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RESEARCH ARTICLE

EFFECTS OF BIOREMEDIATION USING RABBIT DROPPINGS, FOWL DROPPINGS AND NPK FERTILIZER (20:10:10) ON SOME PHYSICOCHEMICAL PARAMETERS IN CHRONICALLY PETROLEUM HYDROCARBON-POLLUTED SOIL IN GIO/B-DERE, OGONILAND, RIVER STATE.

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Authors' contributions
This work was carried out in collaboration between authors. Authors read and approved the final manuscript.

ABSTRACT

Ogoniland, Nigeria, has experienced extensive petroleum hydrocarbon pollution, posing a significant environmental challenge. Bioremediation, the use of biological agents to detoxify contaminants, is a promising approach to restore polluted soil. This study aimed to investigate the effects of bioremediation using fowl droppings, rabbit droppings, and NPK 20:10:10 fertilizer on the physicochemical properties of petroleum hydrocarbon-contaminated soil in Ogoniland. The study was conducted within 28 days. The polluted soils weighing 50kg each were placed into different biocells, and treated with fowl droppings, rabbit droppings and NPK 20:10:10 fertilizer at dose rate of 5%w/w respectively. Unpolluted soil served as the negative control, and untreated polluted soil was the positive control. All treatments and controls were set up in triplicates. Soil samples were collected at day 0, 7, 14, 21, and 28 and analyzed for key physicochemical parameters, including pH, electrical conductivity, nitrate, and organic matter content. The electrical conductivity was significantly higher ($P<0.05$) in the soils treated with fowl droppings (31.20 ± 0.22 dS/m), rabbit droppings (31.20 ± 0.18 dS/m), and NPK 20:10:10 (52.00 ± 0.41 dS/m) compared to the polluted soil (14.00 ± 0.34 dS/m) at day 28. The soil nitrate levels were significantly higher ($p<0.05$) in fowl droppings (51.25 ± 0.35 mg/kg) and NPK 20:10:10 (72.60 ± 0.30 mg/kg) treatment compared to the polluted soil (31.05 ± 0.07 mg/kg) at day 28. The rabbit droppings treatment (27.74 ± 0.30 %) also resulted in significantly higher organic matter content compared to the polluted (19.57 ± 0.07 %) and unpolluted (19.58 ± 0.10 %) soils. This study demonstrates improved soil physicochemical properties, including optimum pH, increased electrical conductivity, nitrate levels, and organic matter content, indicating the potential for enhanced plant growth and agricultural productivity in the remediated soils, which may enhance soil fertility, food security and livelihood opportunities in Ogoniland.

Key Words*:* *Biocell, bioremediation, electrical conductivity, moisture, physiochemical parameters*.

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1.1. INTRODUCTION

Petroleum hydrocarbon pollution is a major environmental concern in Ogoniland and worldwide, leading to soil and groundwater contamination. The toxicity of petroleum hydrocarbon on the soil depends on the chemical and physical characteristics of the various hydrocarbon components, primarily affecting the soil properties through adsorption, biodegradation, and leaching (Clay, 2014; Logeshwaran, Megharaj, Chadalavda, Bowman, & Naidu, 2018). Agricultural soil plays a vital role in providing food for all and maintains balance in the ecosystem environment; however, contamination of soil adversely affects soil fertility, physical and chemical properties (Steliga & Kluk, 2020). The release of petroleum hydrocarbon into topsoil and subsoil has been reported to destroy the soil texture, structure, decreased pore spaces and saturates hydraulic conductivity (Hou et al., 2021; Steliga & Kluk., 2020). It can also affect the soil's biological properties, especially soil microbial population, and enzymatic activities, indirectly affecting plants' nutrient availability. Thus, once petroleum hydrocarbons are released into the environment, biological damage occurs by blocking supply of water, nutrients and light thereby affecting soil fertility, plant growth and germination (Onwurah et al., 2007; Fuentes, Mendez, Aguila & Seeger, 2014). Al-Joumaa (2009) conducted a study to evaluate the impact of petroleum hydrocarbon on soil physical and chemical properties. Results indicated that the polluted soil had relatively higher bulk density and absolute density than unpolluted soils. Percent clay fraction and calcium carbonate content was found low in the contaminated soil. Electrical conductivity (EC) values were higher in the polluted than unpolluted soil. Soluble Ca and SO_4- ions were dominant in unpolluted soil. However, the study of bioremediation mechanisms has the potential to contribute to the environmental rehabilitation of impacted ecosystem, improve the soil health and living conditions of the affected communities, and provide a blueprint for effective and efficient soil remediation strategies in the affected region (Niger Delta).

2.0. MATERIALS AND METHODS

2.1 *Description of Study Area*

The study area is Gio/B-Dere community, located within the Tai Local Government Area of Ogoniland, Rivers State, Nigeria. This area has been subjected to multiple crude oil spill incidents from pipelines and illegal crude oil refining activities over an extended period (Shekwolo, 2020). The contaminated area is situated adjacent to the 28" Nkpoku-Bomu Pipeline, which is part of the Shell Petroleum Development Company (SPDC) right-of-way. The adjoining third-party land is utilized for subsistence farming, with crops such as fluted pumpkin (a type of vegetable), cassava, and yam being cultivated. The impacted soil is situated at latitude 4.7005ºN and longitude 7.24424 ºE, within the Gio/B-Dere communities from which soil samples used in this study were obtained.

Geographically similar areas unaffected by crude oil pollution were chosen as negative control. Throughout the period that preceded sampling, there were no reported cases of oil pollution at the negative control site (Wegwu, Uwakwe & Anobi, 2010).

2.2 *Field Sampling and Source of Materials*

2.2.1. Soil samples collection

Soil samples were collected on November 12th, 2021, between 7am and 10 am to minimize the effect of environmental factors on the samples (Dunn et al., 2007). The epicenter of the chronically petroleum-polluted Gio/B-Dere site was excavated within the polluted depth range of 0.1m to 0.3m using pre-cleaned stainless-steel shovels. The shovels were cleaned with a non-ionic detergent and rinsed with de-ionized water prior to use. The excavated soils were placed into sterilized nylon sealable bags to maintain moisture.

The unpolluted topsoil zone (0.1 to 0.3m) located more than 1km away from the perimeter of the polluted soil area, were also excavated manually using deionized-cleaned stainless-steel shovels and placed in sterilized nylon sealable bags, to prevent leakage and entry of contaminants. The bags were marked with relevant information (FAO, 2020).

The polluted and unpolluted bulk soil samples were transferred to the project site area. The excavated polluted bulk soil and the unpolluted soil were thoroughly mixed to homogeneity respectively using pre-cleaned stainless-steel shovels and trowels (Wegwu et al., 2010). Before use in the treatment study, all soil samples were sieved using a 2mm mesh size to remove pebbles. The sampled soils were then placed in amber bottles and transported in an icebox to the laboratory where they were stored at 4ºC until analysis.

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2.2.2. Rabbit and Fowl Dropping Collection

The rabbit droppings were obtained from University of Port Harcourt Animal Farm in Choba Campus, University of Port Harcourt, Rivers, Nigeria whereas the fowl dung was collected from Local poultry Farm situated at Peter Odili Road, Port Harcourt City LGA, Rivers State. The fowl dung and rabbit droppings were air-dried after which they were ground to powder using electrical grinder and sieved through 2mm standard mesh separately. Representative samples were sent to the laboratory for physicochemical properties to ascertain the remediating properties of the animal derived organic substrates (manure) used

2.2.3. Inorganic Fertilizer (NPK; 20:10:10)

The NPK Fertilizer which composed of Nitrogen, Phosphorus and Potassium was purchased from Indorama Eleme Fertilizer and Chemicals Limited at Eleme, Rivers State, Nigeria.

2.3 *Biocell design and experimental sampling technique*

Biocells of dimension 0.8m (length) x 0.8m(Width) x 0.6m (height) were fabricated locally by the research team. The interior of fabricated biocells were lined with High Density Polyethene (HDPE) liner to prevent nutrients escape and cross-contamination (United Nation Environmental Programme [UNEP], 2011).

Petroleum Hydrocarbon polluted composited soil were divided, each weighing 50kg and placed in each of the biocell except for the negative control (U.S) which was obtained greater than (>) 1km away from the spill site whereas the untreated hydrocarbon polluted soil served as the positive control (P.S).

The Powdered animal derived organic substrates; Rabbit droppings (R.D), fowl dropping (F.D) and inorganic or synthetic Fertilizer (NPK; 20:10:10) were administered accordingly at a one dose rate of 5% w/w petroleum hydrocarbon impacted soil. All treatments and controls were set-up in triplicate.

Using manual means, each of the content in biocell set-up were homogenized and allow for incubation to stimulate microbial activities. Borehole water from Gio/B-Dere locality was analyzed and used for moistening to field capacity of 12 to 30% (US EPA, 2006) in such a manner to prevent leachates from being formed to hamper the process or cause discharge into the environment. Both treated samples and control networks were subjected to the same ambient environmental condition. The various biocells were tilled twice in a week using pre-cleaned trowel and watered to allow for optimum aeration and microbial activities (Ayotamuno, Kogbara & Hart, 2006).

To obtain the initial data, polluted soil samples and controls were taken from the biocells and analyzed for the soil physicochemical parameters at day 0 (the baseline study) before one dose treatment (bioremediation). The effects of animal derived organic substrates and synthetic fertilizer with respect to their controls were analyzed for, at every seven days interval on day 7, 14, 21, and 28.

2.4 *Laboratory Analysis*

2.4.1. Determination of Soil Physicochemical Parameters

The following key physicochemical parameters were determined as follows;

The pH was measured by potentiometer method as described by American Society of Testing and Materials [ASTM], (1995).

The Electrical conductivity was determined by electrometric method as described by Piper (1942).

Carbon/Total Organic Carbon (C/TOC) was determined by chromic acid oxidation method according to Walkey & Black (1934).

The Kjeldahl method (Anderson & Ingram 1996; Omotoso & Shittu, 2007) as described by AOAC (1990) was used for determination of Nitrogen/Total Nitrogen(N/TN).

The Total Organic Matter (TOM) content of each soil was determined by chromic acid oxidation method according to modified Walkey & Black (1934).

Moisture content was evaluated by gravimetric method as described by Black et al. (1965).

Available Phosphorus (P) in soil samples were extracted by the Brays method and determined colorimetrically (Bray & Kurtz, 1945).

Potassium(K) was determined by acid digestion method, as reported by Osam, Wegwu & Uwakwe (2008), and Ademiluyi & Omotoso (2008).

The method of Nicolsky, Romanovsky & Panteleev (2009) was used with slight modification for determination of soil Temperature. Soil Nitrate (NO_3^-) was determined by Brucine method (USEPA, 1971).

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Soil Nitrite was determined by Colometric method (USEPA, 1993).
Soil Phosphate was determined by Colometric method (Amponsah, Etsey & Nagai, 2014).
Soil Sulphate was determined by Nephelometric method (Xue, X & M. Li, 2013)
Calcium and Magnesium were done by mixed acid digestion as described by AOAC (1999).

2.5. *Statistical Analysis*

Data generated were analyzed using students t-test and one-way ANOVA with the aid of Graph pad prism version 5.0 and result were presented as mean +/- standard deviation. Values at P<0.05 with levels of significant difference.

3.0. RESULTS

3.1. *Baseline concentration of physicochemical parameters of polluted and unpolluted soil in Gio/B-Dere before bioremediation.*

The results of baseline physicochemical parameters of polluted soil and unpolluted soil samples from Gio/B-Dere in Ogoniland before bioremediation are presented in **Table 1**. From the results, pH, conductivity, total organic carbon, phosphorus, potassium, nitrate, nitrite, phosphate, sulphate, calcium, moisture content and ash content of polluted soil and unpolluted soil were significant (P<0.05) whereas total organic matter, total nitrogen, temperature, and magnesium of polluted soil and unpolluted soil were not-significant (P>0.05).

Table 1. Physicochemical baseline of soil samples at Day 0.

Parameters	FM Env. STD 1999	Polluted Soil	Unpolluted Soil	T-value	P-value	Comment
pH	6.50	7.60 ± 0.09	7.25 ± 0.07	5.317	0.0060	Significant
Conductivity (µS/cm)	100.00	9.00 ± 0.08	86.00 ± 0.23	547.70	<0.0001	Significant
Total organic carbon (%)	<10.00	6.80 ± 0.05	9.04 ± 0.10	34.70	<0.0001	Significant
Total Organic Matter (%)	NS	99.70 ± 0.20	99.38 ± 0.34	1.405	0.2327	Not significant
Total Nitrogen (%)	NS	0.14 ± 0.01	0.09 ± 0.03	2.739	0.0520	Not significant
Phosphorus (mg/kg)	NS	3.50 ± 0.04	10.00 ± 0.11	96.19	<0.0001	Significant
Potassium, (mg/kg)	>100	5.00 ± 0.09	3.50 ± 0.09	20.41	<0.0001	Significant
Temperature (ºC)	30.00	27.60±0.15	27.60±0.13	0.00	1.000	Not significant
Nitrate (mg/l)	50.00	3.00 ± 0.02	6.80 ± 0.98	6.715	0.0026	Significant
Nitrite (mg/l)	0.30	0.04 ± 0.00	0.08 ± 0.02	3.098	0.0363	Significant
Phosphate (mg/kg)	>100	3.93 ± 0.04	0.63 ± 0.01	138.60	<0.0001	Significant
Sulphate (mg/kg)	100	10.00 ± 0.10	5.00 ± 0.08	67.63	<0.0001	Significant
Calcium (mg/kg)	250.00	9.67 ± 0.59	12.20 ± 0.60	5.208	0.0065	Significant
Magnesium (mg/kg)	100.00	3.78 ± 0.02	3.78 ± 0.09	0.00	1.000	Not significant
Moisture Content (%)	NS	0.87 ± 0.04	1.34 ± 0.02	18.20	<0.0001	Significant
Ash Content (%)	NS	0.29 ± 0.00	0.62 ± 0.04	13.86	0.0002	Significant

Values are mean ± standard deviation of triplicate determinations.
Key: FM.Env. STD-Federal Ministry of Environment standard, NS-Not stated.

3.2. *Post remediation data for physicochemical parameters of Gio/B-Dere polluted soil, unpolluted, NPK treated soil (inorganic fertilizer), fowl and rabbit droppings (organic manure) treated soil.*

The results of day 7 analyses of physicochemical parameters during the bioremediation process are presented in **Table 2**. The results indicated that inorganic fertilizer treated soil (NPK 20-10-10) and organic treated soil (fowl droppings and rabbit droppings, respectively) influenced some of the physicochemical parameters of the chronic petroleum hydrocarbon polluted soils after 7 days

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Table 2. Physicochemical analysis of soil samples at Day 7

Parameters	FM.Env STD 1999	Polluted Soil	Unpolluted Soil	NPK	Poultry dropping	Rabbit dropping
pH	6.50	2.39 ± 0.03 [a]	2.40 ± 0.01 [a]	4.02 ± 0.06 [b]	5.56 ± 0.09[b]	4.31 ± 0.07[b]
Conductivity (µS/cm)	100.00	311.00 ± 0.27[a]	144.00 ± 0.34[b]	167.00 ± 90.89[b]	434.00 ± 8.00[c]	194 ± 2.01 [b]
Total organic carbon (%)	<10.00	11.59 ± 0.11[a]	6.04 ± 0.05[b]	6.94 ± 0.15[b]	10.67 ± 0.25 [a]	10.50 ± 0.40[a]
Total Organic Matter (%)	NS	20.20 ± 0.61[a]	21.48 ± 0.34[a]	18.65 ± 0.40[a]	18.95 ± 0.42[a]	19.80 ± 0.78[a]
Total Nitrogen (%)	NS	0.14 ± 0.00[a]	0.13 ± 0.02[a]	0.65 ± 0.03[b]	0.28 ± 0.01[c]	0.27 ± 0.03[c]
Phosphorus (mg/kg)	NS	6.25 ± 0.07[a]	6.10 ± 0.14[a]	6.10 ± 0.28[a]	7.35 ± 0.78[a]	6.35 ± 0.21[a]
Potassium, (mg/kg)	>100	17.50 ± 3.54[a]	17.50 ± 3.54[a]	12.50 ± 1.54[b]	10.00 ± 0.45[b]	22.56 ± 3.54[a]
Temperature (°C)	30.00	28.60 ± 0.11[a]	27.50 ± 0.08[a]	28.80 ± 0.20[a]	28.80 ± 0.16[a]	26.60 ± 2.12[a]
Nitrate (mg/l)	50.00	1.70 ± 0.06[a]	7.50 ± 0.70[b]	20.30 ± 0.90[c]	19.50 ± 1.30[c]	11.40 ± 1.04[d]
Nitrite (mg/l)	0.30	0.03 ± 0.00[a]	0.04 ± 0.01[a]	0.06 ± 0.01[a]	0.07 ± 0.02[a]	0.07 ± 0.02[a]
Phosphate (mg/kg)	>100	19.20 ± 0.14[a]	18.75 ± 0.35[a]	18.80 ± 0.85[a]	22.60 ± 2.12[a]	19.50 ± 0.85[a]
Sulphate (mg/kg)	100	10.00 ± 0.09[a]	40.00 ± 0.10[b]	10.00 ± 0.08[a]	25.00 ± 0.14 [c]	20.00 ± 0.28 [c]
Calcium (mg/kg)	250.00	5.47 ± 0.59 [a]	3.79 ± 0.60 [b]	8.83 ± 0.59 [c]	7.57 ± 0.09[c]	7.57 ± 0.12[c]
Magnesium (mg/kg)	100.00	9.03 ± 0.51 [a]	9.39 ± 0.70 [a]	18.43 ± 0.51[b]	12.65 ± 0.52[a]	12.28 ± 0.91 [a]
Moisture Content (%)	NS	15.54 ± 0.40[a]	18.75 ± 0.14[a]	14.46 ± 0.11[a]	12.56 ± 0.45[a]	12.01 ± 1.03[a]
Ash Content (%)	NS	79.80 ± 0.61[a]	78.52 ± 0.34[a]	80.86 ± 0.30[a]	80.95 ± 0.28[a]	80.20 ± 0.78[a]

Values are mean ± standard deviation of triplicate determinations. Values with different superscript letters per row are statistically significant ($p<0.05$).
Keys: FMEnv. STD-Federal Ministry of Environment standard, NPK-Nitrogen phosphorus and nitrogen, NS-Not stated.

The results of physicochemical parameters of the polluted soil, unpolluted soil, nitrogen phosphorus and potassium 20-10-10 (inorganic fertilizer) treated soil, poultry dropping (organic nutrient) treated soil and rabbit dropping (organic nutrient) treated soil after 14 days bioremediation are presented in **Table 3.**

Table 3. Physicochemical analysis of soil samples at Day 14

Parameters	FMEnv STD 1999	Polluted Soil	Unpolluted Soil	NPK	Poultry dropping	Rabbit dropping
pH	6.50	3.50 ± 0.04[a]	3.70 ± 0.05 [a]	3.90 ± 0.03[a]	5.60 ± 0.10 [b]	5.00 ± 0.07[b]
Conductivity (µS/cm)	100.00	273.00 ± 3.02 [a]	158.00 ± 4.11 [b]	175.00 ± 3.10 [b]	488.00 ± 4.50 [c]	177.00 ± 3.31 [b]
Total organic carbon (%)	<10.00	10.39 ± 0.05[a]	5.35 ± 0.05[b]	6.39 ± 0.05[b]	9.49 ± 0.05[a]	9.42 ± 0.05[a]
Total Organic Matter (%)	NS	21.36 ± 0.71[a]	30.33 ± 1.62[b]	18.37 ± 0.40[a]	23.43 ± 1.80[a]	24.90 ± 1.21[a]
Total Nitrogen (%)	NS	0.23 ± 0.03[a]	0.14 ± 0.01[b]	0.20 ± 0.04[a]	0.34 ± 0.06[a]	0.20 ± 0.02[a]
Phosphorus (mg/kg)	NS	10.84 ± 1.02[a]	9.40 ± 1.03[a]	7.95 ± 1.02[a]	10.84 ± 1.02[a]	9.76 ± 0.52[a]
Potassium, (mg/kg)	>100	20.00 ± 7.07[a]	12.50 ± 1.54[b]	15.00 ± 7.07[a]	7.50 ± 1.50[c]	22.50 ± 3.54[a]
Temperature (°C)	30.00	29.80 ± 0.09 [a]	29.80 ± 0.10 [a]	29.70 ± 0.07 [a]	28.60 ± 0.11 [a]	29.70 ± 0.13 [a]
Nitrate (mg/l)	50.00	13.60 ± 0.10[a]	ND	37.60 ± 0.13[b]	19.20 ± 0.23[a]	33.20 ± 0.64[b]
Nitrite (mg/l)	0.30	0.01 ± 0.00[a]	ND	0.04 ± 0.01[a]	0.02 ± 0.00[a]	0.05 ± 0.02[a]
Phosphate (mg/kg)	>100	18.45 ± 0.07[a]	18.45 ± 0.07[a]	18.50 ± 0.57[a]	37.70 ±11.46[b]	20.95 ± 2.47[c]
Sulphate (mg/kg)	100	20.00 ± 0.10 [a]	10.00 ± 0.07 [b]	ND	40.00 ± 0.25 [c]	15.00 ± 0.11 [d]
Calcium (mg/kg)	250.00	5.47 ± 0.59[a]	3.79 ± 0.60[a]	8.83 ± 0.59 [b]	7.99 ± 0.59 [b]	7.57 ± 0.61[b]
Magnesium (mg/kg)	100.00	10.84 ± 1.02 [a]	9.40 ± 1.03 [a]	7.95 ± 1.02 [a]	10.84 ± 1.02 [a]	9.76 ± 0.52[a]
Moisture Content (%)	NS	6.00 ± 0.10[a]	6.00 ± 0.09 [a]	6.05 ± 0.21 [a]	12.30 ± 3.82 [b]	6.85 ± 0.78[a]
Ash Content (%)	NS	78.64 ± 0.71 [a]	69.67 ± 1.62[b]	81.64 ± 0.40 [a]	76.58 ± 1.80 [a]	75.10 ± 0.21 [a]

Values are mean ± standard deviation of triplicate determinations. Values with different superscript letters per row are statistically significant ($p<0.05$).
Key: FMEnv. STD-Federal Ministry of Environment standard, ND-Not detected, NPK-Nitrogen phosphorus and potassium, NS-Not stated.

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The results of physicochemical parameters of petroleum hydrocarbon polluted soil, unpolluted soil, nitrogen phosphorus and potassium 20-10-10 (inorganic fertilizer), poultry dropping (organic nutrient) treated soil and rabbit dropping (organic nutrient) treated soil after 21 days bioremediation are presented in **Table 4**.

Table 4. Physicochemical analysis of soil samples at Day 21.

Parameters	FMEnv STD 1999	Polluted Soil	Unpolluted Soil	NPK	Poultry dropping	Rabbit dropping
pH	6.50	3.30 ± 0.02 ^{a}	3.60 ± 0.08 ^{a}	3.90 ± 0.10 ^{a}	5.40 ± 0.12^{b}	4.40 ± 0.09^{b}
Conductivity (µS/cm)	100.00	295.00 ± 5.01^{a}	193.00 ± 3.21^{b}	196.00 ± 2.98 ^{b}	484.00 ± 3.34 ^{c}	158.00 ± 1.96 ^{d}
Total organic carbon (%)	<10.00	9.29 ± 0.05^{a}	4.62 ± 0.08^{b}	6.90 ± 0.10^{c}	10.25 ± 0.05^{a}	9.70 ± 0.05^{a}
Total Organic Matter (%)	NS	25.56 ± 0.36^{a}	32.40 ± 0.48^{b}	18.28 ± 0.23^{a}	21.73 ± 0.78^{a}	28.89 ± 0.49^{b}
Total Nitrogen (%)	NS	0.14 ± 0.01^{a}	0.34 ± 0.02^{b}	0.30 ± 0.03^{b}	0.23 ± 0.03^{b}	0.11 ± 0.05^{a}
Phosphorus (mg/kg)	NS	4.30 ± 0.10^{a}	4.70 ± 0.12^{a}	7.80 ± 0.43^{b}	7.20 ± 0.23^{b}	11.20 ± 0.09^{c}
Potassium, (mg/kg)	>100	10.00 ± 0.20^{a}	10.00 ± 0.13^{a}	ND	15.00 ± 0.45^{b}	5.00 ± 0.07^{c}
Temperature (ºC)	30.00	29.80 ± 0.10^{a}	29.80 ± 0.23^{a}	29.10 ± 0.32^{a}	29.00 ± 0.43^{a}	29.80 ± 0.25^{a}
Nitrate (mg/l)	50.00	25.30 ± 0.12^{a}	73.10 ± 0.32^{a}	25.60 ± 0.23^{a}	46.40 ± 0.54^{c}	20.50 ± 0.21^{a}
Nitrite (mg/l)	0.30	0.05 ± 0.01^{a}	0.10 ± 0.02^{a}	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.04 ± 0.01^{a}
Phosphate (mg/kg)	>100	13.30 ± 0.11^{a}	14.40 ± 0.13^{a}	23.80 ± 0.18^{b}	22.00 ± 0.20^{b}	34.60 ± 0.31^{c}
Sulphate (mg/kg)	100	15.00 ± 0.09^{a}	5.00 ± 0.08 ^{b}	10.00 ± 0.11^{c}	30.00 ± 1.78 ^{d}	5.00 ± 0.07^{b}
Calcium (mg/kg)	250.00	8.41 ± 0.09 ^{a}	7.57 ± 0.07 ^{a}	4.21 ± 0.04 ^{b}	5.89 ± 0.08^{b}	5.05 ± 0.09^{b}
Magnesium (mg/kg)	100.00	18.79 ± 2.00^{a}	16.62 ± 1.20^{a}	20.96 ± 1.98^{a}	23.85 ± 2.01^{a}	27.46 ± 2.96 ^{b}
Moisture Content (%)	NS	20.77 ± 0.14^{a}	26.00 ± 0.21^{b}	14.10 ± 0.08^{c}	15.16 ± 0.57 ^{c}	22.46 ± 0.75 ^{a}
Ash Content (%)	NS	74.45 ± 0.36^{a}	67.60 ± 0.48^{b}	81.72 ± 0.23^{a}	78.28 ± 0.78 ^{a}	71.12 ± 0.49 ^{ab}

Values are mean ± standard deviation of triplicate determinations. Values with different superscript letters per row are statistically significant ($p<0.05$).
Keys: FMEnv. STD-Federal Ministry of Environment standard, ND-Not detected, NPK-Nitrogen phosphorus and potassium, NS-Not stated.

The results of physicochemical parameters of petroleum hydrocarbon polluted soil, unpolluted soil, nitrogen phosphorus and potassium 20-10-10 (inorganic fertilizer), poultry dropping (organic nutrient) treated soil and rabbit dropping (organic nutrient) treated soil after 28 days bioremediation study are presented in **Table 5.**

Table 5. Physicochemical analysis of soil samples at Day 28.

Parameters	FMEnv STD 1999	Polluted Soil	Unpolluted Soil	NPK	Poultry dropping	Rabbit dropping
pH	6.50	6.80 ± 0.13 ^{a}	5.80 ± 0.08 ^{a}	7.00 ± 0.10 ^{a}	6.80 ± 0.08^{a}	6.90 ± 0.11^{a}
Conductivity (µS/cm)	100.00	14.00 ± 0.34^{a}	21.00 ± 0.12^{b}	52.00 ± 0.41^{c}	31.20 ± 0.22 ^{d}	31.20 ± 0.18 ^{d}
Total organic carbon (%)	<10.00	14.49 ± 0.16^{a}	1.93 ± 0.06^{b}	6.90 ± 0.10^{c}	14.63 ± 0.21^{a}	16.28 ± 0.32^{a}
Total Organic Matter (%)	NS	19.57 ± 0.07^{a}	19.58 ± 0.10^{a}	18.41 ± 0.82^{a}	18.89 ± 0.09^{a}	27.74 ± 0.03^{b}
Total Nitrogen (%)	NS	0.21 ± 0.06^{a}	0.16 ± 0.04^{b}	0.09 ± 0.03^{c}	0.24 ± 0.06^{a}	0.25 ± 0.09^{a}
Phosphorus (mg/kg)	NS	3.25 ± 0.21^{a}	3.55 ± 0.49^{a}	3.35 ± 0.35^{a}	4.30 ± 0.57^{a}	3.95 ± 0.17^{a}
Potassium, (mg/kg)	>100	20.00 ± 0.11^{a}	12.50 ± 1.54^{b}	15.00 ± 0.50^{c}	12.50 ± 1.54^{b}	15.00 ± 0.41^{c}
Temperature (ºC)	30.00	31.00 ± 0.10^{a}	31.00 ± 0.12^{a}	31.00 ± 0.09^{a}	31.20 ± 0.30^{a}	31.20 ± 0.17 ^{a}
Nitrate (mg/l)	50.00	31.05 ± 0.07^{a}	70.15 ± 0.27^{b}	72.60 ± 0.30^{b}	51.25 ± 0.35^{c}	22.30 ± 0.14^{a}
Nitrite (mg/l)	0.30	0.04 ± 0.01^{a}	0.05 ± 0.01^{a}	0.06 ± 0.02^{a}	0.05 ± 0.01^{a}	0.04 ± 0.00^{a}
Phosphate (mg/kg)	>100	9.80 ± 0.71^{a}	11.00 ± 1.56^{a}	10.20 ± 1.13^{a}	13.25 ± 1.77^{a}	12.05 ± 0.35^{a}
Sulphate (mg/kg)	100	0.00 ± 0.00 ^{a}	25.00 ± 0.15^{b}	5.00 ± 0.04 ^{c}	70.00 ± 1.65 ^{d}	20.00 ± 0.25 ^{e}
Calcium (mg/kg)	250.00	0.84 ± 0.07 ^{a}	2.52 ± 0.08 ^{b}	1.68 ± 0.05 ^{ab}	6.31 ± 0.60 ^{c}	4.63 ± 0.59 ^{d}
Magnesium (mg/kg)	100.00	27.83 ± 1.52^{a}	24.20 ± 1.54^{a}	25.66 ± 1.50^{a}	23.85 ± 1.63^{a}	27.10 ± 0.51^{a}
Moisture Content (%)	NS	14.23 ± 0.12^{ab}	16.93 ± 0.14^{b}	13.34 ± 0.12^{a}	12.40 ± 0.11^{a}	18.53 ± 0.11 ^{b}
Ash Content (%)	NS	80.43 ± 0.07^{a}	80.42 ± 0.10^{a}	81.59 ± 0.82^{a}	81.12 ± 0.09^{a}	72.26 ± 0.03 ^{b}

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Values are mean ± standard deviation of triplicate determinations. Values with different superscript letters per row are statistically significant ($p<0.05$).
Key: FMEnv. STD-Federal Ministry of Environment standard, NPK-Nitrogen phosphorus and potassium, NS-Not stated.

4. DISCUSSION

The polluted and unpolluted soil pH values indicated that the baseline samples were slightly alkaline (Table 1). From this study, the pH of the treated soils and their controls fluctuated as the days of the treatment progressed with pH of day 28 being slightly decreased than the baseline result (day 0). The pH values of different treatments relative to the controls did not change. Also, the pH values for inorganic treated, organic treated and untreated polluted soils (Positive control) were within the ideal pH range (6.5-8.0) considered to be optimal for bioremediation except for negative control (unpolluted soil) (Vidali, 2001). From this study, the fluctuation in pH may be due to the accumulation of organic acids (metabolites) formed during the degradation of the organic pollutant (petroleum hydrocarbon) in soil under the action of microbial enzymatic activities during the 28 days remediation period. This finding agrees with the reports of (Osuji & Nwoye, 2007; Barua et al., 2011) who attributed the progressive decrease in pH of crude oil polluted soil undergoing bioremediation with time to the accumulation of acidic metabolites resulting from microbial degradation or metabolism of the spilled hydrocarbon in the soils.

Electrical conductivity is a measure of ionic concentration in the soil and is therefore related to dissolve solutes. From this study (Table 1-5), electrical conductivity was found to fluctuate as the days of the treatment progressed with the conductivity of day 28 observed to be lower than those of the other days except for the polluted soil (positive control). The effects of inorganic (NPK) and organic treatment (fowl dropping and rabbit droppings respectively) was noticed, as well as for all the five conditions. However, the values were below the local regulatory (Federal Ministry of Environment, 1999) maximum permissible limit for soil conductivity. The significant fluctuation and decrease can probably be because of exchangeable cations. This observation was in line with the findings of Smith *et al* (2015) in a related study.

The baseline (Day 0) of total organic carbon was represented in Table 1. There was significant increase in the concentration of total organic carbon (TOC) after inorganic and organic treatments as the remediation process advanced. At the end of 28 days there was no change in TOC of organically treated soil compared to the positive control (polluted soil). However, there was a change in TOC of organic treated soil compared to the inorganic (NPK) fertilizer treated soil and the negative control (unpolluted soil). The increase in TOC recorded in the fowl droppings, rabbit droppings, NPK and positive control compared to the day 0 polluted soil may be attributed to the microbial mineralization of hydrocarbons by hydrocarbon-utilizing bacteria which may have degraded the hydrocarbons as a food and metabolized them. As a result, CO_2 and other organic by-products are released during the degradation process, leading to increase in the TOC content of the soils. This affirms the study reported by other researchers (Amadi *et al.*, 2005; Ogboghodo *et al.*, 2005; Onuh, *et al.* 2008a; Mbah *et al*., 2006; 2009). In this study, increase was highest in rabbit droppings followed by poultry droppings and least in NPK treated soil among the treated samples. This may be an indication that the rabbit droppings were rich source of carbon (metabolic feedstock) for soil microbes and an effective agent for remediation.

Organic matter in soil is derived from residual plant and animal materials decomposed by micro-organisms under the influence of temperature, moisture and optimal soil conditions (Amadi *et al*., 1993). The changes in total organic matter (TOM) in the soil samples are represented in Table 1-5. Percentage total organic matter did not change among the treatment options, except for rabbit droppings which increased compared to their controls. But there was a difference between baseline data and the time intervals of 28 days of remediation period. The mean Percentage Total Organic matter values decreased as the remediation progressed in all the five conditions. Mean percentage total organic matter (TOM) values were lower in day 28 soils than the baseline (day 0) soil values. This may be due to various factors that might include the environmental conditions of weathering and climatic predisposition as well as the physicochemical properties like volatilization and chemical oxidation as ascribed by Osuji *et al*., (2006a).

Nitrogen is an essential macronutrient needed for the formation of proteins, nucleic acids and few other cell components attributed in soil chemistry that contribute to soil fertility (Vlassak, 1970). In this present study, the nitrogen content in the contaminated soil was found to be slightly higher than in control. The changes in nitrogen were represented in Table 1-5. The effects of organic treatments (fowl droppings and rabbit droppings) on nitrogen level did not change relative to the polluted soil (positive control). More so, nitrogen value for the organic treated soils increased compared to the unpolluted soil (negative control). The nitrogen of inorganic (NPK) treated soil decreased relative to the positive and negative controls. The slight increase in the nitrogen may have been because of

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anthropogenic inputs of nutrients from the organic manures because organic manures have been reported as being capable of increasing soil nutrients by supplementing the limiting nutrients (Mbah *et al*., 2006; 2009; Tanee & Kinako, 2008).

Phosphorus is needed for crucial component of nucleic acids (sugar-phosphate backbone), phospholipids (for cell membrane), and adenosine triphosphate (ATP) formation (Swindell, Aelion & Pfaender, 1988). Hence, the bioremediation of hydrocarbon impacted soil requires an adequate supply of essential elements, which in turn are harnessed by hydrocarbon degrading microorganisms for active growth and metabolic activities (Van Hamme, Singh & Ward, 2003). The changes in phosphorus in the Gio/B-Dere soil samples are represented in Table 1-5. There was no change across all the five conditions at the end of the 28 days study. Although, there was a slight decrease in phosphorus content for the polluted soil and inorganic treater fertilizer (NPK) compared to their baseline (Day 0) content (Control). Also, there was a decrease in unpolluted soil at Day 28 compared to its baseline (Day 0) content. This decrease in phosphorus concentration over time may be attributed to microbial metabolism and immobilization in biomass (Margesin & Schinner, 2001). However, the increase in phosphorus concentration obtained in organic-treated soils at Day 28; fowl droppings and rabbit droppings, respectively, compared to their baseline (Day 0) concentration may corroborate anthropogenic inputs of this nutrient from the organic manures (fowl and rabbit droppings respectively) which have been reported as being capable of increasing soil nutrients by supplementing the limiting nutrients (Mbah *et al*. 2006; Tanee & Kinako, 2008). This further buttressed that the animal derived organic remediation techniques used was effective.

The dynamics of the potassium is shown in Table 1-5. There were increases in the levels of potassium after the inorganic treatment and organic treatment of polluted soil relative the control soils as the remediation advanced. At the 28 days of the remediation, data evaluated showed that there were changes across the different treatment conditions relative to their controls. This may be attributed to the use of this exchangeable potassium by the microbes present in the Gio/B-Dere soil samples. This microorganism possesses the ability to metabolize hydrocarbons and enhance the mineralization of organic matter. This microbial activity may have resulted in the release of exchangeable potassium from organic matter in the soil, thereby increasing its concentration. This is in tandem with the findings of Mbah *et al*. (2006; 2009).

In all treatments, temperature increased with time. However, the temperature at day 28 of the remediation study did not change. The increase with time effect may be due to the metabolic activity of the biodegrading microbes present in the samples undergoing bioremediation. Energy is released in form of heat during microbial activities. Macromolecules are fragmented with energy liberation which are captured and used for anabolism (Prescott, Harley & Klein, 2004).

Nitrates are essential for normal plant growth. Crops grown in soils with low nitrogen content can exhibit necrosis, wilting and chlorosis (Nwaogu *et al.*, 2012). The trend in nitrate concentration were depicted in Table 1-5. The effects of treatments caused variation during the remediation process for 28 days. There was an increase in the nitrate level in all treatment options and control, but the inorganic treated (NPK) soil option showed the highest concentration due to the presence of inorganic nitrogen composition compared to the individual organic treated soils (fowl droppings and rabbit droppings) and controls (polluted soil and unpolluted soil). This is an indication that the nitrate was produced by the action of nitrifying bacteria present during the bioremediation process. This agrees with the work of Atlas (1994).

The nitrite concentration did not decrease after application of inorganic and organic treatments relative to their controls as experimental period advanced. More so, there was no significant difference for all the five conditions at day 28 of the study. This study showed that nitrite concentration in Gio/B-Dere soil samples were generally infinitesimal and transient. This is in line with the work of Hachiya *et al*. (2016).

Phosphates are required by living organisms for their normal metabolic and physiological processes (Nwaogu *et al*., 2012). The changes in phosphate were depicted in Table 1-5. There was no difference in the phosphate concentration of organic and inorganic treated soil relative to their controls. Although, there was a significant increase in phosphate compared to the baseline (day 0) values in all the treatment options and controls at the end of 28 days study. This finding confirms the unpredictability of the release of phosphate from the inorganic and organic manure.

The effects of organic and inorganic treatment were noticed as the experiment progressed for 28 days period. There was an increase of soil sulphate in organic treated soil over the inorganic treated soil. More so, there was a difference for the three treatment groups compared to their controls. The increase in organic (fowl droppings and rabbit droppings, respectively) treated soil sulphate at day 28 may be due to digestion and metabolism of sulphur-containing components from the animal droppings or microbial action during biodegradation of sulphur component of the petroleum hydrocarbon thereby releasing sulphate as a metabolic byproduct. However, the decrease in soil sulphate in hydrocarbon impacted soil (untreated soil) may be due to soil sulphate adsorption and

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other associated cations (Chao, Harward & Fang, 1963). This phenomenon could be due to chemical bonding or physical entrapment (Kampranth, Nelson & Fitts, 1956).

The fluctuations in calcium concentration in the soil samples were represented in Table 1-5. The effects of treatment decreased the calcium level in all treatment options (inorganic-NPK fertilizer) and organic manure (fowl droppings and rabbit droppings) and controls (polluted untreated and unpolluted soil) as the experiment progressed from day 0 to day 28. At 28th day, the amended options (except for inorganic treated biocell) showed increase in calcium concentration than the controls. This agrees with the study of Steiner et al. (2007). However, the decrease in calcium concentration in inorganic (NPK fertilizer) treated biocell could be as a result of excess inorganic nitrogen (N) released which may have led to acidification and precipitation of exchangeable calcium leading to its decrease. This study was consistent with the study of Fang *el al.* (2012). Also, the decrease in calcium observed in untreated polluted soil sample at day 28 may be because of microbial metabolic activities via natural attenuation (Margesin & Schinner, 2001).

The baseline (day 0) exchangeable Magnesium concentration of Gio/B-Dere polluted soil and unpolluted Gio/B-Dere soil are represented in Table 1-5. As the bioremediation process advanced the magnesium concentration changes. The effect of application of inorganic (NPK; 20; 10; 10) fertilizer, fowl droppings and rabbit droppings, did not cause variation except for inorganic-treated soil which varied when compared to the controls. However, there were increases in exchangeable magnesium level for all the five conditions. This increase can be because of high microbial activity during the bioremediation process. Microbes metabolize hydrocarbons into simpler compounds, including organic acids which can also lead to the release of magnesium from organic matter in the soil, leading to an increase in its concentration. This study corroborates with the assertion of Miller *et al.* (2016) who postulated that decomposition of organic matter increases the cation exchange capacity (CEC) due to an increase in the negatively charged sites on carboxyl and phenolic groups.

Soil moisture serves as the transport medium for soil nutrients and removal of bacterial metabolic waste in the soil media. It affects hydrocarbon bioavailability, aeration status, nature and amount of soluble materials, osmotic pressure, diffusion processes, transfer of produced gases, soil toxicity level, and the pH of the soil (Ibeanuasi, 2012; Njoku *et al*.,2012). Values of % moisture were represented in Table 1-5. There was no difference among treatment options except for rabbit droppings treated Gio/B-Dere soil compared to the controls (except for unpolluted soil) at the end of 28 days bioremediation period. However, there was an increase between the time intervals of bioremediation. The baseline mean percentage (%) moisture contents were lower relative to the 28 days mean % moisture contents. This corroborated to the water added to the soil periodically to create optimal moisture conditions for the hydrocarbon-degrading microorganisms or the biodegradation of organic matter leading to an increase in % moisture in the soil. This is in line with the study of Wu *et al.* (2017).

Soil ash content refers to the inorganic residue remaining in the soil after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a soil. It is an important parameter in understanding the soil chemistry and its impact on the environment. The changes in ash content (%) in the soil samples are represented in the Table 1-5. Ash content (%) values showed no difference among the soil treatment, except for rabbit droppings as compared to their controls (Polluted soil and unpolluted soil respectively). However, difference occurred between the time intervals of remediation for the 28 days period. Thus, ash content (%) values increased with the time of remediation in all the soil options; baseline (Day 0) mean values increased higher than the day 28 soils under the influence of bioremediation. The inorganic and organic amendments containing rich minerals may have led to the increase in ash contents (%).

5. CONCLUSION

It follows from this study that the bioremediation of Gio/B-Dere petroleum hydrocarbon chronic polluted soil using organic fowl droppings, rabbit droppings and inorganic NPK fertilizer respectively, improved the vital physiochemical parameters which was evidenced from increased soil fertility index and enhanced nutrient availability (pH, soil organic matter, electrical conductivity, nitrogen, phosphorus and potassium). This carries significant scientific implications for improved soil health and sustainability, while also holding public significance for agricultural productivity, environmental sustainability, human health, and community livelihoods.

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CONFLICT OF INTEREST

Authors declared no conflict of interest.

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REFERENCES

Ademiluyi, B.O. & Omotoso, S.O. (2008). Comparative evaluation of *Tithonia diversifolia* and NPK fertilizer for soil improvement in maize (Zea mays L.) production in Ado-Ekiti, southwestern Nigeria. *Research Journal of Agronomy*, 2(1): 8-11.

Agarry, S. E, Aremu, M. O. & Aworanti, O. A. (2013a). Kinetic modelling and half-life study on bioremediation of soil co-contaminated with lubricating motor oil and lead using different bioremediation strategies. *Soil and sediment Contam. – An Int. J.* 22 (7): 800- 816.

Al-Joumaa, K. (2009). Effect of pollution with petroleum on some soil characteristics and plant growth. *Arab. Univ. J. Agric. Sci.*, 17 (1): 267–275.

Amadi, A., Dickson, A. A. & Meate, G. O. (1993). Remediation of oil polluted soils, effects of organic and inorganic nutrient supplement on the performance of maize. Soil Pollution, 66(1/2): pp 59-76.

Amadi, E. N, Okol, J. C., & Odu, C. T. I. (2005). Optimizing crude oil degradation in a sandy soil: Effects of urea-nitrogen and phosphoric acid. Phosphorus. *J. Niger. Environ. Soc*., 2(3): 322-329.

American Society of Testing & Materials, (1995). *Annual book of ASTM Standards*, designation D4972 - 95a: Standard test method for pH of soils.

Amponsah, D., Etsey, G., & Nagai, H. (2014). Determination of amount of phosphate and sulphate in soil samples from university of Cape Coast Farm. *International J. Sci. Tech. Res*.3 (7):41-48.

Anderson, J.M., & Ingram, J. S. I. (1996). *Tropical Soil Biology and Fertility: A Handbook of Soil Methods*. 2nd Edition, CAB International Information Press, Eynsham, pp 221.

Association of Analytical Chemistry, (1990). *Methods for Proximate Analysis*, 2217-2280.

Association of Analytical Chemistry, (1999). *Methods of analysis of association of official analytical chemists* (16th ed.). Washington D.C.1:600-792.

Atlas, R., M. (1994). Effect of Temperature and Crude Oil Composition on Petroleum Biodegradation. *Appl. Microbial*. 30:396-403.

Ayotamuno, M.J., Kogbara, R.B., & Hart, B.A. (2006). The combined effect of oxygen, water and nutrient on the remediation of a petroleum polluted agricultural soil. *J Eng*., 16(2): 119-134.

Barua, D., Buragohain, J., & Sarma, S. K. (2011). Certain physicochemical changes in the soil brought about by contamination of crude oil in two oil fields of Assam, NE. India. *European Journal of Experimental Biology*, (3): 154-61.

Black, C. A., Evans, D. D., Whils, J. L., Ensminger, L. E. and Clerk, F. E. (1965). *Methods of chemical analysis of ecological materials.* American Society of Agronomy. Inc Publisher Madson, Winscon USA.

Bray, R.H., & Kurtz, L.T. (1945) Determination of total organic and available forms of phosphorus in the soils. *Soil Science*, 59: 39-45.

Chao, T. T., Harward, M. E., & Fang, S. C. (1963). "Cationic effects on sulphate adsorption by soils," *Soil Sci. Soc. Am. Proc*., 27: 35.

Clay, S. (2014). *Identifying the Fate of Petroleum Hydrocarbons Released into the Environment and Their Potential Biodegradation Using Stable Carbon Isotopes and Microbial Lipid Analysis* (Doctoral Dissertation).

Dunn, R. J. K., Ali, A., Lemekert, C. J., Teasdale, P. R., & Welsh, D.T. (2007). "Short-term Variability of Physico-chemical Parameters and the Estimated transport of filterable Nutrients and Chlorophyll-a in the Urbanized Commbabah Creek System, Southern Moreton Bay, Austraha". *J. Coast'l. Res*., 50, 1062-1068.

Fang, Y., Xun, F., Bai, W., Zhang, W., & Li, L. (2012). Long-term nitrogen leads to loss of species richness due to litter accumulation and soil acidification in a temperate steppe. *Plos*, 1 (7), e47369.

Food and Agriculture Organization, (2020). *Towards a definition of soil health. Intergovernmental Technical Panel on Soils*. Letter no. 1. Rome, Italy, FAO.

Fuentes, S., Mendez, V., Aguila, P., & Seeger, M. (2014). Bioremediation of Petroleum Hydrocarbons: Catabolic genes, microbial communities, and applications. *Appl Microbiol. Biotechnol*, 81: 4781-4794.

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Hachiya, T., Ueda, N., Munenori, K., Hanke, G., Suzuki, A., Hase, T., & Sakakibara, H. (2016). Arabdopsis root-type ferredoxin: NADP(H) oxidoreductase 2 is involved in detoxification of nitrite in roots. *Plant & Cell Physiology*, 57: 2440-2450.

Hou, J., Wang, Q., Liu, W., Zhong, D., Ge, Y., Christie, P. & Luo, Y. (2021). Soil microbial community and association network shift induced by several tall fescue cultivars during the phytoremediation of a petroleum hydrocarbon-contaminated soil. *Sci. Total Environ*. 792, 148411.

Ibeanusi, V. M. (2012). "Putting microbes to work on subsurface contamination", Journal of Bioremediation and Biodegradation, 3 (5).

Kamprath, E. J., Nelson, W. L., & Fitts, J. W. (1956). "The effect of pH, sulphate and phosphate concentrations on the adsorption of sulphate by soils." *Soil Sci. Soc. Am. Proc*., 20: 463.

Kao, C. M., Chen, C. Y., Chen, S. C., Chien, H. Y., & Chen, Y. L. (2008). Application of in situ biosparging to remediate a petroleum hydrocarbon spill site: Field and microbial evaluation. *Chemosphere*, 70: 1492-1499.

Logeshwaran, P., Megharaj, M., Chadalavada, S., Bowman, M., & Naidu, R. (2018). Petroleum hydrocarbons (PH) in groundwater aquifers: an overview of environmental fate, toxicity, microbial degradation and risk-based remediation approaches. Environ. *Technol. Innovat*., 10: 175–193.

Margesin, R. & Schinner, F. (2001). Bioremediation (natural attenuation and biostimulation) of diesel-oil-contaminated soil in an alpine glacier skiing area. *Appl. Environ. Microbiol*., 67: 3127–3133.

Mbah, C. N, Nwite, J. N, & Okporie, O. E. (2006). Effect of organic wastes on some chemical properties and productivity of spent oil polluted ultisol in Abakaliki, Nigeria. *Niger. J. Trop. Agric*., 8:51-56.

Mbah, C. N, Nwite, J. N., & Nweke, I. A. (2009). Amelioration of spent oil contaminated ultisol with organic wastes and its effect on soil properties and maize (*Zea mays* L) yield. *World J. Agric. Sci*., 5(2): 163-168.

Miller, J., Beasley, B., Drury, C., Larney, F., & Hao, X. (2016). Influence of long-term application of composted or stockpiled feedlot manure with straw or wood chips on soil cation exchange capacity. *Compost. Sci. Util*., 24: 54–60.

Nicolsky, D. J., Romanovsky, V. E. & Panteleev, G. G. (2009). Estimation of soil thermal properties using in-situ temperature measurements in the active layer and permafrost. *Cold Regions Science and Technology*, 55: 120-129.

Njoku, K. L., Akintola, M. O. & Oboh, B. O. (2012). Phytoremediation of crude oil polluted soil: effect of cow dung augmentatation on the remediation of crude oil polluted soil by *glycine max*", *Research Journal of Applied Sciences*, 8 (1): 277-282.

Nwaogu, L. A., Ibegbulem, C. O., Ujowundu, C. O. & Alozie S. C. (2012). Impact of Some Heavy Metals in Soil and Oxidative Stress Indices and Chlorophyll Levels of *Telfaria occidentalis* leaves. *Nigeria Journal of Biochemistry and Molecular Biology*, 27(1): 46-52.

Ogboghodo, I. A., Azenabor, U. F & Osemwota, I. O. (2005). Amelioration of Crude oil polluted soil with poultry manure and the effect on growth of maize and some soil properties. *J. Plant Nutr*. 28(1): 21-32.

Omotoso, S.O. & Shittu, O.S. (2007). Effect of NPK fertilizer rates and method of application on growth and yield of okra (Abelmoschus esculentus (L.) Moench) at Ado-Ekiti southwestern, Nigeria. *International Journal of Agricultural Research*, 2(7): 614-619.

Onuh, M. O, Madukwe, D. K., & Ohia, G. U (2008a). Effects of poultry manure and cow dung on the physical and chemical properties of crude oil polluted soil. *Sci. World J*., 3(2): 45 – 50.

Onwurah, I. N.E., Ogugua, V. N., Onyeike, N. B., Ochonogor, A. E., & Otitoju, O. F. (2007). Crude oil spill in the environment, effects and some innovative clean-up biotechnologies. *International Journal of Environmental Research*, 1: 307-320.

Osam, M.U., Wegwu, M.O. & Uwakwe, A.A. (2008). Post-impact assessment of soils polluted by oil spillage in Omoku, Rivers State. *Journal of Nigerian Environmental Society*, 4(4): 27-38.

Osuji, L. C, Iniobong, D. I. & Ojinnaka, C. M. (2006a). Preliminary investigation of Mgbede-20 oil-polluted site in Niger Delta, Nigeria. *Chem. Biodiv*.3: 568-577.

Osuji, L. C. & Nwoye, I. (2007). An appraisal of impact of petroleum hydrocarbons on soil fertility: the Owaza experience. *African Journal of Agricultural Research*, 2(7): 318-324.

Piper, C. S. (1942). Soil and Plant Analyses. University of Adelaide.

Prescott, L.M., Harley, J.P. & Klein, D.A. (2004). *Microbiology*, 6th ed.; McGraw-Hill Science: New York, NY, USA: 154–196. ISBN -13.

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Published online: 16th August, 2024

Shekwolo, P.D.D. (2020). *Guidelines for Characterization and Remediation of Hydrocarbon Contaminated Land, with case studies from Nigeria.*

Smith, E., Tharamani, P., Ramadass, P., Naidu, R., Srivastava, P., & Megharaj, M. (2015). Remediation trials for hydrocarbon-contaminated soils in arid environments: Evaluation of bioslurry and boiling techniques. *International Biodeterioration Biodegradation* 101:56-65.

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., de Macêdo, J.L.V., Blum, W.E. & Zech, W. (2007). Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant Soil*, 291: 275–290.

Steliga, T. & Kluk, D. (2020). Application of *Festuca arundinacea* in phytoremediation of soils contaminated with Pb, Ni, Cd and petroleum hydrocarbons. *Ecotoxicol. Environ. Saf.* 194, 110409.

Swindell, C, M., Aelion, C. M., & Pfaender, F. K. (1988). "Influence of minerals and organic nutrients anaerobic biodegradation and the adaptation response of surface microbial communities". *Applied Environmental Microbiology*, 54(1): 212-217.

Tanee, F. B. G. & Kinako, P. D. S. (2008). Comparative Studies of Biostimulation and phytoremediation in the mitigation of crude oil toxicity in tropical soil. *J. Appl. Sci. Environ. Manage*. 12(2): 143 – 147.

Nations Environment Programme [UNEP], (2011). *Environmental Assessment of Ogoniland: An Independent Study to Determine the Environmental and Public Health Impacts of Oil Contamination in Ogoniland, and Options for Remediation.* ISBN: 978-92-807-3130-9.

United State Environmental Protection Agency [US EPA], (1993). *Determination of Nitrate, Nitrite and Nitrogen by automated colorimetry*. Revision 2.0.

United State Environmental Protection Agency [US EPA], (1971). *Methods for the Chemical Analysis of Water and Wastes* (MCAWW) (EPA/600/4-79/020)

United State Environmental Protection Agency, (2006). Land farming. Retrieved from: http:/www.epa.gov/oust/cat/landfarm.htm.

Van Hamme, J. D., Singh, A. & Ward, O. P. (2003). "Recent advances in petroleum microbiology". Microbiology and molecular *Biology Review*, 67(4), 503-549.

Vidali, M. (2001). 'Bioremediation. An overview'. *Pure and Applied Chemistry*, 73(7): 1163-1172.

Vlassak, K. (1970). Total soil nitrogen and nitrogen mineralization. *Plant soil*, 32: 27-32.

Walkley, A. & Black, I.A. (1934). An examination of the Degtjareff Method for determining soil organic matter and proposed modification of the chromic acid titration method. *Soil Sci*. 37: 29-38.

Wegwu, M. O., Uwakwe, A. A. & Anabi, M. A. (2010). Efficacy of enhanced natural attenuation (land farming) technique in the remediation of crude oil-polluted agricultural land. *Applied Science Research*.2(2): 431-442.

Wu, M., Ye, X., Chen. K., Li., Yuan, J. & Jiang, X. (2017). "Bacterial community shift and hydrocarbon transformation during bioremediation of short-term petroleum-contaminated soil". *Environmental pollution*, 223:657-664.

Xue, X & M. Li (2013). Rapid determination of Sulphate in Water by Turbidimetry. *Chinese Journal of Environmental Engineering* 7(4):1359-1362.

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