



Bioremediation Of Hydrocarbon Contaminated Soil In Eleme Using Poultry Droppings And Cow Dung In An Engineered Biocell

¹Oluka, Mmama Grace & ²Dr Anaele, John Vitus

Department of Chemical Engineering
University of Port Harcourt, Port Harcourt, Nigeria

Email of the Corresponding author: olukammama@gmail.com & john.anaele@uniport.edu.ng

ABSTRACT

Organic waste (dung and droppings) were applied in this study in lowering the intensity of TPH and PAHs in polluted soils of communities in Eleme. An Ex-situ procedure was utilized in an engineered biocell, partitioned into nine smaller cells intended for curing of soil from three spilled unhygienic communities; Ebubu, Ogale, and Alode, and uncontaminated community, Aleto was used as the control station. Poultry droppings and cow dung was applied three times weekly at the rate of 6kg, 12kg, and 18kg to 10kg of soil in each cell for 7, 14, and 21days intervals, and samples were evaluated at the laboratory weekly by GC-FID analysis according to US-EPA method 8015c. Conclusively, the application pace of organic wastes had considerably decreased the concentration of total petroleum hydrocarbon plus PAHs with noteworthy differences of ($P < 0.05$). Similarly, there was a continuing boost in concentration of ph, nitrate, also phosphate amid a corresponding reduction in total organic content (TOC) and sulphate. Intensity of Copper, Zinc, Iron, Chromium and Manganese were gradually reduced for station 1- 3. There was a corresponding increase of the total bacterial count (TBC) as 1.5-2.9 kcfu/g, 1.3-2.1 kcfu/g and 1.6-2.3kcfu/g. Hydrocarbon utilizing bacteria (HUB) similarly increased from 3.0-5.2kcfu/g, 5.0-5.4kcfu/g and 4.1-4.7kcfu/g in stations one to three respectively. Pseudomonas, Achromobacter, Staphylococcus, including Bacillus are isolated bacteria genera. It was observed from the study that cow dung and poultry droppings are excellent biostimulants for the amendment of hydrocarbon-contaminated soil.

Keywords: Bioremediation, Poultry droppings, Cow dung, Engineered Biocell, Contaminated Soil.

INTRODUCTION

Bioremediation is a biological method that encourages beneficial microbes to consume and break down hazardous contaminants as their nutrient source and energy supply. Microorganisms can metabolize toxic chemicals and germs, digesting them and converting them into harmless substances like methane and carbon IV oxide. In certain polluted land formats, the organisms needed for the bioremediation process are already present. Human involvement can accelerate natural remediation processes by promoting microbial activity (Zhao et al., 2018). Al-Turki (2009) asserted that bioremediation is a viable option for the decontamination of soil contaminated with polycyclic aromatic hydrocarbons (PAHs) due to its cost-effectiveness and efficiency, provided the environmental conditions are favorable for the microbial activity. Microorganisms can break down hydrocarbons through biological degradation, which involves various species of bacteria and fungi capable of degrading the hydrocarbons found in oil. According to (El-Hadidi & Al-Turki, 2007), over sixty genera of bacteria and eighty genera of fungi include species with the ability to biodegrade hydrocarbons. Microbes that are capable of breaking down hydrocarbons

have been identified in a wide selection of environments, and they can metabolize them both in the presence/absence of oxygen (Al-Turki, 2007). Certain types of microorganisms can break down aliphatic organic compounds and scent-based particles, although few have the capability to metabolize both types of molecules (Whyte et al., 1996). It has been suggested that complete degradation of PAHs likely necessitates a network of organisms that transfer and convert metabolites as they are broken down stepwise Kelley et al. (1993). Researchers discovered that certain microalgae types possessed the capacity to break down polycyclic aromatic hydrocarbons (Lei et al., 2003). Microbes with the ability to degrade organic compounds aerobically have only been confirmed to a limited degree; most of the existing information relates to the decomposition of limited aromatic molecules like C₆H₆, C₇H₈, as well as C₈H₁₀ (Colberg & Young, 1985).

Effects of Oil Spill on Eleme Soil

The environment of the Niger Delta region, which is plentiful in oil, has been subject to unchecked and lengthy harms, leading to numerous health, social, and economic issues for the people there (Ohanmu et al., 2017). The rise of oil as a major source of energy was partially a result of its comparatively low level of contamination. Nevertheless, the immense scale of oil industry production has produced some challenging ecological issues, which are now apparent in the Eleme region of Nigeria (Ekanem et al., 2010). The condition of soil exposed to crude oil can become less than ideal for plant growth as a result of the hydrophobic layer covering the air-filled pore space (Eneh, 2011) and the elevated oxygen demand caused by organisms that breakdown the oil (Ugbomeh & Atubi, 2010). The contamination of soils by crude oil can deprive them of essential minerals, leading to chlorosis, necrosis and stunting of shoots and roots, which can all result in reduced biomass. (Idodo-Umeh & Ogbeibu, 2010).

Abii and Nwosu (2009) observed a noteworthy decrease in the Calcium, potassium, and Phosphorus levels, as well as a substantial amplification of the sand fraction and the oil spill-influenced soils' content. Previous research has been done by Khamehchian (2007) that have demonstrated how oil contamination of soil affects the diffusion of essential nutrients to plants (Agbogidi & Egbuchua, 2010). This can hinder the plants' uptake of necessary soil elements, thus limiting their growth. The examination discovered that crude oil pollution had a significant effect on the physical/chemical and the microstructure of the soil.

There are two techniques in carrying out bioremediation; these are in situ and ex situ

In Situ Bioremediation Techniques

This method involves managing contaminated elements in the spot where contamination has taken place. It does not need any digging or excavating, so it comes with only a marginal or no impact to soil structure. It is desired that these techniques would be more inexpensive in comparison to ex situ bioremediation techniques, as excavation processes do not come with any additional cost. The primary worry is the cost of designing and putting in place more sophisticated apparatus on-site in order to enhance microbial activities during bioremediation. Enhancement of certain in situ bioremediation techniques might be necessary (bioventing, bioaugmentation, biosparging), while others may proceed without needing any boost (intrinsic bioremediation or natural weakening). The use of in situ bioremediation has been found to be effective in treating certain pollutants in contaminated sites. Studies have illustrated that compounds such as chlorinated solvents, colorants, heavy metals, as well as organic compounds can all be remediated by this process (Folch et al., 2013); (Kim et al., 2014); (Frasconi et al., 2015); (Roy et al., 2015).

Ex-Situ Bioremediation Techniques

This approach involves digging up polluted soil from contaminated locations and afterwards conveying it to a different area for cleansing and remediation. Factors taken into account when selecting this as a treatment option include: expenses for the remediation, depth of the pollution, kind of contaminant, extent of pollution, geography of the soil being remediated, and the geographical environment of the site. In addition, performance criteria for the bioremediation technique of choice have been articulated (Philip & Atlas, 2005). The method can be potentially hazardous, as the transportation of pollution can result in

spillage or spread of contamination. However, there are five-methods typically employed at ex-situ treatment sites which are cultivation of land, composting, biopiles, windrows and bioreactors.

MATERIALS AND METHODS

30kg weight of polluted soil was collected with a shovel between the hours of 8-10 am at depth of 0-15cm in four different synthetic polyethylene bags from each of the four communities Ebubu (Station 1), Ogale (Station 2), Alode (Station 3), and Aleto (station 4, Control) communities of Eleme LGA, R/S. 5kg out of it was collected on the same day using sterile sample bottles properly tagged and transported to the laboratory. 20kg of Poultry droppings and cow dung were collected from the slaughter and poultry farm both located in Aleto Eleme in a sack bag and taken to the location of treatment. 25kg out of the excavated samples from the polluted sites were then measured using a weighing balance CAMRY EMPERORS P/1211/003003/CN/CR1 model. 10kg of the weighed soil were loaded into each cell of the 9-partitioned constructed bio-cell. 2kg each of cow dung and poultry droppings were applied to the soil samples in the biocell using a hand trowel and tilled for aeration.

Sample Analysis

TPH was analyzed using GC-FID analysis according to USEPA 8015C (USEPA, 2007). After the analysis, Chemstation software was used to integrate the results with Acuu Standard Calibration standards. Calculation of the final result is obtained by relating the absorbance to the standard calibration curve plotted. PAHs were done by GC-MS analysis according to US-EPA method 8100.

RESULTS AND DISCUSSION

Table 1: Result of 7 Days Monitoring of Contaminated Soil after Application of 6kg of Nutrient

Organic Pollutants	Ebubu(Station1	Ogale (Station2)	Alode (Station3)
Nitrate	4.56mg/kg	3.73mg/kg	5.85mg/kg
Phosphate	16.50mg/kg	19.20mg/kg	22.95mg/kg
Total Organic content	23.6%	27%	22.5%
Sulphate	4.32mg/kg	5.72mg/kg	3.16mg/kg
pH	5.81	6.22	5.50

Table 1 shows laboratory results of physicochemical parameters of 7 days monitoring of contaminated soil after application of 6kg of organic nutrients in each station, the level of each organic pollutant range as shown for all the stations. The results of each pollutant are plotted against the stations as shown in figures 1 and 2 below.

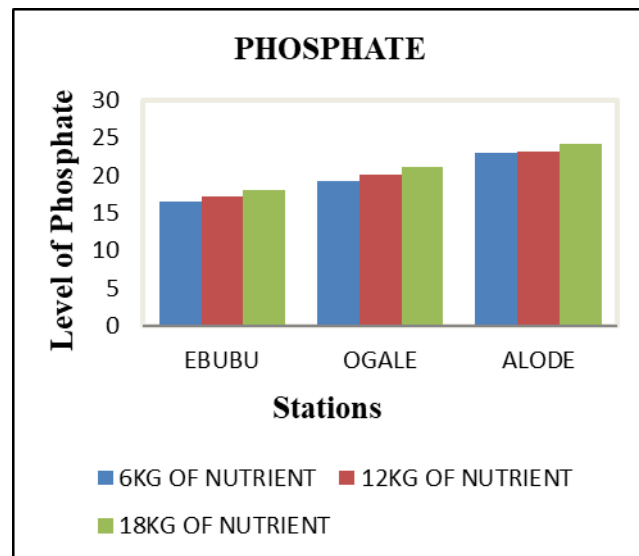
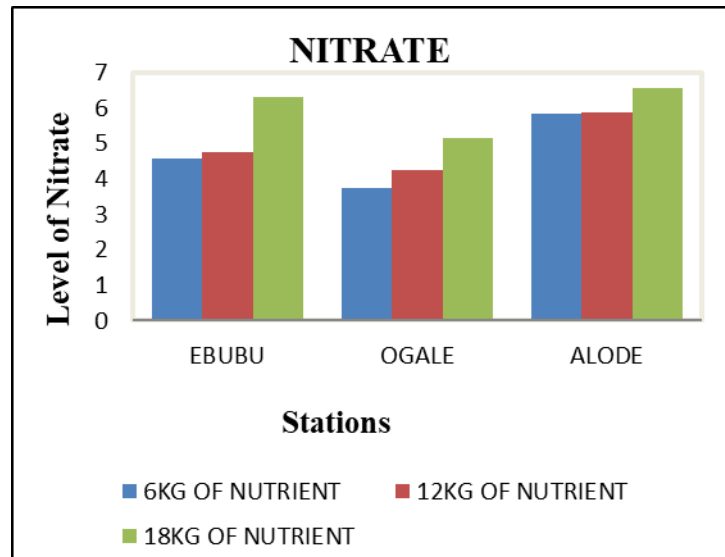


Figure1: Temporal Variation of Nitrate following 21 days of 18kg nutrient application

Figure 2: Temporal variation of phosphate following 21days of 18kg nutrient application

Table 2: Result of 14 Days Monitoring of Contaminated Soil after Application of 12 Kg of Nutrient of (Total Petroleum Hydrocarbon and Polyaromatic Hydrocarbon)

Organic Pollutants	Ebubu (Station 1)	Ogale (Station 2)	Alode (Station 3)
TPH (mg/kg)	ND	1099mg/kg	ND
Acenaphthene (µg/L)	1.20 µg/L	3.21 µg/L	1.40 µg/L
Benzo[b]fluoranthene (µg/L)	0.94 µg/L	ND	ND
Anthracene (µg/L)	0.23 µg/L	1.43 µg/L	-
Benzo[a]pyrene (µg/L)	2.14 µg/L	-	-
Phenanthrene (µg/L)	0.42 µg/L	1.31 µg/L	0.24 µg/L

Table 2 shows the changes in the pollutants after 14 days application of 12kg of nutrient to total petroleum hydrocarbon (TPH) and polyaromatic hydrocarbon (PAHs). The pollutants results for 7, 14 and 21 days are plotted against the stations in figures 3 and 4 below.

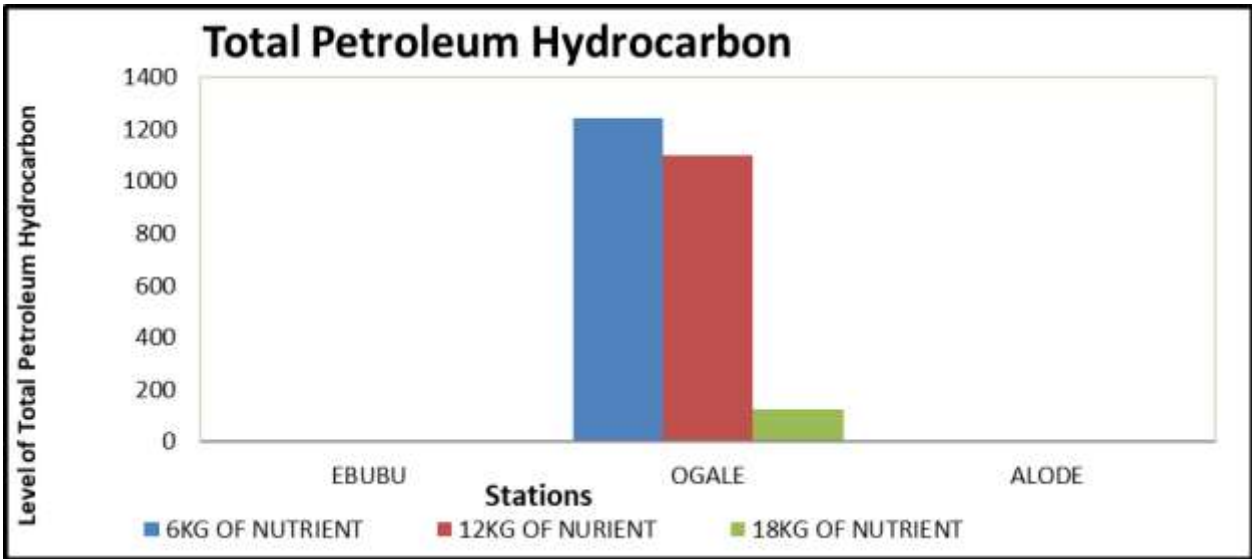


Figure 3: Temporal variation of TPH following 21 days of applying 18kg of nutrient.

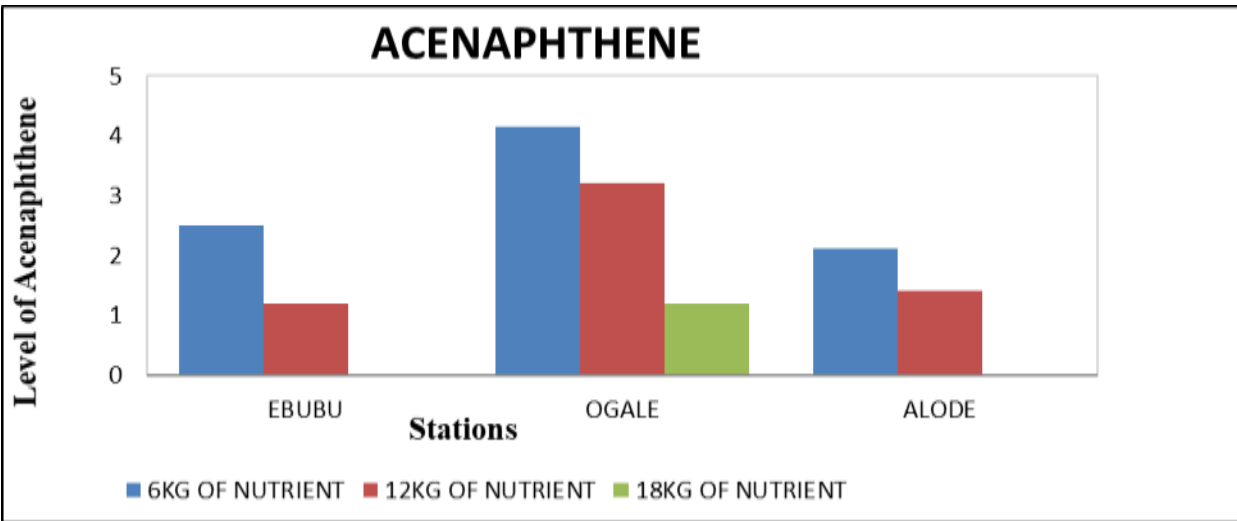


Figure 4: Temporal variation of Acenaphthene following 21 Days of Applying 18kg of Nutrient

Table 3: Result of 21 Days Monitoring of Contaminated Soil after Application of 18 Kg of Nutrient of heavy metals

Organic Pollutants	Ebubu (Station 1)	Ogale (Station 2)	Alode (Station 3)
Copper	<0.001mg/kg	<0.001mg/kg	<0.001mg/kg
Zinc	1.00 mg/kg	<1.00 mg/kg	<1.00 mg/kg
Iron	<0.001 mg/kg	<0.001mg/kg	<0.001mg/kg
Chromium	0.0111 mg/kg	0.0100 mg/kg	0.0102 mg/kg
Manganese	<0.001mg/kg	<0.001 mg/kg	<0.001mg/kg

Table 3 shows the result of heavy metal after 21 days application of 18kg of nutrient. Values of organic pollutants are plotted against the station as shown in figure 5 and 6 below.

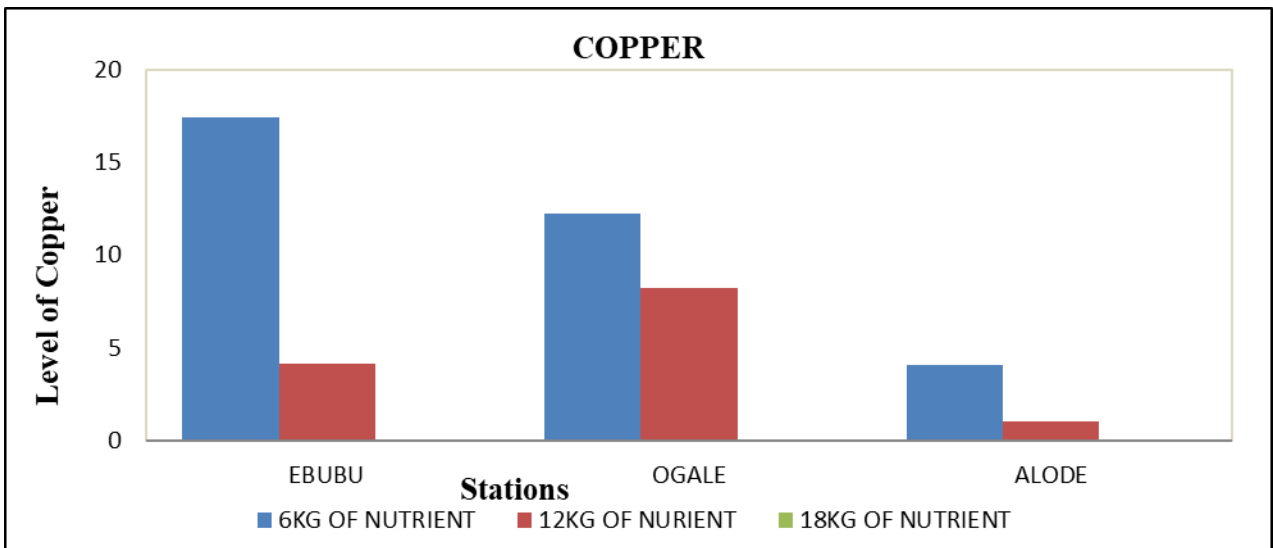


Figure 5: Temporal Variation of Copper after 21 days of 18kg Nutrient Application

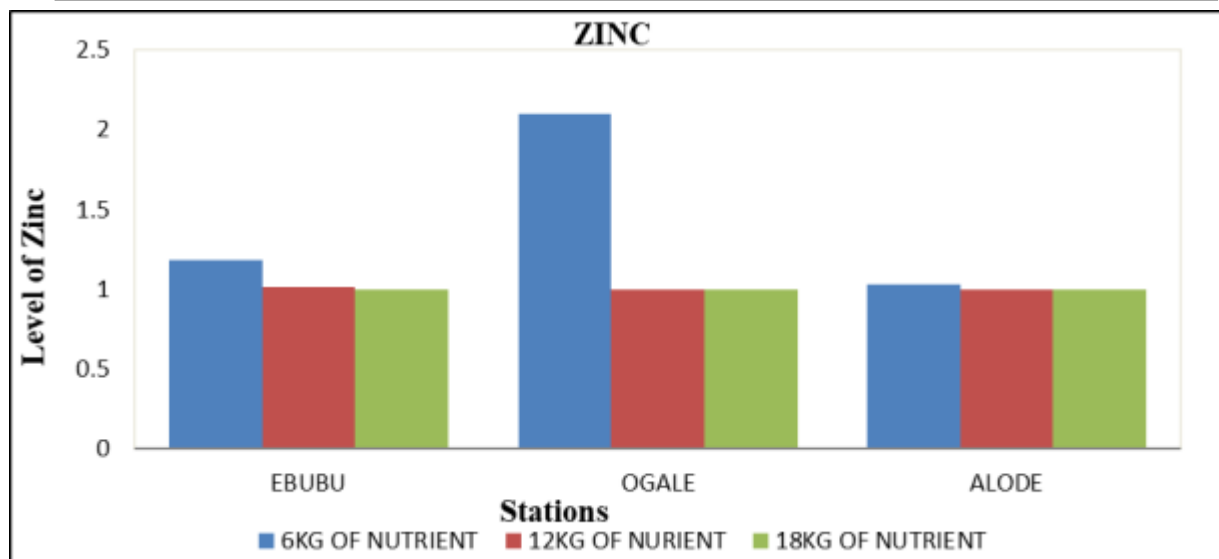


Figure 6: Temporal Variation of Zinc after 21 days of 18kg Nutrient Application

Table 4: Spatial Variation of Microorganisms after Bioremediation

Organic Pollutants	Ebubu (Station 1)	Ogale (Station 2)	Alode (Station 3)	P-value
Total Bacteria Count (kcfu/g)	1.5 - 2.9	1.3– 2.1	1.6– 2.3	0.76460
Hydrocarbon Utilizing Bacteria (kcfu/g)	4.0– 5.2	5.0– 5.4	4.1– 4.7	0.18936

Following the intensive treatment of soil, total Bacteria Count and hydrocarbon utilizing bacteria strains were examined. Station 1, total bacteria count (TBC) ranged from 1.5-2.9 kcfu/g to with a baseline bacteria count of 1.3kcfu/g respectively. In station 2, total bacteria count (TBC) increased as 1.3-2.1 kcfu/g with a baseline bacteria count of 1.1 kcfu/g respectively. In station 3, total bacteria count (TBC) ranged as 1.6- 2.3kcfu/g with a baseline bacteria count of 1.4 kcfu/g also. No notable variation ($P>0.05$) between TBC average figures and locations examined.

Location 1, hydrocarbon utilizing bacteria (HUB) ranged from 4.0- 6.0 kcfu/g with a baseline count of 3.0 kcfu/g respectively. In station 2, HUB ranged from 5.0-5.4 kcfu/g and baseline of 3.5 kcfu/g respectively. In station 3, HUB ranged from 4.1-4.7 kcfu/g with a baseline count of 3.1 kcfu/g respectively. No notable variation ($P>0.05$) between the mean values of HUB average figures and the locations examined.

CONCLUSION

Poultry droppings and Cow dung can efficiently give the essential nutrients to start bioremediation procedure which help to rectify the polluted land to its normal state.

This work has proved that mixture of this organic nutrient to a larger degree can decontaminate petroleum polluted soil since complete rectification of about 89% was achieved judging from the final result on Table 3 above.

ACKNOWLEDGEMENT

Special thanks to BGI laboratories Limited management for their support with the laboratory work.

REFERENCES

- Abii, T. A. and Nwosu, P. C. (2009). “The effects of oil spillage on the soil of Eleme in Rivers State of the Niger Delta Area of Nigerian”, Res J. Environ, pp. 316-320.
- Agbogidi, O. M. and Egbuchua, C.O. (2010). Heavy metal concentrations in soil contaminated with spent engine oil in Asaba, Delta state, Nigeria. Acta Agron. Niger, 10, pp. 65-69.
- Al-Turki, A. (2007). Antibacterial effect of thyme, peppermint, sage, black pepper and garlic hydrolysis against *Bacillus subtilis* and *Salmonella enteritidis*. J. Food Agric. Environ. 5, 92-94.
- Al-Turki, A.I. (2009). Microbial Polycyclic Aromatic Hydrocarbons Degradation in Soil. Research Journal of Environmental Toxicology, 3: 1-8.
- Colberg, P. J. and Young, L.Y. (1985). Aromatic and volatile acid intermediates observed during anaerobic metabolism of lignin-derived. Oligomers. Applied Environ. Microbiol. 49, 350-358.
- El-Hadidi, Y.M. and Al-Turki, A.I. (2007).” Organic fertilizer and biogas production from poultry wastes”, J. Food. Agric. Environ., 5, pp.228-233.
- Ekanem, S. A., Ejue, B. J., Amimi, P.B. and Adalikwu, R.A. (2010). Living with oil: Towards an ethics of the environment in the Niger Delta. Afr. Res. Rev.4, pp.17-30.
- Eneh, O. C. (2011). “A review on petroleum: Source, uses, processing, products and the environment”, J. Applied Sci. 11, pp.2084-2091.
- Folch, A., Vilaplana, M., Amado, L., Vincent R, and Caminal, G. (2013). “Fungal permeable reactive barrier to remediate groundwater in an artificial aquifer”, J. Hazard Mater 262, pp.554–560.
- Frasconi, D., Zanolli, G. and Danko, A.S. (2015). In situ aerobic co-metabolism of chlorinated solvents: a review J. Hazard Mater 283, pp.382–399.
- Idodo-Umeh, G. and Ogbeibu, A. E. (2010). “Bioaccumulation of the heavy metals in cassava tubers and plantain fruits grown in soils impacted with petroleum and non-petroleum activities”, Res. J. Environ. Sci. 4, pp.33-41.
- Jimoh, W. L. O. and Imam M. (2011). Analysis of Iron and Zinc in Soil and Spinach grown in irrigated farmland of Kaduna metropolis Nigeria. International Journal of Research in Chemistry and Environment 1(2), pp.141-146.
- Kelley, I., J. P., Freeman, F. E., Evans, C. E. and Cerniglia, (1993). Identification of metabolites from the degradation of fluoranthene by *Mycobacterium* sp. Strain PYR-1. Applied Environ. Microbiol 59, pp.800-806.
- Khamehchiyan, M. et al. (2007). Effects of crude oil contamination on geotechnical properties of clay and sandy soils. Eng. Geol. 89, pp.220-229.
- Kim, S., Krajmalnik-Brown, R., Kim, J. O. and Chung, J. (2014). Remediation of petroleum hydrocarbon-contaminated sites by DNA diagnosis-based bioslurping technology. Sci Total Environ. 497, pp.250–259.
- Lei, A. P., Wong, Y.S. and Tam, N. F. (2003). Pyrene-induced changes of glutathione-S-transferase activities in different micro algal species. Chemosphere 50, pp.293-301.
- Ohanmu, E., S. Bako and M. Adelanwa, (2017): Effect of Oil Spillage on the Productivity of Pepper (*Capsicum* spp.). Lambert Academic Publishing, Germany.
- Philp, J. C., and Atlas, R. M. (2005). Bioremediation of contaminated soils and aquifers. In Bioremediation: Applied microbial solutions for real-world environmental cleanup. American Society for Microbiology (ASM) Press, pp.139–236. [Google Scholar
- Ugbomeh, B. A. and Atubi, A.O. (2010): The role of the oil industry and the Nigerian state in defining the future of the Niger delta region of Nigeria. Afr. Res. Rev. 4, pp.103-112.
- Roy, M., Giri, A. K., Dutta, S. and Mukherjee, P. (2015). Integrated phytobial remediation for sustainable management of arsenic in soil and water. Environ Int.; 75, pp.180–198.
- Whyte, L. G., Greer C. W. and Inniss, W. E. (1996). Assessment of the biodegradation potential of psychrotrophic microorganisms. Can. J. Microbiol. 42, pp.99-106.
- Zhao, Y., E. Hu, Y. Feng, J. Nai, D. Hu and Lou, X. (2018). Energy Environ. Sci.11, pp.8