Journal of Materials and Environmental Science
ISSN : 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN

J. Mater. Environ. Sci., 2023, Volume 14, Issue 8, Page 913-924

http://www.jmaterenvironsci.com

The Potency of Nutrients- Stimulated Microbial Growth Profile for remediation of Spent Motor Engine Oil Impacted Soil in Uyo, Akwa Ibom State and Ohiya, Abia State, Nigeria

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Received *02 July 2023,*
Revised *04 Aug 2023,*
Accepted *05 Aug 2023*

Keywords:

- ✓ *Total heterotrophic bacteria counts*
- ✓ *total heterotrophic fungal counts*
- ✓ *Spent motor engine oil*
- ✓ *Bioremediation*
- ✓ *Nutrient stimulation*

Citation*: Essien N. E., Ubuoh E. A. and Ogwo P. A. (2023) The Potency of Nutrients- Stimulated Microbial Growth Profile for remediation of Spent Motor Engine Oil Impacted Soil in Uyo, Akwa Ibom State and Ohiya, Abia State, Nigeria, J. Mater. Environ. Sci., 14(8), 913-924*

Abstract: The effects of nutrient enhanced bioremediation of soil contaminated by Spent motor engine oil on total heterotrophic bacterial (THB) and fungal (THF) counts was investigated. Seven kilograms (7 kg) each of soil contaminated by Waste motor engine oil were obtained at eight sampling points in mechanic village, Uyo Akwa Ibom State and Ohiya Abia State respectively. Each soil sample was sieved, sterilized, set up in eight respective plastic vessels and different concentrations of sterile poultry manure, cow dung and $N_{15}P_{15}K_{15}$ fertilizer where added, mixed and 1,000 ml broth culture of eight species of bacteria and seven of fungi were inoculated in each vessel except in their respective controls. Sampling of 1kg bioremediated soil from each vessel of 2 months interval was carried out for one year to evaluate the effect of bioremediation on the microbial growth profile. The results of THB and THF counts indicated a steady increase from 2 months to six months of monitoring with graduate decrease from the 6 months to the 12 months bioremediation process. The highest THB counts of 11 .3 ± 0.02 x 10^7cfu/g and 11.4 ± 0.16 x 10^7cfu/g were found in the 6th month in the soil from Akwa Ibom and Abia State respectively. These occurred in soil enriched with 2kg sterile poultry manure. The lowest TBH counts of 3.8 ± 0.14 x 10^7cfu/g and 4.3 ± 0.10 x 10^7cfu/g respectively in the soil from Akwa Ibom State and Abia State were both found in their controls inoculated with microbial species but no nutrient enrichment. The highest THF counts of 9.3 ± 0.06 x 10^7cfu/g and 9.0 ± 0.13 x 10^7cfu/g respectively were found in the eight and six months in the soil from Akwa Ibom State and Abia State accordingly. Both were in soil enriched with 1kg sterile poultry manure. The lowest TBF counts in soil from Akwa Ibom State and Abia State were 4.6 ± 0.15 x 10^7cfu/g and 5.1 ± 0.09 x 10^7cfu/g respectively, in their controls. significant increase ($P<0.05$) was recorded in total microbial counts in the sixth month bioremediation when compared to the twelfth month treatment. No significant difference ($P>0.05$) was found in the total microbial counts with regards to sample obtained from Akwa Ibom State and Abia State. The results implied that total heterotrophic bacterial and fungal counts were affected by the period of bioremediation and that nutrient enhancement boosted microbial growth profile in the treated soil, especially poultry manure.

1. Introduction

Increase in the use of automobile engines and machines has resulted into rise in use of lubricating (engine) oil hence increase in rates of environmental contamination by used engine oil (Abioye *et al.*, 2012). Used engine oil contains harmful substances such as heavy metals, aliphatic and aromatic hydrocarbons, Benzenes as well as sulphur and nitrogen (Mohammed *et al*., 2011).

The oil is dumped into water bodies, farmlands and open vacant plots use as mechanic workshops hence polluting both soil and water (Ikhajiagbe and Anoliefo, 2011). These substances cause negative effects to the soil and soil micro flora such as abridged growth rate and reproduction, destitute health and mutagenesis, thus alter population dynamic, disrupt tropical relations and edifice of natural groups within ecosystems (Samuel *et al.*, 2015).

Automobile workshop may be regarded as one of the integral parts of the service industry with great implication on the natural ecosystem; leading to seepage of waste and unused engine oil to the environment resulting to ecosystem contamination (Aiyesanmi, 2005; Ekanem *et al*., 2021). Different forms of petroleum products including petrol, engine oil, diesel, lubricant oils, and others are used in mechanic workshop (Udeani *et al.,* 2004). With increasing demand for automobiles mostly in developing countries like Nigeria, automobile workshops proliferate in major cities and towns, with waste generated and dumped indiscriminately on the environment, thereby polluting the aquatic and terrestrial ecosystems causing alteration in the population of flora and fauna forcing some to be extinct (Ikpe et al., 2016; Ikpe *et al*., 2019).

Products in Waste petroleum hydrocarbons tend to persist in the soil, harden or change the texture of the soil, which have effects on both physiochemical and microbiological properties of the contaminated soil (Udo, 2017; Udo *et al*., 2021). Indiscriminate discharge of spent lubricating oil is a major source of diffused or non-point source of oil pollution to the environment. Waste motor engine oil can build up in the environmental media for many years (Nyer, 2001). The oil and greases have been known to cause extensive damage to environment, create risk of contaminating air, water and soil with substantial hazards to animals and plant life, migrating birds, marine life, human life, and a great threat to environment (Kayode *et al.,* 2009; Ikhajiagbe *et al.,*2013; Bantista and Rahman, 2016). Osubor and Anoliefo, 2003, conveyed that approximately 20 million gallons of spent oil are accumulated every year from mechanic workshops across Nigeria and disposed carelessly into the environment. They further reported that oils are release into the milieu from the exhaust systems during engine operations as well as engine leaks. The chemical impurities in spent engine oil are toxic and can cause cancer and mutation in living cells (Udeani*et al.*, 2009; Ajao *et al.*, 2011). These chemicals seep into the water table, contaminate the ground water and subsequently get into the human body through the food web from plants (Adams *et al.*, 2014). The spent engine oil floats as a scum on the surface hence prevent sunlight and oxygen from penetrating the water thereby affecting water animals like fishes, frogs, crabs and water plants (Agarry and Oladipupo, 2012; Adams *et al.*, 2014). Pollution of soil by spent oil results in immobilizations of soil nutrient, loss of water-retaining capacity, low pH, reduced soil catalase enzyme action, as well as inhibition of nitrate reductase activity of plants; thus, soil dependent activities such as agriculture are affected (Imam *et al.*, 2011). Contaminations of exposed vacant spaces and farmland with petroleum product and grease is nowadays widespread than crude oil spill especially in the municipal areas (Osaigbovo *et al*, 2013; Ikpe 2016). The present study aims to use some organic wastes to stimulate bioremediation of soil contaminated with spent engine oil.

Bioremediation is emerging as one of the most promising and environmental-friendly technologies used for the removal of hydrocarbon contaminants from the environment (Adebusoye *et al.,* 2007; Jayashree *et al.,* 2012). A wide range of hydrocarbon degrading microbes useful for bioremediation are found to be in the soil. They include species of bacteria and fungi thriving in petroleum hydrocarbon contaminated environments, since they are reported to be feeding on the contaminants as their energy source (Arotupin and Ogunmalu, 2012). Microbial remediation of hydrocarbon contaminated sites is performed with the aid of a diverse group of micro-organisms most

especially in nutrient boosted environments (Hadelein *et al.,* 2006). However, due to the adverse environmental and public health impacts caused by Waste motor engine oil contamination of the environment, it is justifiable to conduct this investigation on the effect of nutrient enhanced bioremediation of waste oil impacted soil on microbial growth profile in the soil.

2.0 Methodology

2.1 Study Area : The study was carried out in the mechanic village Uyo, Akwa Ibom State and Ohiya, Abia State, Nigeria (Figures 1 & 2). Akwa Ibom State is located on latitude 4°32'N and Longitude 7°25'E and a land mass of about 113.1km^2 (FRNOG, 2007). Uyo recorded mean annual temperature between 20°C and 29°C and mean annual rainfall ranges from 1599 mm to 3855.9 mm. Evaporation is high and annual values range from 1500mm to 1800mm (Akwa Ibom State Government, 2008 Abia State is on Latitude 5°25'N and Longitude 7°30'E and covers an area of about 5243.7km^2.

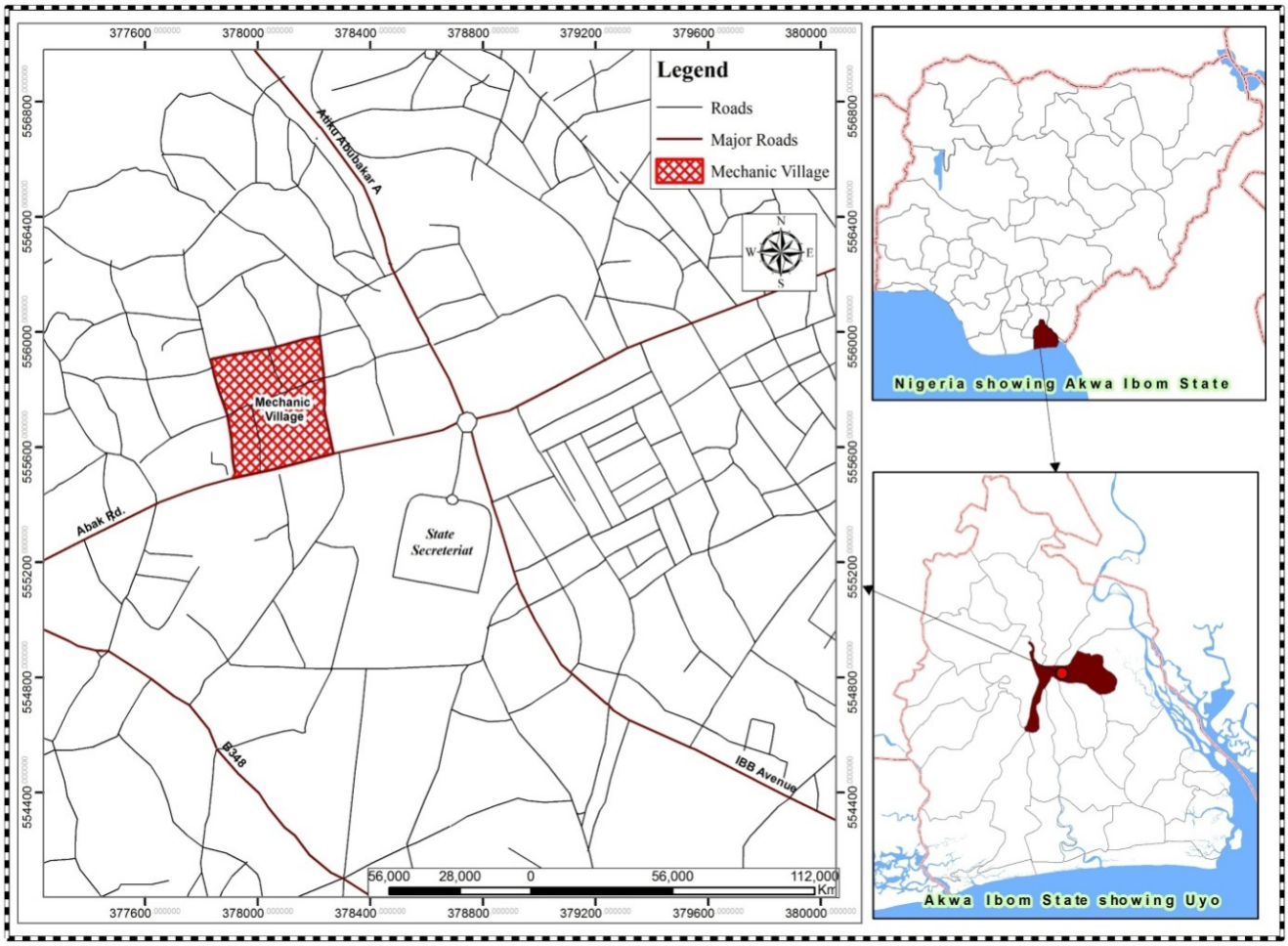

Figure. 1: Scaled map of Nigeria, and Akwa Ibom State showing Uyo, the study location

2.2 Collection of Samples

2.2.1 Spent engine oil contaminated soil

Waste engine oil contaminated soil was sampled at eight different sampling points each from the two study areas at a distance of 50 metres between sampling points. The soil samples were collected at the depth, 0 –20 cm aseptically with the aid of a sterile soil augar into well labelled sterile polythene bags and were taken to the Laboratory for the study.

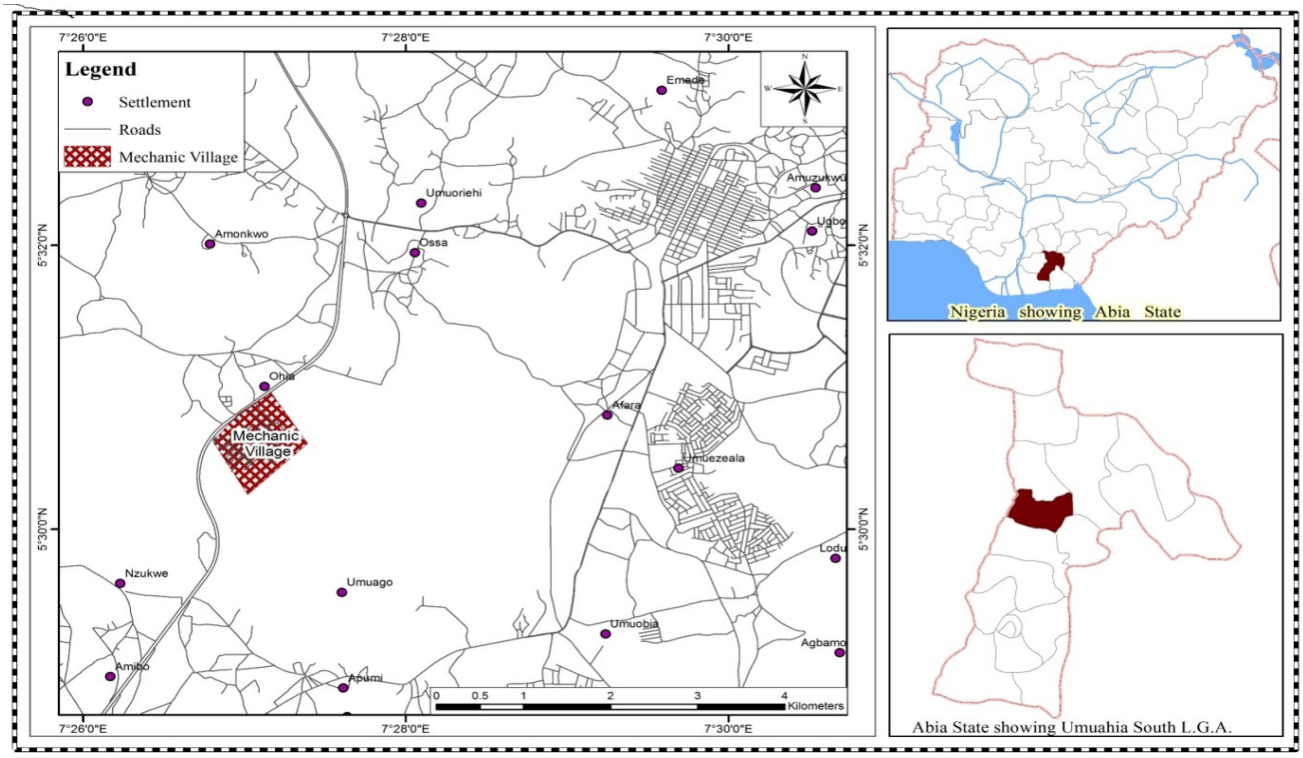

Figure. 2: Scaled map of Nigeria and a map of Abia State showing Ohiya the study location

2.2.2 Animal droppings

Chicken droppings and cow dung were collected from the animal farm, Department of Animal Science, University of Uyo, Uyo, Akwa Ibom State, labelled in a sterile polythene bag and taken to the Laboratory for the work.

2.2.3 Inorganic fertilizer

Ten kilograms (10kg) inorganic fertilizer (N_{15} $P_{15}K_{15}$) was bought from Uyo main market, Uyo, Akwa Ibom State to be used in this study.

2 .2.4 Bacterial cells

Total heterotrophic and hydrocarbonoclastic bacteria were obtained by culturing 10^{-6} tenfold 1g serially diluted spent motor engine oil contaminated soil by pour plate method with nutrient and mineral salt agar respectively.

Characterization using biochemical tests and morphological features were carried out, then, identification was done with schemes of Holt *et al.* (1994) and Barrow and Feltham (2003). More bacterial cells were produced by subculturing the isolated organisms in nutrient broth medium at 32.5 ± 2.5^0C for 2 days (Utah and Essien, 2005). Bacteria identified and used were, *Bacillus mycoides, Staphylococcus aureus, Listeria murrayi, Actinomycetes viscosus, Clostridium sporogenes, Bacillus licheniformis, Corynebacterium ulcerans* and *Clostridium histolyticum.*

2.2.5 Fungal organisms

The total heterotrophic and hydrocarbon clastic fungi were also obtained by culturing 10^{-6} tenfold 1g serially diluted spent oil impacted soil by pour plate technique with potato dextrose and oil agar respectively. Biological examination, microscopy and morphological evaluations were used to characterized the organisms. Identification was with Barnet and Hunter (1987) and Samson *et al.*

(1989) Identification schemes. More fungi cells (yeasts) were produced by subculturing the organisms in potato dextrose broth, while more molds were produced by subculturing the organisms on freshly prepared potato dextrose agar, both at 22.5 ± 2.5°C for 5 days (Itah and Essien, 2005, Ijah and Antai, 2003). Fungi identified were *Penicillium notatum, Aspergillus fumigatus, Rhizopus stolonifer, Rhizopus oryzae, Fusarium oxysporum, Cryptococus terrens* and *Rhodotorulaa mulcilaginosa.*

2.3 Sterilization of samples/ glasswares

Seven kilograms (7kg) each of the spent motor engine oil contaminated soil collected from eight locations from each of the two study areas sieved (2mm mesh size), sterilized at 121°C for 15 minutes at 15 psi in an autoclave and kept for use, sun- dried cow dung was sieved, sterilized at the same temperature and duration in an autoclave and kept for use. Sun-dried poultry manure was sieved and sterilized in a hot- air oven at 160°C for 1 hour, likewise the washed and rinsed glasswares. Workbench was disinfected with absolute ethanol and all aseptic procedures were adhered to strictly.

2.4 Treatment of the spent engine oil contaminated soil with microbial consortium

Seven kilograms (7kg) each of the sterile soil sample from 8 locations from mechanic village, Uyo Akwa Ibom State were respectively dished into eight sterile rectangular rubber containers and label a to age the same was done with soil from mechanic village or here and labelled A - H. The same was done with soil from mechanic village, Ohiya and labelled 1-8. One kilogram (1kg) each of sterile inorganic fertilizer (N_{15} $P_{15}K_{15)}$, poultry manure and cow dung were dispensed into soil in vessels, A, B, C and 1, 2, 3 respectively. Again, 2kg each of sterile N_{15} $P_{15}K_{15,}$ poultry manure and cow dung were introduced into D, E, F and 4, 5, 6 accordingly and were mixed thoroughly. Contents in vessels G, H and 7, 8 were not enriched with soil nutrient. Therefore, 1, 000ml consortium of the broth culture of 48 hours of the eight bacteria and 5 days broth culture of the seven fungi identified from the spent engine oil contaminated soil were inoculated into each of the contents in A to G and 1 to 7 respectively. Contents in vessels H and 8 were not seeded with microorganisms and served as negative controls while G and 7 were seeded but not enriched with soil nutrients served as positive controls. Content in each vessel was tilled twice a week for aeration and moistened with 500ml sterile water twice a month to support microbial growth and bioremediation. One kilogram of soil from each vessel was sampled at two months interval for a period of one year for the determination of total heterotrophic bacterial and fungal counts.

2.5 Determination of microbial counts

2.5.1. Determination of total heterotrophic bacteria counts

Pour plate technique was adopted to isolate and count the total heterotrophic bacteria in each soil collected using 10^{-6} of 1g serially diluted soil sample in nutrient agar supplemented with 50ug/ml Nystatin to inhibit fungal contamination. The organisms were incubated in triplicate at 32.5 ± 2.5°C for 24 hours (Itah and Essien, 2005). Viable colonies on each plate were counted by direct plating technique with a colony counter (Model: TT-02 Techmel/Techmel, USA).

2.5.2. Determination of Total heterotrophic fungi count

Total heterotrophic fungal count was evaluated by pour plate technique. Fungi in 10^{-6} of 1g serially diluted soil sample was cultured in potato dextrose agar supplemented with 50µg/ml streptomycin to inhibit bacterial contamination and was incubated at 22.5 ± 2.5°C for 5 days in triplicates (Ijah and Antai, 2003). Viable colonies were enumerated using colony counter after incubation

Table 1: Mean total heterotrophic bacterial counts in treated soil impacted by spent motor engine oil from the mechanic village, Uyo, Akwa Ibom State and Ohiya, Abia State

Study location	Experimental stand	Treatment period (month) 2	4	6	8	10	12
		(cfu/g)	(cfu/g)	(cfu/g)	(cfu/g)	(cfu/g)	(cfu/g)
MECHANIC VILLAGE, UYO, AKWA IBOM STATE	A	$7.6 \pm 0.08 \times 10^7$	$8.6 \pm 0.02 \times 10^7$	$9.2 \pm 0.04 \times 10^7$	$9.2 \pm 0.02 \times 10^7$	$6.0 \pm 0.05 \times 10^7$	$5.1 \pm 0.13 \times 10^7$
	B	$7.4 \pm 0.10 \times 10^7$	$10.6 \pm 0.11 \times 10^7$	$11.0 \pm 0.11 \times 10^7$	$10.6 \pm 0.15 \times 10^7$	$6.0 \pm 0.03 \times 10^7$	$4.6 \pm 0.04 \times 10^7$
	C	$3.8 \pm 0.03 \times 10^7$	$6.8 \pm 0.05 \times 10^7$	$8.6 \pm 0.01 \times 10^7$	$8.5 \pm 0.06 \times 10^7$	$4.8 \pm 0.6 \times 10^7$	$4.0 \pm 0.01 \times 10^7$
	D	$9.7 \pm 0.11 \times 10^7$	$9.4 \pm 0.02 \times 10^7$	$10.2 \pm 0.05 \times 10^7$	$9.8 \pm 0.01 \times 10^7$	$6.1 \pm 0.7 \times 10^7$	$4.7 \pm 0.12 \times 10^7$
	E	$10.3 \pm 0.03 \times 10^7$	$10.4 \pm 0.11 \times 10^7$	$11.3 \pm 0.02 \times 10^7$	$9.3 \pm 0.04 \times 10^7$	$6.0 \pm 0.12 \times 10^7$	$5.0 \pm 0.08 \times 10^7$
	F	$6.3 \pm 0.16 \times 10^7$	$8.2 \pm 0.01 \times 10^7$	$8.4 \pm 0.10 \times 10^7$	$7.4 \pm 0.10 \times 10^7$	$6.0 \pm 0.01 \times 10^7$	$4.1 \pm 0.03 \times 10^7$
	G	$6.0 \pm 0.12 \times 10^7$	$6.6 \pm 0.21 \times 10^7$	$8.2 \pm 0.11 \times 10^7$	$7.0 \pm 0.13 \times 10^7$	$4.6 \pm 0.11 \times 10^7$	$3.8 \pm 0.14 \times 10^7$
	H (Control (a))	$1.2 \pm 0.04 \times 10^7$	$1.4 \pm 0.05 \times 10^7$	$2.0 \pm 0.04 \times 10^7$	$2.2 \pm 0.3 \times 10^7$	$1.4 \pm 0.05 \times 10^7$	$8.0 \pm 0.11 \times 10^6$
MECHANIC VILLAGE, OHIYA, ABIA STATE	1	$7.4 \pm 0.10 \times 10^7$	$9.4 \pm 0.05 \times 10^7$	$10.5 \pm 0.11 \times 10^7$	$9.1 \pm 0.13 \times 10^7$	$5.5 \pm 0.11 \times 10^7$	$6.1 \pm 0.02 \times 10^7$
	2	$7.3 \pm 0.02 \times 10^7$	$10.5 \pm 0.14 \times 10^7$	$10.8 \pm 0.08 \times 10^7$	$9.4 \pm 0.03 \times 10^7$	$6.1 \pm 0.07 \times 10^7$	$5.3 \pm 0.08 \times 10^7$
	3	$4.8 \pm 0.15 \times 10^7$	$7.7 \pm 0.05 \times 10^7$	$8.6 \pm 0.02 \times 10^7$	$8.7 \pm 0.08 \times 10^7$	$5.4 \pm 0.03 \times 10^7$	$5.0 \pm 0.16 \times 10^7$
	4	$9.9 \pm 0.08 \times 10^7$	$9.1 \pm 0.02 \times 10^7$	$9.6 \pm 0.05 \times 10^7$	$9.2 \pm 0.05 \times 10^7$	$5.0 \pm 0.07 \times 10^7$	$4.4 \pm 0.05 \times 10^7$
	5	$9.9 \pm 0.17 \times 10^7$	$8.7 \pm 0.05 \times 10^7$	$11.4 \pm 0.16 \times 10^7$	$9.9 \pm 0.07 \times 10^7$	$6.8 \pm 0.13 \times 10^7$	$5.7 \pm 0.04 \times 10^7$
	6	$5.2 \pm 0.05 \times 10^7$	$8.0 \pm 0.08 \times 10^7$	$9.0 \pm 0.05 \times 10^7$	$7.7 \pm 0.01 \times 10^7$	$4.2 \pm 0.08 \times 10^7$	$5.3 \pm 0.15 \times 10^7$
	7	$4.7 \pm 0.06 \times 10^7$	$7.6 \pm 0.10 \times 10^7$	$8.9 \pm 0.15 \times 10^7$	$7.5 \pm 0.11 \times 10^7$	$4.1 \pm 0.01 \times 10^7$	$4.3 \pm 0.10 \times 10^7$
	8 (Control (1))	$1.7 \pm 0.02 \times 10^7$	$1.9 \pm 0.01 \times 10^7$	$2.0 \pm 0.02 \times 10^7$	$1.5 \pm 0.08 \times 10^7$	$1.0 \pm 0.01 \times 10^7$	$1.1 \pm 0.05 \times 10^7$

Data are mean of two replications (±)standard deviation

Table 2: Mean total heterotrophic fungial count in treated soil impacted by spent motor engine oil from the mechanic village, Uyo, Akwa Ibom State and Ohiya, Abia State

Study location	Experimental stand	Treatment period (month) 2	4	6	8	10	12
		(cfu/g)	(cfu/g)	(cfu/g)	(cfu/g)	(cfu/g)	(cfu/g)
MECHANIC VILLAGE, UYO, AKWA IBOM STATE	A	5.5 ±0.03 x 10^7	8.3 ±0.10 x 10^7	7.4 ±0.06 x 10^7	7.6 ±0.12 x 10^7	6.4 ±0.01 x 10^7	5.4 ±0.02 x 10^7
	B	6.8 ±0.07 x 10^7	8.0 ±0.03 x 10^7	7.9 ±0.11 x 10^7	9.3 ±0.06 x 10^7	6.8 ±0.05 x 10^7	6.0 ±0.12 x 10^7
	C	5.0 ±0.15 x 10^7	6.4 ±0.09 x 10^7	6.9 ±0.03 x 10^7	6.9 ±0.10 x 10^7	5.7 ±0.08 x 10^7	4.7 ±0.06 x 10^7
	D	6.8 ±0.03 x 10^7	7.3 ±0.11 x 10^7	8.2 ±0.02 x 10^7	8.4 ±0.04 x 10^7	5.8 ±0.12 x 10^7	5.8 ±0.21 x 10^7
	E	6.5 ±0.09 x 10^7	8.0 ±0.05 x 10^7	8.2 ±0.12 x 10^7	8.6 ±0.15 x 10^7	6.6 ±0.06 x 10^7	4.9 ±0.05 x 10^7
	F	5.1 ±0.04 x 10^7	6.2 ±0.01 x 10^7	7.4 ±0.06 x 10^7	7.4 ±0.07 x 10^7	5.8 ±0.07 x 10^7	5.4 ±0.07 x 10^7
	G	5.0±0.10 x 10^7	6.1±0.02 x 10^7	6.7±0.11 x 10^7	6.9±0.10 x 10^7	5.5±0.01 x 10^7	4.6±0.15 x 10^7
	Mean						
	H (Control (a))	1.0 ±0.11 x 10^7	1.1 ±0.03 x 10^7	1.0 ±0.13 x 10^7	1.1 ±0.02 x 10^7	1.5 ±0.10 x 10^7	1.3 ±0.10 x 10^7
MECHANIC VILLAGE, OHIYA, ABIA STATE	1	5.8 ±0.01 x 10^7	6.9 ±0.03 x 10^7	8.1 ±0.07 x 10^7	7.6 ±0.13 x 10^7	7.1 ±0.12 x 10^7	5.6 ±0.02 x 10^7
	2	6.6 ±0.12 x 10^7	7.0 ±0.08 x 10^7	9.0 ±0.13 x 10^7	7.8 ±0.04 x 10^7	6.8 ±0.07 x 10^7	5.8 ±0.11 x 10^7
	3	5.2 ±0.04 x 10^7	6.2 ±0.11 x 10^7	7.6 ±0.07 x 10^7	7.5 ±0.10 x 10^7	5.7 ±0.01 x 10^7	5.1 ±0.09 x 10^7
	4	5.9 ±0.09 x 10^7	7.6 ±0.13 x 10^7	7.9 ±0.01 x 10^7	8.0 ±0.10 x 10^7	6.6 ±0.13 x 10^7	5.8 ±0.03 x 10^7
	5	6.4 ±0.17 x 10^7	6.1 ±0.02 x 10^7	8.1 ±0.06 x 10^7	6.0 ±0.05 x 10^7	6.7 ±0.10 x 10^7	5.8 ±0.01 x 10^7
	6	4.9 ±0.02 x 10^7	5.5 ±0.08 x 10^7	7.2 ±0.11 x 10^7	6.4 ±0.16 x 10^7	5.8 ±0.08 x 10^7	6.0 ±0.10 x 10^7
	7	5.1±0.07 x 10^7	5.9±0.11 x 10^7	7.0±0.02 x 10^7	6.5±0.11 x 10^7	5.6±0.17 x 10^7	5.3±0.01 x 10^7
	Mean						
	8 (Control (1))	1.6 ±0.15 x 10^7	1.8 ±0.10 x 10^7	1.9 ±0.04 x 10^7	1.9 ±0.03 x 10^7	1.1 ±0.03 x 10^7	1.3 ±0.05 x 10^7

Data are mean of two replications (±) standard deviation

2.6. Statistical analyses

Results obtained were subjected to descriptive (mean, standard error of mean and ranges) and inferential (MANOVA and ANOVA) statistics and $P<0.05$ was considered to indicate statistical significant.

3.0 Results and Discussion

3.1 Results

The mean total heterotrophic bacterial counts in bioremediated soil contaminated by Spent motor engine oil from mechanic village Uyo, Akwa Ibom and Ohiya, Abia States are presented in Table 1, while mean total heterotrophic bacterial counts in bioremediated soil contaminated by Spent motor engine oil from mechanic village, Uyo, Akwa Ibom and Ohiya Abia States are presented in Table 2. The effects of nutrients stimulated bioremediation of soil contaminated by spent motor engine oil on microbial growth profile in the soil was investigated. The results of the mean total heterotrophic bacterial (THB) counts (Table 1) and total heterotrophic fungal counts (Table 2) in soil contaminated by Spent motor engine oil from mechanic village Uyo, Akwa Ibom State and Ohiya, Abia State remediated with microbial consortium and monitored at 2 months interval for 12 months are depicted in the respective tables. The results indicated that microbial counts took a steady increase from the second month of bioremediation to the sixth month, but gradually decreased to the twelveth month of treatment as shown in Figure 3 and 4. The highest THB counts of $11.4 \pm 0.16\times10^{7}$cfu/g were found in the sixth month of monitoring in the soil obtained from Akwa Ibom State and Abia State respectively. These were found in soil stimulated with 2kg sterile poultry manure. The lowest THB counts of $3.8 \pm 0.14\times10^{7}$cfu/g and $4.3 \pm 0.10\times10^{7}$ cfu/g respectively in the soil from Akwa Ibom State and Abia State and were in soil inoculated with microbial consortium but was not stimulated with nutrients. Again, the highest THF counts ($9.3 \pm 0.06\times10^{7}$cfu/g and $9.0\pm 0.13\times10^{7}$cfu/g) were in the eight and sixth months of monitoring in the soil from Akwa Ibom State and Abia State respectively. Both were seen in the soil enriched with 1kg sterile poultry manure.

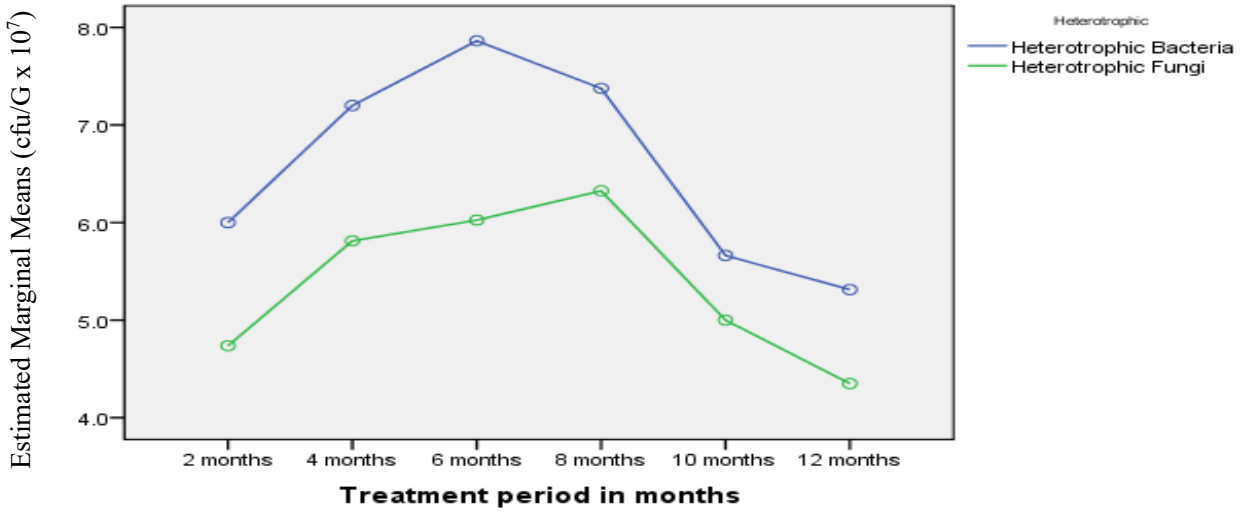

Figure 3: Growth curves of total heterotrophic bacterial (THB) and total heterotrophic fungal (THF) counts in soil impacted by spent motor engine oil from Uyo, Akwa Ibom State treated for 12 months

The lowest THF counts of $4.6 \pm 0.15\times10^{7}$cfu/g and $5.1\pm 0.09\times10^{7}$cfu/g respectively were in the soil from Akwa Ibom State and Abia State. These were found in the soil not stimulated with nutrients, but were inoculated with microorganisms. The result is in tandem with the report that, the count of

hydrocarbon utilizing bacteria in contaminated soil before bioremediation showed 5.7x10^5 cfu/g, indicated that HUB can strive even in extreme conditions and high concentration of TPH (Rahman *et al.*, 2002.Onifade*et al.*, 2007; Omotayo *et al.*, 2012; Adams *et al.*, 2014).

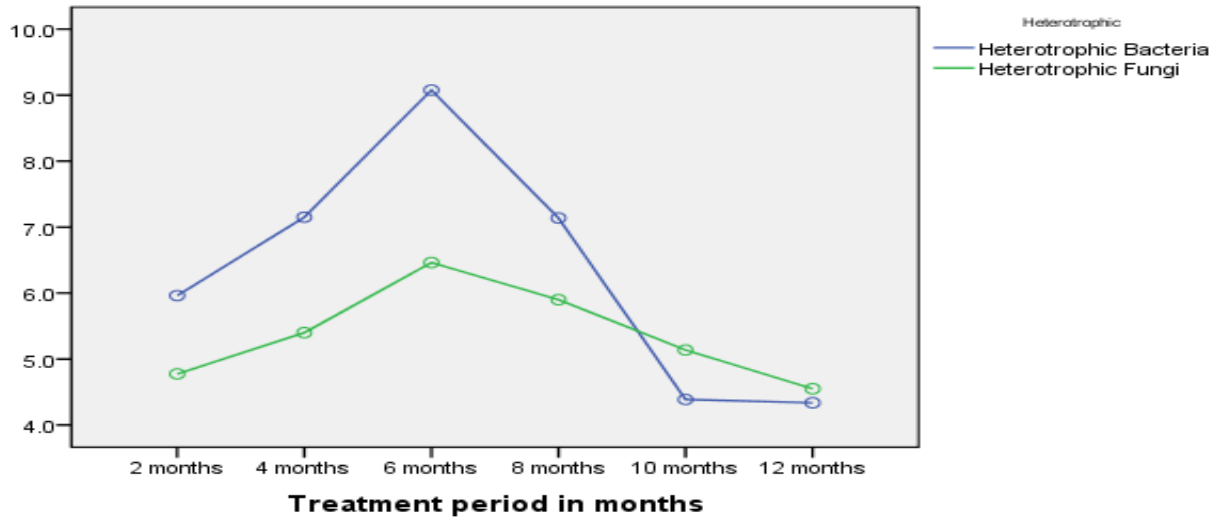

Figure 4: Growth curves of total heterotrophic bacterial (THB) and total heterotrophic fungal (THF) counts in soil impacted by spent motor engine oil from Ohiya, Abia State treated for 12 months

Hydrocarbon degrading bacteria are widely spread in polluted soil, water, and the application of hydrocarbons increases the number of hydrocarbon utilizing bacteria (Leah and Colwell1,1990; Chang *et al.*, 2000, Barathi and Vasudevan, 2001 and Zhuang *et al.,* 2002). Total oil degraders were increased gradually during the experimental period in this work, which was similar to the work of Jane-Francis *et al.* (2008), who reported that oil-degrading bacteria counts ranged from 6×10^4 to 49×10^4 cfu/ml in contaminated samples as against 0 to 14×10^4 cfu/ml in uncontaminated soil, the increase in the oil degrading bacteria counts might be due to the nutrient-induced desorption of hydrocarbons present in the soil sample. Desorption of hydrocarbons in the contaminated soil sample might lead to an increase in the microbial mineralization, either by increasing hydrocarbon solubility or by increasing the contact surface with hydrophobic compounds (Moran *et al.*, 2000, Rahman *et al.*, 2002, Ghulam *et al.*, 2008). Microbial growth can also be enhanced by the addition of hydrocarbons to the soil samples in which the hydrocarbon served as a nutrient to the microorganisms present in the soil samples (Raza *et al.*, 2007). Atlas and Bartha (1972) observed that the application of crude oil to Arctic tundra soil caused overall increase in microbial numbers compared to un-oiled reference (control) soil, in which 7.5×10^5 cfu/g in the un-oiled soil, while 41×10^7 cfu/g was recorded for the soil sample contaminated with crude oil after 14 months. Ghulam *et al.* (2008) also reported that total bacterial count present in the soil contaminated with kerosene increased from 9×10^8 cfu/g at first week of the experiment to 9.6×10^8 by the third week. In this work, it was observed that total bacterial count decreased, while total oil degrader counts increased and this observation was also reported by Ramsay *et al.* (2000) who observed decrease in the heterotrophic bacteria count and increase in the hydrocarbon-degrading bacteria from the soil samples from oiled mangrove and untreated sediments. Olivera *et al.* (2003), also reported an increase of 2.0 × 106 to 1.3×10^8 cfu/ml during the first 24 hrs in the soil contaminated with bilge waste till 17 days when the population had increased to 8.8×10^8 Cfu/ml. Rahman *et al.* (2003) reported an increase in the bacterial population from all the soil samples amended with hydrocarbons, especially the soil amended with 10% petroleum after 56 days of incubation. Increase in the hydrocarbon-degrader

bacteria population from 1×10^5 to 1×10^7 cfu/g, between 4 and 7 days of incubation was observed by Kirsten *et al.* (2005), while the total heterotrophic population of the soil remained relatively unchanged during the incubation period. Abioye *et al.* (2009) reported an increase in the hydrocarbon utilizing bacteria in a soil contaminated with used lubricating oil, which changed from 10.2×10^6Cfu/g to 80.5×10^6 cfu/g, Hanan *et al.* (2009) also reported increase in number of microbes in a consortium used in the biodegradation of petroleum hydrocarbons that ranged from 6.14×10^7 to 3.5×10^8 and Udeani *et al.* (2009) also reported an increase from 1.25×10^4 to 6.25×10^5 in the hydrocarbon degraders present in the soil sample collected from mechanic workshop.

Meanwhile, the mean THB and THF counts revealed lowest growth in the second month of inoculation, which seems to be the lag phase and the twelveth month of bioremediation which seems to be the dead phase in the microbial growth profile. These agreed with the report of Akinjogunla (2016) who reasoned that microbial cells in the lag phase of growth in an enclosed system (experimental stands) tried to acclimatize or adapt to the new growth condition, here, in the spent motor engine oil- polluted soil. They were physiologically and metabolically active; rapidly synthesizing ribonucleic acid, enzymes, coenzymes and other molecules required for cell division; increase in cell size, but no cell division or replication (Hugo and Russell, 2007). The dead phase is characterized with nutrient depletion and accumulation of toxic waste metabolic products in the soil/ oil treatment mixtures which facilitates the organisms to the dead phase. The sixth month bioremediation showed the highest microbial growth. This seems to be the exponential phase in the growth of microbial species in the bioremediated soil. This agreed with the report of Hugo and Russel (2007) and Akinjogunla (2016), that exponential phase in microbial growth in a batch culture (experimental stands) exhibits exponential increase in cells number, cell mass or biomass with time, doubling of cell population at regular interval of time, increase in metabolic activities of the organisms as they rapidly utilized the contaminants (hydrocarbons) In the spent motor engine oil-polluted soils.

Furthermore, the entire results of the THB and THF counts in soil contaminated by Spent motor engine oil from Akwa Ibom State and Abia State revealed low microbial growth in experimental stand that was not stimulated with any nutrient, the stands enriched with sterile cow dung and the stand not enriched nor inoculated with microbial consortium when compared with the ones stimulated with sterile poultry manure and N_{15} $P_{15}K_{15}$ fertilizer. This observation was in agreement with Swannell *et al.* (1999) who found that bioremediation by nutrients enhancement boosted efficient degradation of environmental contaminants in the soil medium. It also agreed with the results of field work by Lee *et al.* (1995) which indicated that although organic fertilizer had a greater effect on total heterotrophic microbial counts, inorganic nutrients were much more effective in stimulating crude oil degradation. According to Macaulay (2015), addition of nutrients adjusts the essential nutrients balance for microbial growth and reproduction as well as having impact on the biodegradation rate and effectiveness. Enim (2013) also reported that nutrient balancing especially the supply of essential nutrients such as nitrogen (N) and phosphorus (P) can improve the biodegradation efficiency by optimizing the bacteria C: N: P ratio. Boopathy (2006) discovered that addition of an appropriate quantity of nutrients is a favourably strategy for increasing the metabolic activity of microorganisms and thus, the bioremediation rate in the soil environment. However, microbial counts are often used to monitor bioremediation process (Das and Chandran, 2011; Thamer *et al.,* 2013). According to Macnaughton *et al.* (1999), the more the numbers, the more quickly the contaminants are degraded. Moreso, the results of this work showed that fungi are slow growers when compared to bacterial growth in the bioremediated soil. This also agreed with Hugo and Russel (2007) who observed that in an enclosed system, bacterial cells replicates faster than the fungi.

Conclusion

Nutrients enhanced bioremediation of soil contaminated by Spent motor engine oil influenced by microbial growth profile by causing steady increase of microbial counts in the first six months of bioremediation, with a gradual decrease as the process continued to the twelveth month. Nutrients addition most especially poultry manure seems to boost microbial counts which might correlate with rapid decontamination of the soil contaminated with spent motor engine oil.

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