EXACT APPROACH TO BIOSTIMULATION OF SOIL CONTAMINATED WITH SPENT MOTOR OIL USING COW DUNG AND POULTRY LITTER IN LAND FARMING MICROCOSM

***Abdulsalam, S., Muhammad, I. M. and Atiku, Y. M**.
Department of Chemical Engineering, Abubakar Tafawa Balewa University Bauchi, Nigeria
*Corresponding Author; email: surajudeen_abdulsalam@yahoo.com

ABSTRACT

Bio-stimulation of hydrocarbon contaminated soil using organic stimulants are usually based on proportion of contaminated soil rather than the concentration of contaminants (exact approach) and most studies carried out on bioremediation of hydrocarbon contaminated soil were based on basic proof of concept and not geared towards development of processes that could lead to development of a realistic large scale treatment technology. In view of the aforementioned, a study was carried out on bio-stimulation of soil contaminated with spent motor oil using cow dung and poultry litter as bio-stimulants employing the exact approach in land-farming microcosms adopting the Box-Behnken design of experiment and based on the design of experiment fourteen microcosms labeled S1 to S13 and a control labeled S14 were investigated. The oil and grease content (O&G) and the total heterotrophic bacteria count (THBC) were used to assess the extent of bioremediation. After eight (8) weeks of bioremediation, the efficiency of degradation in all microcosms (S1 to S14) varied from 68 - 90% except the control (S14) with 35%. S1 showed the highest response to bioremediation of 90% and bio-stimulant efficiency of 61%. The microbial counts increased in the first three weeks (1.18×10^7 – 1.02×10^9) corresponding to the period of fast removal of O&G in all microcosms. Based on the percentage removal of the O&G content (90%), the exact approach showed a great potential for the remediation of spent motor oil contaminated soil at contaminant load range of 6.2 – 12.5% and carbon-nitrogen molar ratio (C:N) chosen (15:1 – 10:1).

Keywords: *biodegradation, bioremediation, carbon-nitrogen ratio, organic stimulants, and oil and grease content.*

INTRODUCTION

Petroleum based products are the major source of energy for our vehicles, industry and daily life. Due to its importance as energy source, it is prone to accidental spill during exploration, production, refining, transport and storage, which is detrimental to public health and environmental safety. Therefore, there is urgent need for the restoration or remediation of hydrocarbon contaminated sites. Global production of crude oil is estimated at more than twelve million metric tons annually, it has been reported that 1.7 to 1.8 million metric tons of petroleum hydrocarbon escapes into the soil and water bodies yearly (Agamuthu and Dadrasnia, 2013). Environment free of pollutants should be the concern of every individual but with industrialization and urbanization, it is difficult to achieve (Abdulsalam *et al*., 2016). Therefore, a form of cleaning technology is inevitable to reduce the concentration of environmental pollutants to acceptable limit.

Spent motor oil is the brown-to black oily liquid removed from a motor vehicle, when the oil is changed, spent motor oil is similar to unused oil, except that it contains additional chemicals that are produced or build up in the oil, when it is used as an engine lubricant at high temperatures and pressures, as it runs due to engine wears and intrusion of dirt. Once this oil get into the soil, the soil is contaminated and therefore, altering its integrity and hence, some form of restoration becomes imperative.

The physicochemical technologies are being used to clean up contamination in the environment (Faisal *et al*., 2004), but such technologies are expensive, not environmentally friendly, destroy soil texture and characteristics and do not always lead to the complete neutralization of contaminants (Abdulsalam *et al*., 2011). Bioremediation, a treatment based on the use of microorganisms can be used to get rid (or reduced to acceptable limits) of environmental pollutants at low cost because of its simplicity in technology, environmental friendliness, and conservation of soil texture and characteristics. Bioremediation can be applied either by stimulating microorganisms present at site called biostimulation which is believed to be more economically or by the addition of genetically grown

microorganisms known as bioaugmentation (Abdulsalam *et al*., 2012). Many research works have been carried out on spent motor oil contaminated soil using biostimulation approach employing either organic or inorganic stimulants but the former has received more patronage in recent time because of its relatively low cost and environmental safety (Abioye *et al*., 2012; Adekunle, 2011).

During the process of bioremediation, which involves the activity of microorganisms to remove pollutants, environmental parameters such as temperature, pH, oxygen and moisture content, are optimized to achieve accelerated biodegradation (Adams *et al.*, 2015). A lot of work has been reported on biostimulation of hydrocarbon contaminated soils using organic wastes as stimulants but application of these stimulants are usually based on proportion of contaminated soil rather than the concentration of contaminants in the soil (exact approach). In view of the aforementioned, a study was conducted on the use of mixture of cow dung and poultry litter blended together for biostimulation of soil contaminated with spent motor oil in landfarming microcosms employing the exact approach.

MATERIALS AND METHOD

Sample Collection

Top soil (0 – 20 cm) contaminated with spent motor oil was collected from Bappah Master Auto-mechanic Workshop located along Railway Road Bauchi, Bauchi State in a polythene bags and kept at the Biochemical Engineering Laboratory, Department of Chemical Engineering, Abubakar Tafawa Balewa University, Bauchi. The soil prior to microbial analysis was kept at 4°C in a refrigerator. The stimulants: cow dung and poultry litter were collected from Bappah Poultry Farm located at old G.R.A Bauchi, and cattle settlement along Gubi Dam Bauchi, Bauchi State Nigeria respectively.

Methods

The contaminated soil sample was subjected to physicochemical and microbial analyses. The soil texture was determined according to method of Day (1953), pH was determined according to method of Bates (1954), organic carbon was determined according method of Walkley and Black (1934), bulk density, particle density and porosity were determined according to method of Brandy and Weil (1999), oil and grease (O&G) was determined according to method of Chang (1998), total organic content was determined according to method of Camobre *et al*. (1996) and total heterotrophic bacteria counts (THBC) was determined according to method of John (1982). All analyses were carried out in triplicate. Details of methods for determining O&G and THBC are described as follows:

Determination of oil and grease content

Five grams (5 g) each of soil sample was weighed on an electronic weighing balance and transferred into a test tube, 5 ml of n-hexane was then added. The sample mixture was shaken vigorously for 5 min, after settling, the solvent and extract was decanted into a pre-weighed 50 ml beaker. This procedure was repeated three times to bring the total solvent volume to 20 ml, the extract and the solvent obtained was evaporated on a heating mantle. The O&G residue, which is the extract, was allowed to cool and weighed on a sensitive balance to four decimal places (Chang, 1998). Results obtained was presented in mg/kg or ppm using Eq. (1):

$$O\&G(ppm\ or\ mgkg^{-1}) = \left(\frac{Weigth\ of\ oil\ in\ soil\ sample(g)}{Weigth\ of\ soil\ sample\ taken\ (g)} \times 10^{6}\right) \quad \ldots \quad (1)$$

Percentage (%) degradation (D) was calculated using the modified form of equation in Samuel (2013):

$$D = \frac{O\&G_i - O\&G_r}{O\&G_i} \times 100 \quad \ldots \quad (2)$$

where $O\&G_i$ and $O\&G_r$ are the initial and residual O&G concentrations respectively.

More so, the percentage (%) biostimulant efficiency (B.E) was calculated at the end of day-56 using modified form of equation in Samuel (2013) presented in Eq. (3).

$$\%\ B.E = \frac{O\&G_s - O\&G_u}{O\&G_s} \times 100 \quad \ldots \quad (3)$$

where $O\&G_u$ is the removal of spent motor oil in the unamended soil, and $O\&G_s$ is the removal of spent motor oil in the amended soil.

Determination of total heterotrophic bacterial count

The enumeration of the total heterotrophic bacterial count in the microcosms were determined using standard plate counting technique. One gram (1 g) of the content in S1 was placed in a test tube and diluted

to 10 fold dilution: dilutions 10^{-4}, 10^{-5} and 10^{-6} were used and later changed to 10^{-5}, 10^{-6} and 10^{-7} past week "3". Zero point one milliliter (0.1 ml) of inoculum each from each of the selected dilutions was pipetted and plated using nutrient agar (NA) and incubated at (34±2℃) for 24 h. Plate with growth within the range of 30 – 300 colonies were counted (John, 1982). The THBC was calculated using Eq. (4).

$$THBC\ (cfu/g) = \frac{number\ of\ colonies \times reciprocal\ of\ the\ dilution\ factor}{weight\ of\ contaminated\ soil} \quad (4)$$

Experimental Design and Treatment

The Box-Behnken design of experiment was employed: three independent variables or factors at three levels (-1, 0, +1) were considered. The dependent variables are oil and grease content (O&G) and total heterotrophic bacteria counts (THBC). The values and levels of the independent variables are presented in Table 1 and coded Box Behnken design for the three independent variables and their responses are presented in Table 2.

Table 1: Experimental Factors and Levels of Variables

Factor	Level		
	Low (-1)	Average (0)	High (+1)
Cow dung (C: N)	7.5:1	6.25:1	5:1
Poultry litter (C: N)	7.5:1	6.25:1	5:1
Moisture content (%)	20	25	30

The contents in Table 2 were based on calculated amounts of various parameters in Table 1 taking 5 kg of contaminated soil sample as basis.

Table 2: Coded and Actual Values of Box Behnken Design of Experiment for the Three Independent Variables Using the Exact Approach

Run	Cow Dung (kg)	Poultry Litter (kg)	Moisture Content (kg)
1	-1 (1.95)	-1 (0.98)	0 (1.98)
2	+1 (2.93)	-1 (0.98)	0 (2.23)
3	-1 (1.95)	+1 (1.47)	0 (2.11)
4	+1 (2.93)	+1 (1.47)	0 (2.35)
5	-1 (1.95)	0 (1.18)	-1 (1.63)
6	+1 (2.93)	0 (1.18)	-1 (1.82)
7	-1 (1.95)	0 (1.18)	+1 (2.44)
8	+1 (2.93)	0 (1.18)	+1 (2.73)
9	0 (2.36)	-1 (0.98)	-1 (1.66)
10	0 (2.36)	+1 (1.47)	-1 (1.77)
11	0 (2.36)	-1 (0.98)	+1 (2.50)
12	0 (2.36)	+1 (1.47)	+1 (2.65)
13	0 (2.36)	0 (1.18)	0 (2.14)
14	-	-	-

Bioremediation Experiment

Five kilograms (5 kg) of contaminated soil sample sieved and homogenized using 2 mm sieve were transferred into a container, calculated amounts of blends of cow dung and poultry litter based on their nitrogen contents and organic carbon content in the contaminated soil as suggested by Box Behnken design of experiments were used to attain the desired carbon to nitrogen molar ratio (C:N). The calculated amounts of cow dung and poultry litter were added to the container and mixed thoroughly. The well mixed content of the container was then transferred into the microcosm labelled S1 (Figure 1). The above procedure was repeated for the remaining twelve microcosms (S2 – S13). S14 was the control, therefore, no stimulant was added. Periodic sampling of content in each of the microcosms was performed on weekly basis for the eight weeks of study. Parameters determined were the oil and grease content (O&G), total heterotrophic bacterial counts and the pH regime. In addition, the moisture content in each microcosm was determined on 2-weekly basis and where the moisture content in any of the microcosms fell below the initial amount as presented

in Table 2, such value was adjusted to its initial value using distilled water.

Figure 1: Microcosms stacked with various treatments described in Table 2

RESULTS AND DISCUSSION

Physicochemical Properties of Soil and Organic Wastes

The physicochemical properties of the spent motor oil contaminated soil and that of the stimulants used for bioremediation are presented in Table 3. The high value of total organic carbon (8.19±0.31%) and O&G content (188 273.33±253.25 ppm) was indication that the soil was highly contaminated with spent motor oil. The O&G content was 377 folds greater than the safe limit of 500 mg/kg set by the Nigerian Federal Ministry of Environment (Abdulsalam, 2011), hence the need for the restoration of the contaminated soil for environmental safety and public health in general.

The soil pH (6.83±0.05) was within the acceptable limit of 5.5 – 8.5 for effective bioremediation (Vidali, 2001). The soil moisture content (1.700±1.015 %) was out of the range of 12 – 25% required for optimum growth and proliferation of microbes (Adams *et al*., 2015), hence there was need to argument the moisture content to acceptable limits.

The nitrogen content of the poultry and cow dung were found to be 1.4±0.00% and 0.7±0.00% respectively (Table 3). These nitrogen contents were used to obtain the exact amounts of cow dung and poultry litter added to the contaminated soil to attain the desired C:N for all the microcosms.

Table 3: Physicochemical Properties of the Samples

Parameter	Value/inference		
	Soil	Poultry litter	Cow dung
Texture	Loamy sand		
Organic Carbon (%)	8.19±0.31		
Moisture (%)	1.70±1.02		
Porosity (%)	47.98±20.14		
Water absorption capacity (%)	38.48±0.56		
Particle density (g/cm^3)	2.00±0.06		
Bulk density (g/cm^3)	1.04±0.37		
Total organic content (%)	26.00±1.36		
Oil and grease (ppm)	188 273.33±253.25		
Organic carbon (%)	8.19±0.31		
pH	6.83±0.05		
Nitrogen (%)		1.4±0.00	0.7±0.00
Phosphorus (ppm)		2396.514±34.014	393.743±93.422

Microbiological Analysis

The THBC in the test soil was found to be $6.2\pm2.52\times10^6$ cfu/g which was above the minimum microbial population of 10^5 required for effective bioremediation (Forsyth *et al.*, 1995). Considering the microbial population in the test soil, biostimulation strategy was the most appropriate option. In addition, the five microbial types identified (Table 4) were hydrocarbon degrading ((Das and Chandran, 2011; Subathra *et al*., 2013; Drzewiecka, 2016).

Table 4: Microbiological analysis of spent motor oil contaminated soil

Parameter	Value/inference
Total heterotrophic bacterial count (cfu/g)	$6.23\pm2.52\times10^6$
Bacterial identity	*pseudomonas* spp, *klebsiella* spp, *bacillus* spp, *micrococcus* spp, *proteus* spp

Bioremediation Experiments

Oil and Grease Content (O&G) for the Microcosms

According to Abdulsalam (2011), the O&G content is one of the best indices usually used to quantify biodegradation of spent motor oil since the C-H bond in the oil is low as a result of its breakdown during usage in motor engine. Hence, the O&G content was used to assess the extents of degradation in this study. The variation in O&G content for all the microcosms with time are presented in Figure 2. All the profiles were characterized with period of fast decreased in O&G contents (week 0 - 4), followed by period of slower activities (past week 4). After week 4, the O&G content started fluctuating with ± (increase/decrease) in the residual O&G contents as shown in Figure 2, this could be attributed to uneven distribution of nutrients in the microcosms. In week 4 the residual O&G contents for S1 – S14 were 14 000.0, 14 666.7, 24 000.0, 20 000.0, 29 333.3, 14 000.0, 19 333.3, 16 000.0, 24 000.0, 18 000.0, 17 333.3, 13 333.3, 36 000.0 and 83 333.3 ppm respectively corresponding to 85, 79, 74, 69, 53, 81, 78, 71, 63, 68, 65, 86, 48 and 33% biodegradation. At the end of the 56 day, the biodegradation efficiency varied from 68 – 90% except the control (S14) with 35%. In addition, bio-stimulant efficiency (B.E) varied from 48 – 61%. The control had 0% B.E since it is on its basis the biostimulants efficiency is calculated.

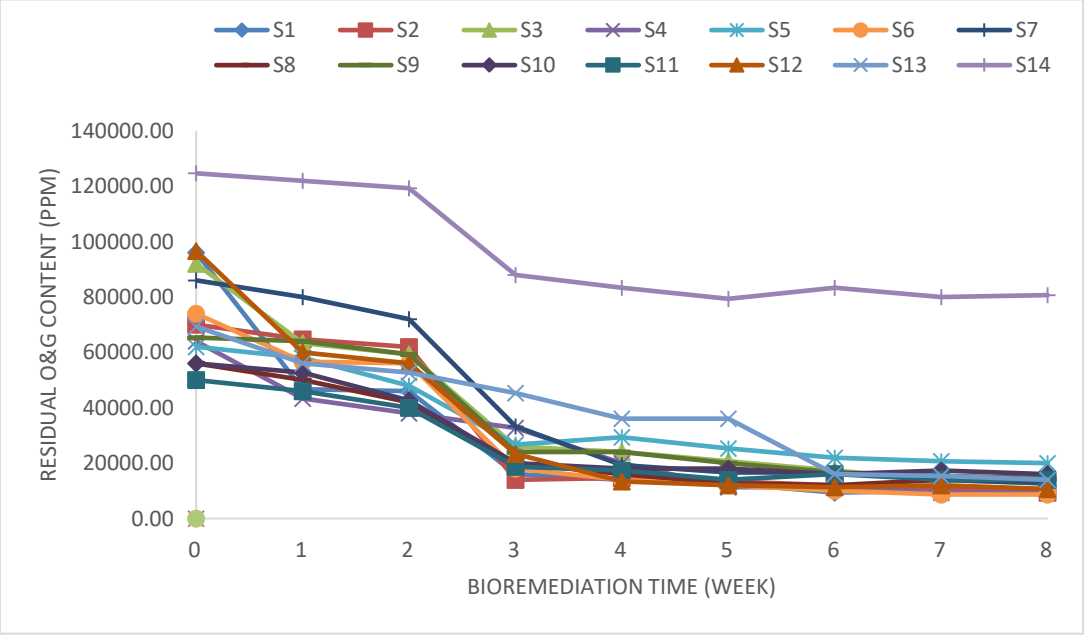

Figure 2: Variation in oil and grease content with bioremediation time

From the percentage oil and grease degradations and biostimulant efficiencies, it could be deduced that the indigenous microorganisms were able to utilize the O&G content in the spent motor oil contaminated soil as their source of carbon and energy thereby leading

to biodegradation of the oil content. This observation is in line with the findings of Samuel (2013) and Ajao *et al.* (2011).

The bioremediation and bio-stimulant efficiencies obtained in this study were superior to most of the previous studies that employed the proportion of contaminant approach: Adekunle (2011) obtained 40% degradation on soil artificially contaminated with spent motor oil at contaminant load of 5 – 12%w/w and 12%w/w organic compost over a period of 21 days. The amount of organic compost added was 0.12 weight stimulant per weight contaminated soil. In addition, Agarry and Ogunleye (2012) carried out research on bio-stimulation of spent motor oil contaminated soil using poultry manure as stimulant at contaminant load of 10%w/w over a period of forty-two (42) days. Results showed that 67.76% degradation was achieved and the amount of manure added was 0.24 weight stimulant per weight contaminated soil.

The stimulant amount used in this study ranged from 0.586 to 0.88 weight stimulant per weight contaminated soil. This stimulant range was obtained by using the carbon to nitrogen molar ratio in the range 15:1 – 10:1. From the summary of the two previous studies above, the amount of organic stimulants applied were low, although, the nitrogen content in different organic wastes are varies but using the exact approach (or C:N approach) and taking appropriate carbon-nitrogen molar ratio, the exact amount of organic stimulant required for effective degradation can be computed as was done in this study.

pH and Microbial population in microcosms

The pH values in all the microcosms ranged from 6.90±0.00 to 8.80±0.00 for the fifty-six days of bioremediation. These pH values were within the range required for effective bioremediation. Therefore, pH was not a limiting factor in this study (Vidali, 2001).

The variation in THBC and bioremediation time is presented in Figure 3. From this figure, it could be observed that the microbial growth profiles obtained for all microcosms followed typical microbial growth pattern depicting the lag, exponential, stationary and death phases (Bailey and Ollis, 1977; Abdulsalam, 2011). It could also be observed that there were fluctuations in the growth phases past week 4 which could be attributed to non-uniformity in mixing leading to un-even distribution of nutrients within the microcosms. Hence, un-even utilization of available nutrients by microorganisms, consequently the un-even growth pattern observed. This observation is in line with the finding of Samuel (2013).

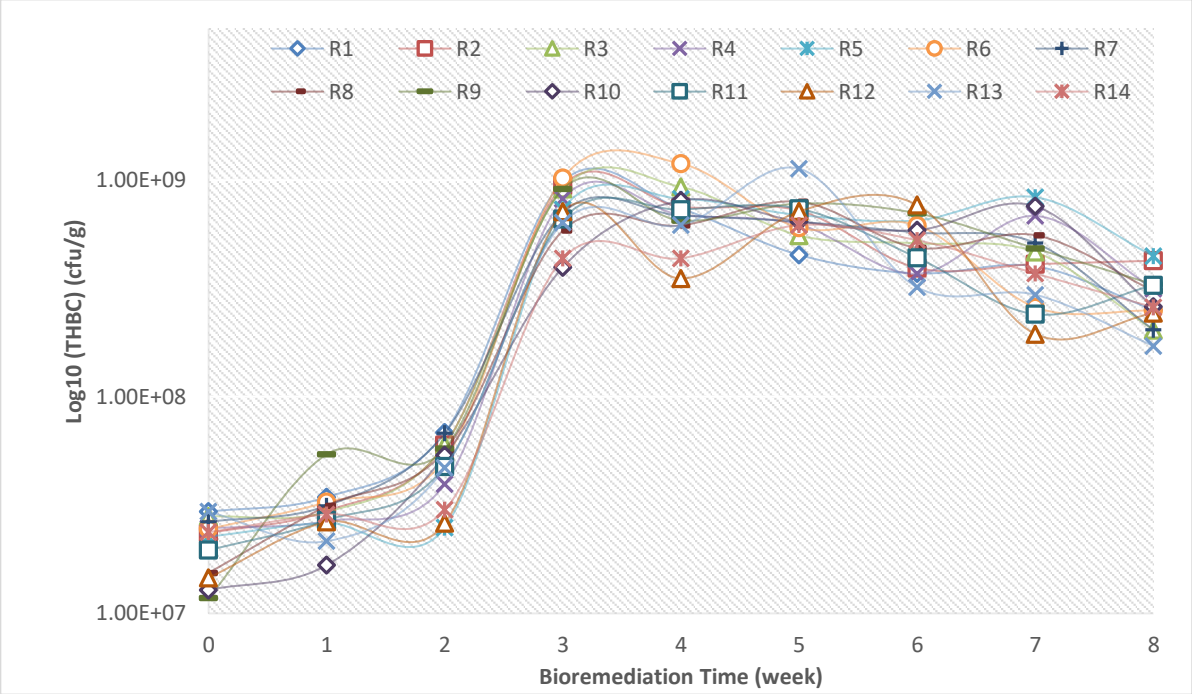

Figure 3: Variation in THBC with bioremediation time

In all the microcosms, a similar trend of lag phase or period of adaptation of microorganisms which lasted

for two weeks (week 0 – 2), followed by one week of exponential growth (between week 2 and 3) which corresponds to period of maximum microbial growth in all microcosms. The THBC for all the microcosms in week 4 (period of maximum microbial growth) ranged from 3.51×10^7 – 1.19×10^8 cfu/g, with S6 having the highest microbial growth. This period of maximum microbial growth corresponds with the period of fast removals of the O&G contents in all microcosms. Hence, it is clear that the microbial species present in the microcosms: *pseudomonas* spp, *klebsiella* spp, *bacillus* spp, *micrococcus* spp, *proteus* spp utilized the oil and grease contents contained in spent motor oil contaminated soil effectively as their source of carbon and energy. This observation agrees with the findings of Kamaluddeen *et al*. (2016).

From the aforementioned, judging from the results of O&G contents and THBC in all the microcosms, percent degradation could be improved by addition of more nutrients (i.e. two stages application of nutrient) since the activities of degradation past week "4" were very sluggish due to the slow release nature of organic wastes or stimulants. This was attested to by the profiles of O&G content attaining plateau (Figure 1) and the fact that there were no significant difference in biodegradation at past week "6" at 10% confidence level. All factors required for effective bioremediation were still present at the end of the eighth week: microbial populations (1.72×10^8 – 4.46×10^8 cfu/g), pH regime (7.04 – 7.30), residual O&G content (8 666.67 – 80 666.67 ppm) but nutrient was a limiting factor. Therefore, addition of more nutrients could drive the reaction forward and improve percent degradation.

CONCLUSIONS

Bioremediation of soil contaminated with spent motor oil contaminated soil was conducted using cow dung and poultry litter as organic stimulants at a soil contaminant load of 6.2 - 12.5%w/w and carbon to nitrogen molar ratio in the range of 15:1 – 10:1. Microbiological analyses of the test soil revealed that indigenous microorganisms were present in required quantity ($>10^5$ cfu/g) and type (*pseudomonas* spp, *klebsiella* spp, *bacillus* spp, *micrococcus* spp, *proteus* spp). Bioremediation studies using the Box Behnken designed of experiment revealed that the available microorganisms in all the microcosms were able to use the oil and grease content in spent motor oil contaminated soil as their sole source of carbon and energy. The biodegradation efficiency ranged from 35 – 90% and biostimulant efficiency varied from 48 – 61%. Therefore, the exact approach is an effective technique of remediating spent motor oil contaminated soil.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of Tertiary Education Trust Fund, Institutional Based Research (IBR) grant TETFUND/DESS/ATBU/BAUCHI/RP/VOL. V. Also, they would like to thank the management of Abubakar Tafawa Balewa University Bauchi-Nigeria for providing other necessary supports.

REFERENCES

Abdulsalam, Surajudeen (2011). Bioremediation of Soil Contaminated with Used Motor Oil; Concept, Process Development and Mathematical Modeling, LAP Lambert Academic Publishing GmbH & Co. KG Germany, ISBN: 978-3-8443-1234-8.

Abdulsalam, S., Adefilia, S.S., Bugaje, I.M., and Ibrahim, S., (2012). Bioremediation of soil contaminated with used motor oil in closed system .*J Bioremed, **4**(172):2155-6199.*

Abdulsalam, S. Adefia, S. S. Bugaje, I. M. and Ibrahim, S., (2011). Comparison of biostimulation and bioaugmentation for remediation of soil contaminated with spent motor oil, *Int. J. Environ. Sci. Tech.*, **8** (1): 187–194.

Abdulsalam, S., (2012). Mathematical Modeling of Bioremediation of Soil Contaminated With Spent Motor Oil. Journal of Emerging Trends Engineering and Applied Science, **3**(4):654-659.

Abdulsalam, S., Sulaiman, A.D.I., Musa, N.M., and Yusuf, M., (2016). Bioremediation of Hydrocarbon and some Heavy Metals polluted Waste Water effluent of a Typical refinery, World Academy of Science, Engineering and Technology International Journal, **10** (2):245-249.

Abdulyekeen, K. A., Muhammad, I.M., Giwa, S.O., Abdulsalam, S. (2016). Bioremediation of Used Motor Oil Contaminated Soil Using Elephant and Horse Dung as Stimulants *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) e-ISSN: 2319-2402, p-ISSN: 2319-2399, **10**(12) 73-78* www.iosrjournals.org DOI: 10.9790/2402-1012027378.

Abioye, O.P., Agamuthu, P., and Abdul-Aziz, A.R. (2012). Biodegradation of Used Motor oil in Soil

using Organic Waste Amendments. Biotechnology Research international, **8**(10):1-8.

Adams, G. O., Fufeyin, P. T., Okoro, S. E. and Ehinomen, I. (2015). Bioremediation, Biostimulation and Bioaugmention: A Review, International Journal of Environmental Bioremediation & Biodegradation, Vol. 3, No. 1, 28-39 Available online at http://pubs.sciepub.com/ijebb/3/1/5 © Science and Education Publishing DOI:10.12691/ijebb-3-1-5

Adekunle, I. M. (2011). Bioremediation of Soils Contaminated with Nigerian Petroleum Products Using Composted Municipal Wastes, *Bioremediation Journal*, 15(4): 230 – 241. ISSN: 1088 – 9868, DOI: 1080/10889868.2011.624137.

Agamuthu, P., and Dadrasnia, A. (2013). Potential of Biowastes to Remediate Diesel Fuel Contaminated Soil, *Global Nest Journal, **15**(4):* 474-484.

Agarry, S. E. and Ogunleye, O. O. (2012), Box-Behnken Design Application to Study Enhanced Bioremediation of Soil Artificially Contaminated with Spent Engine Oil using Biostimulation Strategy, *International Journal of Energy and Environmental Engineering*, Vol. 3, No. 31, pp. 1-14.

Ajao, A.T., Oluwajobi, A.O., and Olatayo V.S., (2011) .Bioremediation of soil microcosm from Auto-mechanic workshops. J. APP. SCI. Environ. Management, **15**(3):473-477, ISSN: 1119-8362.

Bates, B.G., (1954).Electronic pH Determinations John Wiley and Sons, Inc. New York.

Bailey, J. E. and Ollis, D. F. (1977).Biochemical Engineering Fundamentals, International student Edition, Mc Graw- Hill Kogakusha.LTD.Tokyo:335-356.

Brandy, N.C and Weil, R.R., (1999).The Nature and Properties of Soil, 12th Prentice-Hall Inc, U.S.A:145.

Camobreco,V.J., Richard, B.K., Steenhnis, T.S., Peverty, J.H., MC-Bride, M.B., (1996). Movement of Heavy Metals through Undisturbed and Homogenized Soil Columns. Soil Science: An Interdisciplinary Approach to Soil Research ***161**(11):*740-749.

Chang, R. (1998). *Chemistry*, 6th edition. 24: 962-963, McGraw-Hill Companies, Inc.

Das, N. and Chandran (2011), Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview, *Biotechnology Research International*, Volume 2011 (2011), Article ID 941810. Available online @ http://dx.doi.org/10.4061/2011/941810

Drzewiecka, D. (2016). Significance and Roles of *Proteus* spp. Bacteria in Natural Environments, *Microbial Ecology*, Volume 72, Issue 4, pp 741–758.

Faisal, I.K., Tahir, H., and Ramzi, H., (2004).An overview and analysis of Site Remediation Technologies. Journnal of Environmental Management. ***71**(2004):* 95-122.

Forsyth, J. V., Tsao, Y. H. and Bleam, R. D. (1995). *Bioremediation: when is bioaugmentation needed?*, pp. 1-14. *In* R. E. Hinchee, J. Fredrickson and B. C. Alleman (ed.) Bioremediation for site remediation. Battelle Press, Columbus, Ohio.

John, P.E., Anderson (1982) .Soil Respiration In: Page A.L., Miller, R.H., and Keeney D.R. (Ed) Method of Soil Analysis, Part: Chemical and Microbial Properties, 2nd Edition American Society of Agronomy. Inc. Madison, Wisconsin USA: 832-871.

Kamaluddeen, K., Yerima, M.B., Abu, T.R., and Deeni, Y., (2016). Biostimulatory Effect of Processed Sewage Sludge in Bioremediation of Engine Oil Contaminated soils. *International Journal of Scientific and Technology Research, **5**(2):* 203-207, ISSN 2277-8616.

Samuel, E. A. (2013). Application of Carbon-Nitrogen Supplementation from Plant and Animal Sources in In-situ Soil Bioremediation of Diesel Oil: Experimental Analysis and Kinetic Modeling. *Journal of Environment and Earth Science, **3** (7):* 51-61, ISSN 2224-3216.

Subathra, M. K., Immanuel, G. and Suresh, A. H. (2013). Isolation and Identification of hydrocarbon degrading bacteria from Ennore creek, *Bioinformation*. 2013; 9(3): 150–157. doi: 10.6026/97320630009150.

Walkley, A., and Black, I.A., (1934). An Examination of the Degtjarelf Method for Determining Soil Organic Matter and proposed Modification of the Chromic Acid Method. Soil. Sci 87, 29-39.

Vidali, M. (2001). Bioremediation*:* An overview, *Journal of Applied Chemistry*, Vol. 73, No. 7, pp. 1163-1172.