International Journal of Innovative Research and Advanced Studies (IJIRAS)
Volume 5 Issue 12, December 2018

ISSN: 2394-4404

Bacterial Population Of Soil Samples From Selected Mechanic And Non-Mechanic Sites In Makurdi, Benue State

Iyoula, F.I

Gberikon, G.M

Department of Microbiology, Federal University of Agriculture, Makurdi

Abatyough, M.T

Department of Chemistry, Bingham University, Abuja

Abstract: ***Investigations were carried out to ascertain bacterial population of soil samples from selected mechanic and non - mechanic sites in Makurdi, Benue state of Nigeria. A total of forty (40) soil samples (20g each) were collected from different mechanic shops namely; New garage, Kanshio and North bank. Soil not contaminated with petroleum products was also collected alongside petroleum contaminated soil at the depth of 8-10 cm by using a clean and dry sterile spatula in clean polythene bags. Serial dilution and pour plate technique was used for microbial analysis. The bacteria present were identified using cultural, morphological and biochemical techniques. The results showed that one hundred and fifty two (152) bacteria strains belonging to ten (10) genera were isolated. Counts ranged from 2.40 x 10^5 Cfu/g to 8.23 x 10^5 Cfu/g for Mechanic sites and for non – mechanic site (control), counts were 1.23 x 10^4 Cfu/g to 9.00 x 10^4 Cfu/g. Mechanic sites 63(41.45%)) had the highest occurrence of hydrocarbon degrading organisms while soil not contaminated with petroleum products (control) had the lowest percentage of 16(10.53%) The genera Pseudomonas, Staphylococcus, Proteus and Escherichia were the predominant petroleum hydrocarbon degrading bacteria. Therefore indiscriminate setup of mechanic workshops should be prohibited by appropriate agencies, so as to reduce proliferations of microorganisms that can use hydrocarbons as source of nutrients and will later evolve traits that could pose serious health challenges to the populace.***

Keywords: ***Bacterial population, Bacterial counts, Mechanic sites, Non-Mechanic site.***

I. INTRODUCTION

Petroleum hydrocarbons can be degraded by microorganisms such as bacteria, fungi, yeast and microalgae (Bundy *et al*., 2004). In recent years, many microbial ecologists have identified various microbial species that are effective degraders of petroleum hydrocarbons in natural environments. Bacterial population can be influenced by a number of factors in the environment which is chemical and physical. The environment in which some organisms grows will be injurious to others. A typical example of a bacterial that can thrive in a very harsh environment is *Bacillus* species. However, bacteria play the central role in hydrocarbon degradation (Oteyza *et al.,* 2005). Hydrocarbon degraders regardless of the environment from which they were isolated have all the traits necessary to cause a wide variety of human infections (Rojo and Martinez, 2010). Studies of Susceptibility of hydrocarbon degraders to antibiotics have been undertaken previously. For instance Okoh (2003) reported multiple antibiotic resistances of hydrocarbon degraders to ten antibiotics out of twelve antibiotics tested. Mathe *et al*. (2012) described a significant correlation between antibiotic resistance and the hydrocarbon biodegradation potential of bacteria isolated from long-term contaminated soils.

II. MATERIALS AND METHODS

SAMPLE COLLECTION AND SAMPLE SIZE

A total of 40 soil samples (20 g each) were collected from different mechanic shops located in three parts in Makurdi

metropolis: New garage mechanic site, mechanic village Kanshio and mechanic site North bank. Soil samples were also collected from soil that was not contaminated with petroleum products and used as control. Twenty (20) gram of each soil sample was collected at the depth of 8-10 cm by using a clean and dry sterile spatula in clean polythene bags, labelled and transported in ice box for preservation to prevent changes in microbial load for basic isolation at the advanced biological science laboratory, University of Agriculture Makurdi (Sudhir *et al.*, 2014).

MEDIA USED FOR ISOLATION

Nutrient agar (NA), Mueller Hinton agar, Peptone water and Normal Saline were used to culture bacteria. It was prepared under aseptic conditions according to the manufacturers specifications (Olukunle, 2013).

ISOLATION AND ENUMERATION OF HYDROCARBON UTILIZING BACTERIA

Vapour phase transfer method was adopted for enumeration of hydrocarbon utilizing bacteria. Serial dilution of the soil samples was achieved by shaking 1.0 g of the soil samples in 9 ml of distilled water in test tubes and dilution was done up to 10^{-5}. Aliquot (1 ml) of dilutions was plated out in triplicates on a sterile Nutrient Agar (oxoid) as described by Olukunle (2013). Sterile Whitman No. 1 filter paper saturated with crude oil was placed on the inside cover of each of the Petri dishes and incubated with the agar side up. The filter supplied the microorganisms with hydrocarbon by vapour phase transfer to the inocula. The plates were placed in the incubator (Gallenkamp Duostat Incubator size 2 England) at 37 ^{0}C for 2 days after which colonies were counted to determine titre (viable counts per unit) of soil samples (Ichor *et al.*, 2014). This was repeated to all 40 samples collected.

PURIFICATION OF BACTERIA ISOLATES

The streak method was used to subculture the bacteria isolate that grew on the Nutrient Agar. An inoculating loop was sterilized using hot flame, it was allowed to cool before it was used to take part of the grown bacterial colonies from the cultured agar and streaked on the surface of a fresh Nutrient Agar. The pure strains obtained were preserved on nutrient agar slants at 4 ^{0}C for further studies as described by Olukunle (2013).

CHARACTERIZATION OF BACTERIA ISOLATES

Individual bacteria colonies were identified by cultural, morphological and biochemical techniques using the taxonomy scheme of Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). The cultural characterization of bacterial colonies isolated was done by observing the colonies for color, shape, edge, elevation and surface appearance displayed on Nutrient Agar. Biochemical test for bacteria identification included Gram staining, spore staining, catalase test, oxidase test, Indole test, coagulate test, urease test, methyl red and Citrate test.

STATISTICAL ANALYSIS

The data was analyzed using one-way ANOVA with assistance of SPSS version 20 and means were compared using Duncan multiple range test (DMRT). The results were expressed in terms of mean ± standard deviation. All data presented are mean values of triplicate measurement (n=3) obtained from the separate runs.

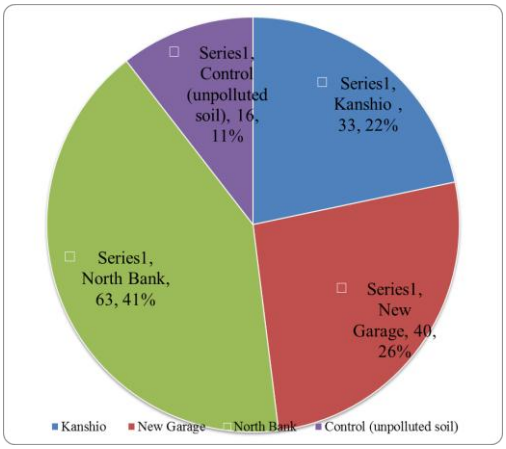
III. RESULTS AND DISCUSSION

Sample No.	Kanshio	North Bank	New Garage	Control
1	2.40×10^5	5.86×10^5	4.16×10^5	1.33×10^4
2.	2.73×10^5	7.33×10^5	2.53×10^5	7.66×10^4
3.	2.60×10^5	5.20×10^5	3.83×10^5	2.33×10^4
4.	3.00×10^5	7.80×10^5	2.83×10^5	9.00×10^4
5.	6.30×10^5	8.16×10^5	2.43×10^5	1.83×10^4
6.	7.50×10^5	8.23×10^5	2.70×10^5	2.00×10^4
7.	2.93×10^5	2.90×10^5	6.40×10^5	1.23×10^4
8.	4.13×10^5	5.20×10^5	3.26×10^5	1.60×10^4
9.	6.66×10^5	6.26×10^5	5.46×10^5	1.90×10^4
10.	3.13×10^5	4.63×10^5	2.36×10^5	2.16×10^4

Table 1: Bacterial Population of Various Soil Samples from some Selected Mechanic Sites and Non-Mechanic Site (Control) in Makurdi

Bacteria Isolates	Kanshio	New Garage	North Bank	Control	Percentage of Isolate
Acinetobacter spp	0	5	4	0	9(5.92%)
B. cereus	3	0	3	2	8(5.25%)
B. subtilis	2	0	2	2	6(3.95%)
Escherichia coli	5	5	6	2	18(11.84%)
Enterococcus spp	0	4	3	2	9(5.92%)
K. pneumonia	3	0	4	0	7(4.60%)
K. oxytoca	0	2	3	0	5(3.29%)
Micrococcus spp	4	0	2	1	7(4.60%)
P. vulgaris	3	6	5	0	14(9.21%)
P. mirabilis	2	2	3	0	7(4.60%)
P. aeruginosa	4	4	5	3	16(10.53%)
P. fluorescens	3	0	3	1	7(4.60%)
P. stutzeri	0	5	9	0	14(9.21%)
S. aureus	4	7	8	3	22(14.47%)
Salmonella spp	0	0	3	0	3(1.97%)
	33 (21.71%)	40(26.31%)	63(41.45%)	16(10.53%)	152(100%)

Table 2: Percentage of Bacteria Isolates from Soils from Mechanic and non-Mechanic sites



ISSN: 2394-4404

Figure 1: Frequencies of Bacteria Isolates in Different Locations Sampled

Bacteria Isolates	NorthBank	Kanshio	New Garage	Control	Means ± SD
Acinetobacter spp	4.00± 0.00^{c}	0.00± 0.00^{a}	5.00± 0.00^{d}	0.00± 0.00^{a}	2.25± 2.63
B. cereus	3.00± 0.00^{c}	3.00 ± 0.00^{c}	0.00± 0.00^{a}	2.00± 0.00^{b}	2.00± 1.41
B. subtilis	2.00± 0.00^{b}	2.00± 0.00^{b}	0.00± 0.00^{a}	2.00± 0.00^{b}	1.50± 1.00
E. coli	6.00± 0.00^{d}	5.00± 0.00^{d}	5.00± 0.00^{d}	2.00± 0.00^{b}	4.50± 1.73
Enterococccus spp	3.00± 0.00^{c}	0.00± 0.00^{a}	4.00± 0.00^{c}	2.00± 0.00^{b}	2.25± 1.71
K. pneumonia	4.00± 0.00^{c}	3.00± 0.00^{c}	0.00± 0.00^{a}	0.00± 0.00^{a}	1.75± 2.06
K. oxytoca	3.00± 0.00^{c}	0.00± 0.00^{a}	2.00± 0.00^{b}	0.00± 0.00^{a}	1.25± 1.50
Micrococcus spp	2.00± 0.00^{b}	4.00± 0.00^{b}	0.00± 0.00^{a}	1.00± 0.00^{a}	1.75± 1.71
P. vulgaris	5.00± 0.00^{d}	3.00± 0.00^{c}	6.00± 0.00^{d}	0.00± 0.00^{a}	3.50± 2.65
P. mirabilis	3.00± 0.00^{c}	2.00± 0.00^{b}	2.00± 0.00^{b}	0.00± 0.00^{a}	1.75± 1.26
P. aeruginosa	5.00± 0.00^{d}	4.00± 0.00^{c}	4.00± 0.00^{c}	3.00± 0.00^{c}	4.00± 1.82
P. fluorescens	5.00± 0.00^{d}	5.00± 0.00^{d}	0.00± 0.00^{a}	1.00± 0.00^{a}	2.25± 2.22
P. stutzeri	9.00± 0.00^{d}	0.00± 0.00^{a}	5.00± 0.00^{d}	0.00± 0.00^{a}	3.50± 436
S. aureus	8.00± 0.00^{d}	4.00± 0.00^{c}	7.00± 0.00^{d}	3.00± 0.00^{c}	5.50± 2.38
Salmonella spp	3.00± 0.00^{c}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.75± 1.50
	4.20± 2.08^{c}	2.33± 1.92^{ab}	2.67± 2.58^{b}	1.02± 1.16^{a}	2.57±2.25

Bacteria Isolates F (14, 42) = 2.41, P= 0.014.
Study Sites F (3, 42) = 8.42, P< 0.001.
Means with same alphabets are not significantly different.
Means with different alphabets are significantly different.

Table 3: Percentage Frequencies of Bacteria Isolated in Respect to Location of the Study Areas in Makurdi

IV. DISCUSSION

The Bacterial population obtained from this study showed that the counts from non - mechanic site (control) were lower than those of the Mechanic sites as shown in Table 1. This confirms the work of Olukunle (2013) on the characterization of indigenous microorganisms associated with crude oil-polluted soils and water using traditional methods and these could be attributed to the nutrient status in the soils. All the bacteria strains isolated were aerobic and belong to ten genera which are *Acinetobacter, Bacillus, Escherischia, Enterococcus, Klebsiella, Micrococcus, Proteus, Pseudomonas, Staphylococcus* and *Salmonella* which confirm the work carried out by Kafizadeh *et al.* (2011) where 80 bacteria strains belonging to 10 genera were isolated and identified. *Pseudomonas, Staphylococcus, Proteus and Escherichia* spp were the most frequently occurring degraders in the order of high occurrence. *Salmonella* spp was the least isolated bacteria found only in samples collected from North bank Mechanic site.

According to Pirnay *et al*. (2005) most opportunistic organisms are successful in environments contaminated by human activities. These organisms can cause morbidities such as intestinal disorders etc. The difference in percentage frequency and mean frequency between the study sites indicated that bacterial isolated from the mechanic sites contaminated with petroleum hydrocarbon products had more degraders as compared to the control. This was expected because of the pollution that had existed.

V. CONCLUSION

It was concluded from findings of this study that microbial population were highest at contaminated soils from mechanic sites as compared to the non – mechanic site. Hydrocarbons from contaminated soils could also serve as nutrients hence the increased bacterial population. It was established from results of this study that predominant petroleum hydrocarbon degrading bacteria in Makurdi metropolis were from the genera *Pseudomonas, Staphylococcus, Proteus* and *Escherichia.*

REFERENCES

[1] Bundy, J. G., Paton, G. I. and Campbell, C. D. (2004). Combined microbial community level and single species biosensor responses to monitor recovery of oil polluted soil. Soil Biology & Biochemistry. 36:1149-1159.

[2] Holt, J. G., Krieg, N. R., Sneath, P. H. A., Stanley, J. T., and William, S. T. (1994) Bergey's Manual of Determinative Bacteriology. Baltimore, USA: Williamand Wilkins.

[3] Ichor, T., Okerentugba, P. O., and Okpokwasili, G. O. (2014). Molecular Characterization of Aerobic Heterohophic Bacteria Isolated from Petroleum Hydrocarbon Polluted Brackish Waters of Bodo Creeks, Rivers State Nigeria. Open Journal of Ecology, 4, 715-722

[4] Kafizadeh, F., Houshang Shiva, A. and Malekpour, R. (2011). Determination of polycyclic aromatic hydrocarbons (PAHs) in water and sediment of the Kori River, Iran. Middle East Journal of Science Research.10:01-07.

[5] Mathe, I., Benedek, T., Tancsics, A., Palatinszky, M., Lanyi, S., Marialigeti, K. (2012). Diversity, activity, antibiotic and heavy metal resistance of bacteria from petroleum contaminated soils located in Harghita County (Romania). International Biodeterioration and Biodegradation. 73:41e9.

[6] Okoh, A. I. (2003). Biodegradation of Bonny light crude oil in soil microcosms by some bacterial strains isolated from crude oil flow stations saver pits in Nigeria. African Journal of Biotechnology. 2(5):104-108.

[7] Olukunle .O. F, (2013) Characterization of indigenous Microorganisms associated with crude oil-polluted soils and water using Traditional techniques. Microbiology journal, 3:1-11.

[8] Oteyza de, T. G., Grimalt J. O., Lliros M. and Esteve I. (2005). Microcosm experiments of oil degradation by microbial mats. Science of the Total Environment. Article in press.

[9] Pirnay, J. P., Matthijs, S., Colak, H., Chablain, P., Bilocq, F., Van Eldere, J., De Vos, D., Zizi, M., Triest, L., and Cornelis, P. (2005). Global Pseudomonas aeruginosa biodiversity as reflected in a Belgian river. Environmental Microbiology. 7:969– 980.

[10] Rojo, F., and Martınez, J. L. (2010). Oil Degraders as Pathogens, Handbook of Hydrocarbon and Lipid

Microbiology, K. N. Timmis (ed.), DOI 10.1007/978-3-540-77587-4_250 Springer-Verlag Berlin Heidelberg.

[11] Sudhir, K. S., Jai Godheja and Modi, D.R. (2014) Hydrocarbon Bioremediation Efficiency by five Indigenous Bacterial Strains isolated from Contaminated Soils. International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 4 Number 3 pp. 892-905 http://www.ijcmas.com