HOMOLOGY-DEPENDENT METAGENOMICS STUDY OF AGRICULTURAL SOILS INAKWA IBOM STATE, NIGERIA

Mfoniso P. Uko*, Ime R. Udotong [1], and Senyene I. Umana

Department of Biological Sciences, Akwalbom State University, IkotAkpaden, Nigeria.
[1]Department of Microbiology, University of Uyo, Uyo, Akwalbom State, Nigeria

ABSTRACT

The soil is highly complex and constitutes diverse populations of bacteria and archaearesponsible for the several soil functions andplantsgrowth. Prokaryotic study of two agricultural soils was carried out. One soil surrounds an aviation fuel-contaminated lentic ecosystem in InuaEyetIkot village, Ibeno, and another surrounds an uncontaminated lentic ecosystem in Shelter Afrique, Uyo, both in Akwalbom State, Nigeria. Samples of the surface (0-15cm) soils were collected using hand-held auger into well labeled sterile containers. MetagenomicDNA was extracted from both samples using ZYMO soil DNA extraction Kit. The extracted DNA fragments were purified by electrophoresis and amplified by Polymerase Chain Reaction with the aid of 16S rRNAuniversal primers785F (GGA TTA GAT ACC CTG GTA) forward and 805R (GAC TAC CAG GGT ATC TAA TC) reverse primers. Sequence homology of the 16S rRNAgene was performed using Nucleotide BLAST program on NCBI software. A large data of bacterial and archaeal sequences were detected in the analysis with bacteria outnumbering archaeain both soils. The bacterial population in the contaminated soil was 0.55% higher than their counterpart in the uncontaminated soil. Sequences of members of Proteobacteria, Actinobacteria, Firmicute, Acidobacteriaand those designated 'unknown' showed dominance in both soils with little variations. Euryarchaeotal and Crenachaeotal sequences were detected and were the only archaeal representativesfound in both soils. The most dominant genus in the contaminated soil was Nitrospira. *Nitrospira* sp.-Y14643.1and *Chromobacterium*sp.-AY701878.1 are highly associated with the soil around the aviation fuel-contaminated ecosystem. *Bradyrhizobium* sp.-AJ558030.1 predominates in the uncontaminated soil. Both soils have high composition of Gram-negative cells. Years (17) after the ecosystem was contaminated with aviation fuel the soil surrounding it constitute higher prokaryotic communities compared to the uncontaminated ecosystem.The group 'unknown'show higher occurrence at all levels of classification as well as the 'uncultured' and 'unidentified' at the species level, in both soils. Soil microbial study by sequence analysis reveals invaluable information on the rich microbial diversity of the soil, the kind that would remain hidden despite routine cultivation. Molecular ecology is therefore an important approach to discovering new organisms.

Keywords: Homology, Metagenomics, Soil, Aviation Fuel, Contamination.

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INTRODUCTION

Quest for wealth has led to activities that hamper the ecosystems. The soil, water, and air become characterized withunconducive living conditions and only species that are able to bear stress predominate leading to reduced biodiversity. In a report by Menta, natural ecosystems undergo continuous regulation and flows of energy as well as nutrients. The driving force behind this regulation is an undisturbed community of the soil biological diversity (Menta**,** 2012**).** Soil microbial biomass mediates soil activities (Hirsch *et al*., 2010) and is an important agent in biogeochemical cycling and mineralization (Douglas and Green, 2015) that lead to soil fertility and promotion of plants health (Hirsch *et al*., 2010). Substances, whose presence in the environment affects these microbial activities, also adversely affect plant growth, as well as detoxification of organic pollutants (Douglas and Green, 2015) in the ecosystem.

Majority of soil microorganisms have not been fully characterized, because they have not been readily culturable on standard cultivation media (Lee and Lee, 2013). Molecular study of microorganisms by polymerase chain reaction targeting the 16S rRNA gene has been useful in the investigation and identification of procaryotes diversity (Attallah, 2014). Microbial genomes hold a vast amount of information and analysis of these genetic resources enables investigation and successful discovery of such information.

Deoxyribonucleic acid (DNA)- or ribonucleic acid (RNA)-based methods allow better characterization of microorganisms. Molecular biological methods which practically involve isolation of the total DNA, amplification of microbial signature rRNA sequences (Sharma *et al*., 2013) and DNA sequencing of the rRNA sequences (Lee and Lee, 2013) to obtain material(s) for further analysis have revealed an enormous reservoir of unexplored microbes over the years (Sharma *et al*., 2013). Among these are the metagenomic approaches which involve the extraction of DNA from soil (Delmont *et al*., 2011) known as the soil metagenome. Metagenomics which isthe genomic study of microorganisms involves collective investigation of microbial genomes from a mixed population of microorganisms (Neelakanta and Sultana,2013). The method increases access to the genetic resources contained in the soil (Ghazanfar*et al*., 2010) in soil analyses. The recovered gene sequences are used to identify organisms (Fakruddin and Mannan, 2013) and also their functions.

Hirsch *et al*., (2010), suggested that for proper management and minimization of the negative environmental impacts, there is a need for detailed and predictive understanding of the microbial communities of the soil. From estimate, over 90% of species that constitute the microbial communities in the environment obviously do not form colonies or escape cultivation using conventional techniques (Chikere*et al*., 2011).The opportunities for

the discovery of new organisms and the development of resources based on microbial diversity are greater (Jurgens, 2002).In this study, a survey was carried out on two soils ecosystemsusing metagenomicsapproaches with the objective to identify the communities of prokaryotes present in them.The first soil surrounds an aviation fuel-contaminated lentic ecosystem while the second one surrounds a lentic ecosystem with no history of contamination. This study findings will serve as a reference material on the soil microbial diversity.

Materials and methods

Collection of Sample

Samples of soil surrounding an aviation fuel-contaminated lentic system on 04° 32.647' N, 007° 59.951' E, Ibeno and another soil on 04° 58.519' N, 007° 57.908' E behind Graceland High School at Shelter Afrique, Uyo, were collected by removing the weeds on the surface to obtain the top soil (0-5cm). The samples were collected using a hand-held auger into different sterile and well labeled containers. The soil around the contaminated ecosystem was labeled 'contaminated soil'. The second soil was labeled 'uncontaminated soil'. Both were transported to the laboratory on ice.

Community DNA Extraction and Metagenomics Analysis

Total DNA was extracted from the samples using the ZYMO Soil DNA Extraction Kit (Model D 6001, ZymoResaerch, USA). Individual soil's crude DNA extract was purified by electrophoresis on a 0.7 % low melting agarose gel at 70V for 3hrs. The purified extracts underwent amplification by Polymerase Chain Reaction with the aid of 16S rRNA 785F (GGA TTA GAT ACC CTG GTA) forward and 805R (GAC TAC CAG GGT ATC TAA TC) reverse primers. The programme of amplification consisted of denaturation at 94 °C for 1 min, and 30 cycles of 94 °C for 20 s, annealing at 53 °C for 25 s, and extension at 68 °C for 45 s, with a final extension at 68 °C for 10 min. PCR products were separated electrophoretically in 1% agarose gel as described by Sambrook*et al*., (2000) and visualized with the aid of ethidium bromide under ultraviolet illumination. The PCR amplicons of the 16S rRNA genes were sequenced by Next-Generation Sequencing Technologies (NGSTs) using the MiseqIllumina platform. Amplified and sequenced 16S rRNA gene products were analyzed for sequence homology using Nucleotide BLAST program on NCBI software (http://www.ncbi.nlm.nih.gov/BLAST).

Results and Discussion

Metagenomics 16S rRNA gene sequence analysis of the two soilsmetagenomerevealed enormous communities of bacteria and archaea. The contaminated soil had 99.82% bacterial community, 0.15% unknown and 0.03% archaeal community while the uncontaminated soil had 99.27% bacteria, 0.59%unknown and 0.14% archaea. Top and representative members of each taxon are presented in figure 2 to 6. Table 1 and 2present members of the bacteria and archaeaincluding cyanobacteria

from the contaminated and uncontaminated soil respectively at species level.

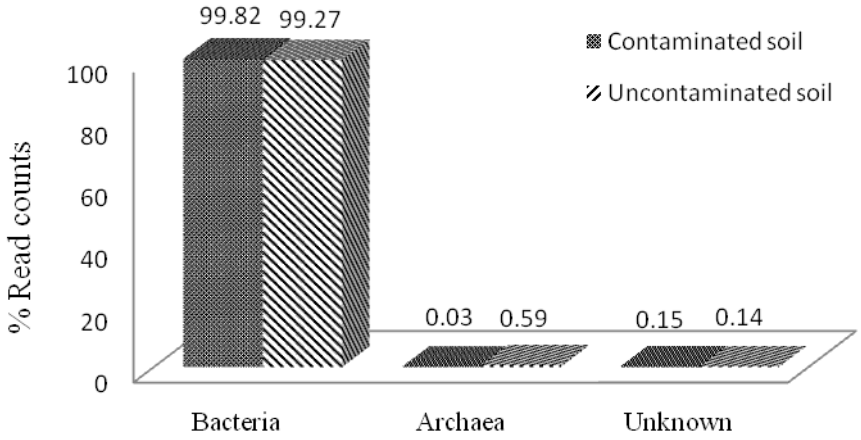

Figure 1: Kingdom classifications of gene sequences detected in the contaminated and uncontaminated soils.

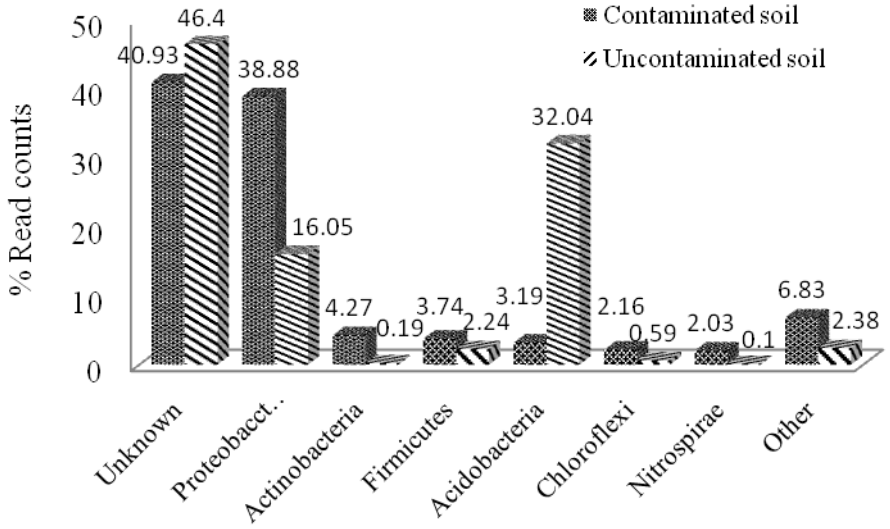

Figure 2: Relative abundance of top 7 and 5 out of 23 and 21 prokaryotic phyla detected in the contaminated and uncontaminated soils respectively (Other is the sum total of all classifications with percentage read of <1%)

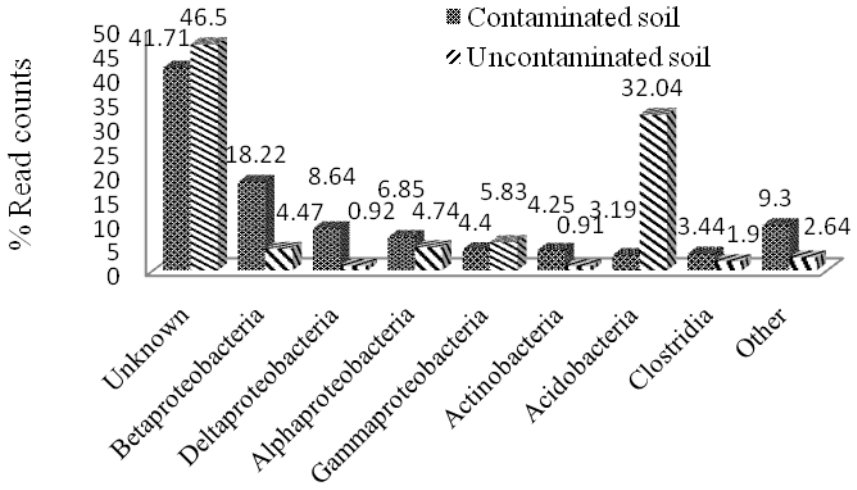

Figure 3: Relative abundance of top 8 out of 32 and 34 classes of prokaryotes detected in the contaminated and uncontaminated soils respectively (Other is the sum total of all classifications with percentage read of <1%)

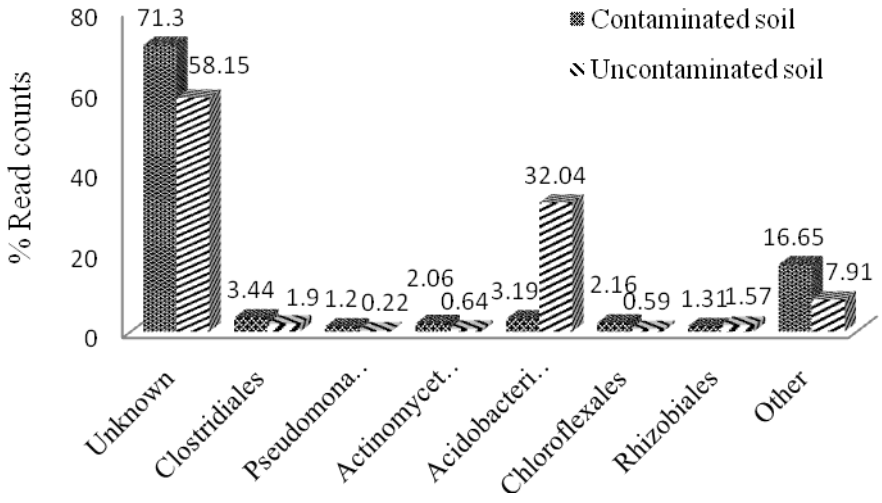

Figure 4: Relative abundance of top 7 and 4 out of 59 and 65 orders of prokaryotes detected in the contaminated and uncontaminated soilsrespectively (Other is the sum total of all classifications with percentage read of <1%).

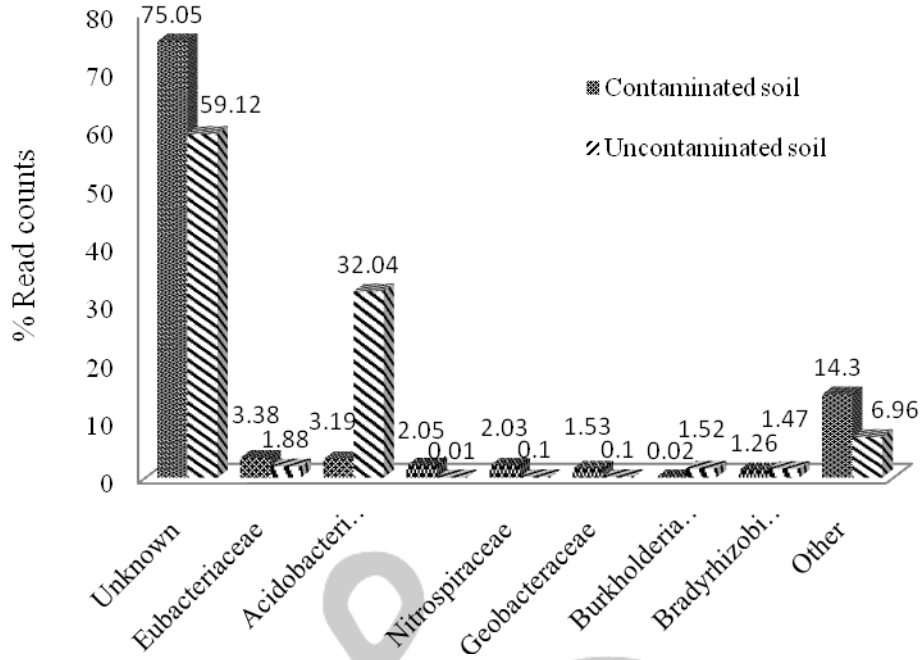

Figure 5: Relative abundance of top 7 and 5 out of 95 and 105 families of prokaryotes detected in the contaminated and uncontaminated soils respectively (Other is the sum total of all classifications with percentage read of <1%)

Table 1: Top 25 of prokaryotic species detected in the soil around the contaminated lentic ecosystem.

Species Diversity	% Read Count	Percentage nucleotide identity match	Accession number
Uncultured bacterium	26.94	96	AJ548899.1
Uncultured acidobacteria	12.77	96	AM884631.1
Unidentified bacterium	3.80	96	AJ518257.1
*Nitrospira*sp.	1.68	93	Y14643.1
*Chromobacterium*sp.	1.15	97	AY701878.1
Unidentified eubacterium	1.63	97	AJ232828.1
Uncultured Rhodospirillaceae	1.43	96	AM159320.1
Uncultured *Geobacter* sp.	1.37	84	AM159295.1
Acinetobacter sp.	0.74	92	AJ410290.1
Achromobacterxylosoxidans	0.50	91	AY189752.1
Bdellovibriobacteriovorus	0.44	91	AF148941.1
Gemmataobscuriglobus	0.41	98	X85248.1
Defluviicoccusvanus	0.37	96	NR_041771.1
*Delftia*sp.	0.34	92	AB164685.1

Frankia sp.	0.28	93	U60287.1
Enterobacter cloacae	0.25	93	AF030416.1
Kouleothrixaurantiaca	0.11	91	AB079638.1
Klebsiellaoxytoca	0.22	90	U78183.1
Pseudomonas putida	0.20	89	AE015451.1
*Methylobacterium*sp.	0.20	85	AY904733.1
Polyangiumcellulosum	0.13	90	AF387629.1
Bacillus sp.	0.12	93	AY159884.1
*Dermatophilus*sp.	0.12	94	AJ244775.2
Streptomyces sp.	0.11	90	AB123037.1

Table 2: Top 26of prokaryotes detected in the soil around the uncontaminated ecosystem.

Species Diversity	% Read Count	Percentage nucleotide identity match	Accession number
Unculturedbacterium	29.89	91	AJ534633.1
Uncultured acidobacterium	25.65	98	KF225943.1
Bradyrhizobium sp.	1.20	95	AJ558030.1
Uncultured eubacterium	1.15	97	AJ292907.1
Burkholderiacepacia	0.91	91	AB114607.1
Uncultured*Verrucomicrobia*	0.41	90	AY694604.1
Delftia sp.	0.31	90	AB164685.1
Uncultured *Legionella* sp.	0.30	87	AY924076.1
Uncultured*Holophaga*sp.	0.09	94	AJ519371.1
Planctomyces sp.	0.05	92	Y14640.1
*Nevskia*sp.	0.05	78	DQ242479.1
Brochothrixthermosphacta	0.05	97	M58798.1
Achromobacterxylosoxidans	0.04	91	AY189752.1
Bacillus gelatini	0.04	83	AJ586347.1
Streptomycescoelicolor	0.03	93	AL939130.1
Uncultured*Chlorobi bacterium*	0.03	90	AJ519647.1
Enterococcus raffinosus	0.01	91	AJ301838.1
*Dechlorosoma*sp.	0.01	90	AY171616.1
Roseatelesdepolymerans	0.01	96	AB003626.1
Rhizobium sp.	0.01	98	AY500261.1
Synechococcuselongates	0.01	89	CP000100.1
Hahellachejuensis	0.01	85	CP000155.1
Flavobacteriumgelidilacus	0.01	90	AJ871226.1
Rubrivivaxgelatinosus	0.01	90	AJ871464.1
Sphingomonas sp.	0.01	90	AY694604.1

Prokaryotic diversity:

Analysis of the rRNA genes revealed a large data of bacterial, archaeal and cyanobacterial sequences. Sequences affiliated with bacteria predominated in both soilsas seen in Figure 1.The bacterial population in the contaminated soil was 0.55% higher than their counterpart in the uncontaminated soil. The archaeal members were less by 0.11% in the contaminated soil than the uncontaminated soil. This predominance nature of bacteria has been observed previously (Udotong*et al*., 2015; 2017).Taxonomic classification of the sequences detected from the contaminated soil revealed prokaryotic representatives from 23 phyla, 32 classes, 59 orders, 95 families and 151 genera (Figure 2 through 6).As observed in the figures, the group "Unknown" leads inthe compositions. Similar observation has been recorded in other ecosystems (Udotong*et al*., 2018). The percentage occurrence of members in each taxon is higher in the contaminated soil than the uncontaminated soil.The microbial diversity of the contaminated soil based on taxonomic analysis were classified into 2 known kingdoms (Fig. 1), 23 phyla (Fig. 2), 32 classes (Fig. 3), 59 orders (Fig. 4), 95 families (Fig. 5), and 151 genera (Fig. 6). These numbers were quite higher than observed in the uncontaminated soil. Actinobacteria (4.27%) followed next in abundance after the Unknown (40.93%) and Proteobacteria (38.88%) before Firmicute(3.74%) and Acidobacteria (3.19%) in the contaminated sample (figure 2). However, Acidobacteriafollowed (figure 2) in abundance before Proteobacteria (16.05%)in the uncontaminated sample and represented22214of the reads at 32.04%. Similar compositions at phyla level have been recorded by Nair *et al*., (2013) in the study ofmangrove soil. Lee and Lee (2013) and Delmont (2011) through culture-independent analysis have reported that majority of the soil microbial diversity also belongs to these phyla.Actinobacteria are Gram positive bacteria. They are dominant in terrestrial and aquatic ecosystems and are highly involved in the decomposition of organic matter and making nutrients available for plants uptake (Servin*et al*., 2008).In a report by Jurgens in 2002, the application of new molecular approaches has led to the discovery of high numbers of novel and unexpected "non-extreme" archaeal phenotypes.The presence of Euryarchaeotal and Crenarchaeotal sequenceswere detected in both soils in this study and a similar finding has been documented by Jurgens, (2002). The low relative abundance of archaeain this study validates report by Fierer*et al*., (2012).Betaproteobacteriawas relatively higher in the class (Figure 3) lineage in the contaminated soil while members of Alphaproteobacteria were the highest in the uncontaminated soil. Both have a common ancestor, Proteobacteria and are active in fixing nitrogen in plants where the Betaproteobacteria, especially, oxidize ammonium to nitrate. Acidobacteria occurred most in the uncontaminated soil (22,214 reads at 32.04%). These groups of bacteria are physiologically diverse in the soil environment (Eichorst*et al.,* 2007). Clostridiales (3.44%) occurred most in the

contaminated soil at the class level while Acidobaceriales (32.04%) was the highest in the uncontaminated soil (Figure 3). Most representatives of Clostridiales are known to be saprophytic in the environment.Aside members of the Uncultured and Unidentified, the genusNitrospira (1.69%) and Chromobacterium (1.15%) as seen on Table 1 predominate as they contain high numbers of species from the contaminated soil, and this gives evidence that these species are probably the most active members in this environment.The uncontaminated soilhad most species to belonging to the genusBradyrhizobium(1.20%) (Table 2). All phyla, class, order, family and genus members with relative abundanceless than 1% were grouped and designated 'other' in the figures.

Species diversity:

A large percentage ofsequences affiliated to sequences of organisms denoted uncultured and unidentified were detected in the soils. These includesUncultured bacteriumwith the accession number AJ548899.1, Uncultured acidobacterium -KF225943.1, Uncultured *Geobacter* sp.-AM159295.1, Uncultured *Sterolibacterium* sp.-DQ279355.1, etc. (Table 1) and Uncultured bacterium-AJ534633.1, Uncultured eubacterium-AJ292907.1, Uncultured Legionella sp.-AY924076.1, Uncultured Holophaga sp.-AJ519371.1, etc. (Table 2). The relatively high abundance of these groups of organisms suggests that a vast number of sequences belonging to soil microorganisms have affiliation with organisms of no scientificallyspecified genus.The soilaround the contaminated ecosystem showed the presence of *Nitrospira* sp.-Y14643.1and *Chromobacterium*sp.-AY701878.1in higher percentage than*Acinetobacter* sp.-AJ410290.1, *Achromobacter xylosoxidans*-AY189752.1, *Bdellovibrio bacteriovorus*-AF148941.1, *Gemmata obscuriglobus*-X85248.1, *Defluviicoccus vanus*-NR_041771.1, *Delftia* sp.-AB164685.1, *Frankia* sp.-U60287.1, and many more on Table1. The uncontaminated soil showed lower abundances of *Acinetobacter* sp.-AJ410290.1, *Nevskia*sp.-DQ242479.1, *Brochothrixthermosphacta*- M58798.1, *Enterococcus raffinosus*-AJ301838.1, *Delftiatsuruhatensis*-AJ606337.1, *Achromobacter xylosoxidans*-AY189752.1,*Bacillus gelatini*-AJ586347.1, *Streptomyces coelicolor*-AL939130.1, *Dechlorosoma* sp.-AY171616.1, *Synechococcus elongates*-CP000100.1, etc. A large number of these species are Gram negative bacteria which demonstrate their position as the dominant and most active bacterial communities in the soil ecology.*Nitrospira* sp. are nitrite-oxidizers. *Chromobateriu*mis the soil's normal flora and antibiotics producers. The species *C.violaceum*produces theviolacein antibiotics (Kodache*t al.,*2006).*Bradyrhizobium* species form symbiotic relationships with leguminous plants in the soil where they fix nitrogen in exchange for carbohydrates from the plants. The detection of sequences affiliated with *Synechococcuselongates* in the uncontaminated soil confirms the present of the group, cyanobacteria which is an important component of the

prokaryotic community.*S. elongates* is a photosynthetic bacteria and therefore responsible for primary production (Scanlan and Nyree, 2002) in the environment.

Conclusion

Soil study using sequence analysiswas carried out for the first time on two agricultural soils. BLAST results showed sequences affiliated with species of bacteria, archaea and cyanobacteria. Sequences affiliated with bacterial community predominated in both soils. The contaminated soil displayed higher number of organisms than observed in the uncontaminated soil.The presence of Euryarchaeotal and Crenarchaeotal sequences were detected in both soils. Cyanobacterial sequences were also found in the uncontaminated soil.The contaminated soil revealed the presence of more than 75.37% of species designated 'uncultured' and 'unidentified'. This includedUncultured bacterium (26.94%) and Unidentified bacterium (3.80%).Both soils are high in Gram negative bacteria.Molecular ecology is an important approach to discovering new organisms.

REFERENCES

Attallah, A. G., EL-Shaer, H. F. A. and Abd-El-Aal, S. K. (2014). 16S rRNA Characterization of a Bacillus Isolates From Egyptian Soil and its Plasmid Profile. *Research Journal of Pharmaceutical, Biological and Chemical Sciences-RJPBCS,* 5(4), 1590-1604.

Chikere, C. B; Surridge, K. J; Cloete, T. E; Okpokwasili, G. C. (2011). Phylogenetic diversity of dominant bacterial communities during bioremediation of crude oil-polluted soil. Ambi-agua, Taubaté, 6 (2), 61-76.

Delmont, T. O., Robe, P., Cecillon, S., Clark, I. M., Constancias F, Simonet P, Hirsch, P. R. and Vogel, T. M. (2011). Accessing the soil metagenome for studies of microbial diversity. *Appl. Environ. Microbiol.*77:1315-1324.

Douglas, S. I. and Green, D. I. (2015). Microbial Communities found in a Diesel Contaminated Soil. *International Journal in Physical & Applied Sciences-IJPAS*, 2 (4), 38-47.

Eichorst, S. A., Brenak, J.A. and Schmidt, T M. (2007). Isolation and characterization of soil bacteria that define *Terriglobus* gen. nov., in the Phylum Acidobacteria. *Applied Environmental Microbiology*, 73 (8), 2708-2717.

Fakruddin, M. D. and Mannan, K. S. B. (2013). Methods for Analyzing Diversity of Microbial Communities in Natural Environments. *Ceylon Journal of Science (Bio. Sci.)* 42(1): 19-33.

Fierer, N., Leff, J. W., Adams, B. J., Nielsen, U. N., Bates, S. T., Lauber, C. L., Owens, S., Gilbert, J. A., Wallh, D. H. and Caporaso, J. G. (2012). Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *PNAS*, 109 (52): 21390–21395.

Ghazanfar, S., Azim, A., Ghazanfar, M. A., Anjum, M. I. and Begum, I. (2010). Metagenomics and its application in soil microbial community studies: biotechnological prospects. *Journal of Animal & Plant Sciences,* Vol. 6, Issue 2: 611- 622.

Hirsch, P. R., Mauchline, T. H. and Clark, I. M. (2010). Culture-independent molecular techniques for soil microbial ecology. *Soil Biology & Biochemistry,* 42, 878-887.

Isitekhale, H. H. E., Aboh, S. I., Edion, R. I. and Abhanzioya, M. I. (2013). Remediation of Crude Oil Contaminated Soil with Inorganic and Organic Fertilizer Using Sweet Potato as a Test Crop. *Journal of Environment and Earth Science*, 3 (7).

Jurgens, G. (2002). Molecular phylogeny of archaea in boreal forest soil, freshwater and temperate estuarine sediment. *Academic Dissertation in Microbiology*. Helsinki University Printing House, Helsinki, Finland.

Kadafa, A. A. (2012). Environmental Impacts of Oil Exploration and Exploitation in the Niger Delta of Nigeria. *Global Journal of Science Frontier Research Environment & Earth Sciences,* 12 Issue 3 (1).

Kodach, L. L.,Bos, C. L., Duran, N., Peppelenbosch, M.P.,Ferreira, C. V., Harwick, J.C. (2006). Violacein synergistically increases 5-fluorouracil cytotoxicity, induces opoptosis and inhabit Akt-mediated signal transduction in human colorectal cancer cells. *Carcinogenesis*, 27(3):508-516.

Lee, M. H. and Lee, S. (2013). Bioprospecting Potential of the Soil Metagenome: Novel Enzymes and Bioactivities. Genomics & Informatics, 11(3):114-120

Menta, C. (2012). Soil Fauna Diversity – Function, Soil Degradation, Biological Indices, Soil Restoration. In: Lameed, G. A. (Editor).*Biodiversity Conservation and Utilization in a Diverse World*. 59-94.

Nair, H. P., Vincent, H. and Bhat, S. G. (2013). Culture independent analysis of the soil microbiome to assess microbial diversity of mangrove soil. Bio-Genetics Journal, 1: 1-4

Neelakanta, G. and Sultana, H. (2013).The Use of Metagenomic Approaches to Analyze Changes in Microbial Communities. *Microbiology Insights*, 2013:6 37–48. Nigeria. Med. Clin. Res., 3(1): 1-5.

Obire, O. and Anyanwu, E. C. (2009). Impact of various concentrations of crude oil on fungal populations of soil. *Int. J. Environ. Sci. Tech.,* 6 (2), 211-218.

Sambrook, J, Fritsch, E. F. and Maniatis, T. (2000). *Molecular Cloning: a laboratory manual.* 3rd Edition. Cold Spring Harbor Laboratory Press, New York.

Scanlan, D. J. and Nyree, J. W. (2002). "molecular ecology of marine cyanobacterial genera Prochlorococcus

and Synechococcus ." FEMS Microbiology Ecology, 40:1-12.

Servin, J. A, Herbold, C. W., Skophammer, R. G. and Lake, J. A. (2008). Evidence excluding the root of the tree of life from the actinobacteria. *Molecular Biology and Evolution*, 25 (1), 1-4.

Sharma, N., Singh, G. and Sudarsan, Y. (2013). Assessment of microbial diversity under arid plants by culture-dependent and culture-independent approaches. *Afr. J. Biotechnol. Afr. J. Biotechnol.* 12(40), 5860-5868.

Udotong, I. R., Uko, M. P. and Udotong, J. I. R. (2015). Microbial diversity of a remote aviation fuel-contaminated sediment of a lentic ecosystem in Ibeno, Nigeria. *J. Environ. Anal. Toxicol.*, 5, 320.

Udotong, I. R., Uko, M. P. and Udotong, J. I. R. (2018).Procaryotic Diversity of a Remote Aviation Fuel-Polluted Lentic Ecosystem in Ibeno,*Med Clin Res*, 3, 1- 5.

Udotong, I., Uko, M.and Udotong, J. (2017). Use of 16S rDNA Sequencing to Determine Procaryotic Diversity of a Remote Aviation Fuel-Polluted Lentic Ecosystem in Ibeno, Nigeria. *J Environ Anal Toxico.* 7: 493.