International Journal of Engineering Science Invention (IJESI)
ISSN (Online): 2319-6734, ISSN (Print): 2319-6726
www.ijesi.org ||Volume 9 Issue 3 Series. I || Mar.2020 || PP 55-66

Comparative Enumeration of the Effect of Soil Particle Sizes on Bioremediation of Crude Oil from Exploration and Pristine Regions of Nigeria

*[1]Abdullahi, A; [1]Idiris M; [1]Muhd F and [2]Ishaq S.A.
[1,](Department of Scie. Lab. Tech; School of Technology, Kano state Polytechnic, Nigeria.)
[2,] (Department of Microbiology, Fedral University Gusau, Zamfara Nigeria.)
correspondence author: Abdullahi

Abstract: *Biodegradation of hydrocarbon-contaminated soils, which exploits the ability of microorganisms to degrade organic contaminants, has been established as an efficient, economic, versatile and environmentally sound treatment. The ability of hydrocarbon degrading bacteria was tested in soil contaminated with crude oil. Soil samples were analyzed to isolate and identify population of aerobic heterotrophic bacteria (AHB) and hydrocarbon degrading bacteria (HDB) in soils of Warri Delta State and Kura Kano State, from where bacillus subtilis was isolated. The Bacillus subtilis isolated from the regions where the soils have been collected was inoculated again into the same soils of exploration region and pristine region of soil particle sizes 0.6mm,0.3mm and 0.15 mm after sterilizing and contaminating the soils with crude oil and the degradation potential was evaluated using FourierTransmission Infrared spectroscopic analysis (FTIR) in twelve (12) day , at four (4) days and eight (8) days intervals respectively. The bacterial strain isolated seem to have potentiality or capability to remediate the contaminated soil. Results of the FTIR obtained were subjected to statistical analysis one way (ANOVA) using DMRS version 20.0 did not differ significantly ($p<0.05$).*

Keywords - *Biodegradation, Bacillus sutilus, Petroleum products, Pristine region, Exploration Region,*

Date of Submission: 28-02-2020 Date of Acceptance: 11-03-2020

I. INTRODUCTION

Soil accommodates one of the most complex and dynamic microbial assemblies in the entire biosphere [1]. Different sizes of soil particles and aggregates in infinite combination result in a highly diverse physical environment with heterogeneity readily displayed at very fine scale [2].The diversity of physical characteristics of soil can harbor a large diversity of microorganisms in close proximity due to the minute size of microorganisms. Furthermore, the relationships between microorganisms and soil particles are fully interactive: while soil particles control the survival and biological activity of microorganisms [2].Soil microorganisms influence soil structure by modifying the soil particles' arrangement or providing cohesive materials to help form and stabilize aggregates [3].

Soil texture and soil structure are both unique properties of the soil that will have a profound effect on the behavior of soils, such as water holding capacity, nutrient retention and supply, drainage, and nutrient leaching. The texture of soil is determined primarily by the size of the mineral particles that make up the soil. The mineral particles of a soil are present in a wide range of size and in association with organic matter. Fine earth fraction includes all soil particles that are less than 2 mm. Soil particles within this fraction are further divided into three (3) separate size classes, which includes sand, silt, and clay. The size of sand particles range between 2.0 and 0.05 mm; silt, 0.05 mm and 0.002 mm; and clay, less than 0.002 mm [4]

Crude oil is a naturally occurring liquid found in formations in the earth, it is a heterogeneous liquid consisting of hydrocarbons and elements such as nitrogen, sulphur, and oxygen, all of which constitute less than 3% (v/v) . Hydrocarbons are compounds made up of mainly carbon and hydrogen atoms with different variations in the individual atoms. The most common source of the hydrocarbons is the crude petroleum. Crude oil is composed of complex mixture of the three main classes of hydrocarbons; aliphatic, alicyclic, and aromatics [5].

Petroleum products remains the principle source of energy, however because of its importance and extend to which it is used on land, crude petroleum spill has continued to be one of the most devastating environmental pollutant globally [6][7]. Soil contamination by hydrocarbon is one of the world's most common environmental problems [8].

Crude oil spillage has been a challenge, most especially in Nigeria. This is borne out of crude oil exploration and exploitation activities particularly in the oil rich Niger Delta States of Nigeria, which can be located between Longitude 3° 19' and 15° 11' East and Latitude 40° 30' and 14° 34' North. This region is very

rich in hydrocarbon deposits, and these results in more than eighty percent of the Nation's Foreign Exchange earnings.

Petroleum based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during exploration, production, refining, transport, and storage of petroleum and petroleum products[9] .Soil contamination with hydrocarbons can enter food chain and seriously affect animals and human health causing death or mutations [10] .The oil spill which penetrates to a depth of about 10-20cm has negative impact on agricultural activities, resulting in the loss of soil fertility and also initiates environmental degradation making it impossible for the soil to produce at its optimal capacity as a result of hardening of the soil structure by the oil .

Soils with predominantly large particles tend to drain quickly and have lower fertility. Very fine-textured soils may be poorly drained. Soils with a medium texture and a relatively even proportion of all particle sizes are most versatile .Hence, the effect of soil particle sizes on bioremediation of crude oil of contaminated and uncontaminated soil need to be investigated.

The high incidence and frequency of crude oil spill have been a great source of environmental contamination especially in the Niger Delta Region of Nigeria .The Federal Government of Nigeria through the Nigerian National Petroleum Corporation (NNPC) had recently announced the discovery of crude oil in commercial quantities along Chad Basin, which cut across some states in Northern Nigeria and planed to commence exploration activities in earnest. There is the possibility of greater incidence of oil spill in those areas because hydrocarbon contamination are known to be widespread in oil producing areas and are of public health concern. Thus, the importance of this study has no other better time than now, as it could contribute to a better understanding of the interactions between soil particle sizes and hydrocarbons that can be explored for the remediation of crude polluted soils. Many research activities have been conducted on the physiochemical and microbial characteristic of various soils, but not much has been done on the effects of soil particle sizes on bioremediation. Therefore, results of this study will also assist in generating information and data required to aid in bioremediation strategies of crude oil spills particularly in soil with different textural properties.

II. Materials And Methods

2.1 Sample Collection

Fifty (50kg) soil sample was collected from of Kura local government of Kano State in north western region of Nigeria. Another Fifty (50kg) soil samples was collected from the contaminated soil of crude oil exploration area of Warri local government of Delta state in south western region of Nigeria. All samples were collected using standard procedures, stored in a clean polythene bag and labeled. They were then transported to the Biology/Microbiology laboratory of Science Laboratory Technology (SLT), School of technology, Kano State polytechnic for the analysis. Crude oil used for this study was obtained from the Kaduna Refining and Petrochemical Company (KRPC), Kaduna State.

2.2 Sieve analysis

The soil samples were air dried and sieved using sieves of different pore sizes; 0.6mm, 0.3mm, and 0.15mm respectively. Soil particles that passed through the various sieve mesh were weighed and stored in a sterile plastic container for further analysis.

2.3 Set up of Laboratory Bioremediation Experiment

Exactly twenty five (25) grams each of the sieved soil particle sizes; 0.3mm, 0.6mm and 0.15mm were weighed and poured into a mason jar bottle containing 25ml of Bushnell- Haas broth medium (composed of (g/l): $MgSO_4.7H_2O$, 0.2; K_2HPO_4, 1.0; KH_2PO_4, 1.0; $FeCl_3$, 0.05; NH_4NO_3, 1.0; $CaCl_2$, 0.02; pH to 7.2). One 1ml of crude oil was also added (to serve as the sole carbon source), and incubated at 37^0C for 12 days. The samples were replicated in triplicates for each of the soil particle sizes, with each having control.

The experiment was repeated for the remaining soil particle sizes, 0.6mm, 0.15mm respectively. These procedure was done for both sample from Warri Delta and Kura Kano at 4 days and 8 days interval respectively, the residual crude oil was extracted from the soil using Soxhlet method and the level of microbial degradation of the oil was determined using Fourier Transform Infrared Spectroscopy (FTIR) analysis.

2.4 Enumeration of total aerobic heterotrophic bacteria and hydrocarbon utilizing bacteria.

Exactly one gram (1g) each of the samples was weighed aseptically and placed in test tube containing 9ml of distilled water the test tube was shaken vigorously in order to dislodge the microorganisms that adhered to the soil particles. The content of the tube was serially diluted from 10^1 to 10^7. Aliquot (0.1ml) from dilution (10^{-7}) was plated in triplicates on to sterile Nutrient agar (NA) and (10^{-5}) on oil agar (OA) for the enumeration of total aerobic heterotrophic bacteria and crude oil utilizing bacteria respectively. The plates were incubated at room temperature (30 ± 2^0C) for 48 hours and 5 days for OA, respectively. The colonies, which developed on the

plates was then counted and recorded as colony forming units per gram (cfu/g) of soil [11]. Pure cultures of the isolates were obtained by repeated sub-culturing on media used for primary isolation. The pure isolates were maintained on agar slants for further characterization and identification [12].

2.5 Identification of bacterial Isolates

Bacteria colonies were isolated and identified to their species level using conventional morphological, physiological, biochemical test and molecular analysis as described by[13][14]

2.5.1 Gram Staining

A smear of the culture of the test organism was prepared on a clean glass slide and heat fixed. It was stained with crystal violet solution for 60 seconds and washed with tap water. It was then flooded with lugos iodine solution for 30 seconds and washed with tap water after which it was decolorized by adding alcohol in drop wise manner until all free colors had been removed, and again washed with water. The slide was flooded with safranin (counter stain) for 30 seconds, rinsed with water and allowed to air dry. The slides were then examined under oil immersion objective (×100) [14].

2.5.2 Coagulase test

Two drops of physiological saline was placed on a clean glass slide. A colony of the test organism (previously checked by Gram Staining) was emulsified on the drop of the physiological saline. Loop full of plasma was added to the suspension and mixed gently. Clumping of organisms within 10 seconds indicated positive Coagulase test while absence of clumping within 10 seconds indicated negative Coagulase test [14].

2.5.3 Catalase test

Catalase test was done by putting a drop of 3% hydrogen peroxide on a clean glass slide. With the edge of another slide a colony of the organism was picked and placed in the 3% hydrogen peroxide. Presence of bubbles indicates positive reaction while absence of bubbles shows a negative reaction [14].

2.5.4 Indole test

Exactly One percent (1%) of tryptophan broth in a test tube was inoculated with bacterial colony. After incubation at 37℃ for 48hours one milliliter of chloroform was added to the broth. The test tube was gently shaken. Then 2ml of Kovac`s reagent was added and this was also shaken gently and allowed to stand for 20 minutes. The formation of red coloration at the top layer indicates positive reaction while yellow coloration shows a negative result [14].

2.5.5 Methyl red/Voges-proskauer (mrvp) test

This was carried out by inoculating Five milliliters (5ml) of methyl red- Voges-proskauer broth with the test organism and incubated at 35℃ for 48hours. After the period of incubation, one milliliter (1ml) of the broth was transferred into a test tube and five drops of methyl red reagent were added. A red color on addition of the indicator signified a positive methyl red reaction while yellow color signified a negative test [14].

2.6 Measurements of rates of crude oil biodegradation by isolates

The basis for the assessment of biodegradation in this study was the determination of the total petroleum hydrocarbon (TPH) residue after the periods of incubation. The residual crude oil was extracted from the soil using Soxhlet extraction method and the level of microbial degradation of the oil was determined using Fourier Transformed Infrared Spectroscopic analysis of oil extracts (FTIR) analysis.

III. Result And Discussion

3.1 Result

Table 1 Present the result of gram staining of soil obtained from delta and Kano state. From the result a gram positive rod shape Bacillus subtilis was isolated from both pristine region and exploration region. Biochemical test was further carried out to confirm the identification of the bacteria isolated from the exploration region and pristine region. From the result Bacillus sp was Indole negative, methyl red negative and Catalase and Coagulase positive respectively (Table 2).

The FTIR analysis of different particles size of soil (0.6, 0.3 and 0.15) obtained from exploration region of Delta at zero; four and 12 days interval respectively were conducted (Table3). Soil of 0.6mm particle size (Fig 1) revealed band at 2951-2854 cm^1 indicating -C-H stretching in aliphatic compounds after four days; However, the FTIR spectrum (Fig 2) after 12 days of microbial incubation also revealed band at 2951-2851 cm^1 indicating -C-H stretching in aliphatic compounds, new band at 1640-1380 cm were revealed indicating C=O stretching and at 1037 cm^1 related to stretching of primary alcohols; at 795.4 cm signifying the presence of alkyl

halides. It was observed that there is significant change in transmittance which increased after 12 days of incubation with bacteria representing degradation of complex mixtures. This is in agreement with the work of [15].

Meanwhile after 4 days of incubation the soil of particle size 0.3mm (Fig 3) revealed bands at 3294 cm^{1} bands corresponding to hydroxyl stretching of alcohols and phenols; at 2921 cm^{-1}, 2925 cm^{-1} and 2854 cm^{-1} indicating -C-H stretching in aliphatic compounds; at 1640 cm^{-1} due to the presence of carboxylates; 1037 cm^{-1} corresponding to stretch of primary alcohol . However, the FTIR spectrum after 12 days of microbial incubation(Fig 4) revealed bands at 3692 cm^{-1} corresponding to hydroxyl stretching of alcohols and phenols; at 2921 cm^{-1} , 2925 cm^{-1} and 2854 cm^{-1} indicating -C-H stretching in aliphatic compounds; new bands at 1871 cm^{-1} cm,780 cm^{-1} , 1164 cm^{-1} were revealed and at 695 cm^{-1} revealed the presence of aryl halides [16]. These bands could be associated with new carbonyl groups arising from the microbial oxidation. The Besides the per cent transmittance increased which shows the high degradation of hydrocarbon compounds by the treatment with the microbial strains.

However, The FTIR spectrum (Fig.5) of the soil of particle size 0.15 mm obtained from exploration region of Delta state after 4 days incubation period revealed bands at 2951cm^{-1}, 2921 cm^{-1}, and 2854 cm^{-1} indicating -C-H stretching in aliphatic compounds; at 1037 cm^{-1} corresponding to stretch of primary alcohol; at 3346 cm^{-1} revealed bands. However after 12 days of incubation period the FTIR spectrum (Fig 6) revealed the bands at 2951 cm^{-1}, 2921 cm^{-1} and 2854 cm^{-1} indicating -C-H stretching in aliphatic compounds respectively. There was disappearance of some bands present in the soil after 4 days incubation and appearance of new bands at 1640 cm^{-1}, 1462 cm^{-1} and 1380 cm^{-1}. This may be due to degradation action by hydrocarbon degrading bacteria in the soil.

The FTIR analysis of different particles size of soil (0.6, 0.3 and 0.15) obtained from pristine region of Kano at zero, four and 12 days interval respectively were also analyzed (Table4). The FTIR spectrum (Fig.7) of the soil particle size 0.6 mm obtained from pristine region of Kano state after 4 days incubation period revealed bands at 2951cm^{-1}, 2921 cm^{-1}, and 2854 cm^{-1} indicating -C-H stretching in aliphatic compounds; at 1037cm-1 corresponding to stretch of primary alcohol; at 3335 cm^{-1} indicating hydroxyl stretching in phenols, alcohols and new bands were revealed at 1458 cm^{-1} and 1380 cm^{-1} . Meanwhile after 12 days Interval (Fig 8) also revealed bands at 2951cm^{-1}, 2921 cm^{-1}, 2854 cm^{-1} indicating -C-H stretching in aliphatic compounds. Then with disappearance of bands at 3335 cm^{-1} and appearance of new bands at 1704 cm^{-1}, 1380 cm^{-1}, 1462 cm^{-1} and 1607 cm^{-1}. This may be due to the microbial oxidation. Also the transmittance increased significantly after the treatment with bacterial strains.

FTIR spectrum (Fig.9) of the soil of particle size 0.3 mm obtained from pristine region of Kano state after 4 days incubation period revealed bands at 2951cm^{-1}, 2921 cm^{-1}, and 2854 cm^{-1} indicating -C-H stretching in aliphatic compounds; soil revealed bands at 3335 cm^{-1} corresponding to hydroxyl stretching in phenols, alcohols; other bands at 1011 cm^{-1} cm and at 1033 cm^{-1} corresponding to stretch of primary alcohol respectively, and another new bands at 1380 cm^{-1} . However, the FTIR spectrum after 12 days of microbial incubation(Fig 10) indicated bands at 2951cm^{-1}, 2921 cm^{-1}, and 2854 cm^{-1} indicating -C-H stretching in aliphatic compounds; and at the same soil revealed bands at 3335 cm^{-1} corresponding to hydroxyl stretching in phenols, alcohols. Other bands indicated after 4 days incubation was also revealed in addition to new bands at 1458 cm^{-1} and at 877 cm^{-1}. This may be due to microbial degradation and also the transmittance increased significantly after the treatment with bacterial strains.

FTIR spectrum (Fig.11) of the soil of particle size 0.15 mm obtained from pristine region of Kano state after 4 days incubation period revealed bands at 2951cm^{-1}, 2921 cm^{-1}, and 2854 cm^{-1} indicating -C-H stretching in aliphatic compounds; other bands at 2125 cm^{-1} corresponding to C-C, C N (Nitriles); and at 1037 cm^{-1} corresponding to stretch of primary alcohol, at 1640 cm^{-1} cm indicating C=O stretching and another new bands at 1380 cm^{-1} cm. However, the FTIR spectrum after 12 days of microbial incubation(Fig 12) indicated bands at 2951cm^{-1}, 2921 cm^{-1}, and 2854 cm^{-1} indicating -C-H stretching in aliphatic compounds; and at the same soil revealed bands at 3692 cm^{-1} and 3346 cm^{-1} cm corresponding to hydroxyl stretching in phenols, alcohols respectively. These bands could be associated with new carbonyl groups arising from the microbial oxidation of the crude oil. Besides the per cent transmittance increased which shows the high degradation of hydrocarbon compounds by the treatment with the bacterial strains.

At given environmental conditions, the degree of hydrocarbon biodegradation is mainly affected by the type of hydrocarbons in the contaminant matrix [17] Of the various petroleum fractions, n-alkanes and branched alkanes of intermediate length (C10–C20) are the preferred substrates to microorganisms and tend to be most readily degradable. Longer chain alkanes (>C20) are hydrophobic solids and are difficult to degrade due to their inherent recalcitrance and their poor water solubility [18]

Results obtained were statistically analyzed by one way (ANOVA) using SPSS version 20.0. DMRS was used to separate means of significant difference ($p<0.05$).

Table; 1 Result of Gram staining of soils obtained from Delta and Kano states.

S/n	Region	Colonial morphology after 24hrs incubation at 37^0c	Gram reaction	Presumptive identification
1.	Warri(Delta)	White spherical growth glassy colonies	Gram positive rod	Bacillus subtilis
2.	Kura(Kano)	White spherical growth glassy colonies	Gram positive rod	Bacillus subtilis

Table: 2 Biochemical test carried out for the identification of the bacteria isolated from the regions.

Region	Organism	Shape	Gram reaction	Catalase	Coagulase	Indole	Methyl red
Delta	Bacillus subtilis	Rod	Gram +ve	+	+	-	-
Kano	Bacillus subtilis	Rod	Gram +ve	+	+	-	-

Key:+ = positive and - = negative

Table 3Result of Hydrocarbon degradation of Bacillus subtilis from soil collected in Delta state

Soil Size	Treatment/incubation Time	Wave number(cm^{-1})
	Soil (control)	2704 ± 2.82^a
0.6		
	S + C+O	2864 ± 1.88^a
	After 4 days	2767 ± 1.25^a
	After 12 days	2778 ± 3.98^a
	Soil (Control)	2715 ± 2.17^a
	S + C + O	2864 ± 4.52^a
0.3		
	4 days	2823 ± 4.83^a
	12 days	7057 ± 9.44^a
	Soil (control)	2437 ± 1.19^a
	S + C + O	2847 ± 5.41^a
	After 4 days	7580 ± 3.92^a
0.15		
	After 12 days	3060 ± 4.87^a

Key; S + C+O = Soil+ Control + oil

Values are means and Standard deviation, values with the same superscripts along a column did not differ significantly ($P<0.05$).

Table 4 : Result of Hydrocarbon degradation of Bacillus subtilis from soil collected in Kano state

Soil Size	Treatment/incubation time	Wave number(cm^{-1})
	Soil (control)	2887 ± 9.40^a
0.6		
	S + C+O	2812 ± 8.65^a
	After 4 days	7374 ± 1.65^a
	After 12 days	7925 ± 1.11^a
	Soil (control)	2715 ± 2.44^a
0.3		

	S + C + O	2864 ± 7.92[a]
	4 days	2789 ± 9.40[a]
	12 days	2834 ± 2.76[a]
	Soil (control)	3060 ± 4.34[a]
0.15	S + C + O	2978 ± 1.70[a]
	After 4 days	2851 ± 4.00[a]
	After 12 days	3060 ± 4.89[a]

Key; S + C+O = Soil+ Control + oil

Values are means and Standard deviation, values with the same superscripts along a column did not differ significantly (P<0.05).

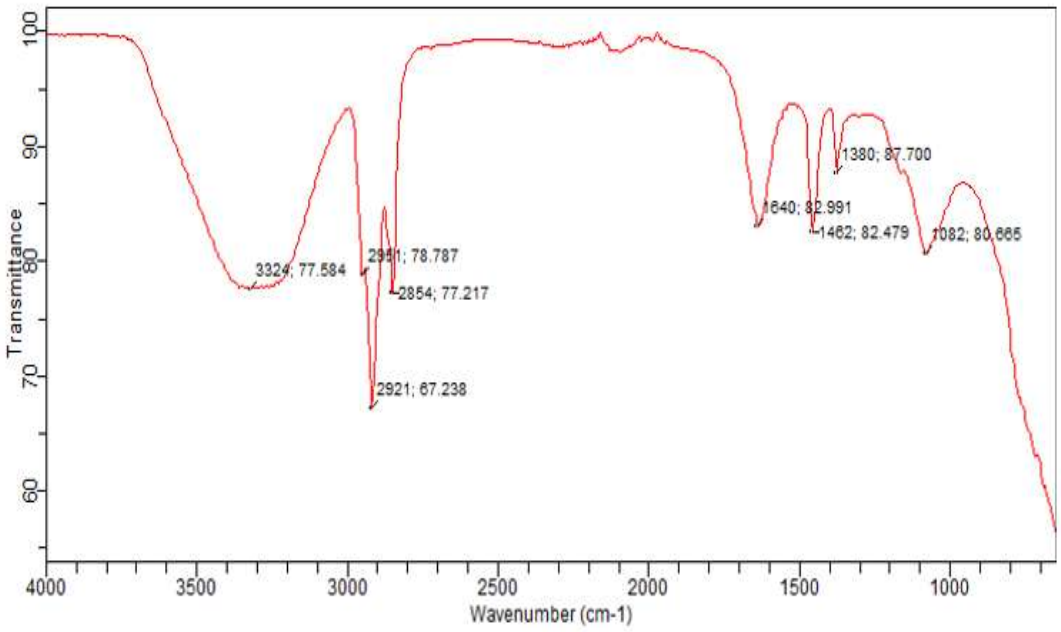

Fig 1. FTRI analysis of 0.6mm soil obtained from Warri Delta after 4 days incubation period.

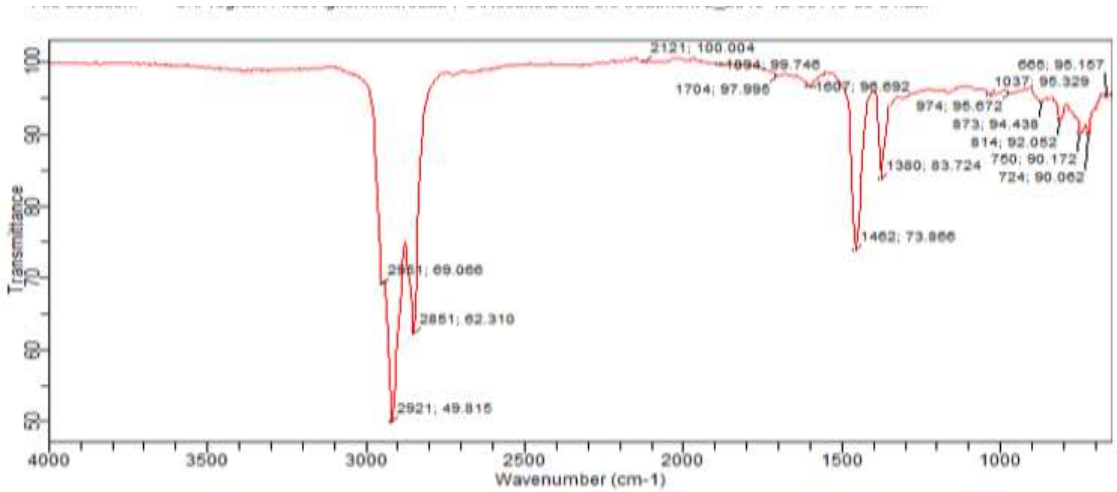

Fig 2. FTRI analysis of 0.6mm soil obtained from Warri Delta after 12 days incubation period.

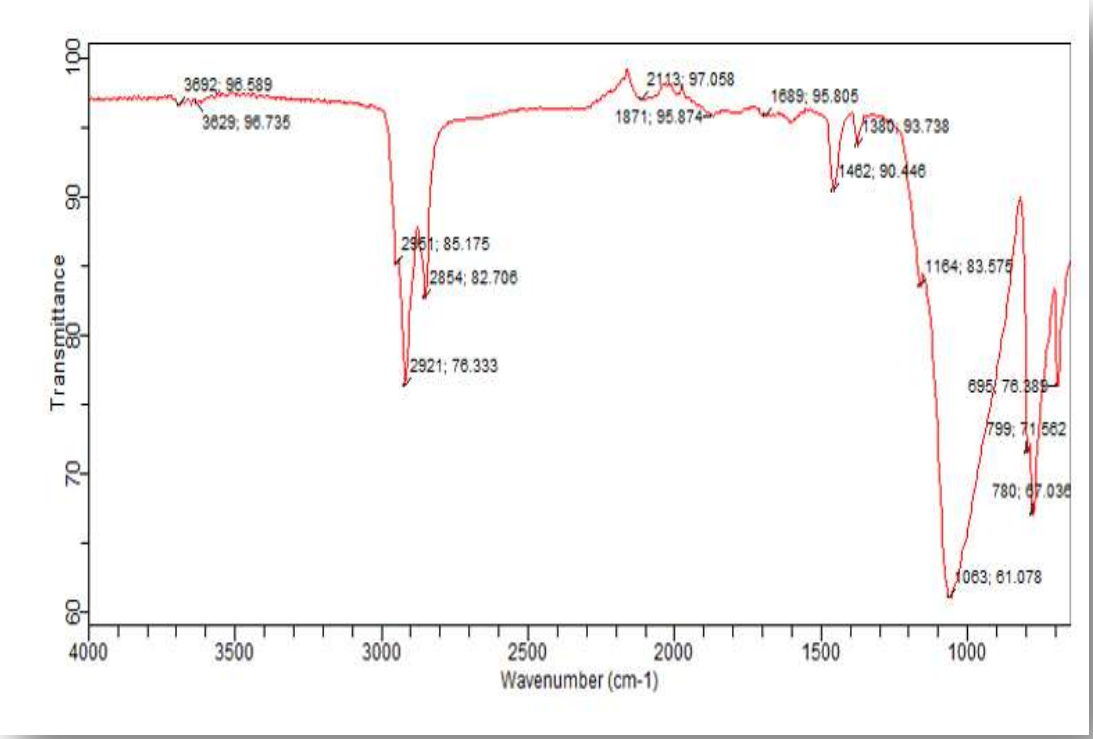

Fig 3. FTRI analysis of 0.3 mm soil obtained from Warri Delta after 4 days incubation period.

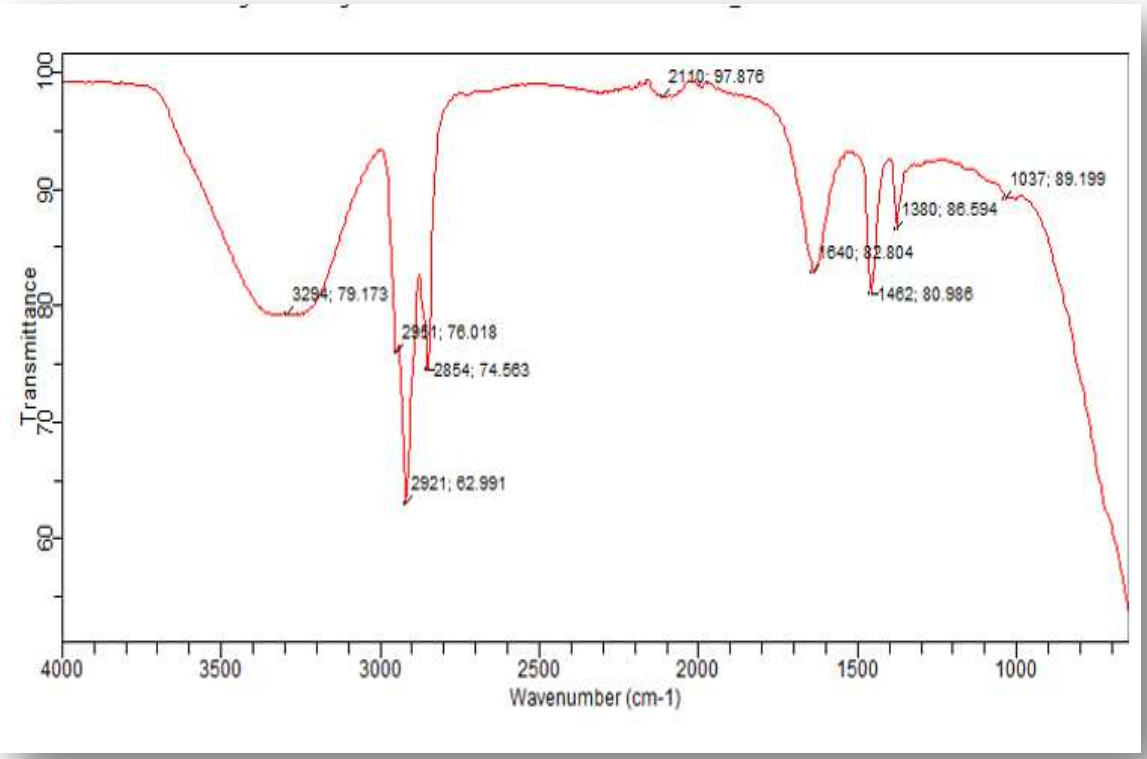

Fig 4. FTRI analysis of 0.3 mm soil obtained from Warri Delta after 12 days incubation period.

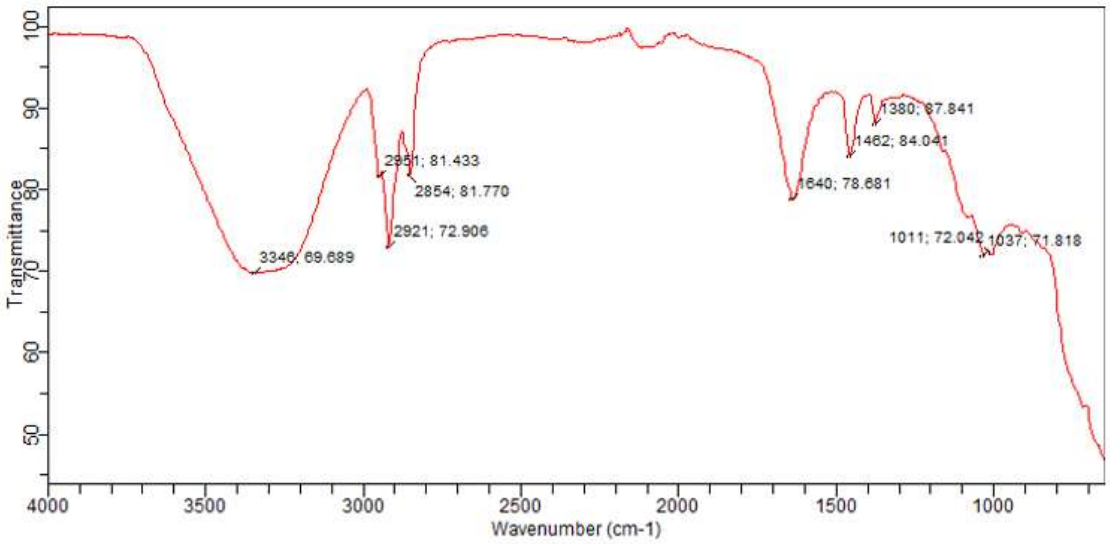

Fig 5. FTRI analysis of 0.15 mm soil obtained from Warri Delta after 4 days incubation period

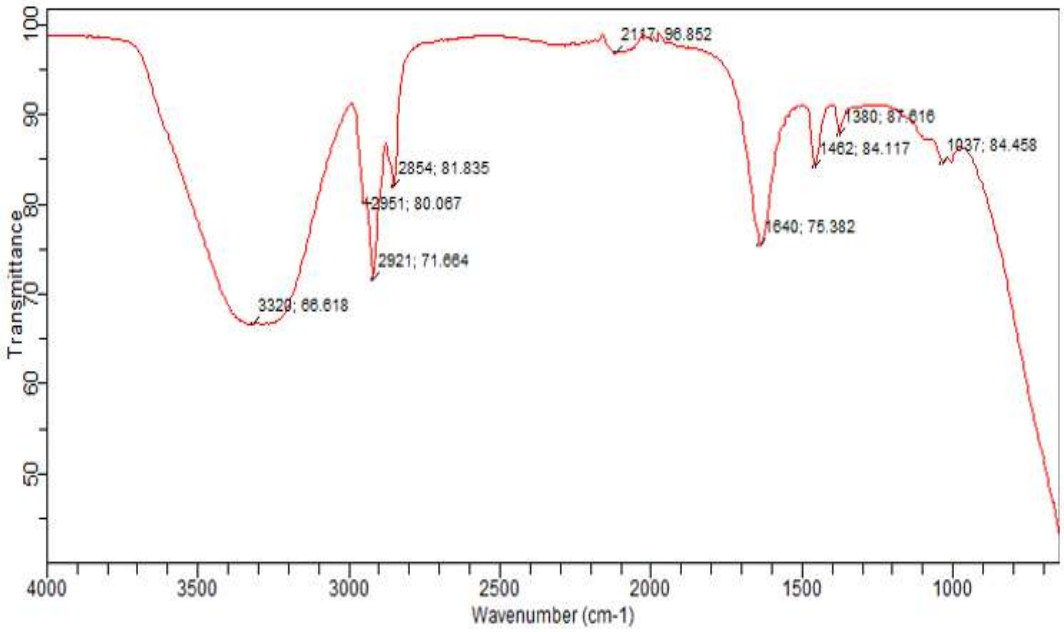

Fig 6. FTRI analysis of 0.15 mm soil obtained from Warri Delta after 12 days incubation period

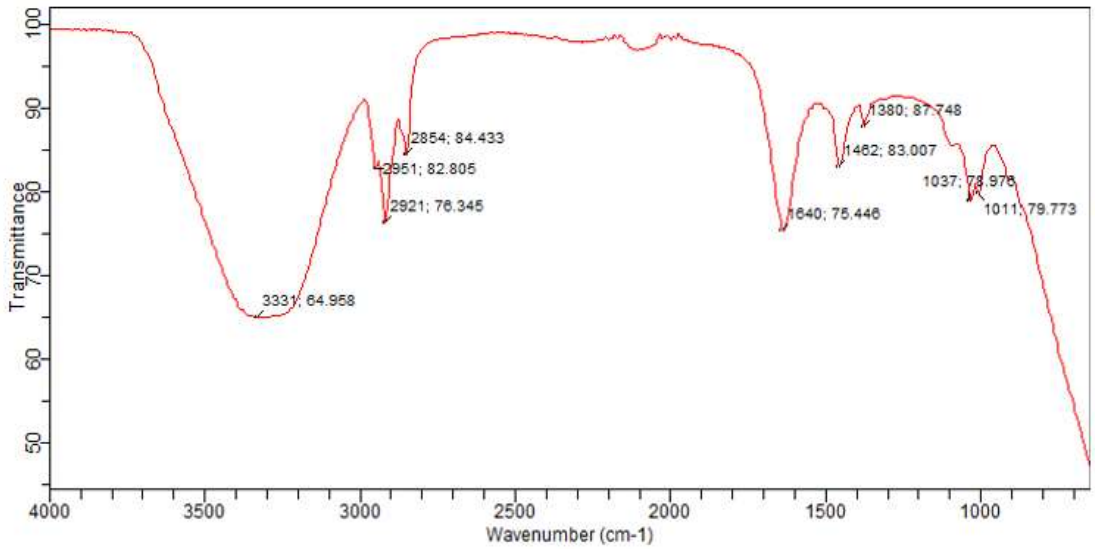

Fig 7. FTRI analysis of 0.6 mm soil obtained from Kura Kano after 4 days incubation period

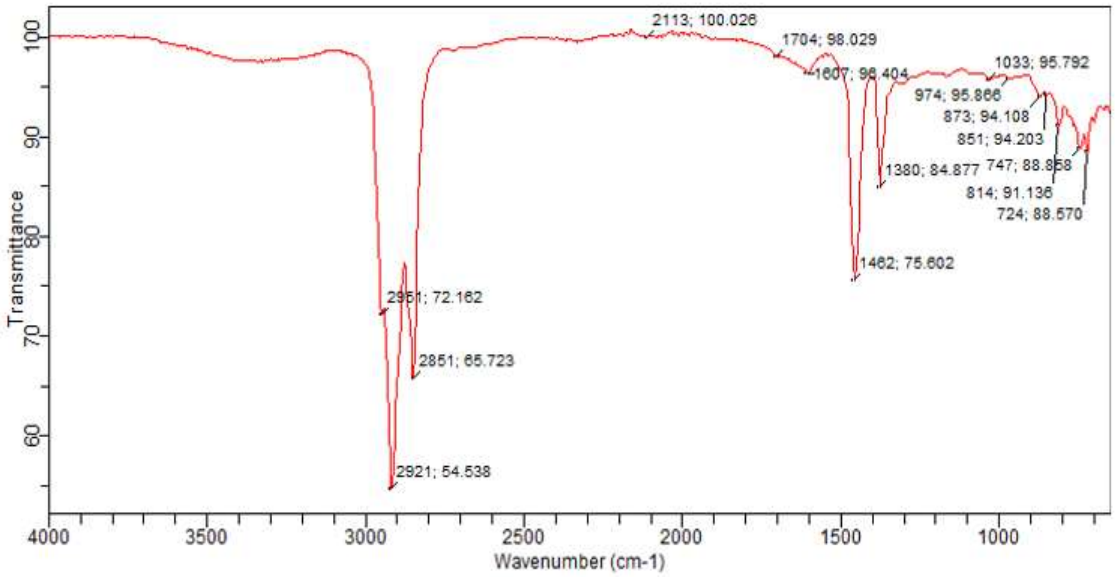

Fig 8. FTRI analysis of 0.6 mm soil obtained from Kura Kano after 12 days incubation period

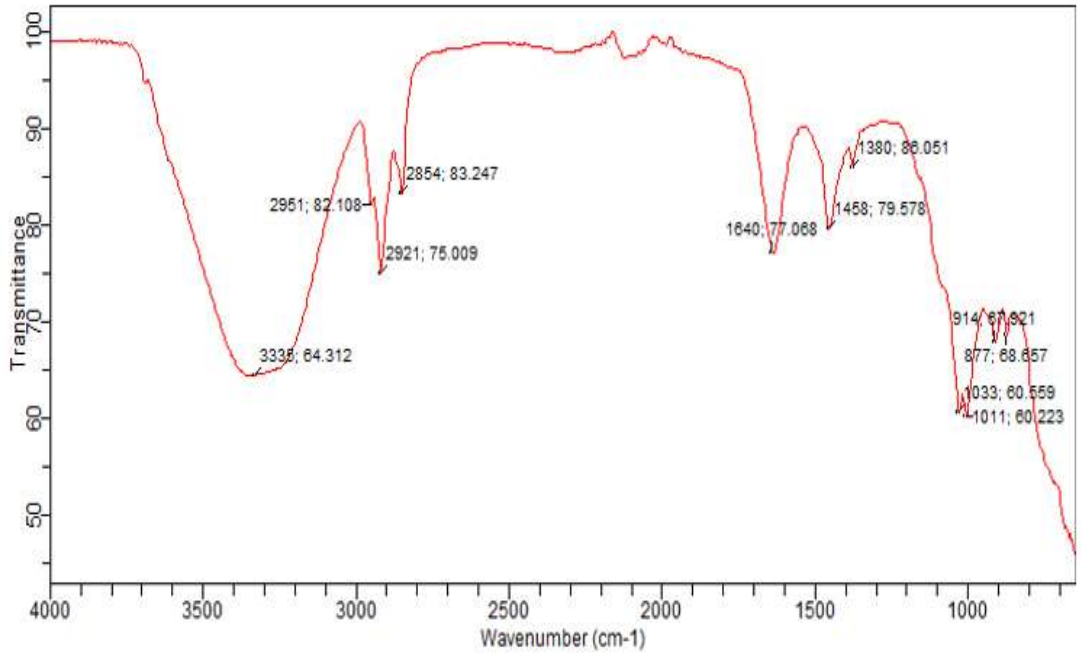

Fig 9. FTRI analysis of 0.3 mm soil obtained from Kura Kano after 4 days incubation period

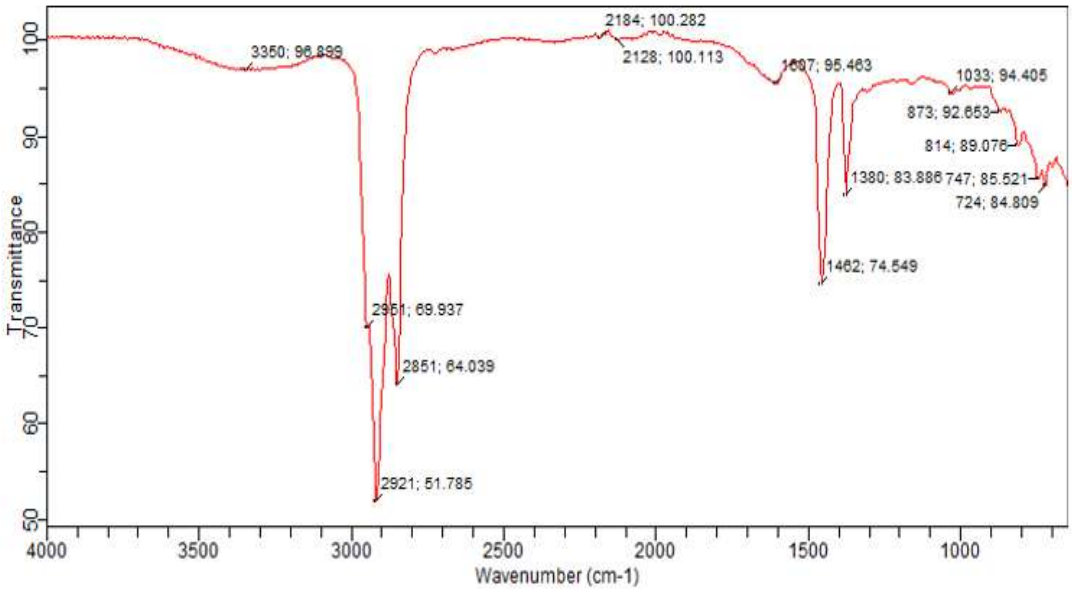

Fig 10. FTRI analysis of 0.3 mm soil obtained from Kura Kano after 12 days incubation period

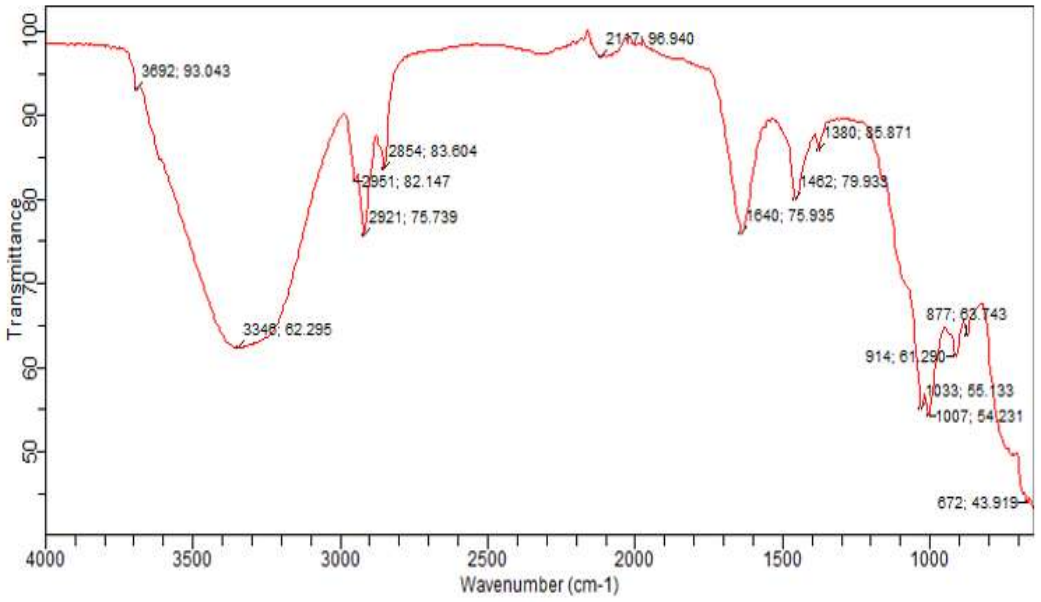

Fig 11. FTRI analysis of 0.15 mm soil obtained from Kura Kano after 4 days incubation period

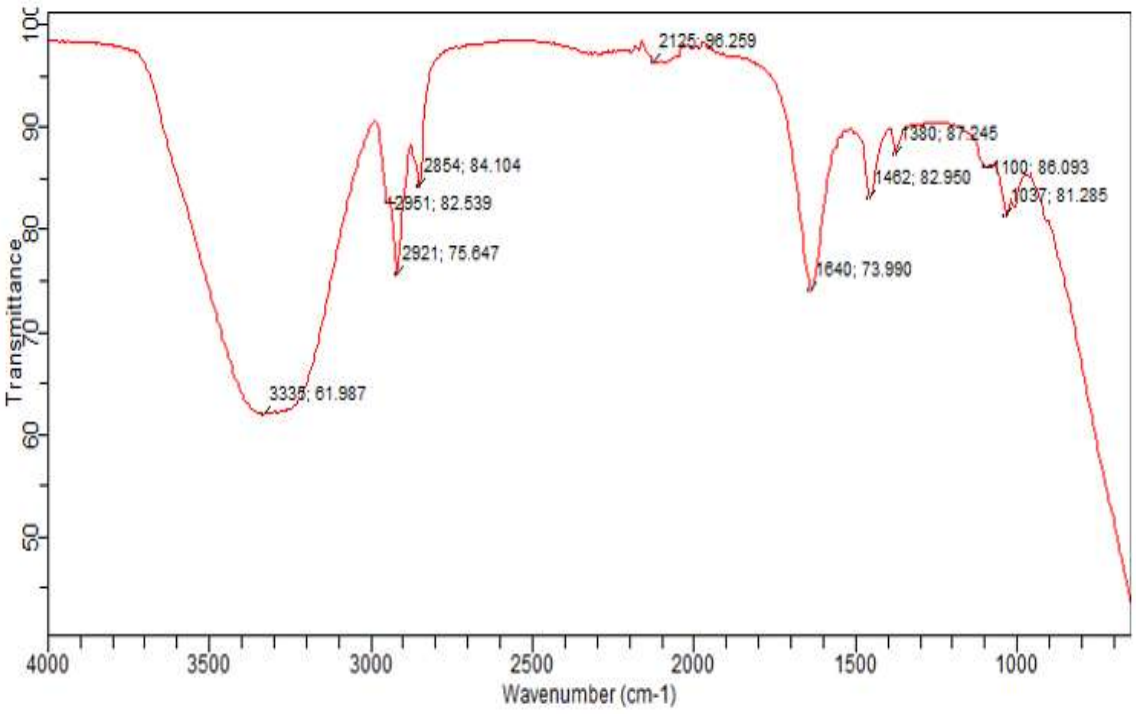

Fig 12. FTRI analysis of 0.15 mm soil obtained from Kura Kano after 12 days incubation period

IV. CONCLUSION

The FTIR spectra recorded at different time intervals delivered a good indication of hydrocarbon degradability in soil by the isolated bacterial strains. The results suggest that the bacterial strains prefer C-H aliphatic and aromatic stretches for degradation of long chain alkanes present in soil contaminated with crude oil. It is therefore concluded that by using the isolated bacterial strain, the soil which has lost its fertility because of the oil may be remediated and be turned back as fertile soil. Moreover, FTIR spectroscopy can be a very useful tool in performing preliminary tests in order to predict remediation performance so as to select an appropriate approach for clean- up technologies.

The research recommends that, further studies be carried out on contaminated soils of varied particle sizes for better and the most efficient bioremediation. Combined practice of bio augmentation and bio stimulation will help to improve the rate of degradation of crude oil polluted soils. Sponsorship into the technological researches of this kind by Governments and other agencies should be encouraged in order to achieve better output of the result.

Acknowledgements

The authors gratefully acknowledge the Tertiary Education Trust Fund (TETFund) for providing research grant. The department of Science Laboratory Technology are acknowledged with thanks for enormous assistance by dedicating of Laboratory for the conduct of the research and other facilities as the case maybe.

REFERENCES

[1]. Curtis, T.P., Sloan, J.T. and Scannell, W.T.. Estimating prokaryotic diversity and its limits. Proceedings of the National Academy of Sciences of the United States of America 99: 10494-10499, 2002.
[2]. Bashir, A.H.Diversity and morphology of bacterial community characterized in topsoil samples from the Gaza Strip, Palestine. Research Journal of Microbiology 7(6): 309-318, 2012.
[3]. Bossuyt, H., Frey, R. M. and Paustian, K.. Influence of microbial populations and residue quality on aggregate stability. Applied Soil Ecology 16: 195-208 2001.
[4]. Silva, J. and Uchida, R.S. Plant Nutrient Management in Hawaii's Soils: Approaches for Tropical and Subtropical Agriculture. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu 2000.
[5]. Kafilzadeh, F., Sahragard, P., Hooshang, J. and Tahery, Y. Isolation and identification of hydrocarbon degrading bacteria in soil around shiraz refinery. African Journal of Microbiology Research 4(19): 3084-3089 2011.
[6]. Chikere, C. B. 2006. Microbial diversity and physicochemical properties of a crude oil polluted soil. Nigerian journal of microbiology, 20(2); 1039-1046.
[7]. Plohl K, L. H. 2000, Biological degredation of motor oil in water. Acta Chim. Slovenica. 49; 279-289.
[8]. EPA, U. 2000. Introduction to phytoremediation.Environmental Protection Agency, p; 5.
[9]. Antai, S. P. 1990. Biodegradation of Bonny light crude oil by Bacillus sp. and Pseudomonas sp. Waste Management 10: 61-64.
[10]. Das, K., Mukherjee, AK. 2013. "Crude petroleum-oil biodegradation efficiency of Bacillus subtitles and Pseudomonas aerations strains isolated from a petroleum-oil contaminated soil from North-East India." Bioresearch Technology; 98(7):1339–1345, 2011.
[11]. Bashir, A.H. Diversity and morphology of bacterial community characterized in topsoil samples from the Gaza Strip, Palestine. Research Journal of Microbiology 7(6): 309-318. 2012.
[12]. Abioye, O. P., Agamuthu, P. and Abdul Aziz, A. R. (2012). Biodegradation of used lubricating oil by microbes' isolateds from pristine soil environment Malaysian Journal of Science 31 (1): 1-7.d.
[13]. Cheesborough, M. Biochemical tests to identify bacteria in laboratory practice in tropical countries.(Cheesborough M. (ed).Cambridge Edition. pp. 63-872002.)
[14]. Oyeleke, S.B. and Manga, S.B.. Essential of Laboratory Practical First edition, (Best Publisher, Minna, Nigeria pp. 36-63 2008.)
[15]. Bhat, M.M., S. Shankar, Shikha, M. Yunus and R.N. Shukla, 2011. Remediation of Hydrocarbon Contaminated Soil through Microbial Degradation- FTIR based prediction. Advances in Applied Science Res., 2(2): 321-326.
[16]. Elena, D.R. and P. John, 2003. Chemical characterization of fresh, used and weathered motor oil via GC/MS, NMR and FTIR techniques. Proceedings of Indiana Academy of Sci., 112: 109-116.
[17]. Huesemann, M.H., 1995. Predictive model for estimating the extent of petroleum hydrocarbon biodegradation in contaminated soils. Environ. Sci. Technol., 29: 7-18. 28.
[18]. Facundo, J.M., H. Vanessa and M.L. Teresa, 2001. Biodegradation of diesel oil in soil by a microbial consortium. Wat. Air Soil Pollu., 128: 313-320.

Abdullahi "Comparative Enumeration of the Effect of Soil Particle Sizes on Bioremediation of Crude Oil from Exploration and Pristine Regions of Nigeria." International Journal of Engineering Science Invention (IJESI), Vol. 09(03), 2020, PP0 55-66.