05)

Table 3: Mean and Percentage Microbial Counts (log_{10}cfu/g) of 10% Crude Oil Contaminated Soil during Bioremediation

Experimental units	Treatments	Total heterotrophic bacteria	Fungi	Hydrocarbon utilizing bacteria	Hydrocarbon utilizing fungi	Percentage (%) hydrocarbon utilizing bacteria	Percentage (%) hydrocarbon utilizing fungi
EU 1	US (control1)	7.39 ±0.71	4.17 ±0.07	3.68 ±0.15	3.57 ±0.15	49.79	85.61
EU 27	10%CS (control 3)	7.56 ±1.37	4.15 ±0.08	4.13 ±0.08	4.06 ±0.10	54.63	97.83
EU 28	10%CS+GM	7.85 ±1.61	4.22 ±0.13	4.17 ±0.09[a]	4.14 ±0.15[ab]	53.12	98.10
EU 29	10%CS+FW	8.03 ±1.76	4.13 ±0.16	4.15 ±0.10[a]	4.04 ±0.09[a]	51.68	97.82
EU 30	10%CS+BC	7.89 ±1.65	4.14±0.21[ab]	4.16 ±0.28[a]	3.92 ±0.11[a]	52.72	94.69
EU 31	10%CS+CM	7.76 ±1.54	4.11 ±0.14	4.06 ±0.07[a]	4.01 ±0.10[a]	52.32	97.57
EU 32	10%CS+GM+FW	8.25 ±1.95	4.14±0.11	4.17 ±0.10[a]	4.11 ±0.04[a]	50.54	99.28
EU 33	10%CS+GM+CM	8.11 ±1.83	4.16 ±0.16	4.17 ±0.10[a]	4.10 ±0.09[a]	51.42	98.56
EU 34	10%CS+GM+BC	8.22 ±1.92	4.17 ±0.14	4.11 ±0.04[a]	4.04 ±0.09[a]	50.00	96.88
EU 35	10%CS+FW+CM	8.27 ±1.96	4.18 ±0.11	4.17 ±0.09[a]	4.05 ±0.08[a]	50.42	96.89
EU 36	10%CS+FW+BC	7.86 ±1.62	4.11 ±0.27[ab]	4.10 ±0.04[a]	3.85 ±0.09[a]	52.16	93.67
EU 37	10%CS+CM+BC	8.25 ±1.94	4.18±0.16	4.15 ±0.08[a]	4.04 ±0.09[a]	50.30	96.65
EU 38	10%CS+GM+FW+BC	8.12 ±1.84	4.23 ±0.09	4.24 ±0.14[a]	4.08 ±0.12[ab]	52.22	96.45
EU 39	10%CS+GM+FW+CM	8.22 ±1.92	4.24 ±0.15	4.21 ±0.13[a]	4.09 ±0.12[ab]	51.22	96.46

Key: US-Uncontaminated soil;GM:goatmanure; FW:fishwastes,BC:*Bacillus amiloliquefaciens*,CM:*Comamonas testosteroni* Not significantly different (p>0.05)

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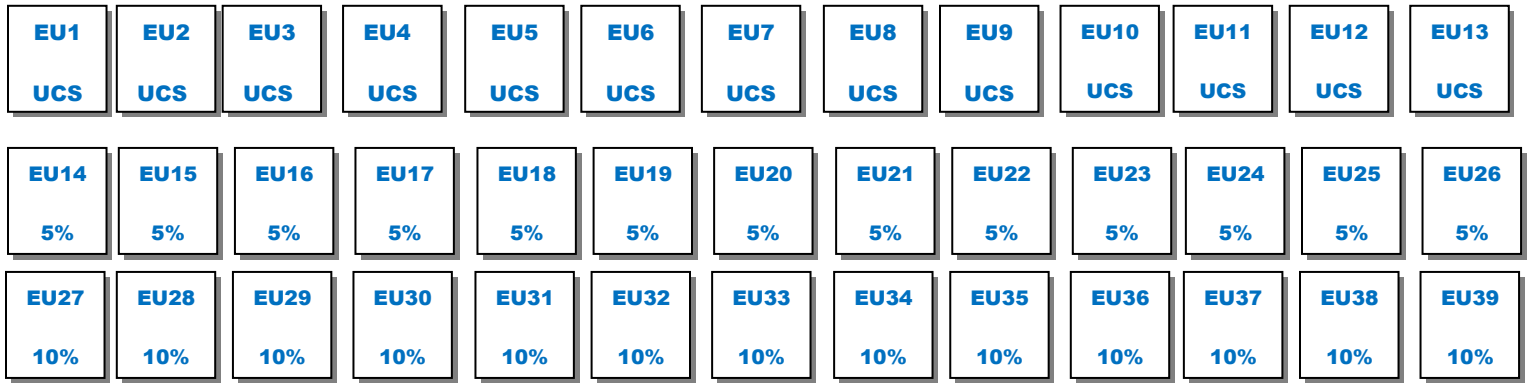

Fig. 2: Experimental units layout design

Key: EU-experimental unit, US-uncontaminated soil, CS- contaminated soil

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Results of total petroleum hydrocarbon (TPH) contents of the uncontaminated soil, 5% and 10% contaminated soil samples during bioremediation during bioremediation are as shown in Tables 4 to 6. The control sample of the uncontaminated soil, (ctrl I) recorded an initial TPH value of 88.15mg/kg while, the final TPH values of the various treatments ranged between 18.90mg/kg and 82.43mg/kg. The control sample of the 5% contaminated soil (ctrl II) recorded an initial TPH value of 6548.06mg/kg while, the final TPH values of the various treatments ranged between 1557.94mg/kg and 4707.48mg/kg. The control sample of the 10% contaminated soil (ctrl III) recorded an initial TPH value of 10328.03mg/kg while, the final TPH values of the various treatments ranged between 2713.28mg/kg and 8067.01mg/kg.

Table 4: TPH Values of Uncontaminated Soil (US) Samples

Setup	Treatment	Initial TPH Value (mg/kg)	Final TPH Value (mg/kg)	Amt (mg/kg) of TPH Remediated	Percentage Bioremediated (%BR)
EU1	US (CTRL 1)	88.15	82.43	5.66	6.43
EU2	US + GM	88.15	44.40	43.70	49.60
EU3	US + FW	88.15	34.23	53.86	61.14
EU4	US + BC	88.15	66.58	21.51	24.42
EU5	US + CM	88.15	62.43	25.66	29.13
EU6	US+GM+FW	88.15	35.12	52.98	60.14
EU7	US + GM + BC	88.15	35.12	52.98	60.14
EU8	US+GM+CM	88.15	25. 14	62.96	71.46
EU9	US + FW + BC	88.15	42.10	46.00	52.21
EU10	US+ FW + CM	88.15	23.75	64.35	73.04
EU11	US+ BC+ CM	88.15	46.52	41.58	47.20
EU12	US+ GM+ FW+ BC	88.15	28.27	59.82	67.90
EU13	US+GM+FW + CM	88.15	18.90	69.19	78.54

Key: CTRL: control, US: uncontaminated soil, GM: goat manure, FW: fish wastes, BC: *Bacillus amiloliquefaciens*, CM: *Comamonas testosteroni*

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Table 5: TPH Values of Five Percent Crude Oil Contaminated Soil (5%CS) Samples

Setup	Treatments	Initial TPH Value (mg/kg)	Final TPH Value (mg/kg)	Amt (mg/kg) of TPH Remediated	Percentage (%BR) Bioremediated
EU 1	US (CTRL 1)	88.15	82.43	5.66	6.43
EU14	5%CS (CTRL 2)	6548.06	5984.54	563.51	8.60
EU15	5%CS + GM	6548.06	2939.21	3608.84	55.11
EU16	5%CS + FW	6548.06	2391.56	4156.49	63.47
EU17	5%CS + BC	6548.06	4308.18	2239.87	34.20
EU18	5%CS + CM	6548.06	4707.48	1840.57	28.10
EU19	5%CS + GM + FW	6548.06	2197.36	4350.69	66.44
EU20	5%CS + GM + BC	6548.06	2685.31	3862.74	58.99
EU21	5%CS + GM+ CM	6548.06	1572.94	4975.11	75.97
EU22	5%CS + FW+ BC	6548.06	3096.42	3451.63	52.71
EU23	5%CS + FW + CM	6548.06	1557.94	4990.11	76.20
EU24	5%CS + BC+ CM	6548.06	3688.15	2859.90	43.67
EU25	5%CS + GM+FW +BC	6548.06	2190.94	4357.11	66.54
EU26	5%CS + GM+FW+CM	6548.06	1265.31	5282.74	80.67

Key: CTRL: control, US: uncontaminated soil, CS: crude oil contaminated soil, GM: goat manure, FW: fish wastes, BC: *Bacillus amiloliquefaciens*, CM: *Comamonas testosteroni*

Table 6: TPH Values of Ten Percent Crude Oil Contaminated Soil (10%CS) Samples

Setup	Treatments	Initial TPH Value (mg/kg)	Final TPH Value (mg/kg)	Amt(mg/kg) of TPH Remediated	Percentage (% BR) Bioremediated
EU 1	US (CTRL 1)	88.15	82.43	5.66	6.43
EU27	10%CS (CTRL 3)	10328.03	10202.32	125.71	1.21
EU28	10%CS + GM	10328.03	5905.28	4422.74	42.82
EU29	10%CS + FW	10328.03	4785.87	5542.15	53.66
EU30	10%CS + BC	10328.03	8472.28	1855.74	17.96
EU31	10%CS + CM	10328.03	8067.01	2261.01	21.89
EU32	10%CS + GM + FW	10328.03	4159.37	6168.65	59.72
EU33	10%CS + GM + BC	10328.03	5344.21	4983.81	48.25
EU34	10%CS + GM + CM	10328.03	3014.55	7313.47	70.81
EU35	10%CS + FW+ BC	10328.03	5985.21	4342.81	42.04
EU36	10%CS + FW+ CM	10328.03	2713.28	7614.74	73.72
EU37	10%CS + BC+ CM	10328.03	7006.79	3321.23	32.15
EU38	10%CS+ GM+FW+BC	10328.03	4099.38	6228.64	60.30
EU39	10%CS+GM+ FW+CM	10328.03	2227.43	8100.59	78.43

Key: CTRL: control, US: uncontaminated soil, CS: crude oil contaminated soil, GM: goat manure, FW: fish wastes, BC: *Bacillus amiloliquefaciens*, CM: *Comamonas testosterone*

The results of the physical growth parameters analyzed for bioassay for ecotoxicity test of remediated soil are presented in Figures 3 to 5 and 6 to 8 for maize and okra plants respectively where, the effects of normal soil, different concentrations of remediated soil and contaminated soil on plant performance characteristics were statistically compared.

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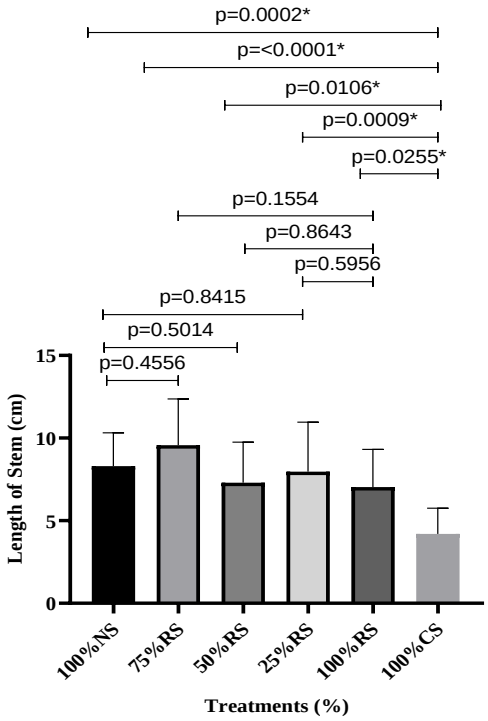

Fig. 3: Length of Stem of maize plant during Ecotoxicity Test
Key: NS- Normal Soil, RS- Remediated Soil, CS-Contaminated Soil

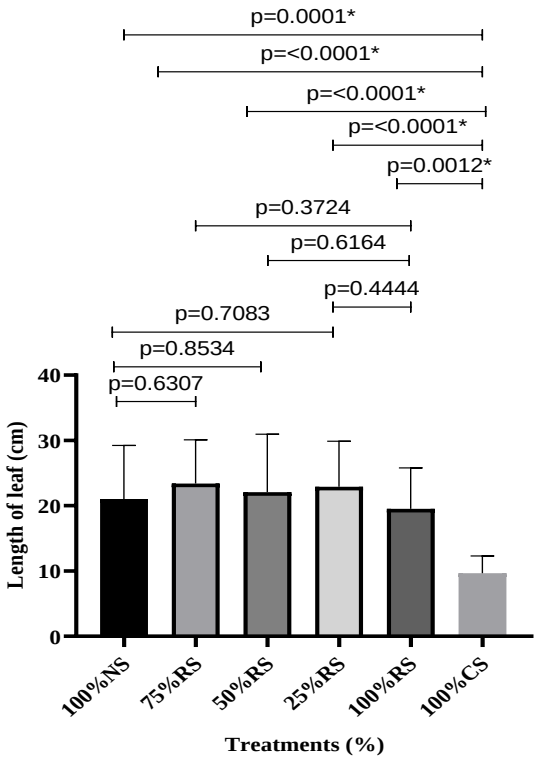

Fig. 4: Length of Leaf of maize plant during Ecotoxicity Test
Key: NS- Normal Soil, RS- Remediated Soil, CS-Contaminated Soil

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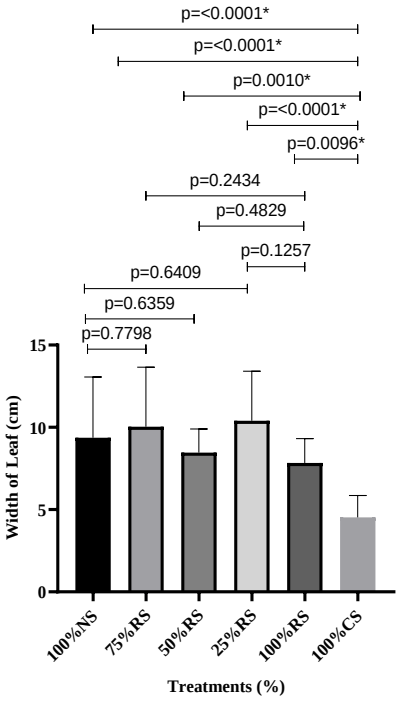

Fig. 5: Width of Leaf of maize plant during Ecotoxicity Test
Key: NS- Normal Soil, RS- Remediated Soil, CS-Contaminated Soil

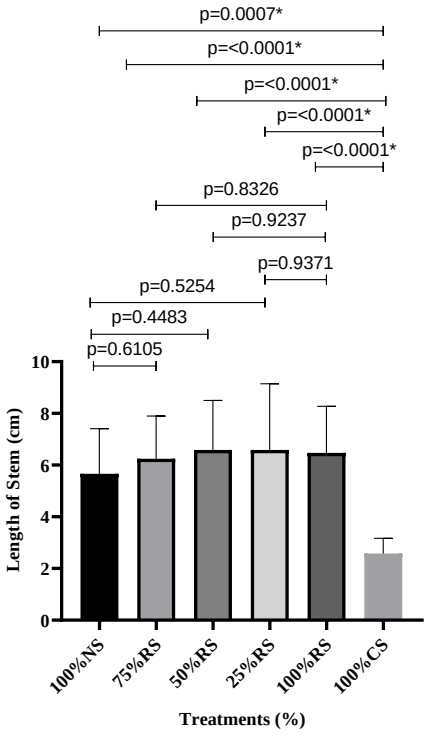

Fig. 6: Length of Stem of okra plant during Ecotoxicity Test
Key: NS- Normal Soil, RS- Remediated Soil, CS-Contaminated Soil

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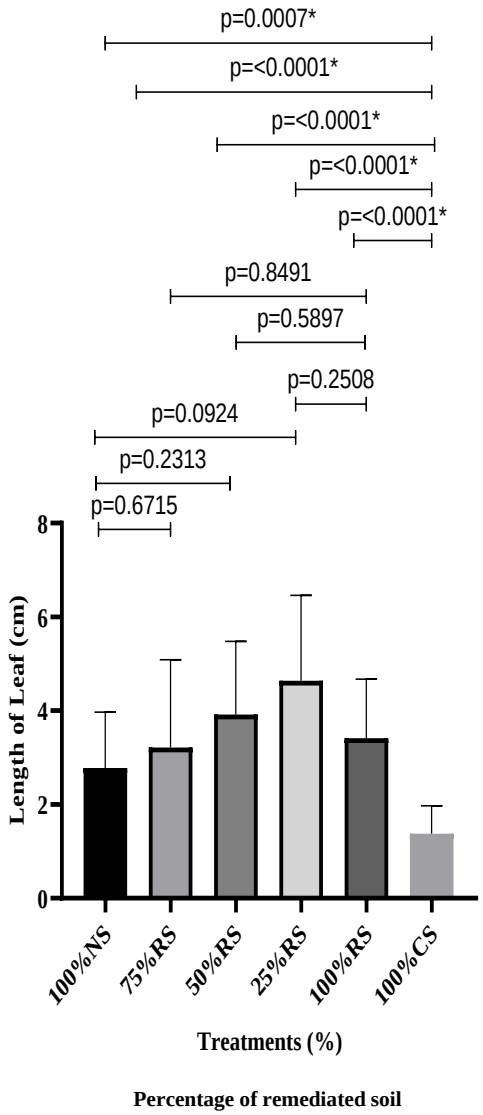

Fig. 7: Length of Leaf Characteristics of Okra Plant during Ecotoxicity Test
Key: NS- Normal Soil, RS- Remediated Soil, CS-Contaminated Soil

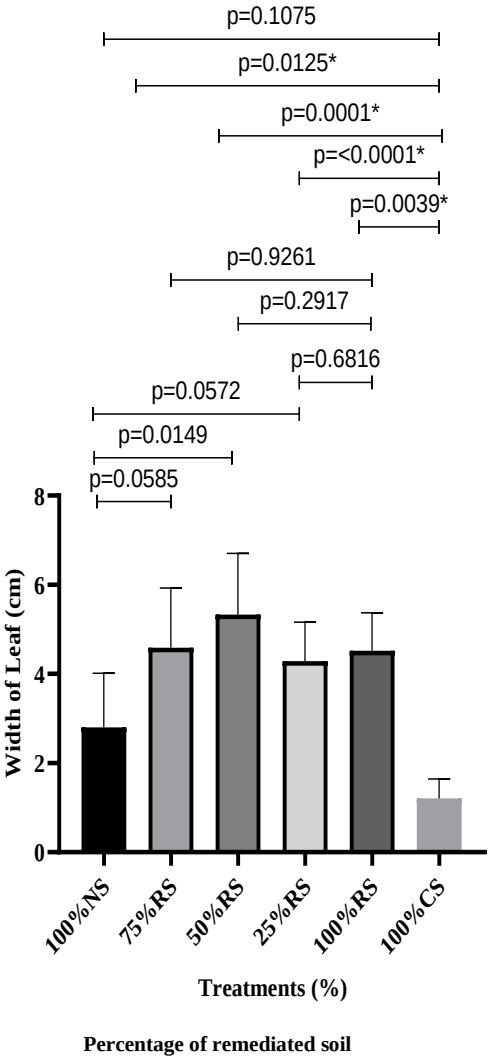

Fig. 8: Width of Leaf Characteristics of Okra Plant during Ecotoxicity Test
Key: NS- Normal Soil, RS- Remediated Soil, CS-Contaminated Soil

Discussion

Crude oil pollution has increased in the last few decades in the Niger Delta areas of Nigeria. This has caused massive degradative impact on the soil, thereby, making it difficult for agricultural usage (Ogbonna *et al.*, 2012; Awari *et al.*, 2020). However, there are challenges on the effective elimination and enrichment of the soil that has been polluted with hydrocarbon. This study exploits the use of some common wastes simply known as organic nutrients or stimulants obtained in the environment together with bacteria that have the ability to degrade hydrocarbon in order to remediate soil contaminated with crude oil. Thereafter, evaluate the ecotoxicity of the remediated soils using some common plants including maize (*Zea mays*) and okra (*Abelmoshus esculentus*) plants. Hence, restoring the initial status of the soil for agricultural and sustainability purposes (Menkit and Amechi, 2019). The mean microbial counts were used to compare the various treatment samples with the control samples in the different microbial parameters for uncontaminated soil, 5%CS and 10%CS samples, so as to ascertain for any significant difference in all the samples analyzed.

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There were no significant differences (P>0.05) in the uncontaminated soil samples for all the parameters analyzed for uncontaminated soil. Also, there were no significant differences (P>0.05) in all the treatments setup samples observed in both five and ten percent contaminated soil samples for the THBC and FC. However, there were significant differences (P<0.05) in the HUB and HUF counts for 5% and 10% contaminated experimental unit samples.

Results of the present study showed that lower microbial counts were observed in all the control samples than in the other treated contaminated soil samples for all the microbial parameters analyzed, except for the total fungal count which was slightly higher in the control sample than in few of the experimental unit samples. These results of the microbial analyses agrees with work done by Menkit and Amechi (2019) who reported lower heterotrophic microbial counts in the control samples than in the treated soil samples. The reason for the lower fungal count (FC) in some of the samples could be attributed to the fact that crude oil contaminated soil is often poor in organic nutrients and generally low in the microbial population, thus, may lack essential nutrients to support growth and multiplication of some microorganisms (Hamamura *et al.*, 2006). This result is also consistent with the reports of some other researches on bioremediation by Chikere *et al* (2009), Ogbonna *et al* (2012), and Nrior and Echezolom, (2016) who emphasized that, the significant differences observed in the counts of the hydrocarbon utilizing microbes in the samples could be due to the presence of crude oil in the contaminated soil. According to Albert and Tanee (2011), the presence of hydrocarbon in the crude oil contaminated soil depletes the nutrient level in soil. Also, environmental factors and or weather conditions could have contributed to the drastic increase in the microbial counts as there was heavy rainfall between the sampling days. These findings supported the reports of Ekpo and Ebeagwu (2009); Antai *et al.*, (2014) who, in their studies stated that, microbial population may show rapid increase due to presence of crude oil and the prevailing weather conditions. The results of the higher microbial counts in normal soil than in the crude oil contaminated soils showed that, bacteria and fungi were higher in normal soil than in the crude oil contaminated soil, thereby, implying that, these microorganisms grow better in nutrient sufficient conditions. Furthermore, this result is similar to the study performed by Ra and Zheng (2019) who recorded higher THBC and FC in the unpolluted soil than polluted soil and recorded higher counts of HUB and HUF in the soil collected from a polluted site than the unpolluted site. Their results indicated that, crude oil contamination shifts the dynamics of microbial population towards crude oil degrading microbes (Ikuesan *et al*., 2017). Our result concurs with research by Antai *et al.*, (2014) who reported significant differences between the heterotrophic microorganisms and oil-degrading microorganisms of crude oil polluted soil and pristine soil. Nonetheless, oil pollution of soil leads to its degradation which has been suggested by Albert and Tanee (2011) to cause decrease in agricultural productivity and alterations in the number and types of environmental microbes.

However, the increased counts of the hydrocarbon utilizers suggestively represents an immediate response to the added organic carbon present in the petroleum hydrocarbon soil which must have acted as additional carbon substrate for microbial growth, activity and multiplication.

Results of this study also revealed that the addition of the organic nutrients stimulated the activities of the indigenous microbial population and resulted in lesser amounts of TPH concentration. These results are in line with several other researchers like; Bento *et al* (2005), Ogbonna *et al* (2012), Awari *et al.*, (2020) who reported a reduction in TPH level of polluted soils and sites after the introduction of organic nutrient formulations. The results are also in line with works done by Antai *et al.*, (2014) who in their research, stated clearly, that the introduction of mixed bacterial isolates could have supported the degradation process and ensured faster and effective remedy for clean-up of crude oil contaminated soils or sites. Also, Wemedo *et al* (2018) reported an initial TPH value on the first day of a hydrocarbon contaminated soil as 74.81mg/kg and when soil was treated with mixed culture of bacteria and treated with only *Chryseobacterium* specie, the TPH value recorded 30.44mg/kg and 51.08mg/kg on the last day of the experiment, indicating that mixed bacteria produced more effective treatment with lesser TPH value as against the single bacteria. According to Wemedo *et al* (2018), the reason for the reduction in TPH level could be due to the lignolytic features of these hydrocarbon utilizing microorganisms to produce extracellular enzymes, that breakdown the pollutants and help in metabolism of the different compounds. Bioassays such as measurement of seed germination

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and early growth parameters have been used to monitor treatments effect and restoration of crude oil contaminated sites (Sverdrup *et al*., 2003). In this study, bioassay for ecotoxicity test of bioremediated soil was carried out in order to evaluate the potentials of treatments applied to agricultural purposes and to ascertain if the soil can encourage growth of some plants after bioremediation. This was conducted by planting maize and okra, differently, on the bioremediated soil, normal and crude oil contaminated soil. Thereafter, some vegetative growth parameters like; size of stem, length and width of the maize (Figures 3-5) and okra (Figures 6-8) plants were measured after 2 weeks of planting and at weekly interval in order to evaluate the effect of the remediated soil on the growth performance characteristics in the normal uncontaminated soil, different percentages of remediated soil and remediated soil and also, to statistically compare the plants for any significant differences in the different soil conditions. At the end of the bioassay study, it was observed that the mean values of stem length ranged between (6.23±0.25 cm - 12.83±0.29 cm), leaf length ranged between (13.67±1.15 cm - 32.17±1.04 cm) and leaf width ranged between (6.47±0.64 cm - 14±1.32 cm) in the different soil conditions, for maize plants. While, the mean values of stem length ranged between (3.2±0.2 cm - 8.83±0.91 cm), leaf length ranged between (2.07±0.31 cm - 7±1.32 cm) and leaf width ranged between (1.77±0.25 cm - 6.6±1.81 cm) in the different soil conditions for okra plants. The results showed that, the bioremediated soil supported plants growth. However, no significant differences ($P>0.05$) were observed between the plants grown on the normal uncontaminated soil, the remediated soil as well as on the other percentages of remediated soil used for the planting. Hence, the similarities in the plant performance characteristics indicated that most of the pollutants were degraded by the hydrocarbon degrading microbes (Baek *et al.*, 2004). The indifferences noted in the present study agrees with studies done by Ogbonna *et al* (2012) who reported indifferences in the remediated and fertile soil used in comparism. In the research by Baek *et al* (2004) on; effects of crude oil, oil components and bioremediation of plant growth; toxicity of crude oil was tested on two plants (red bean and corn) and results showed that the growth of both plants appeared normal in two different concentration of remediated crude oil hydrocarbons contaminated soils and uncontaminated soil, although corn growth was slightly reduced in soil planted on the higher concentration of the remediated soil.

Results of this present study are also consistent with previous reports on improved germination and plant growth parameters after bioremediation. Saterbak *et al* (2000) who carried out ecotoxicity test on remediated soil using lettuce and oat plants and observed improved root elongation of plants grown on soil after bioremediation exercise. Also, Salanitro and Dorn (2000) observed that seed germination and plant growth using corn, wheat and oats differed in normal soil and in soils with crude oil combinations before and after bioremediation as there were improvements in plants grown on soil after bioremediation.

Furthermore, Baek *et al* (2004) reported that *Acinetobacter* specie treated soil permitted better germination and growth of mung beans as evidenced by better plant length, leaf width and leaf chlorophyll content and stated clearly that, *Acinetobacter* specie applied to the crude oil contaminated soil was capable of reducing the crude oil content during the biodegradation experiment as observed from the result on the plant performance characteristics. The ecotoxicity evaluation of the remediated soil monitoring analyses revealed a continuous gradual increase in seeds planted as no notable difference was observed with time in seeds grown on normal and remediated soils and no significant differences in the vegetative growth parameters monitored in the normal uncontaminated soil and bioremediated soils.

In conclusion, the bacteria and the organic nutrient mixtures used for bioremediation in this study were efficient and confirmed to have a high hydrocarbon degrading capacity as it provided the soil with nutrients required for plant growth. The amendments detoxified the soil and improved the quality of the soil, since the remediated soils supported the growth of maize and okra plants. The study explored the ecologically friendly bacteria and nutrient formulations that significantly enhanced bioremediation of crude oil contaminated soil within a short period of time. It is therefore recommended that, soils and environments contaminated with crude oil hydrocarbons in the Niger Delta States in Nigeria, can still be treated and the natural quality of the soils be restored which can equally be used for agricultural purposes producing similar output to improve agricultural sustainability.

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