International Journal of Academic Engineering Research (IJAER)
ISSN: 2643-9085
Vol. 5 Issue 2, February - 2021, Pages: 22-27

Combined Effects of Spent Mushroom Substrate (SMS) and NPK Fertilizer in the Remediation of Crude Oil Polluted Soil

[1]Raymond Alex Ekemube, [2]Davidson Dimabo Davis, [3]Bernard Beabu Dumkhana, [4]Tamunokuro Oswald Amgbara

1Department of Agricultural and Environmental Engineering, Rivers State University, Port Harcourt, Nigeria
raymond.ekemube@ust.edu.ng
2Department of Agricultural and Environmental Engineering, Rivers State University, Port Harcourt, Nigeria
davis.davidson@ust.edu.ng
3Department of Agricultural and Environmental Engineering, Rivers State University, Port Harcourt, Nigeria
beabu.dumkhana1@ust.edu.ng
4Department of Civil Engineering, 4Kenule Beeson Saro Wiwa Polytechnic, Bori, Nigeria
amgbara.tamunokuro@kenpoly.edu.ng

Abstract: *This study focused on biostimulation of crude oil polluted soil by means of activated nutrients through spent mushroom substrate (SMS), and NPK fertilizer on the degradation of hydrocarbon pollutant. Soil pH, temperature, nitrogen, organic carbon, moisture content, total petroleum hydrocarbon (TPH), and changes in total heterotrophic bacteria (THB) counts were monitored from the baseline to the 62 days. In this study, 1500g of crude oil contaminant were sprinkled on 4000g agricultural soil with different applications of SMS and NPK fertilizer during the study period. The concentration of TPH was 6323 mg/kg prior to treatment, after 31 days of enhanced crude oil contaminants with the biostimulating agents. On the averaged, there was 63% reduction of TPH concentration across treatment samples. The results showed microbial degradation of TPH loss across treatment cells from 81% to 84% monitored at the end of 62 days period. There was significant increase in THB counts of 3.30 x10^6, 4.52x10^6, and 5.35x10^6 across control cell P, with the highest increase of 5.42 x10^6 to 5.87 x10^6 recorded in 62 days across the treatment cell. It is concluded that the combination of SMS and NPK fertilizer 20:10:10 is an effective stimulant in the degradation of densely hydrocarbon polluted soil and its associated risk in the environment.*

Keywords — Biostimulants; Crude Oil Polluted Soil; NPK fertilizer; Remediation; SMS; TPH Degradation

1. INTRODUCTION

It is well-established that crude oil when spilled in the environment even at very low concentration is a serious concern. In the case of exposure can cause harmful effects on physical, chemical properties, organisms, populations and can raise inhibitory actions on metabolism [1; 2]. Oily contaminated soil lead to reduction in oxygen flow, and poor soil fertility thereby causing ecological and toxicological effects on plants and alteration of the natural state of the oilfield [3]. Oil soaked soil causes pollution load thereby smothering soil particles and blocking air diffusion in the soil pores, and affects soil microbial communities [4; 5; 6]. The fact remains that crude oil even at low levels of pollution causes damage [3] due to their persistence in natural environment and biological toxicity to plant. As it contain complex combination of hydrocarbons of aliphatic, alicyclic and aromatic hydrocarbons with little amount of nitrogen, oxygen, and sulfur compounds.

Biodegradability of hydrocarbons and their degree of persistence in natural environment is much longer with increase mortality of plant, aquatic life, and other soil depending organisms [7; 8] than most conventional carbon sources. The interference with the soil aeration and water retention is due to the toxicity of oil constituents. In a contaminated site where organic pollutants in high molecular weight are saturated with soil, available nutrients can extensively amend the hydrocarbon structure and toxicological properties of such contaminants. These organic pollutants usually caused disruptions of soils and sediments as described by Okerentugba et al. [9]. Furthermore, Tanee and Albert [10] explains that transportation of contaminates takes unforeseeable phenomena with effects that are harmful to the environment, thus inhibiting seed germination and outright death of plants.

The presence of viable microbial population able to degrade hydrocarbon under natural environmental conditions for optimal microbial degradative activities to detoxify pollutant [11].This explains the postulate of Kumar and Gopal [12] on biostimulation strategy as the addition of appropriate spent mushroom substrate as a stimulant to the growth of indigenous microbes on a polluted soil. Okerentugba et al. [9] reported that the most widely used and practiced inorganic fertilizer suitable for oil-loving environment is the NPK, KNO_3, and HN_4NO_3 with different application level on soil amendment in order to achieved bioremediation. Therefore, it is very important to biostimulate contaminated soil with nutrients that will ameliorate microorganisms utilizing bacterial to degrade the hydrocarbon contaminant. The amount of nitrogen and phosphorus required to establish bioremediation is a sites specific value. However, the hydrocarbons appeared to be degraded more quickly in comparison to natural attenuation processes, probably because of the increased number of microorganisms induced by the greater amount of nutrients

provided to the contaminated soil. The aim of this study was to evaluate the combined effects of spent mushroom substrate (SMS) and NPK fertilizer in the degradation of oily laden polluted soil.

2. MATERIALS AND METHODS

2.1. Description of study area

This experiment was carried out at the research and demonstration farm of the Rivers State University, Port Harcourt, Nigeria located on a GPS coordinate of latitudes 4°45' – 4°60' E and longitudes 6°55' – 7°56' N (Figure 1). The experimental site was carried out in an open space but shielded from the rain and sun to control the moisture content in the reactor.

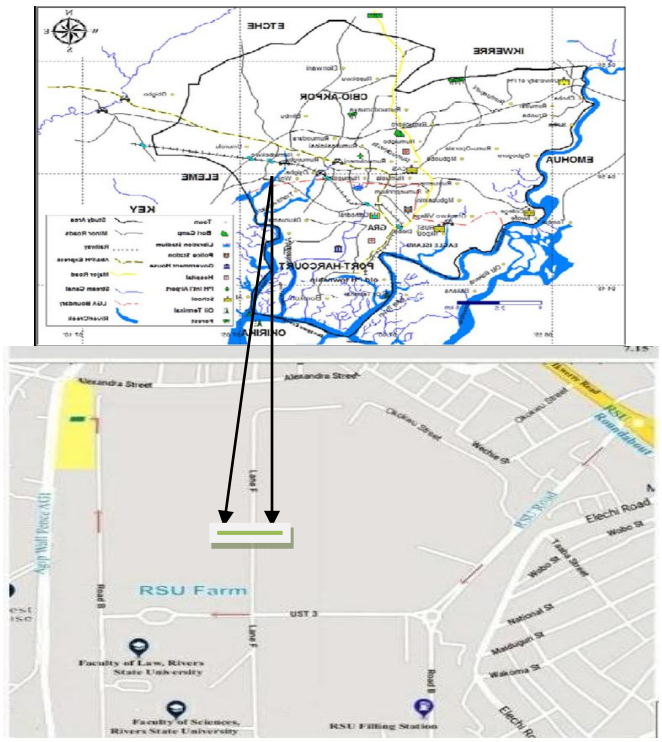

Figure 1: Map of the Study Area using GPS Coordinate

2.2. Sample collection and contamination

Agricultural soil was collected from Rivers State University demonstration farm, Port Harcourt, Nigeria at 0 - 30cm depth with 9-inch hand auger capable of obtaining uniform cores of equal volume. Crude oil was obtained from AGIP facility in the Niger Delta, Nigeria. Spent mushroom substrate used as organic stimulant was collected from Dilomat Mushroom Production Centre, Rivers State University, Port Harcourt. NPK fertilizer 20:10:10 was obtained from a commercially available market.

2.3. Experimental Layout

Cell P: Control 4000g agricultural soil + 1500g crude oil without any form of treatment
Cell Q: 4000g agricultural soil + 100g SMS, + 100g NPK fertilizer 20:10:10 + 1500g crude oil
Cell R: 4000g agricultural soil + 150g SMS, +150g NPK fertilizer 20:10:10 + 1500g crude oil.
Cell S: 4000g agricultural soil + 200g SMS + 200g NPK fertilizer 20:10:10 + 1500g crude oil.
Cell T: 4000g agricultural soil +250g SMS + 250g NPK fertilizer 20:10:10 + 1500g crude oil.
The contaminated-soil was left undisturbed for 3 days to ensure the performance of microbes' interaction on the hydrocarbon before remediation.

2.4. Preparation and Addition of Biostimulant

A combination of five treatment cells inclusive of a control aimed at accelerating the biodegradation of hydrocarbon contaminated soil was experimented. Crude oil (contaminant) of 1500g was sprinkled using a perforated can on each of the Bioreactor treatment cells containing 4000g agricultural soil, SMS and NPK fertilizer 20:10:10 are homogeneously mix. The essence for homogeneity is because they may be a significant variation of contaminants in depth. A commercially available 20:10:10 NPK fertilizer was employed alongside spent mushroom substrate (SMS) as stimulant to existing microbes in the soil. Water application of 0.5 liter twice weekly, especially on treatment cell has been greatly use in bioremediation study [13; 14] in other to keep the soil under moist condition.

2.5. Tilling

The bioreactor treatment cell were not tilled during the study period since tillage modifies soil environmental conditions, available microorganisms were in turn affects their ability to release nitrogen [15]. This also revealed that specific conditions such as optimal temperature, microbial growth, soil population, and moisture provides proper situation that could decrease the contaminant level due to oxidation of easily degraded petroleum component.

2.6 Laboratory Analysis

Test protocols for soil physicochemical and biological parameters include moisture content (MC), soil pH, electrical conductivity (EC); total nitrogen (TN), total organic carbon (TOC), total heterotrophic bacterial (THB) counts, as well as total petroleum hydrocarbon (TPH) were collected and analyzed after 3 days, 31 days, and 62 days of the study period.
The moisture content (w/w), on dry basis, was determined by oven-dry method using an oven maintained at 105 ± 5°C for 24 hours. The moisture content was calculated using equation (1).

$$MC = \frac{W_w - W_d}{W_w} \times 100 \quad (1)$$

Where,

MC is the moisture content (%) of the sample
Ww is the wet weight of the sample
W_d is the weight of the sample after oven-drying
The total nitrogen was determined by the Kjeldahl method following the guideline described in Bremner and Mulvaney [16]. The total organic carbon was determined using 1g of dried soil sample placed in a 500ml conical flask, thereafter 10ml of $1NK_2Cr_2O_7$ solution was added and mixed thoroughly with 20ml concentrated H_2SO_4 . The flask was swirl 2 to 3 times for reaction to be completed. 200ml of distilled water was poured into the flask to allow the suspension of sample. 10ml of orthophosphorus acid or 0.5g of NaF was added and 1ml of diphenylamine indicator was use to titrate with 0.5N ferrous ammonium till the colour changes from violet to blue and finally bright green colour. Organic carbon was calculated using Walkley–Black combustion method as cited in [17] expressed in equation (2).

$$R = \frac{(X-Y)\times N\times 0.\times 003\times 100}{W} \times C \qquad (2)$$

Where,
R= % of organic carbon in soil
W= Weight of sample
X= Black titre reading
Y = Sample reading
N= Normality of $K_2Cr_2O_7$ (i.e. 1N)
C= Correction factor (1.724)
The pH value of the soil was measured in a 20 mL beaker stirred with 8 g of soil in a deionised water suspension of ratio 1:1.5 (m/v) soil to water suspension and allowed to equilibrate for about 1 hour in line with ISO 10390 [18]. The pH of the agricultural soil and treatment cell was then measured using a Hach pH meter calibrated with standardized pH buffer solutions.

2.6.1. Determination of total petroleum hydrocarbon (TPH) in treatment sample

The TPH analyses were conducted in accordance with the operating procedure of USEPA 8015B. A 5g of treated (or untreated) drill cuttings samples were weighed into clean extraction containers thoroughly mixed with 10ml dichloromethane (dcm) extraction solvent. The extract were then cleaned and concentrated using a hypodermic syringe, 1uL of the concentrate was injected through a rubber septum into a calibration solution mixed. The calibration solution mix was made up with TPH stock solution mix within a series of specific carbon ranges (C8 - C40) and surrogate standard (1-Chlorooctadecane) solution mix for the aliphatics [19].

2.6.2. Sterilization / Preparation of Dilute and Bacteria Enumeration

In order to sterilize the treatment-soil media and all diluents, an autoclave at 121^{o}C for 15min was used. All glass wares were sterilized in a dry saline, 0.85g of Nacl (Sodium chloride) was weighed out and transferred into 100ml of distilled water and mixed thoroughly. The solution was then dispersed in 9ml volume into a test tube, after which it becomes the solution for serial dilution of soil samples. Hydrocarbon-degrading and heterotrophic bacteria were estimated from agricultural soil and treated-soil using plate count agar by serially diluting the samples in line with the method APHA 8015B in accordance to the procedures of APHA [19]. 1g of soil samples were aseptically transferred into 9ml sterile peptone water to give a 10-fold serial dilution. The test tubes containing peptone water and the soil samples were shaken vigorously with care to ensure proper mixture and homogenization.

2.7. Statistical Analysis

Data were statistically analyzed using Data analysis tool pack of Microsoft Office Excel 2010 (Microsoft, New York, NY, USA) for Analysis of variance (ANOVA). This was employed without replication and also the drawing of graphs and bar charts with respect to pH, TN, TOC, TPH, and microbiological analysis of crude oil polluted soil amended with SMS and NPK fertilizer, and control cell for 3, 32, and 62 days of treatment respectively. Significance differences were evaluated at $p \leq 0.05$ level using the appropriate degrees of freedom for each source of variability.

3 Results and Discussion

The soil from the demonstration farm of Rivers State University, Port Harcourt is predominantly loamy soil
(Sand 75.6 %, Silt 16. 9%, and Clay 7.5%). Table 1 shows the baseline data from the soil physicochemical and microbiological parameters including moisture content (MC), soil pH, electrical conductivity (EC); total nitrogen (TN), total organic carbon (TOC), changes in total heterotrophic bacterial (THB) counts, as well as total petroleum hydrocarbon (TPH) before contamination and 3 days after different application of treatment. The results obtained before bioremediation treatment from uncontaminated soil on pH, electrical conductivity, organic carbon, and total nitrogen were 5.63, 50.42 (μS/cm), 0.98% mg/kg, and 0.084 mg/kg respectively. The initial characterization of TPH concentration was 6323 mg/kg.

Table 1: Physicochemical and Microbiological Property before and after Contamination

Parameters	Uncontaminated Soil	Contaminated Soil (Days)
		3
pH	5.63	5.17
Electrical conductivity (μS/cm)	50.42	50.86

Total Nitrogen (mg/kg)	0.084	0.23
Total Organic Carbon (mg/kg)	0.98	2.61
Total Petroleum Hydrocarbon (mg/kg)	22	6323
Moisture content (%)	10.48	12.75
Sand (%)	75.6	-
Silt (%)	16.9	-
Clay (%)	7.5	-
Total Heterotrophic bacteria count ($x10^6$ cfu/g)	2.90	3.30

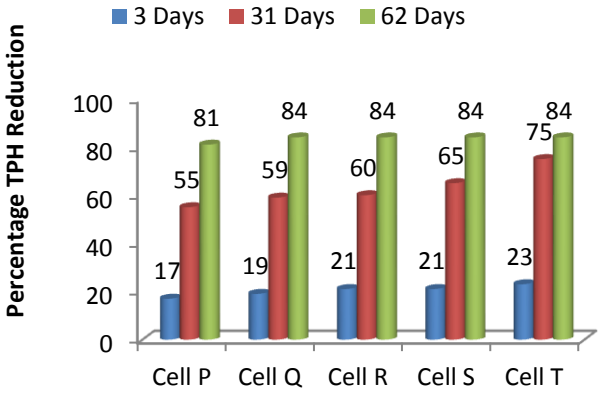

Figure 1: TPH Percentage Reduction Treated Sample

Figure1 showed the percentage TPH reduction in the treatment samples during the 62 days of study. The control cell P had the least TPH reduction of 55% at 31 days amongst other treated samples. At the end of the study, there was further decrease in TPH reductions with respect to time in control cells 81 and 84% across various cells, when compared with the baseline data of 6323 mg/kg. The TPH losses in the treatments cell are supported by changes in microbial activities and pH value shown in Figures 1, 2 and 6 respectively. Although the reduction occurred across all treatment cells, it was higher with increase amount of SMS and NPK fertilizer application. This finding agreed with the findings of Adenipekun and Ojunjobi [20] who observes the high percentage loss of 90% TPH reduction with the application of SMS on soil planted by Pleurotus tuber. This explains the posit of Gbarakoro and Chukumati [21] that spent mushroom substrate increased the efficacy in degrading hydrocarbon especially as they are nutrient activator. In the experimental oil study, the concentration of TPH in the crude oil-contaminated soil decreased with high amount of SMS and NPK fertilizer application across treatment cell. From the results, it revealed that TPH losses were highly significant ($P \leq 0.05$) over time in the treatment cell Q, R, S, and T at the 62 days period. In comparing this study with a related results obtained by Agarry et al. [22] and Agarry [23] in their study of enhanced bioremediation of soil artificially contaminated with Bonny light crude oil showed that soil amended with NPK fertilizer, and Tween 80 mixture recorded 83.79% reduction of TPH content.

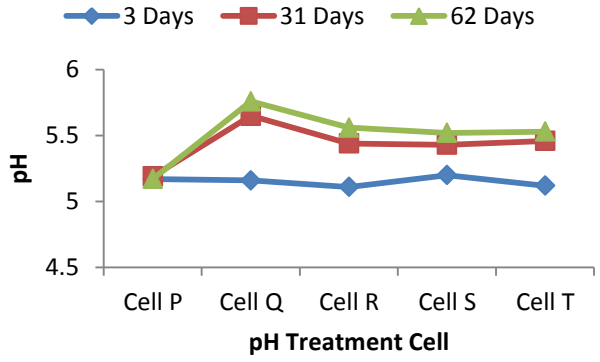

Figure 2: pH on Various Treated Sample

Figure 2 showed the pH value of the soil range from 5.11 to 5.17 after 3 days of remediation, and 5.17 to 5.76 at 62 days of the study. This showed that the soils are acidic despite the different levels of SMS and NPK fertilizer application. This explains the view of the Department of Environment and Heritage Protection [24] that optimum pH requirement for most agricultural soils is 5.50 to 7.50 with plant thriving under varying conditions.

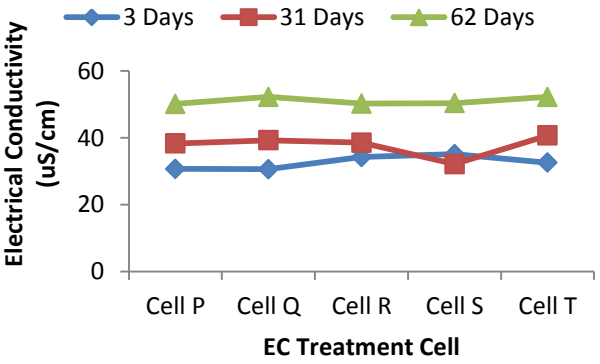

Figure 3: Electrical Conductivity on Various Treated Sample

The results in figure 3 indicated slight increase in electrical conductivity value of the various treatments cell from 30.69 to 35.10 (µS/cm) in 3days, and 32.12 to 40.70 (µS/cm) at 31days of study. At the end of 62 days of the

study there was considerable increase from 50.12 to 52.25 (µS/cm) across treatment cells.

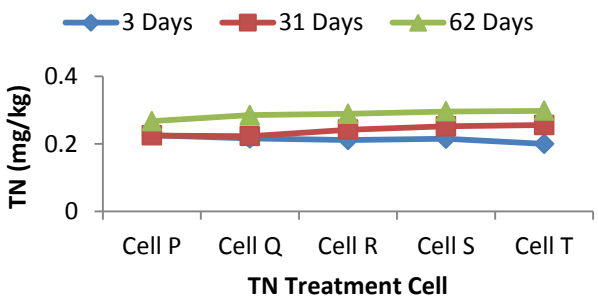

Figure 4: Total Nitrogen on Various Treated Sample

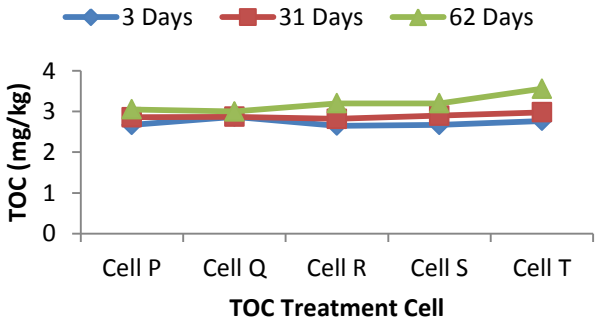

Figure 5: Total Organic Carbon on Various Treated Sample

Figure 4 showed that total nitrogen increases as the application rate of SMS and NPK fertilizer increases across the treatment cells from 0.225 to 0.256 mg/kg in the 31 days of treatment and 0.267 to 0.298 mg/kg at the end of 62 days of study. The most increase was recorded in treatment cell T (0.298 mg/kg) whereas the control cell P (0.267 mg/kg) recorded the least amongst treatment cells. Despite the presence of total petroleum hydrocarbon (TPH) content in the treatment cells containing total organic carbon (TOC). Figure 5 showed considerable increase of organic carbon in control cell P of 2.67 to 3.05 mg/kg, whereas treatment cell T recorded the highest increase of 2.77 to 3.56 mg/kg of organic carbon at the end of the study. This could be attributed to high amount of SMS and NPK fertilizer application rate in the treatment cell. Total nitrogen was highly significant ($P \leq 0.05$) over time at the end of the study period in Cell Q, R, S, and T than in the agricultural soil control cell P.

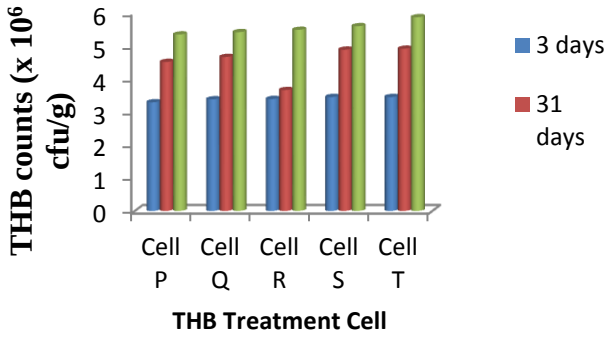

Figure 6: Changes in Bacteria Counts in the Treated Sample

Figure 6 showed the degrading activities of bacteria counts which recorded 4.52 to 4.92 (10^8 cfu/g) at the 31 days of the study. It was observed that the number of microbes at 62 days of study in the control cell P increase to 5.35 (10^8 cfu/g) whereas other treatment cells where within 5.42 to 5.87 (10^8 cfu/g) respectively. Two ways Anova without replication shows non-significant difference ($P \leq 0.05$) across treatment cells. Although, there was significant difference ($P \leq 0.05$) over time in the treatment cell due to interaction between bacteria counts. This is enhanced by the application of SMS and NPK fertilizer in the treatment cell where the substrate biostimulate soil microbes and increased their ability in degrading hydrocarbon content. This agrees with the report of Gbarakoro & chukumati (2016) that biodegrading activities are enhanced by spent mushroom substrate (SMS) responsible for the recovery of its normal soil profile. In a related study by Jain et al. [25] stated that biodegradation of hydrocarbons by natural populations of microorganism represent one of the primary mechanism by which petroleum and other hydrocarbon pollutants are eliminated from the environment.

4. Conclusion

This study shows the effectiveness of spent mushroom substrate SMS and NPK fertilizer application as bioremediation agents in soil polluted with crude oil. The TPH reduction across various treatment cells compare to the baseline data is an indication of successful detoxification on agricultural cells. The different amount of SMS and NPK fertilizer was responsible for the removal of total petroleum hydrocarbon (TPH) concentration across treatment cells. The changes in microbial activities pinpointed the highest hydrocarbon degradation supported by increase in total organic carbon and total nitrogen. This research implies that increase amount of spent mushroom substrate and NPK fertilizer derived nutrient when compare with the control cell P restore soil and inhabiting organisms. Therefore, recommended the combined use of SMS and NPK fertilizer in the remediation of soil contaminated with crude oil.

REFERENCES

[1] Abowei, M. F. N. (1996). Prediction and Consequences

of Petroleum Spills into the Nigerian Aquatic Environment in the Year 2000. *International Journal of Environment and Pollution, 6*: 306 - 316.

[2] Tanee, F. B. G., & Albert, E. (2011). Reconnaissance Assessment Of Long – Term Effects Of Crude Oil Spill On Soil Chemical Properties And Plant Composition at Kwawa, Ogoni. Journal of Environmental Science and Technology, 8(6): 320 – 329.

[3] Yu, Y., Zhang, Y., Zhao, N., Guo, J., Xu, W., Ma, M., & Li, X. (2020). Remediation of Crude Oil-Polluted Soil by the Bacterial Rhizosphere Community of Suaeda Salsa Revealed by 16S rRNA Genes. *International Journal of Environmental Research and Public Health. 17*: 2 – 18.

[4] Labud, V., Garcia, C., & Hernandez, T. (2007). Effect of Hydrocarbon Pollution on the Microbial Properties of a Sandy and a Clay Soil. *Chemosphere*, 66(10): 186 3–1871.

[5] Sutton, N. B., Maphosa, F., & Smidt, H. (2013). Impact of Long-Term Diesel Contamination On Soil Microbial Community Structure. *Applied and Environmental Microbiology*, 79(2): 619 – 630.

[6] Ying. W., Jiang, F., Qianxin, L., Xianguo, L., Xiaoyu, W., & Guoping, W. (2013). Effects of Crude Oil Contamination on Soil Physical and Chemical Properties in Momoge Wetland of China. *Chinese Geographical Science, 23*(6): 708–715.

[7] Lin, Q., & Mendelssohn, I. A. (2012). Impacts and Recovery of the Deepwater Horizon Oil Spill on Vegetative Structure and Function of Coastal Salt Marsh in the Northern Gulf of Mexico. *Environmental Science and Technology, 46*(7): 3737–3743.

[8] Ali, N., Dashti, N., Khanafer, M., Al-Awadhi, H., & Samir, R. (2020). Bioremediation of Soils Saturated With Spilled Crude Oil. *Scientific Report*, l0: 11 – 16.

[9] Okerentugba, P. O., Orji, F.A., Ibiene, A.A., & Elemo, G.
N. (2015). Spent Mushroom Compost for Bioremediation of Petroleum Hydrocarbon Polluted Soil: A Review. *Global Advanced Research Journal of Environmental Science and Toxicology, 4*(1): 001-007.

[10] Tanee, F. B. G., & Albert, E. (2011). Post-Remediation of Crude Oil Polluted Site at Kegbara-Dere Community, Gokana L.G.A. of Rivers State, Nigeria. *Journal of Bioremediation and Biodegradation, 2:* 122 – 129.

[11] Chikere, C. B., Okpokwasili, G. C., & Chikere, B. O. (2011). Monitoring of Microbial Hydrocarbon Remediation in the Soil. *Biotechnology, 1*: 117 – 138.

[12] Kumar, B. L., & Gopal, D. S. (2015). Effective Role of Indigenous Microorganisms for Sustainable Environment. *Journal of Biotechnology,* 5(6): 867 – 876.

[13] Kogbara, R. B., Dumhana, B. B., Ayotamuno, M. J., & Okparanma, R. N. (2017). Recycling Stabilized/Solidified Drill Cuttings for Forage Production in Acidic soils. *Chemosphere, 184*: 625 663.

[14] Dumkhana, B. B., & Ekemube, R. A. (2020). Performance of Elephant Grass and Maize Plant admixture of Stabilised/Solidified Drill Cuttings with Loamy Sand Soil. European *Journal of Earth and Environment, 7*(1): 13 – 28.

[15] Lobb, D. A. (2008). Movement by Tillage and Agricultural Activities. *Ecological Engineering, 4:* 3295 – 3303.

[16] Bremner, J. M., & Mulvaney, C. S. (1982). Nitrogen Total. In Methods of Soil Analysis, Part 2. Chemical and Microbiological properties; page, A.L., Miller, R.H,
Keeney, D.R. Eds., American Society of Agronomy and Soil Science Society of American: Madison, W.I, 595 - 624.

[17] Sato, J. H., de Figueiredo, C. C., Marchao, R. L., Madari, B. E., Benedito, L. E. C. Busato, J. G., & De Souza, D. M. (2014). Methods of Soil Organic Carbon Determination In Brazilian Savannah Soils. *Science Agricultural (piracicaba) Brazilian, 71*(4): 1-8.

[18] ISO (2005). Soil quality-Determination of pH International Organization for Standardization Geneva, Switzerland.2005. ISO 10390.

[19] APHA (1998). Standard methods for the Examination of Water and Wastewater, twentieth ed. American Public Health Association. Washington. DC.

[20] Adenipekun, C. O., & Ogunjobi, A. A. (2011). Bioremediation of Cutting Fluids Contaminated Soil by Pleurotus Tuber Region. *The Environmentalist. 32*: 11-18.

[21] Gbarakoro, T. N., & Chukumati, J. (2016). Impact of Spent Mushroom Substrate on Soil Microarthropods in Spent Automobile Lubricant Habitat-Types at University Farm, University of Port Harcourt, Rivers State, Nigeria. *International Journal of Environment and Pollution Research, 4*(4): 13-23.

[22] Agarry, S. E, Owabor, C. N., & Yusuf, R. O. (2012). Enhanced Bioremediation of Soil Artificially Contaminated with Kerosene: Optimization of Biostimulation Agents through Statistical Experimental Design. *Journal of Petroleum Environment and Biotechnology. 3:* 120.

[23] Agarry S. E. (2018). Evaluation of the Effects of Inorganic and Organic Fertilizers and Activated Carbon on Bioremediation of Soil Contaminated with Weathered Crude Oil. *Journal of Applied Science Environment and Management. 22*(4): 587 – 595.

[24] Department of Environment and Heritage Protection (2017). Annual Report – Department of Environment and Heritage Protection, Queensland Government. Retrieved from www.ehp.qld.gov.au (Accessed 4th February, 2021)