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Enhanced Biodegradation of Spent Lubricating Oil Contaminated Soil Using Poultry Litter

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Authors' contributions

This work was carried out in collaboration between both authors. Author ES designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author OTT managed the literature searches. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Enhanced biodegradation of spent lubricating oil contaminated soil using (40% w/w) poultry litter was studied for a period of 56 days. The bacterial count ranged from 1.7×106 cfu/ml – 4.0×106 cfu/ml for oil free soil (OFS), 1.7×106–9.0×105 for oil polluted soil (OPS) and 1.7×105 – 1.0×106 for Poultry Amended oil polluted soil (PAOPS) while the fungal count ranged from 1.2 x105cfu/ml to 5.0 x 105cfu/ml OFS, 4.0x10 -5.0 x105 for OPS and 1.2x105 - 5.0x 105 for PAOPS. The result revealed higher bacterial counts in poultry litter amended soil (PAOPS) compared to oil polluted soil (OPS) and oil free soil (OFS). The fungi counts were low in all treatments (OFS, OPS and PAOPS) .Ten organisms were isolated in the course of this study. The bacteria were *Bacillus spp*, *Micrococcus spp*, *Pseudomonas spp*, *Proteus* spp and *Staphylococcus spp* while the fungi include yeasts, *Aspergillus niger*, *Aspergillus flavus*, *Mucor Spp, Penicillium spp*. PAOPS had higher nitrate and phosphorus content compared to OFS and OPS. This result indicates that poultry litter could be used as biostimulating agent to enhance the biodegradation of spent lubricating oil contaminated soil.

Keywords: Biodegradation; poultry litter; biostimulating agent; oil free soil.

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1. INTRODUCTION

Soil and water contamination by spent lubricating oil is a common occurrence in most developing countries. This has been shown to have harmful effects on the environment and human beings at large [1].

Contamination of soil by spent lubricating oil is rapidly increasing due to global increase in the usage of petroleum products and has attracted much attention in recent decades [2,3]. The illegal dumping of used motor oil is an environmental hazard with global ramifications [3]. Used motor oil contains metals and heavy polycyclic aromatic hydrocarbons (PAHs) that could contribute to chronic hazards including mutagenicity and carcinogenicity [4].

Lack of essential nutrients such as nitrogen and phosphorus is one of the major factors affecting biodegradation of hydrocarbon by microorganisms in soil and water environment. Therefore, the addition of inorganic or organic nitrogen-rich nutrients (bio stimulation) is an effective approach to enhance bioremediation process. Positive effects of nitrogen amendment on microbial activity and /or petroleum hydrocarbon degradation have been widely demonstrated [5]. Concentration of petroleum hydrocarbon determines to a great extent the rate of breakdown of the hydrocarbons from soil environment. High concentration of hydrocarbon can be inhibitory to microorganisms, and concentration at which inhibition occurs varied with the compound [6]. Rahman et al. [7] reported high degradation of hydrocarbons in soil contaminated with 10% and 20% crude oil compared to those contaminated with 30% and 40% crude oil which experience partial degradation of hydrocarbons within a period of 12 months. The investigators further reported that degradation by mixed bacterial consortium decreased from 78% to 52%, as the concentration of crude oil increased from 1 to 10%.

According to Ijah and Antai [6], poultry litter contains hydrocarbon degrading microbes of the genera *Proteus, Micrococcus, Bacillus, Pseudomonas, Aspergillus and Rhizopus*. The authors also reported that poultry litter enhanced the degradation of crude oil by stimulating the organism present in the soil. Poultry litter is an Agro-based waste rich in nutrient and microorganism, cheap and readily available. Due to the contamination of soil caused by spent lubricating oil, there is the need to reclaim spent lubricating oil using materials that are inexpensive and biodegradable. The significance of this work lies in the use of inexpensive and readily available poultry litter; a biodegradable organic material obtained from poultry farms to enhance the degradation of spent lubricating oil polluted soil.

2. MATERIALS AND METHODS

2.1 Study Area Description

The experiment was conducted in the botanical garden of the Kogi State University, Anyigba, Nigeria. Anyigba lies between latitudes 7º291 North and longitude 7º111 East and falls within the rain forest belt of Nigeria with an annual mean rainfall of about 1600mm. The average temperature of the experimental area is 25ºC [8].

2.2 Sample Collection

Plot measuring 3m by 1m was divided into 3 plots each measuring $1m^2$. The first plot served as control (without lubricating oil and poultry litter). The second contained only spent

lubricating oil while the third contained both spent lubricating oil and poultry litter. Each plot was polluted by 10 litres of spent lubricating oil and amended with 40% (4kg) poultry litter. Sampling was carried out bi weekly for a period of eight weeks.

2.3 Laboratory Statistical Methods

pH of the soil was determined at ambient temperature using glass electrode pH and conductivity meter (Hannia, Italy) in 1:1 water to soil ratio. Nitrogen was determined by the micro Kjedahl method as described by Ibitoye [9]. Phosphorus was determined by the Murphy and Riley [10] method. The ignition method of Akinsanmi [11] was used to determine the organic matter content while the dry weight method of Tropical Development Institute, TDI [12] was used to determine the moisture content. Microbiological analysis was carried out following the procedure described by Harrigan and McCane [13]. Descriptive statistics and analysis of variance (ANOVA) was performed using procedure of SPSS version 16 (2007). Experimental precision achieved was reported at $p \leq 0.05$ level.

3. RESULTS

Fig. 1 shows the total aerobic bacteria counts obtained from spent lubricating oil polluted soil undergoing biodegradation. Linear growth was observed in oil free soil (OFS). Higher bacteria counts were observed in poultry litter amended oil polluted soil (PAOPS) than oil polluted soil (OPS). The highest count was observed in PAOPS at day 14 and declined at day 28 in both PAOPS and OPS. However, the bacteria counts were higher in OPS and PAOPS at day 56. There was no significant difference between OFS, OPS and PAOPS at 5% probability level.

Fig. 2 shows the fungal count in spent lubricating oil polluted soil undergoing biodegradation. The fungi counts ranged from 1.2 $x10^5$cfu/ml to $5.0x10^5$cfu/ml OFS, $4.0x10^4$ -$5.0x10^5$ for OPS and 1.2 $x10^5$ -$5.0x10^5$ for PAOPS. The highest fungi count was observed in OPS at day 14. There was an observed decline in OFS and PAOPS after day 0 which later picked up at day 28. The counts in OFS and PAOPS were higher than OPS at the end of the study (Day 56). There was no significant difference between OFS, OPS and PAOPS at 5% probability level.

Fig. 3 shows the changes in the pH of spent lubricating oil polluted soil undergoing biodegradation. The pH ranges from 5.3 to 7.0. The highest pH was observed in PAOPS while the least pH was observed in OPS. There was an increase in the pH of PAOPS at day 14 compared to OPS and OFS. General increased in pH was observed in the treatments after day 28 till the end of the experiment. There was no significant difference in pH between OFS, OPS and PAOPS at 5% probability level.

The change in nitrate concentration of the spent lubricating oil polluted soil is shown in Fig. 4 higher nitrates concentration was observed in PAOPS and OFS than OPS. Decline in Nitrate level/concentration started at day 0 in OPS which continued until day 28. This trend was also observed in PAOPS and OFS after day 14 but increased from day 28 till the end of the study. There was no significant difference in nitrate concentration between OFS, OPS and PAOPS at 5% probability level.

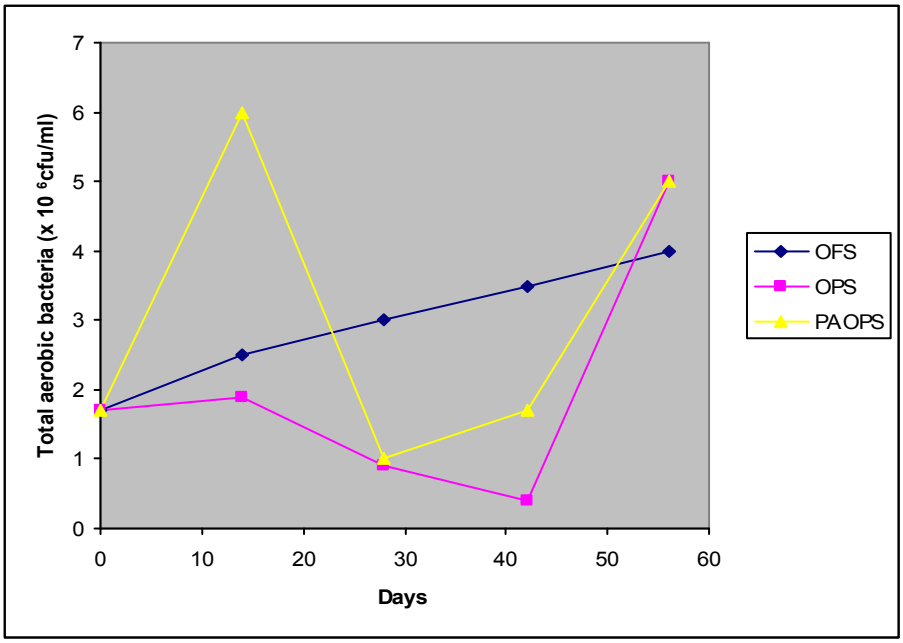

Fig. 1. Total aerobic bacteria in spent lubricating oil polluted soil undergoing biodegradation

OFS: Oil free soil, OPS: Oil polluted soil, PAOPS: Poultry litter amended oil polluted soil

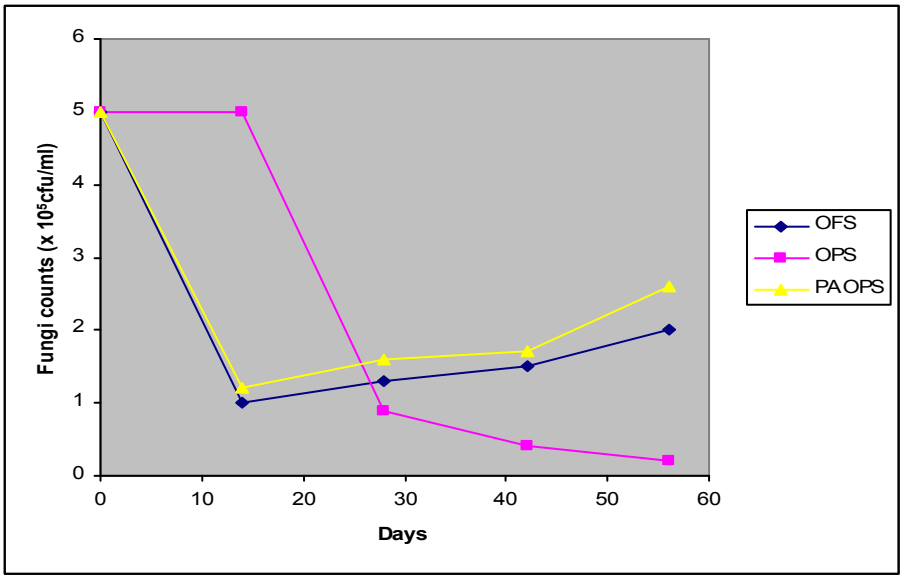

Fig. 2. Fungi counts in spent lubricating oil polluted soil undergoing biodegradation

OFS: oil free soil, OPS: oil polluted soil, PAOPS: poultry litter amended oil polluted soil

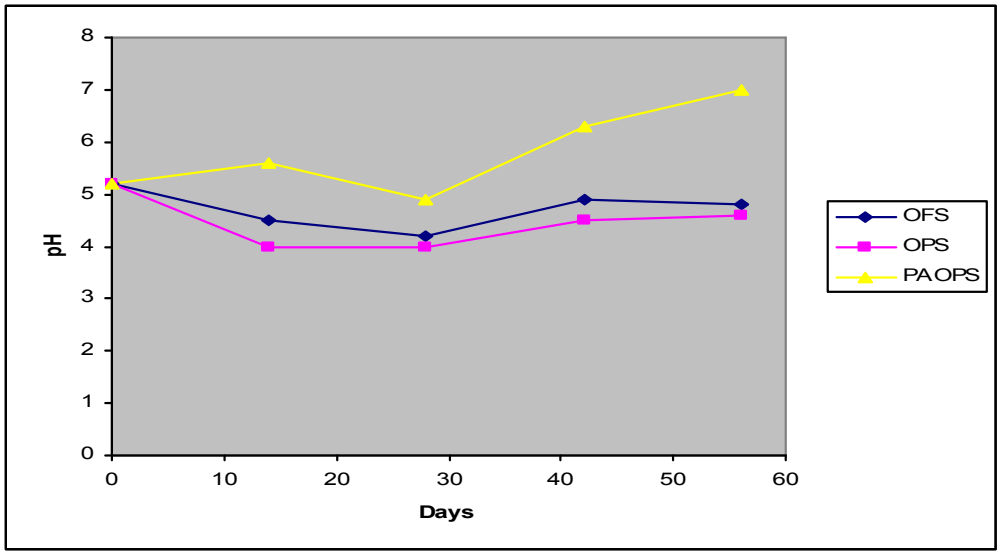

Fig. 3. pH of spent lubricating oil polluted soil undergoing biodegradation
OFS: Oil free soil, OPS: Oil polluted soil, PAOPS: Poultry litter amended oil polluted soil

Fig. 5 shows the phosphorus content on the spent lubricating oil polluted soil undergoing biodegradation. The phosphorus contents were higher in PAOPS and OPS than OFS from day 14 till day 28. However, the phosphorus level declined at day 42 in PAOPS and OPS while an increased was observed in OFS till the end of the study. There was no significant difference in phosphorus content between OFS, OPS and PAOPS at 5% probability level.

Table 1 shows the physicochemical properties of spent lubricating oil polluted soil undergoing biodegradation. The mean moisture content ranged from 9.26±0.46 to 12.00±0.84%. Higher moisture was observed in PAOPS compared to OFS and OPS. There was a significant difference in the moisture content of OFS, OPS and PAOPS at 5% probability level.

The mean organic carbon was low in OFS compared to OPS and PAOPS. It ranged from 1.37±0.15 to 2.65±1.26%. There was no significant difference in the organic carbon between OFS, OPS and PAOPS at 0.05 probability level.

Organic matter content was higher in OFS and PAOPS compared to OPS. The mean organic matter content ranged from 1.90±0.25 to 2.24±0.30 %. There was no significant difference in the organic carbon between OFS, OPS and PAOPS at 0.05 confidence interval. The electrical conductivity, (EC) was low in all treatments throughout the period of study. Higher EC was observed in PAOPS followed by OPS and OFS. There was no significant difference in the electrical conductivity between OFS, OPS and PAOPS at 0.05 confidence interval.

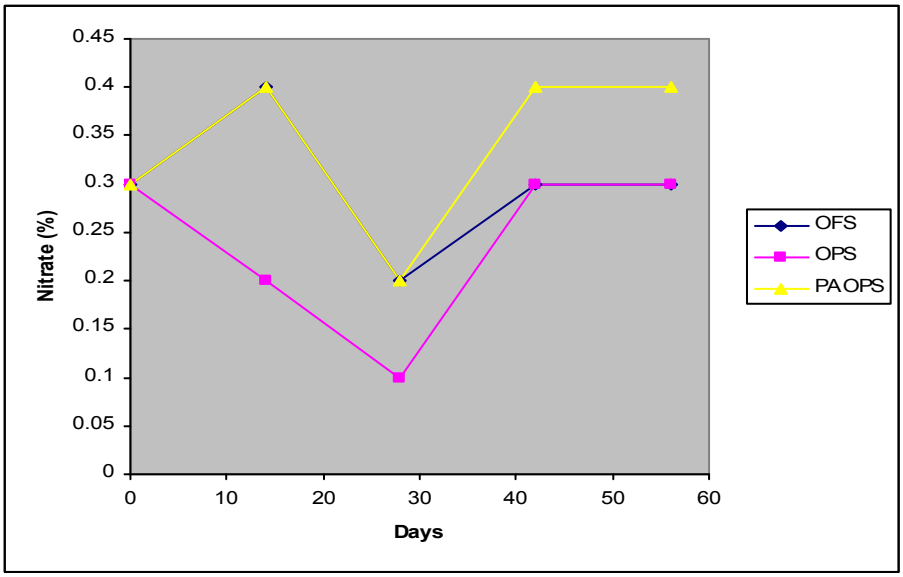

Fig. 4. Nitrate concentration of spent lubricating oil polluted soil undergoing biodegradation

OFS: oil free soil, OPS: oil polluted soil, PAOPS: poultry litter amended oil polluted soil

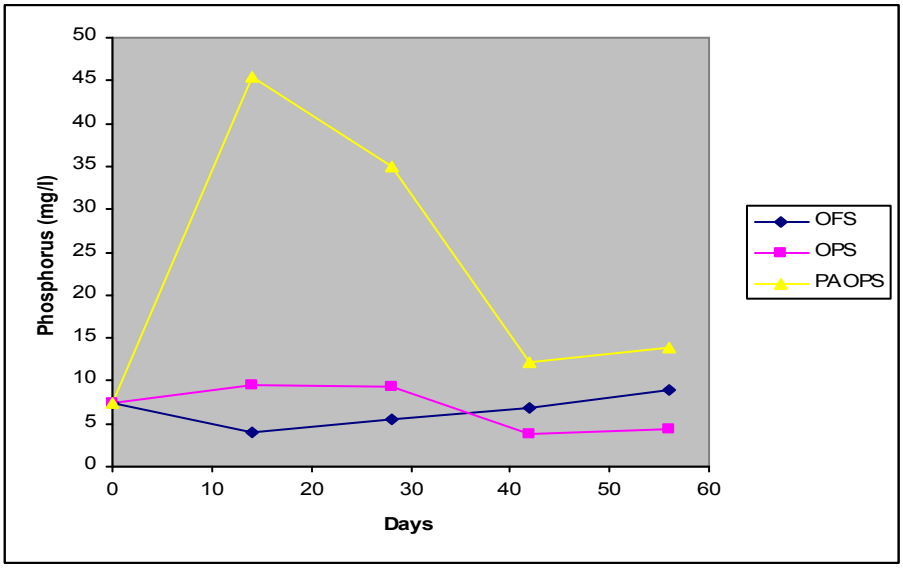

Fig. 5. Phosphorus content of spent lubricating oil polluted soil undergoing biodegradation

OFS: oil free soil, OPS: oil polluted soil, PAOPS: poultry litter amended oil polluted soil

Table 1. Physicochemical properties of spent lubricating oil polluted soil undergoing biodegradation (mean ± S.E)

Parameters	OFS	OPS	PAOPS
Moisture (%)	9.26±0.46[b]	9.60±1.01[a,b]	12.00±0.84[a]
O. C (%)	1.37±0.15[a]	1.10±0.33[a]	2.65±1.26[a]
O.M.C (%)	2.36±0.25[a]	1.90±0.25[a]	2.24±0.30[a]
EC (Ms/cm)	0.04±0.02[a]	0.09±0.03[a]	0.83±0.60[a]

[a,b,]: means denoted by different superscripts along the same row are significantly (p<0.05) different. O.C: Organic Carbon, O.M.C: Organic Matter content, EC: Electrical conductivity, %: percentage, Ms/cm: Microsecam per centimetre, S.E: standard error

4. DISCUSSION

The microbiological results revealed higher bacteria counts in poultry litter amended soil (PAOPS) followed by oil polluted soil (OPS) compared to the oil free soil (OFS). The highest bacterial counts /population in PAOPS could be due to the presence of the organic matter in PAOPS compared to OPS and OFS. This is in agreement with Stephen et al. [3] who observed similar results in Diesel polluted soil amended with cowpea shaft. The decline observed in OPS till day 42 may be due to reduction in the metabolic activity of bacteria as a result of the spent lubricating oil in the soil [3,6].

The fungi population did not follow the pattern observed in the bacterial population. The counts in PAOPS and OFS were lower than that of OPS until day 28. This may be due to the time it used to produce extracellular enzymes to degrade the oil as well as the poultry litter. This result is in agreement with earlier work by Stephen et al. [3,14]. They observed higher fungi counts in amended soil compared to oil polluted soil and oil free soil.

The organisms isolated were *Bacillus spp, Staphylococcus spp, Pseudomonas spp Micrococcus spp, Proteus spp, Aspergillus flavus, Aspergillus niger, Penicillium, Mucor spp and Yeast.* These organisms have been isolated by many researchers including Atagana [15], Stephen et al. [3,14] from hydrocarbon polluted soil undergoing biodegradation.

The pH range of 5.3-7.0 observed in this study has been reported to favour biodegradation of hydrocarbon [16,8]. PAOPS had the highest pH compared to OFS and OPS. This may be due to the amendment and the metabolic activity of the organisms present in the poultry litter amended soil [17,18].

The nitrate content was higher in PAOPS compared to OFS and OPS. This may be due to the presence of high nitrogenous compounds in the poultry litter compared to the oil free soil and the oil polluted soil. A similar result was reported by Atagana [15] who worked on compost manure. This result also agrees with the findings of Okwute and Ijah [17] who observed higher nitrate contents in palm oil mill effluent soil (POME) amended with chicken droppings.

The phosphorus content of the soil was also higher in PAOPS than OPS and OFS. This may be due to the presence of organic manure. However, the high phosphorus observed in OPS relative to OFS may result from the spent lubricating oil. This is in agreement with Stephen and Egene [8] who observed similar result in spent lubricating oil polluted soil in Anyigba. Phosphorus and nitrates are used by microorganism during biodegradation process [6,8].

The moisture content was high in all treatments. This may arise from the rain since this study was carried out during the rainy season (April - June, 2013). This is in agreement with Stephen and Egene [8] who observed high moisture content in spent lubricating oil polluted soil during the rainy season.

The organic carbon and organic matter contents were low in all treatments. Carbon and organic matter are utilized by microorganisms for their growth and metabolic activities. Carbon also serves as source of nutrients and also required for biodegradation.

The electrical conductivity was low in all treatments but however higher in PAOPS. This may be due to acid radical produced in the course of biodegradation especially in the poultry litter amended soil.

5. CONCLUSION

The result of this study revealed higher bacterial counts in poultry litter amended soil compared to oil free soil and oil polluted soil. There was higher pH, nitrate, phosphorus, moisture content, and lower electrical conductivity, lower organic carbon and organic matter contents in polluted soil amended with poultry litter than oil free soil and oil polluted soil. This is an indication that poultry litter can be used in amending soil polluted with spent lubricating oil and also enhanced the recovery of the soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Molina-Barahona L, Vega-Loyo L, Guerrero M. Ecotoxicological evaluation of diesel-contaminated soil before and after a bioremediation process. Environmental Toxicology. 2005;20(1):100-109.
2. Okoh IO. Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants, Biotechnology and Molecular Biology Reviews. 2006;1(2):38-50.
3. Stephen E, Job OS, Abioye OP. Study on Biodegradation of Diesel contaminated Soil Amended with Cowpea Chaff. Journal of Science and Multidisciplinary Research. 2013;2(1):14-18.
4. Abioye O P, Agamuthu P, Abdul Aziz AR. Biodegradation of motor oil in soil using organic waste amendments. Biotechnology Research International. DOI: 10.1155/2012/587041.
5. Adesodun JK, Mbagwu JSC. Biodegradation of waste-lubricating petroleum oil in a tropical alfisol as mediated by animal droppings. Bioresource Technology. 2008;99(13):5659-5665.
6. Ijah UJJ, Abioye OP. Assessment of physicochemical and Microbiological Properties of soil 30 months after kerosene spill. Journal of Research in Science and Management. 2003;1(1):24-30.
7. Rahman KSM, Thahira-Rahman J, Lakshmanaperumalsamy P, Banat IM. Towards efficient crude oil degradation by a mixed bacterial Consortium, Bioresource Technology. 2002;85(3):257-261.

8. Stephen E, Egene UM. Microbiology and physicochemical properties of soil polluted with lubricating oil in Anyingba, Kogi State, Nigeria. Nigerian Journal of Technological Research. 2012;7(2):49-52.
9. Ibitoye AA. Laboratory Manual on Basic Soil Analysis (2nd ed). Foladave Nigeria Limited, Akure. 2006;30-37.
10. Murphy J, Riley JP. A modified Single Sowton method for the determination of phosphorus in natural water. Analytical Chemistry. 1962;27:31-36.
11. Akinsanmi O. Certificate Agricultural Science. Longman, Nigeria. 1975;104-112.
12. Tropical Development Institute, TDI. Outlined method for the determination of organic matter content; 1984.
13. Harrigan WF, McCane ME. Laboratory methods in Food and Dairy Microbiology, 8th ed. Academic press, London; 1990.
14. Stephen E, Ekwetafia BE, Esemikose EE, Akogu EA, Abioye OP. Microbiology and physicochemical properties of mechanic workshop polluted soil amended with cowpea chaff in Anyingba, Kogi State, Nigeria. Malaysian Journal of Sciences. 2013;32(1):3-8.
15. Atagana HI. Compost bioremediation of hydrocarbon contaminated soil inoculated with organic manure. African Journal of Biotechnology. 2008;7(10):1516-1525.
16. Bossert I, Bartha R. The fate of petroleum in soil ecosystem. In: Petroleul Microbiology, Macmillan, New York, U.S.A. 1984;435-473.
17. Okwute LO, Ijah UJJ. Changes in Microbial Population of Palm Oil Mill effluent polluted soil amended with Chicken droppings and Cow dung. British Biotechnology Journal. 2014;4(3):279-288.
18. Tanee FGB, Kinako PDS. Comparative studies of biostimulation and phytoremediation in the mitigation of crude oil toxicity in tropical soil. Journal of Applied and Environmental Management. 2008;12(2):146-147.

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