RIVERS STATE UNIVERSITY, PORT HARCOURT

MICROBES: INDICATORS AND REMEDIATORS OF POLLUTED ENVIRONMENTS

AN INAUGURAL LECTURE

By

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MICROBES:

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An

Inaugural Lecture

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Dear God, what marvels there are in so small a creature!

-----Leeuwenhoek 1693

Never underestimate the power of the microbe

---- Jackson W. Foster

BACTERIA MAKE THE WORLD GO ROUND

The bacteria particularly are still more important than any other organism. Omnipresent in infinite varieties, they release the carbon and nitrogen held in the dead bodies of plants and animals including humans which would ---without bacteria and yeasts ---- remain locked up forever in useless combinations, removed forever as further sources of energy and synthesis. Incessantly busy in swamps and fields, these minute benefactors release the frozen elements and return them to the common stock, so that they may pass through other cycles as part of other living bodies----

Without the bacteria to maintain the continuities of the cycles of carbon and nitrogen between plants and animals, all life would eventually cease----Without them, the physical world would become a storehouse of well preserved specimens of its past flora and fauna----useless for the nourishment of the bodies of posterity

---Hans Zinsser, 1935

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Dedication

TO

MY PARENTS

PRINCE JACKSON MAJEMITE OBIRE

AND

MRS EKPETIUDI ERHONVIGE OBIRE

(NEE ONOKURHEFE OBUKUHWO)

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The Vice-Chancellor, Sir,

Members of the Governing Council here present,

Deputy Vice-Chancellor,

Principal Officers of the University,

Dean of Postgraduate School,

Dean, Faculty of Science,

Deans of Other Faculties,

Provost, College of Health Sciences

My Fellow Professors and Other Academic Colleagues,

Directors and Heads of Departments,

Members of the University Community,

Great Students of Rivers State University,

Friends of the University,

My Family Members and Friends,

Distinguished Ladies and Gentlemen,

I humbly welcome you to this Inaugural Lecture titled **"Microbes: Indicators and Remediators of Polluted Environments"**.

PREAMBLE

Mr. Vice-Chancellor Sir, It is a great pleasure and honour for me to deliver the 54th Inaugural Lecture in the Series of this Great University and the very first from the Department of Microbiology.

I thank the Vice Chancellor especially for his kind approval in making this possible. I am standing before this distinguished gathering outside my regular lecture halls and classrooms to deliver

my inaugural lecture to mark the installation of my Professorship to the chair of the subject of Environmental Microbiology. It is therefore expected that I should deliver this lecture in a manner that both academic and non-academic persons would comprehend. I shall be talking as a microbio-logist or to be more precise, as an Environmental Microbiologist.

The term Environment has evolved over time. Today, many of us use the word **"Environment"** to mean different things. Many of us discharge wastes into the environment believing that **"The solution to pollution is dilution".** This is a disaster that can only be avoided through efficient and scientific management of our environment.

THE ENVIRONMENT - The Life Support System

What is meant by the term Environment?

The term environment, stripped of all rhetoric, simply means the life-support system. All other aspects of the environment are ancillary to the life support system, which we can define as those items that are absolutely essential to the sustenance of aerobic life: water, air, and food (from soil and water).

This life-support system, food, air, and water, (for human protection and control) is maintained by two major natural cycles and accompanying mineral cycles. The two major cycles are the hydrologic cycle (water source) and the carbon-oxygen cycle (food and air source). Since the organic food source contains, in addition to carbon, some nitrogen, phosphorus, sulfur, and other elements, the cyclic migrations of these elements in the biosphere (the sphere of living organisms) are also vital to the life - support system.

According to the Holy Bible (NIVB, 1985), God's creation (in Genesis 1:1-25) is composed of the earth, sky and natural elements found therein such as rocks, minerals, soil, water, air,

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vegetation and fish and wildlife. This Natural creation is referred to in short as "nature" or "the environment."

I will like to remind this august gathering that, Life also comes out of the environment (water, air and the earth) as stated in **Genesis 1: 20, 24 -** Then God commanded, *"Let the waters bring forth abundantly (be filled with) many kinds of living beings (Sea monsters and all kinds of creatures), and let the air be filled with birds* (NIVB, 1985), *.....Then God commanded, "Let the earth produce all kinds of animal life: domestic and wild, large and small" and it was done (GNB, 1979).*

God also created microorganisms before man was created on the sixth day; Genesis 1:24; Then God commanded, "Let the earth produce all kinds of animal life: domestic and wild, large and **small**". This is not to say that there were no microbes in the waters before that pronouncement by God because, we had *many kinds of living beings (Sea monsters and all kinds of creatures)!!!.* The Almighty in His wisdom was simply preparing the environment to support human life hence man was the last of God's creation.

There are also rules about the environment.

> Numbers 35 v 33 – 34: *"You shall not pollute the land in which you live, in the midst of which I dwell, for I the Lord dwell in the midst of the people."*

When we cherish the water, air and soil (environment), respect and honour them both as the gifts of God that are freely given to us, we will find our reservoirs full. If we continue to take the water, air and soil (environment), for granted and contaminate them, waste or pollute them, surely, we will have difficulties in our physical bodies.

There are rules in the Bible that govern how to dispose of human excrement in order to help combat diseases that may arise from contact with human waste.

Keeping the Camp (City) clean Deuteronomy 23 v 12 – 13

> "You are to have a place outside the camp where you can go when you have to relieve yourselves. Carry a stick as part of your equipment, so that when you have a bowel movement you can dig a hole and cover it up".
>
> Covering the excrement makes obvious sense. It helps cover the smell, which while odious to humans, is also detected by unwanted animals and insects. Covering the excrement also assists in avoiding everyone's pedestrian nightmare: stepping in the excrement.

The Faeco-Oral Route of Transmission

The three elements of the life-support system-water, food, and air-are essential for human survival, but they are also potential vehicles for the spread of disease-causing microorganisms as well as toxic chemicals. Infectious diseases are spread by three major routes: (1) ingestion of water or food containing pathogenic microorganisms; (2) inhalation of pathogens; and (3) transmission by animal vectors, usually insects. Except in certain cases, there is little the environmental technologist can do to prevent respiratory spread of disease, since this occurs usually through very localized contamination of the air by sneezing or coughing.

Some bacteria, protozoa, helminths and viruses, are directly infectious for man as they are passed in the faeces. The most important pattern of transmission is the passage of infective material from human faeces into the mouth of a new host and this is known as "Faeco-oral" or "intestino-oral transmission.

Faeco-oral transmission occurs mostly through inapparent faecal contamination of food, water and hands- the three main items which regularly make contact with the mouth (Figure 1).

I appreciate my in-laws who stabilized my stay in Rivers State. Most worthy of mention are His Royal Majesty, the Amanyanabo of Nembe, Mingi XI Justice Ambrose Allagoa and his Queen, Lady Carol Allagoa, Alabo (Dr) Eric. D. Mangete (Former Chief Medical Director of UPTH), His Highness, The Ada of Twon Brass Chief Alfred Spiff, Chief James Spiff (Chief Douglas), Professor Clifford T. I. Odu (Chief Cameron of Twon Brass and former Commissioner of Agriculture of Rivers State), Dr Eddy Spiff (former Commissioner of Agriculture of Rivers and of Bayelsa States), Flight Captain Seigha Spiff, Mr Rollins Ekenyo Alagoa, Chief Kombo Igbeta, Mrs Doris Dokubo, Mrs Elsie Somiari, Aunty Inara Spiff, and a host of others too numerous to mention. May God continue to grant you all peace and love also.

Many thanks beyond measure to my mother Ekpetiudi and to my daughter Rachel for their ever-loving support.

Finally, to you distinguished Audience for your esteem presence. Without you, there would have been no Inaugural lecture. A BIG THANK YOU to you for your presence and thank you for listening. May God Almighty Bless You All. Amen.

friends within the Faculty of Science and the University Community at large, space and time will not permit me to name you one by one. I cannot but mention Alabo Professor Bob-Manuel for his time and concern with regards to the delay in my promotion. Alabo Sir, I am very grateful for your effort and for your time. To His Royal Majesty, King Professor T.J.T. Princewill, the Amayanabo of Kalabari, a former Dean of the Faculty of Science and a friend of Professor T. I. Francis; Your Majesty, I still remember your words "*Karo, in spite of all odds, you have done very well*". His Majesty has asked after me and my welfare till date. Long May You Reign Your Majesty!!!.

To all my past and present undergraduate and postgraduate students, home and abroad, I say thank you. Together, we have made this day. I appreciate you all.

To my husband, Professor Theodore Idibiye Francis (Chief Ogiriki I of the King Ockiya Group of War Canoe Houses of Nembe) grandson of Rev. Daniel Ogiriki Ockiya who translated the Holy Bible into the Izon Language. Professor T. I. Francis was the foundation Provost of the College of Medicine, University of Port Harcourt, the foundation Chief Medical Director of University of Port Harcourt Teaching Hospital, and the two term foundation Vice Chancellor, Federal University of Technology, Akure); an epitome of humility, what else can I say? You insisted that I should not do a change of name for reasons best known to you. You believed in my potentials and wanted me to make a mark using my family name **OBIRE** and insisted that I should only be addressed as Mrs. Francis when it has to do with legal matters relating to the family. You taught me how to type with an electric type-writer (1991) instilling it on me that there are confidential issues that must be only between me and the receiver. I was too naïve to understand that you were preparing me for the inevitable. You shook the world with those words "Whatsoever, wheresover, absolutely". You braced up to it. Thank you for your courage.

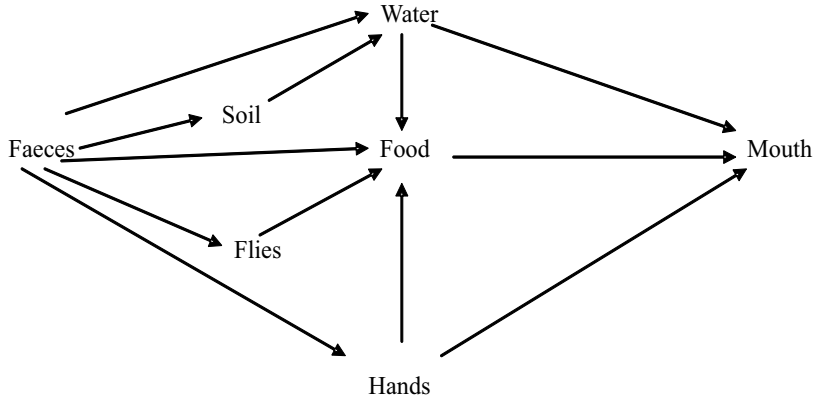

Fig. 1: Pathway of faeco-oral transmission. Source (Obire, 2017)

It should be noted that minute quantities of faeces can carry the infectious dose of various pathogens. Thus, dangerously polluted water may appear sparking clear, contaminated food may be free of objectionable odour or taste, and apparently clean hands may carry and transmit disease.

As shown in the diagram, food occupies a central and important position. Not only can it be contaminated directly by faeces but also indirectly through polluted water, dirty hands, contaminated soil and filth flies. Water may be polluted directly by faeces; faecal material may be washed in from the polluted soil on the river bank. There are many opportunities for the contamination of hands: the person may contaminate his hands on cleaning after defaecation or in touching or handling contaminated objects including soil. Filth flies, in particular, the common housefly, spread faecal material and play a role in the transmission of gastro-intestinal infections.

The housefly mechanically transfers faecal pollution by carrying faeces on its hairy limbs, or through "vomit drop" or by defaecating on the food; its faeces may contain surviving organisms derived from human faeces.

Some of the infections which are acquired through the gastro-intestinal tract characteristically occur in epidemic form, e.g. cholera and infectious hepatitis. The vehicle of infection may be water. The water-borne epidemic is typically explosive; it may affect people over a wide area who have no other traceable connection but the use of the same source of water supply.

The mode of disease transmission through ingestion of food or water that contains pathogens is the major concern. The two sources of infection- food and water are related. Since water is used in growing and processing most foods and often can be the source of food contamination. The common use of water resource as the repository for human waste demands that special attention be focused on the potential danger to public health that results from this practice and the need for improved monitoring techniques and strict enforcement of sanitary regulations for water used for drinking, food preparation, or recreation. The use of a common water supply by very large numbers of people, in contrast to the relatively smaller number likely to be exposed to a source of contaminated food, means that water presents the greatest potential for the spread of disease to the greatest number of people.

Moisture on our planet undergoes continuous circulation, supplying the water all living things need. It enters the earth's atmosphere by evaporation from lakes, streams, and oceans, and by transpiration from the leaves of plants. Then it precipitates back onto the earth in the form of snow, hail, and rain. While a drop of water may appear simple, it really is quite complex, often containing chemicals and microorganisms of many kinds. These micro-

To my colleagues in the Department of Microbiology and Applied and Environmental Biology of old and of Biological Sciences of old, I am very grateful. Professor I.K.E. Ekweozor my office mate and long time friend, Prof. B.A. Okwakpam, Dr. E.N.U. Okpon, Prof. T.G. Sokari, Prof. E.N. Amadi, Prof. (Mrs.) O.K. Ogbalu, Prof. G.C. Akani, Prof. E.R. Daka, Prof. C.K. Wachuku, Prof. (Mrs.) Blessing Green, Prof. (Mrs.) E.E. Orlu, Prof. (Mrs.) E.C. Chuku, Dr. N.J. Abby-Kalio, Dr. Onwuteaka, Dr. (Mrs.) Emylia. T. Jaja, Dr. D.N. Ogbonna, Dr. A.B. Nwauzoma, Dr. S.A. Wemedo (Ag. HOD), Dr. E.C. Amadi, Dr. N. Ebere, Dr. (Mrs.) M.T.V. Adeleke, Dr. (Mrs.) A.O. Ugbomeh, Dr. (Mrs.) N.P. Akani, Dr. Moslen Miebaka, Dr. (Mrs.) J.O. Williams, Dr. (Mrs.) S.I. Douglas, Dr. Karibi Bob-Manuel, Dr. R.R. Nrior, Mr. G.C. Diesegha, Mrs. Obulor-Godwin, Ms G. Baeka, Mrs Baranu and Mr. I.P. Peekate. To the General office staff Mrs Grace Amadi, Miss Rachael A. Enyinnah and to the Biology Lab staff, Mr. L.D. Jaja (Chief Technologist), Mrs. Augustina Tasie, Mr. G.C. Nwokocha, Mr. A.S. Oroatankpo, Mrs Cecilia Nyeche, Mr. A. Gbule, and Mr. Sunebari you are just wonderful. I thank God for your personalities. The FULL ACREDITATION of the National Universities Commission (NUC) which the then Department of Applied and Environmental Biology gained in 2008 during my tenure as the Head of the Department was also as a result of the cooperation which I enjoyed from all of you. I thank you all.

My special gratitude goes to Engineer Emma Okon Umoette for his absolute care for my cars and to Miss Amafina Isoboye Micheal, Miss Berembo Beremboba Benedicta, Miss Beauty Fiberesima, Mr Isreal Jaja and Fidelis Dombe. Thank you for all the stress and laughter.

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publication fees were required. Ramesh is based in the United States and interestingly is also a member of the Sigma XI Scientific Research Society of the United States of America. He took interest in my online environmental impact publications in 2008 and has kept in touch ever since. Ramesh, I am just grateful.

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organisms can change the chemical substances found in water; they also provide nutrients for other aquatic organisms.

The profound and sweeping involvement of microbes in the environment is inescapable. Although our daily encounters with microbes usually go unnoticed, human and microbial lives are clearly intertwined on many levels. It is no wonder that long ago humans realized the power of microbes and harnessed them for specificmetabolic tasks and as a new source of products and processes for the benefit of society. For example, alcohol produced by fermentation of grain, fruits and vegetables have become a new source of fuel (gasohol). New varieties of microorganisms, produced by genetic engineering can produce important medicinal substances such as human insulin which can now be produced in unlimited quantities by a genetically engineered bacterium (unlike insulin extracted from the pancreas of calves which some patients could not use) (Pelczar *et al*., 1993).

Microorganisms act as early alarm system to warn us of impending danger. Microorganisms occur in a great variety of forms with a diverse array of enzymes capable of degrading or detoxifying and mineralizing virtually any substance to gas (CO_2) and water. The metabolic activities of microorganisms are employed for the decomposition of wastes. Microorganisms assist us in the cleanup of polluted environments- from the decomposition of petroleum compounds in oil spills to the decomposition of pesticides (herbicides and insecticides) used in agriculture. In fact, specific varieties of microorganisms are in use, and others are being developed, to replace chemicals presently being used to control insects (Atlas, 1997). The microbial degradation of waste and pollutants is essential for maintaining environmental quality.

Since the three elements of the life-support system-water, food, and air-are essential for human survival, but they are also potential vehicles for the spread of disease-causing microorganisms as well as toxic chemicals, one of our major concerns must be the protection of the public health by preventing contamination of the life-support system with harmful organisms and materials; with the role of microorganisms in the prevention of pollution through their activities in both the natural and engineered processes, necessary for the degradation of waste organic material and the recycling of carbon and other elements.

Accordingly, the topic of my discussion **"Microbes: Indicators and Remediators of Polluted Environments"** is aimed at

1. Reminding us of the importance of microorganisms in the environment
2. Discussing my own research output on environmental pollution and bioremediation and
3. Making some suggestions on how we can preserve our environment through improved management techniques.

It is my hope that the absolute necessity for the existence (and the control) of microorganisms will become apparent during the course of this inaugural lecture.

my mentors, Professor C. Cooper and Professor M. L. Lockhart. To you all, I remain ever grateful.

I remain grateful to our formal Vice Chancellor, Emeritus Professor S. C. Achinewu for granting me first, a short study leave which he also converted to a sabbatical leave. Sir, I remember your phone call from the Rivers State Government House to me in the United States. You are a caring leader indeed. Prof Sir, I am just grateful. I also remember with amusement that Professor T. G. Sokari, my then Head of Department of Biological Sciences endorsed my application form for this said leave in front of the Amphitheatre! Thank you Prof.

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INTRODUCTION

What are microorganisms?

Microorganisms which we simply abbreviate as microbes are very small organisms (microscopic), so small that they cannot be seen with the unaided eye. Therefore you need an instrument such as a microscope to view them. There are several major groups of microorganisms. They are bacteria, viruses, algae, protozoa, fungi, and parasitic worms (helminthes). They all have different biological characteristics that are distinguishing traits or features that can be used to describe or define them. Microbes are found nearly everywhere in nature and therefore are a force in the environment.

Microbiology is the study of microorganisms and their activities such as physiology, metabolism, distribution and their beneficial and harmful effects on man to mention a few. Scientists conclude that microorganisms originated an estimated 4 billion years ago from complex organic materials in ocean waters, or possibly in vast cloud banks surrounding our primitive earth. As the first life form on earth, microorganisms are thought to be the ancestors of all other life forms (Pelczar *et al*., 1993).

Microorganisms have emerged as part of the mainstream of biological sciences. One of the reasons for this are the concept of "unity with biochemistry," which means that many of the biochemical processes of microorganisms are essentially the same in all forms of life including humans, and the more recent discovery that all of the genetic information of all organisms, from microbes to human beings, is encoded in DNA (Talaro and Talaro, 2002).

The Impact of Microbes on Earth: Small Organisms with a Giant Effect

Microorganisms have a profound influence on all aspects of the earth and its residents. For billions of years, microbes have extensively shaped the development of the earth's habitats and evolution of other life forms. They are the foundation of the biosphere controlling the biogeochemical cycles and affecting geology, hydrology, local and global climates. All life is completely dependent on them.

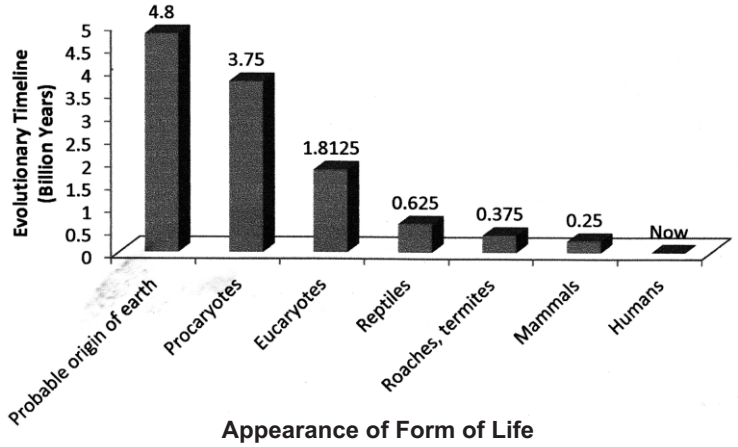

Fig. 2: Appearance of form of life with evolutionary timeline
Source: Adapted from Talaro (2005)

Humans cannot survive without the rich diversity of microbes but most species can survive without humans" (American Academy of Microbiology, 2001). It is understandable that scientists searching for life on other planets first look for signs of microorganisms.

Mother Professor Marie Pauline Eboh, Lady Harmony Nwosu (President CWO) and all my Sacred Heart Members especially Miss Cecilia Japheth and her family. I am very grateful to the parishioners and members of the League of Sacred Heart of Jesus, St Joseph's Parish Borokiri for their prayers and concern about my welfare at all times and especially to our then Rev. Father John. They have always checked on my at my home in Borokiri, when I was in the hotel for three (3) years (2007 to 2010) and up till today in my residence here on campus. Worthy of mention are Rev. Sister Patricia, Rev. Sister Geraldine, Rev. Sister Theresa, Mrs Omiunu (mother of our Rev. Fr. Thomas), Mrs. Emmanuella Dim and Mrs Dumbere. I appreciate your prayers. Thank you all.

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an inaugural lecture. She is a mother par excellence. She devoted her time and resources to the care and education of her children and relations. We were never sent home for lack of payment of school fees and I tend to wonder when some persons say that they lacked shoes, etc. My mother also sold school supplies such as blankets, bed sheets, towels, etc to other parents on "credit" which they paid on installments so that their children can attend school. Today, I feel happy and proud to be the inaugural lecturer but I am pretty sure that my mother's heart is full of joy and fulfillment. My father (Alias Oshare-royonvwi-neya) too will be turning in his resting place this moment. Papa was one of the very first surveyors in the then Mid-Western Region. One of his pet projects was the surveying of the Koko road which is one of the roads constructed by the expatriates in the Mid-Western Region. Papa gave me selected passages from his Newspapers to read to his hearing when I was in Primary school and would nod as I read along. Though my father wanted me to read law and become a lawyer, he had believed that I will one day become a Professor. Papa would always tell me "***Perseverance Conquers All Difficulties***". Thank you Papa! I deeply appreciate the support and prayers of my siblings, Beauty, Eyaride, Felix, Francis, Orisemudia, Clara, Mamuyonvwi, and Ejiro and their spouses.

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I sincerely appreciate my spiritual father, Rev. Fr. Innocent Obi and the CWO of the Holy Cross Catholic Parish, Afiesere Ughelli and members of the League of Sacred Heart of Jesus worldwide for their devotion to the Sacred Heart. I am grateful to the Parishioners of Our Lady Seat of Wisdom Chaplaincy RSU especially our very dear Rev.

Bacteria-type organisms have been on this planet for about 3.5 billion years, according to fossil records. It appears that they were the only living inhabitants on earth for almost 2 billion years. At that time (about 1.8 billion years ago) a more complex type of single-celled organism arose, of a eukaryotic (true nucleus) cell type which gives a hint that those first inhabitants, the bacteria, had no true nucleus. For that reason they were called prokaryotes (pre-nucleus). The early eucaryotes were the precursors of the cell type that eventually formed multicellular animals including humans. As seen in Fig. 2, Humans seemed to have just appeared on the time scale.

The bacteria preceded even the earliest animals by 3 billion years. This is a good indication that humans are not likely to, nor should we try to eliminate bacteria from our environment. **We have to learn to live with them because we cannot live without them.** They have survived and adapted to many catastrophic changes over the course of their geologic history.

Human and microbial lives are clearly intertwined on many levels. It is likely that from the earliest history, humans noticed that when certain foods spoiled they became inedible or caused illness, and yet other "spoiled" foods did not harm and even had enhanced flavor and taste and even become safer to consume (e.g., fermented cassava, locust bean, etc). It is no wonder that long ago humans realized the power of microbes and harnessed them for specific metabolic tasks and as a new source of products and processes for the benefit of society. For example, alcohol produced by fermentation of grain, fruits and vegetables have become a new source of fuel (gasohol). New varieties of microorganisms, produced by genetic engineering can produce important medicinal substances such as human insulin which can now be produced in unlimited quantities by a genetically engineered bacterium (unlike insulin extracted from the pancreas of calves which some patients could not use) (Pelczar *et al*., 1993).

Microorganisms have great potential for assisting in the cleanup of the environment- from the decomposition of petroleum compounds in oil spills to the decomposition of pesticides (herbicides and insecticides) used in agriculture. In fact, specific varieties of microorganisms are in use, and others are being developed, to replace chemicals presently being used to control insects (Atlas, 1997).

Life-styles of microorganisms

The majority of microorganisms live a free existence in habitats such as soil and water, where they are relatively harmless and often beneficial. A free-living organism can derive all required food and other factors directly from the non-living environment. Some microorganisms require interactions from other organisms. One such group termed **parasites**, are harbored and nourished by other living organisms, called **hosts**. A parasite's action cause damage to its host through infection and disease, they make up only a small proportion of microbes.

Microorganisms are found in nearly every environment on earth (ubiquitous), including environments in which no other life forms can survive. They are found on the permafrost of the arctic regions, deep seas, hot deserts, acid mines, thermal springs and ocean vents with temperatures nearing boiling point. These are extreme environments that ordinarily will not support life. Microorganisms are found in the air we breathe, the food we eat, the soil where food is grown, and the water we drink. Every part of the human body is a habitat to well established distinct microflora. No living and non-living thing is free from colonization by them and you cannot escape their influence.

Microbes can exist in a great many environments because they are small and easily dispersed, occupy little space, need only small quantities of nutrients, and are remarkably diverse in their

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I am grateful to the various Vice Chancellors of this great university from the days of Professor Banigo to the present Vice Chancellor Professor Blessing C. Didia. I am particularly grateful to the Present Vice Chancellor of Rivers State University, Professor Blessing C. Didia for the approval granted me to deliver the 54th inaugural lecture of this great university without which, there would have been no inaugural lecture.

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I wish to thank the ministers of God here present.

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The removal of antibiotics as growth promoters and over the counter drugs is recommended to reduce the risk of the continuous emergence and spread of microbial resistance to antibiotics.

10. Government should provide health facilities and medical personnel to the communities and provide affordable if not free health care to the citizens to prevent self medication and proliferation of antibiotic resistant microorganisms.

11. Qualified Microbiologist/Toxicologist should be engaged by government and private production or manufacturing companies to carried out routine monitoring of both physiochemical and microbiological parameters of soil, water and air so that any alteration of the constituent from the standard acceptable limit will be discovered and appropriate remedial actions immediately taken.

12. The Niger Delta region should benefit from development of indigenous microbes for bioremediation. The use of bioremediation technology will help to remove the huge heaps of solid wastes in our cities and elsewhere and remove oil spills from our land and waters. This will not only give us a cleaner environment, but it will also create employments leading to improved economic status of the people and the Government.

13. Microbes are a vast resource waiting to be exploited for the benefits of mankind. Unlike plants and animals which can be handled by any person, only those who have the knowledge of microorganisms can handle or manipulate them.

14. To maximize the use of this resource, there is need to step up the study of microbes. To this end, the Government should properly fund the teaching and research in Microbiology as this has great potential to improve the environment and the nation's economy.

nutritional requirements, and have great capacity for adapting to environmental changes. For almost any substance, there is some microbe that can metabolize it as a nutrient, for almost any environmental change, there is some microbe that can survive the change. A single microbial cell is a complete life unit. Wherever a microbial cell finds itself (environment) it seeks to express the characteristics of life (metabolism) geared towards growth, multiplication and survival strategies of the microorganism (Talaro, 2008).

Locations of Enzymes and Extracellular Digestion and Absorption in Microbes

Enzymes are protein catalysts that speed up chemical processes by lowering the required energy. Enzymes are involved in activities that synthesize, digest, oxidize, and reduce compounds, and convert one substance to another and are useful in the growth and survival of the organism. Most microorganisms move a variety of small molecules across their cell or plasma membranes and metabolize them. These substances include glucose, amino acids, small peptides, nucleosides, and phosphates as well as various inorganic ions. In addition to the endoenzymes that are produced for use within the cell, many bacteria (and fungi) produce *exoenzymes* and release them through the cell or plasma membrane. These enzymes include **extra-cellular enzymes,** usually produced by gram-positive rods, which act in the medium around the organism, and **periplasmic enzymes,** usually produced by gram-negative organisms, which act in the periplasmic space. Most exoenzymes are hydrolases; they add water as they split large molecules of carbohydrate, lipid, or protein into smaller ones (catabolism) i.e. carbohydrates to monosaccharide, proteins to make amino acids, fats to fatty acids and glycerol that can be absorbed. There occurs a decrease in the amount of large molecules and transient increase in the amounts of smaller molecules because these are absorbed into

the cells of the organisms for the synthesis of cellular components. As these activities proceed the microorganisms produce and excrete enzymes and metabolites into the surrounding medium. An illustration of the extracellular digestion and absorption in bacteria and fungi is shown in Figure 3.

Microbial enzymes may be constitutive (always present in constant amounts within the microbial cell). Others may be referred to as regulated or induced enzymes. These are produced in response to the presence of a substrate. The concentration of induced enzymes varies with the substrate.

A food substance is considered fermented when one or more of its constituents have been acted upon by selected microorganisms (whether in single pure culture populations or in combination of selected mixed populations or by naturally occurring mixed populations) or their enzymes to produce a significantly altered final product desirable for human consumption (Robert-Nout *et al.,* 2007).

The acceptability of any food substance depends on the perceived acceptable impression of the food as determined by taste and smell. The acceptability of food may be enhanced by the addition of food flavors. Microorganisms are well known to impart flavors in fermented foods that contribute to the characteristic taste and aroma of the food product. Nigeria is blessed with so many indigenous fermented foods such as *Garri*, *Fufu*, *Lafun*, *Akamu* (pap), *Tapioka*, *Fura de Nonu*, African salad/*Ugba*, *Dadawa*, local fermented beverages such as *Pito*, *Oyorkpor*, Palm wine and *Burukutu* to mention a few (Obire and Ekundayo, 1985). There is a whole lot of varieties of fermented foods across the globe indigenous to the countries worldwide. The major food flavors produced by microorganisms are organic compounds which impart their characteristic flavors as a result of volatile acids, alcohols, esters,

5. Government should provide state-of-the-art waste disposal trucks for use and waste handlers should be well kitted with the appropriate personal protective gear such as overall, eye goggles, nose mask, hand gloves, safety shoes and hard hat. At present, this is not done. Rain boot is not safety shoes.

6. Government and every waste generating institution or industry should provide waste treatment facilities for the adequate treatment of both solid and liquid wastes generated from domestic, agricultural, abattoir and industrial operations to ensure decontamination as to avoid the dangers such as the contamination of soil, surface and ground water resources and air pollution that are associated with discharging untreated wastes into the environment.

7. The degradation efficiency of microorganisms in the municipal solid waste dump and abattoir wastes should be properly harnessed to accelerate the degradation and bioconversion of organic solid waste and abattoir wastes into biogas, compost/organic fertilizer for use in gardening, agriculture and horticulture and the protection of the environment. Other means of processing cow hide is therefore advocated as to avert health hazards associated with roasting of cowhide with plastic wastes and used tyres.

8. The process of producing bio-fuel (ethanol) from the waste fruits is not only ecologically sound but also it can enhance income and impart higher level of sustainability. The Government at all levels should encourage this effort as this will provide employment, generate income as well as reduce potential environmental hazards when the waste fruits are left to ferments in waste dumps.

9. Antimicrobial resistance to antibiotics is a dangerous phenomenon and a potent threat to the treatment of diseases.

RECOMMENDATIONS

Proper waste management will promote waste reduction, recovery and recycling practices, create new resource markets, encourage innovation in waste to energy (WTE) technologies, generate new revenue resources and provide gainful employment for many persons.

It is therefore recommended that pollution control strategies should include: -

1. Outreach and enlightenment campaigns to educate the public on proper hygiene practices, awareness of methods of transmission of pathogens and diseases and organized waste disposal methods.
2. There should be proper dug well water location, construction and protection. There should be control of human activities in the vicinity of water bodies to prevent sewage from entering drinking water sources (dug wells, springs, streams, rivers, etc.,) as to avoid microbial contamination.
3. Each community needs an adequate and safe supply of water. Borehole water without treatment is not potable/drinkable water. Government should therefore as a matter of urgency, provide water treatment facilities or water-purification plants complete with microbiological treatment processes to provide potable water.
4. The government and the public should display some discipline and respect towards the environment. Wastes should be sorted and systematically collected and disposed off. The industries, government and the public should avoid indiscriminate dumping of wastes into creeks, rivers, gutters, ditches, unprotected borrow pits or open unused piece of land.

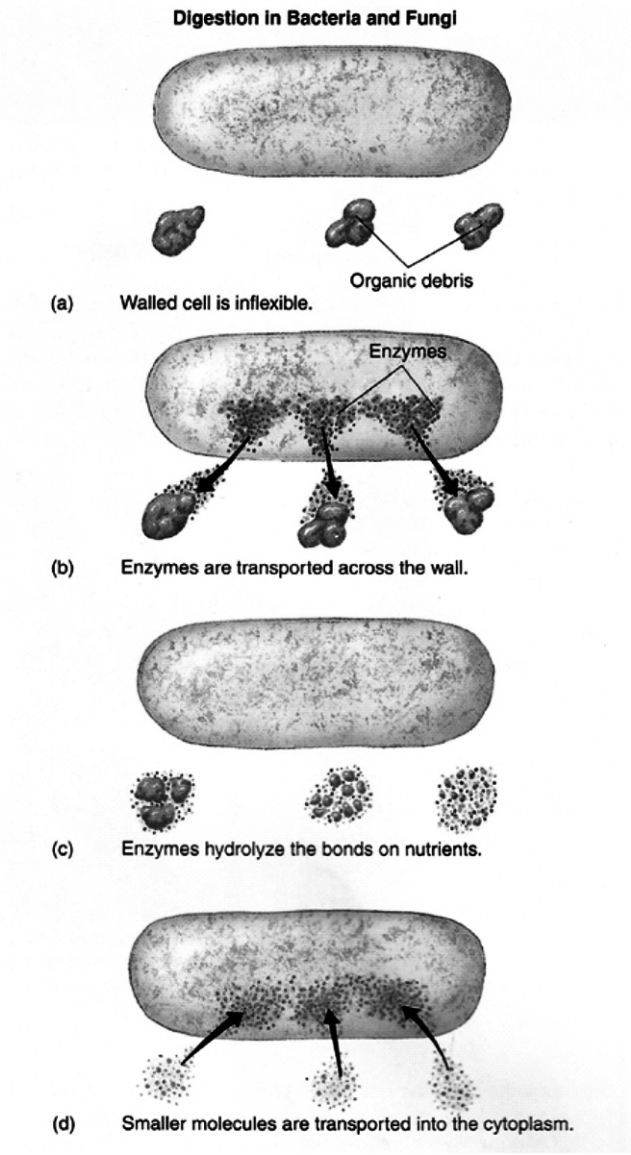

Fig. 3: Extracellular digestion and absorption in Bacteria and fungi
Source: Talaro (2005)

furanones, phenolic compounds and carbon dioxide (Ricke *et al*., 2007, Robert-Nouth *et al*., 2007). All the happiness in any fermented food or beverage is as a result of microbial activities as approved by God. *Ecclesiastes 9:7 Go ahead -- eat your food and be happy; drink your wine and be cheerful for it is alright with God. Good News Bible*.

> *1 Timothy 5:23 "No Longer drink only water, but use a* ***little*** *wine for your stomach's sake and your frequent infirmities (illnesses)"* KJV. 1 *Timothy 5:23 "Do not drink water only, but take a* ***little*** *wine to help your digestion since you are ill so often". Good News Bible*.

We learn to appreciate the invisible world of microorganisms daily. Many microbes benefit humans, whereas only a few are harmful in that they may cause diseases of humans, other animals, and plants. Some synthesize antibiotics that kill or restrict the growth of other microorganisms. In a sense, therefore, microorganisms cure diseases as well as cause them. To control disease, we need to know how to control microorganisms in air, food, soil, and water. To do that, we need to understand the roles of microorganisms in the environment. It is believed by most microbiologists that all naturally occurring materials and all but a very few synthetic materials are subject to microbial attack. Leather, textile, wood, paper, metals, and even electrical conduit boards and optical equipment are subject to deterioration caused by the growth and metabolism of microorganisms. But many more are very important in bringing about changes in the environment which are essential for the maintenance of life on planet Earth (Atlas, 1997). Microorganisms, particularly bacteria, are able to perform this role in nature because they exist in an almost infinite variety of species with different metabolic requirements and capabilities. One reason for studying microbiology is that microorganisms are part of the human environment and are therefore important to human health.

Although developed countries have commercially produced microbial cultures for oil cleanup, the Niger Delta region falls short of researches for commercially produced cleanup strains. The ultimate goal of waste management is the protection of the environment in a manner commensurate with public health, economic, social and political concerns. As the Government in collaboration with multinational companies ceaselessly expands the development of crude oil exploration activities in the Niger Delta, Government must also address the environmental issues associated with this exploitation.

In the "environmental (natural) economy", human use and management of the environment can have positive or negative consequences. We should therefore avoid exceeding the capacity of soil, air and water resources to absorb and disperse pollution as to prevent air and water becoming unsafe to consume.

waste and pollutants is essential for maintaining environmental quality.

Microorganisms readily reveal the effects of a pollutant in their habitat through a shift in their numbers and the elimination of some certain types thereby acting as an alarm system or indicators of pollution.

Since the three elements of the life-support system -water, food, and air-are essential for human survival, but they are also potential vehicles for the spread of disease-causing microorganisms as well as toxic chemicals, one of our major concerns must be the protection of the public health by preventing contamination of the life-support system with harmful organisms and materials; with the role of microorganisms in the prevention of pollution through their activities in both the natural and engineered processes, necessary for the degradation of waste organic material and the recycling of carbon and other elements.

The complexity of the Niger Delta is too frequently used as an excuse for failure to take action in line with international good practice and standards to prevent and address pollution and environmental damage and protect the human rights of communities affected by oil operations.

Bioremediation is a necessary and cost-effective means of removing hydrocarbon pollutants and other environmental pollutants that adversely affect human health or environmental quality. The worldwide market potential for the application of waste treatment facilities and pollution abatement projects that employ microbial biodegradation has been estimated to be over $75 billion.

Microbes can grow under environmentally stressed conditions such as low pH and poor nutrient status and are easy to transport, genetically engineer, and produced in large quantities. These attributes makes microorganisms the organisms of choice in bioremediation.

A positive aspect of microbial deterioration is the degradation of pollutants that may accumulate in the environment. Commercial processes called bioremediation have been developed that use massive numbers of microorganisms known to decompose pollutants such as polychlorinated biphenyls (PCBs). These simple examples illustrate the need for an understanding of the growth habits of microorganisms and the control of their activities in their natural habitats.

Microbial involvement in energy and nutrient flow

Microbes are deeply involved in the flow of energy and food through the earth's ecosystems (Figure 4). They are particularly important because of their unique role of trophic dynamics in aquatic and terrestrial ecosystems.

Microorganisms can be producers, consumers, or decomposers in ecosystems. Producers include photosynthetic organisms among bacteria, cyanobacteria, protists, and eukaryotic algae. Most people are aware that plants carry out photosynthesis, which is the light-fuelled conversion of carbon-dioxide to organic material, accompanied by the formation of oxygen. But microorganisms were photosynthesizing long before the first plants appeared.

Although green plants are the primary producers on land, microorganisms fill this role in the ocean. In fact, they were responsible for changing the atmosphere of the earth from one without oxygen, to one with oxygen. Today photosynthetic microorganisms (including algae and cyanobacteria) account for more than 50% of earth's photosynthesis, contributing the majority of oxygen to the atmosphere.

Some consumers feed directly and exclusively on producers, the most abundant living energy source in any ecosystem. Consumers include heterotrophic bacteria, protists, and microscopic

fungi. Viruses are also consumers since they divert a cell's energy to the synthesis of new virus. Many microorganisms act as decomposers. In fact, they play a greater role in the decomposition of organic substances than larger organisms do.

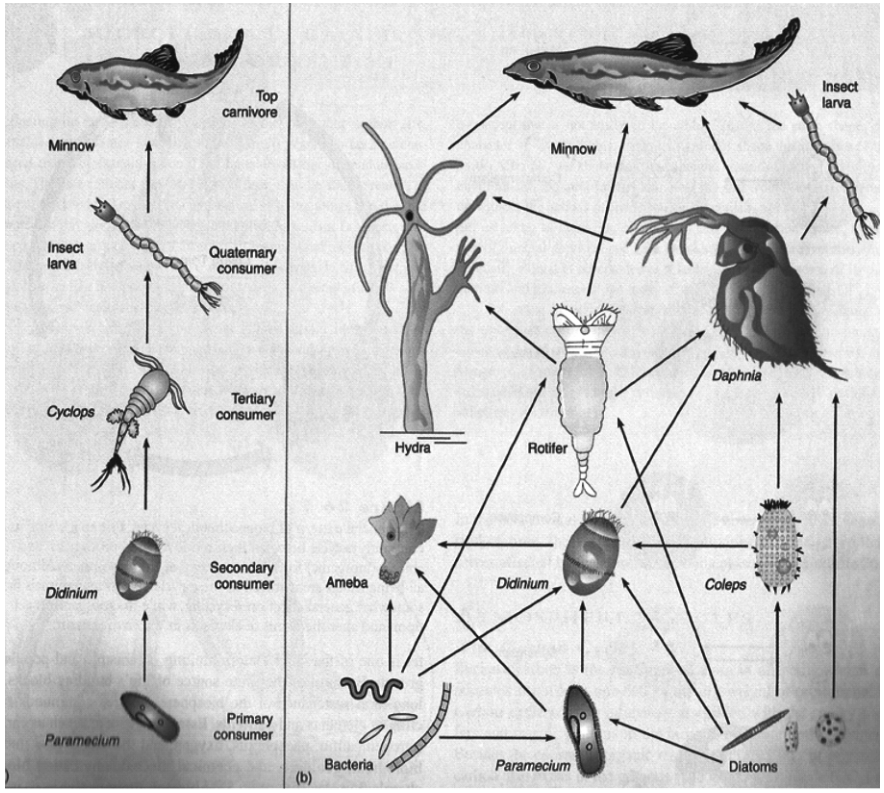

Fig. 4: An aquatic food chain and food web
Source: Talaro and Talaro (1999)

dysentery, and other diseases are preventable. However, poverty-stricken citizens and workers suffer a higher incidence of disease than people from upper class. This difference is attributed to the abominable living conditions. Therefore, humans can shape their environment and can also eliminate diseases of filth by doing away with filth.

Despite the tremendous natural and human resources base, the potential for sustainable development in the Niger Delta region remains unfulfilled and its future is being threatened by diverse environmental problems. While there are other sources of pollution in the Niger Delta, the oil industry is by far the largest and major contributor, and moreover, has been for over half a century. Frequent oil spills are a serious problem in the Niger Delta. Much of the pollution and damage that has contributed to serious abuses of human rights is foreseeable and avoidable. Where problems do occur, timely and effective action can mitigate the consequences.

Pollution by crude oil and its associated oilfield wastewater causes extensive damages ranging from the destruction of plants and animals to biomagnifications of the toxic components of the pollutant, conversion of arable land to barren land, ground water contamination and the destruction of the aesthetic quality of terrestrial and aquatic environments. The effect on microbial populations affects ecosystem dynamics and agro soil fertility problems that would create food scarcity and food insecurity.

Microbes can exist in a great many environments and are deeply involved in the flow of energy and food through the earth's ecosystems. They are particularly important because of their unique role of trophic dynamics in aquatic and terrestrial ecosystems. They provide a source of enriched particulate organic carbon by utilization of both dissolved and particulate organic carbon due to their possession of a wide array of enzymes. The microbial degradation of

CONCLUSION

The dense human and animal population has resulted in an astronomical increase in the amount of waste that is generated. Ecological imbalances have occurred where the natural assimilative capacity has been exceeded because the amount of wastes generated is now beyond the local ecosystems biodegradative threshold. The inability of most administrations to manage the wastes has resulted in serious environmental pollution and epidemic outbreaks of diseases. Public health control measure is vital and we all have to realize that waste has to be collected and disposed off in a sanitary manner to control rodents, flies, and other vectors of diseases.

Government also does not possess the expertise, equipment and technology to enforce the requirements of a fair collection of legislation on waste management and have allowed local contractors to adopt the most convenient method of waste evacuation, use of open, unhygienic waste dumps and the burning of refuse. Wastes as handled in Nigeria now constitute hazardous waste and liability.

The majority of the populace in the Niger Delta does not have access to potable (drinkable) water and therefore, depend on well, spring, stream and river water for domestic and industrial use. Their poverty, and its contrast with the wealth generated by oil, has become one of the world's starkest and most disturbing examples of the "resource curse".

The greatest threat posed to water resources arises from microbiological contamination. The microbial populations of faecal and non-faecal coliform of the various water sources used directly for drinking in communities in the Niger Delta are not within permissible limits of international standards for drinking water by World Health Organization (WHO) and therefore are unfit for drinking. Diseases such as typhoid fever, cholera, tuberculosis,

Hydrothermal vent communities

In 1977 a new and unusual source of nutrients, the basis of a spectacular undersea community, was discovered in the deep ocean. Geologists exploring the Galapagos Rifts (an area in the Pacific floor where the plates that form the Earth's crust are separating) found vents emitting superheated water, black with sulfur and minerals. Surrounding these vents was a rich community of pink fish, blind white crabs, enormous mussels, giant white clams, sea anemones, and giant tube worms (Audesirk *et al*., 2005). Scientists have now identified vent communities in many deep-sea areas where tectonic plates are spreading apart and material from the Earth's interior is spewing forth to form new crust.

In this unique ecosystem, sulfur bacteria serve as the primary producers. They harvest energy from an unlikely source that is deadly to most other forms of life—hydrogen sulfide discharged from the cracks in the Earth's crust. This process, called chemosynthesis, replaces photosynthesis in the vent communities, which flourish more than a mile below the ocean surface. The growth rate of bacteria is equal to that found in productive, sunlit coastal waters. Both bacteria and archaea proliferate in the hot water surrounding the vents, covering nearby rocks with thick, mat-like colonies. These colonies provide the food on which the animals of the vent community thrive. Many vent animals consume the microorganisms directly (Audesirk *et al*., 2005).

Microorganisms as decomposers

Another process that helps keep the earth in balance is the process of **biological decomposition** and nutrient recycling. Decomposition involves the breakdown of dead matter and wastes into simple compounds that can be directed back into the natural cycles of living things. The decomposers are primarily fungi and bacteria that digest

food outside their bodies by secreting enzymes into the environment. They absorb the nutrients they need and release the remaining nutrients.

Through the activities of decomposers, the bodies and wastes of living organisms are reduced to simple molecules such as carbon dioxide, water, minerals, and organic molecules that return to the atmosphere, soil and water. If it were not for multitudes of bacteria and fungi, many chemical elements would become locked up and unavailable to organisms. By liberating nutrients for reuse, decomposers form a vital link in nutrient cycles of ecosystems.

The carbon cycle (Figure 5) depends on photosynthesis and respiration; the Nitrogen cycle (Figure 6) relies heavily on bacteria while the Phosphorus cycle (Figure 7) depends on the weathering of rocks (Campbell *et al*., 2000). In the long term scheme of things, microorganisms are the main forces that drive the structure and content of the soil, water and atmosphere.

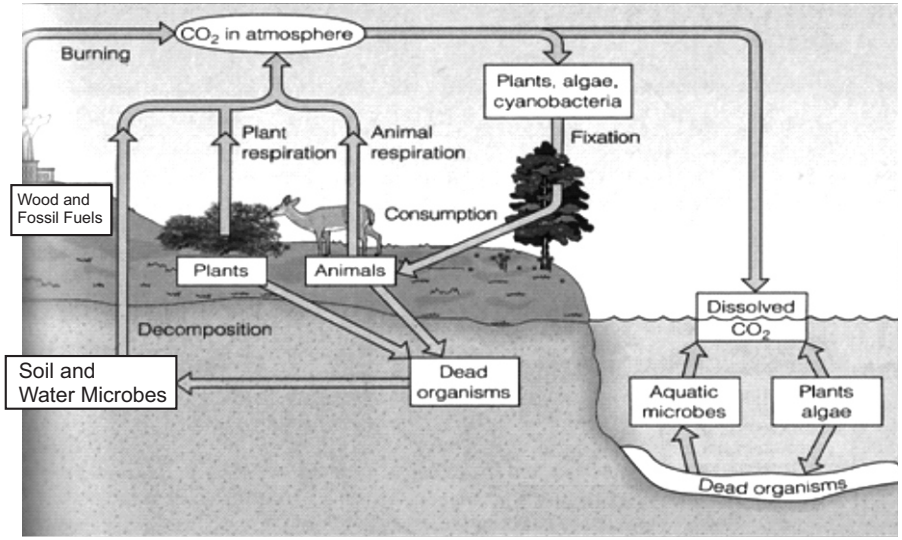

Fig. 5: The Carbon cycle (Source: Campbell *et al.,* 2000).

prop roots of the mangrove trees are usually the natural obstacles during oil recovery efforts in this region; through which heavy machinery cannot be moved for oil recovery efforts. Bioremediation is therefore the only answer to the removal of oil spilled in these areas and the best means of remediation in such ecosystems. The specific steps need to be taken to start the implementation of microbes for bioremediation in Nigeria.

No information is yet available regarding the commercial production of microbial inocula in Nigeria for use in bioremediation of oil polluted environments. Efforts should therefore be focused on developing indigenous microbes for use in large scale operations in the Niger Delta. The data in Table shows that between 66% and 100% of crude oil is lost after recovery efforts. This quantity of oil is lost because of the geographical terrain of the Niger Delta, which is mostly a mangrove swamp and marsh. The soft flowing mud of the swamps and prop roots of the mangrove trees are usually the natural obstacles during oil recovery efforts in this region; through which heavy machinery cannot be moved for oil recovery efforts. Bioremediation is therefore the only answer to the removal of oil spilled in these areas and the best means of remediation in such ecosystems. The specific steps need to be taken to start the implementation of microbes for bioremediation in Nigeria.

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Although there have been reports of commercial production of fungal and bacteria inocula for treatment of oil spills in developed countries (Bartha and Atlas, 1977), species of microorganisms are habitat specific (Obire, 1988). One region that should benefit from bioremediation is the Niger Delta because of incessant oil spills. The Table below shows the oil spillage data in the Eastern Operations in Nigeria. No information is yet available regarding the commercial production of microbial inocula in Nigeria for use in bioremediation of oil polluted environments. Efforts should therefore be focused on developing indigenous microbes for use in large scale operations in the Niger Delta. The data in Table shows that between 66% and 100% of crude oil is lost after recovery efforts. This quantity of oil is lost because of the geographical terrain of the Niger Delta, which is mostly a mangrove swamp and marsh. The soft flowing mud of the swamps and

Table 15: Oil Spillage in the Eastern Operations in Nigeria (1989 – Feb. 2000)

Year	Total No. of Incidents	Approx. No. of Barrels Spilled	Approx. No. of Barrels Recovered	Approx. No. of Barrels Lost
1989	92	6,147.51	1,467.25	4,680.34
1990	119	15,264.50	5,172.50	10,091.61
1991	117	155,031.33	1,402.25	153,629.08
1992	184	27,161.54	721.00	26,440.54
1993	251	7,310.34	1,973.50	5,336.64
1994	270	32,259.70	1,692.25	30,567.45
1995	245	67,561.41	8,846.39	58,715.02
1996	264	43,841.35	0.92	43,840.43
1997	266	74,749.52	1,243.50	73,506.02
1998	133	69,338.68	383.50	68,955.18
1999	260	28,013.72	100.80	27,912.92
Jan-Feb 2000	51	10,179.75	NIL	10,179.75
Total	2252	536,858.84	23,003.86	513,854.98

Source: Department of Petroleum Resources, cited by Azaiki, 2009.

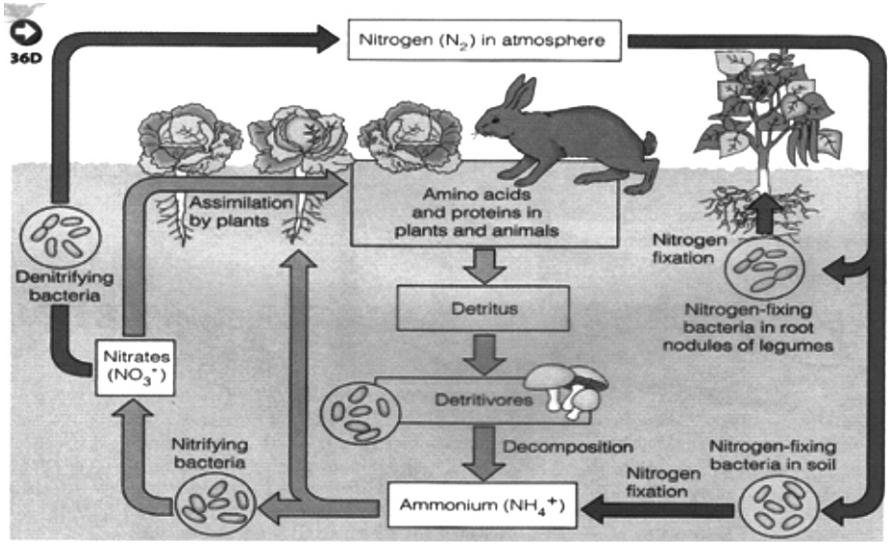


Fig. 6: The Nitrogen cycle (Source: Campbell *et al.,* 2000).

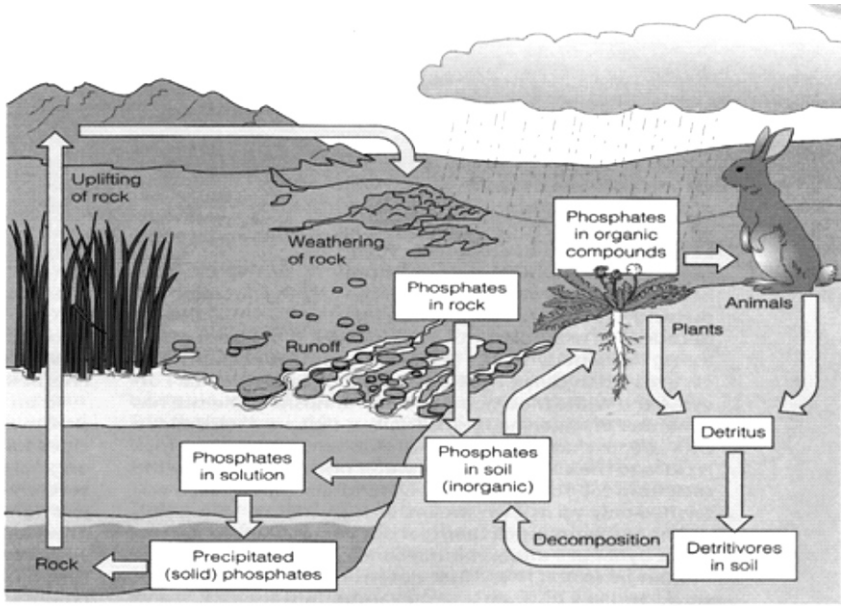


Fig.7 : The Phosphorus cycle (Source: Campbell *et al.,* 2000).

Microbes have also developed symbiotic associations with other organisms that are highly beneficial to the participating members. Bacteria and fungi live in complex associations with plants that assist the plants in obtaining nutrients and water and may protect them against disease. Microbes form similar interrelationships with animals, notably represented by the stomach of cattle, which harbor a rich assortment of bacteria to digest the complex carbohydrates of the animals' diets.

Others, such as the giant tube worm (which lacks a digestive tract) in ocean vent community, harbor the bacteria within special organs in their bodies. In this mutualistic association, the bacteria provide high energy carbon compounds, and the tube worm provides hydrogen sulfide (Audesirk *et al*., 2005). Other microbes become normal flora that serve as barriers to infectious diseases.

Table 14: Saprophytic and crude oil utilizing fungi (moulds and yeasts) isolated from cow dung and poultry droppings

Cow Dung		Poultry Droppings	
Saprophytic fungi	**Crude oil utilizing fungi**	**Saprophytic fungi**	**Crude oil utilizing fungi**
Alternaria sp.	*Aspergillus* sp.	*Alternaria* sp.	*Aspergillus* sp.
Aspergillus sp.	*Cephalosporium* sp.	*Aspergillus* sp.	*Cladosporium* sp.
Cephalosporium sp.	*Cladosporium* sp.	*Cladosporium* sp.	*Fusarium* sp.
Cladosporium sp.	*Geotrichum* sp.	*Fusarium* sp.	*Geotrichum* sp.
Geotrichum sp.	*Mucor* sp.	*Geotrichum* sp.	*Mucor* sp.
Monilia sp.	*Penicillium* sp.	*Mucor* sp.	*Penicillium* sp.
Mucor sp.	*Candida* sp.	*Penicillium* sp.	*Trichoderma* sp.
Penicillium sp.		*Trichoderma* sp.	*Candida* sp.
Rhizopus sp.		*Candida* sp.	*Rhodotorula* sp.
Sporotrichum sp.		*Rhodotorula* sp.	
Thamnidum sp.		*Torulopsis* sp.	
Candida sp.		*Trichosporon* sp.	
Rhodotorula sp.			
Torulopsis sp.			

Source: Obire *et al.*, (2008d)

Microbes for bioremediation in the Niger Delta

In petroleum-producing regions of Nigeria, Obire (1988) isolated several species of oil-degrading aquatic fungi in the genera *Candida*, *Rhodotorula*, *Saccharomyces* and *Sporobolomyces* (yeasts) and, among filamentous fungi, *Aspergillus niger*, *Aspergillus terreus*, *Blastomyces* sp., *Botryodiplodia theobromae*, *Fusarium* sp., *Nigrospora* sp., *Penicillium chrysogenum*, *Penicillium glabrum*, *Pleurofragmium* sp., and *Trichoderma harzianum*.

We investigated the population and types of saprophytic and crude oil degrading fungi from cow dung and poultry droppings monthly for a period of four months using standard methods. Our results revealed that although the counts of total saprophytic fungi were higher in the poultry droppings than the cow dung, the cow dung however, supported the growth of a greater variety of fungal species.

Our result revealed that the cow dung and poultry droppings possess a mixed culture of petroleum degrading fungi. The result suggests that the addition of cow dung or poultry manure as bioremediating agents to polluted soils is beneficial because they can enhance the proliferation of both population and diversity of fungi (total saprophytic and petroleum-utilizing fungi) that may be suppressed by the addition of crude oil as to enhance bioremediation of polluted sites (Figure 35 and Table 14).

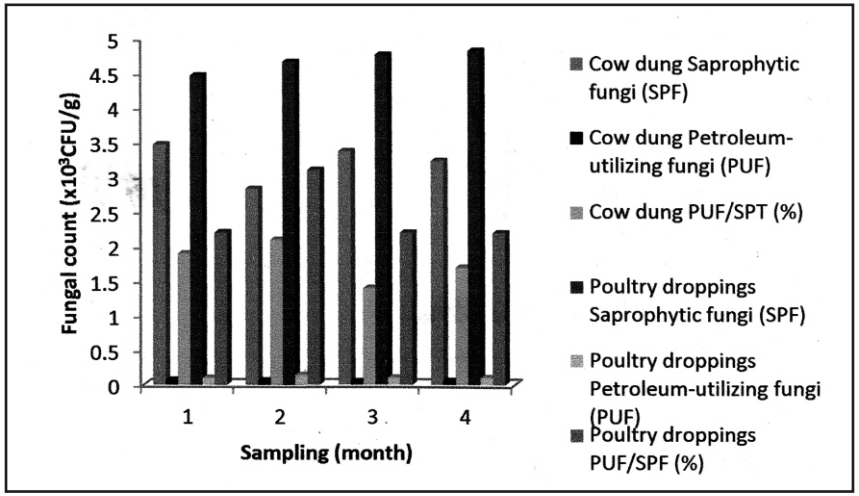


Fig. 35: Counts of total saprophytic fungi, petroleum-utilizing fungi and of petroleum-utilizing fungi expressed as a percentage (%) of total saprophytic fungi in cow dung and poultry droppings

Source: Obire *et al.*, (2008d).

THE GENERAL CHARACTERISTICS OF MICROORGANISMS

Cellular organization

The two basic cell lines which appeared during evolutionary history are termed procaryotic cells and eukaryotic cells. These two cell lines differ primarily in the complexity of their cell structure.

In general, prokaryotic cells are smaller than eukaryotic cells, and they lack special structures such as a nucleus and organelles. Organelles are small membrane-bound cell structures that perform specific functions in eukaryotic cells.

All procaryotes are microorganisms, but only some eucaryotes are microorganisms. The bodies of most microorganisms consist of either a single cell or just a few cells.

Viruses are subject to intense study by microbiologists. They are small particles that exist at a level of complexity somewhere between large molecules and cells. Viruses are much simpler than cells; they are composed essentially of small amount of hereditary material wrapped up in a protein covering. Some biologists refer to viruses as parasitic particles; others consider them to be very primitive organisms. One thing is certain— they are highly dependent on a host cell's machinery for their activities.

Mr Vice-Chancellor Sir, there are various groups of microorganisms. They are the algae, bacteria, fungi, helminthes, potozoans and viruses. I will be focusing on the bacteria, fungi and viruses because these are the microbes I have handled during the course of my academic carrier.

The Structure of a Generalized Prokaryotic Cell

The typical bacterial cell appears simple and featureless under a light microscope. At higher magnifications (Figure 8), a bacterial cell has a cell wall. External to this, some cells may have some of these structures; flagella, pilli, fimbriae, glycocalyx, capsule and slime layer. On the inside of the cell wall are the cell membrane, the cytoplasm and the nuclear material. The flagella when they occur are used for motility while the pilli and fimbriae are used by the organisms for attachment onto surfaces. The slime layer protects against dehydration and dessication while the capsule contributes to the pathogenicity and virulence of the organism. The cell wall determines the shape and integrity of the cell against osmotic pressure and lysis. Structurally, the cell wall is made up of peptidoglycan composed of N-acetyl-glucosamine and N-acetyl-muramic acid.

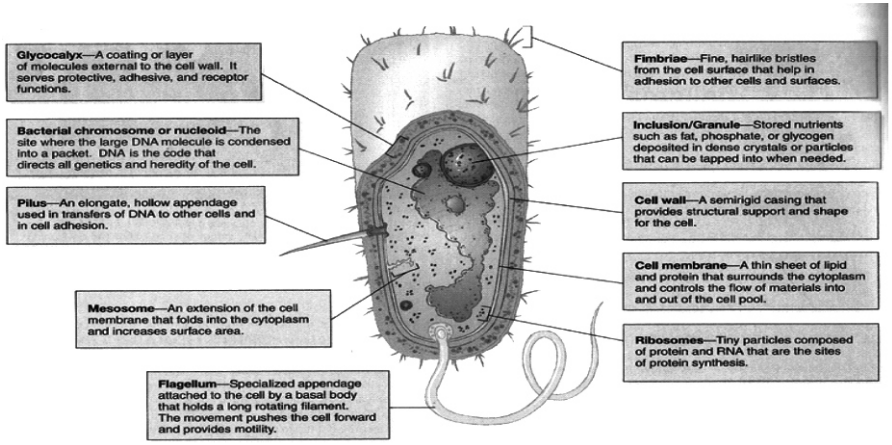


Figure 8: Structure of a procaryotic cell (typical rod shaped-bacterium)
Source: Talaro and Talaro (2002)

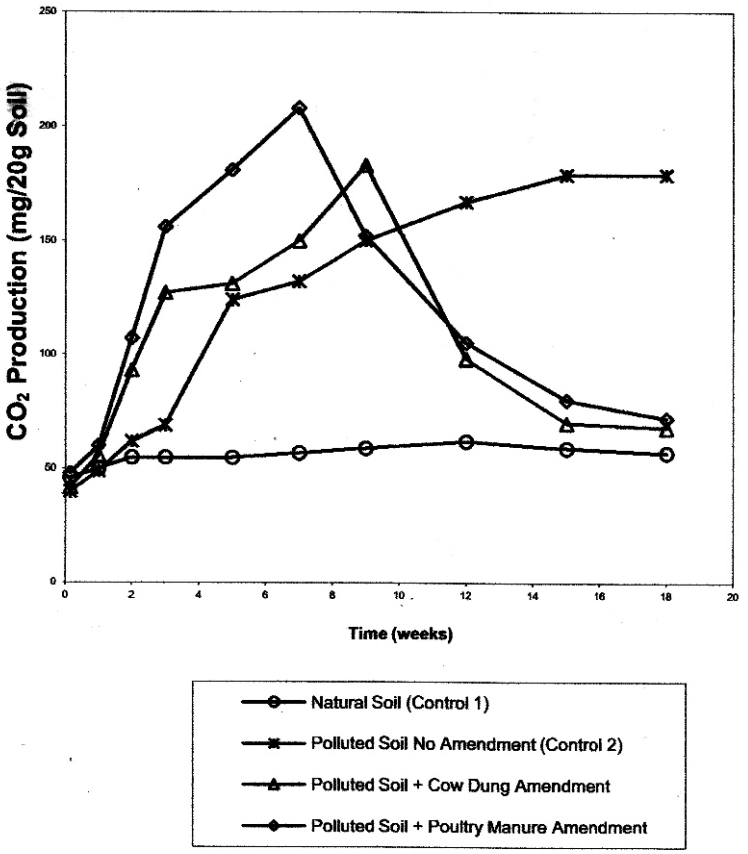


Fig. 34: Carbon (IV) oxide evolution in 20g of the controls and oil polluted bioremediated soils

Source: (Obire and Akinde, 2006).

Complex mixtures of components are contained in the petroleum hydrocarbon contaminants and microbial degradation differs in the susceptibility of each component. Therefore, naturally mixed microbial populations degrade crude oils and hydrocarbons better than single isolates from the mixed populations.

Crude Oil Degradation:

Percentages of crude oil degraded in the control and oil polluted bioremediated soils are presented on Figure 3. Percentages of crude oil degraded ranged from 17.8 – 84.4% in the polluted soil without nutrient, from 13.3 – 88.9% in polluted soil treated with cow dung, and from 15.6 – 92.1% in polluted soil treated with poultry manure.

The natural soil (control 1) maintained average optical density value of 0.063 during the incubation period with baseline concentration of $9cm^3/13{,}500cm^3$ soil. This implied that the source of hydrocarbon to the soil and its breakdown was constant, and no degradation was done beyond the baseline value which indicated a balanced or stable ecosystem.

Carbon (IV) Oxide Evolution:

The values of carbon (IV) oxide evolved from 20gm of the controls and oil polluted bioremediated soils are as shown on Figure 4. Values ranged from 46 – 62mg in the natural soil, from 40 – 179mg in polluted soil without nutrient, from 38 – 186mg in polluted soil treated with cow dung, and from 40 – 197mg in polluted soil treated with poultry manure.

The disappearance of oil was maximal in polluted plots amended with poultry manure (92.1%) followed by polluted plots amended with cow dung (88.9%). Generally, addition of nutrient increased CO_2 production in polluted soils. However, the amount of CO_2 liberated in polluted soil amended with poultry manure (48 – 208mg/20g soil) was generally higher than in polluted soil amended with cow dung (42 – 183mg/20g soil). The evidence revealed that amelioration of oil polluted soil with cow dung or poultry manure facilitates the disappearance of crude oil in the soil thereby increasing the rate of soil recovery.

The lipid portions of the outer membrane of some bacteria are basically endotoxins that cause fever and shocks when released into the host bloodstream during an infection. The cell membrane (CM) is a semipermeable, bilayer fluid with proteins embedded in them. The cell membrane serves various functions in the transportation of molecules in and out of the cell as well as providing the site for energy reactions. Enclosed within the CM is a dense solution- the cytoplasm. It is the site of metabolic activities. It is a matrix rich in nutrients, organic molecules, salts, cell inclusion and storage bodies. The single circular chromosome which carries the genetic information of the microbial cell is also found in the cytoplasm (Talaro and Talaro, 2002).

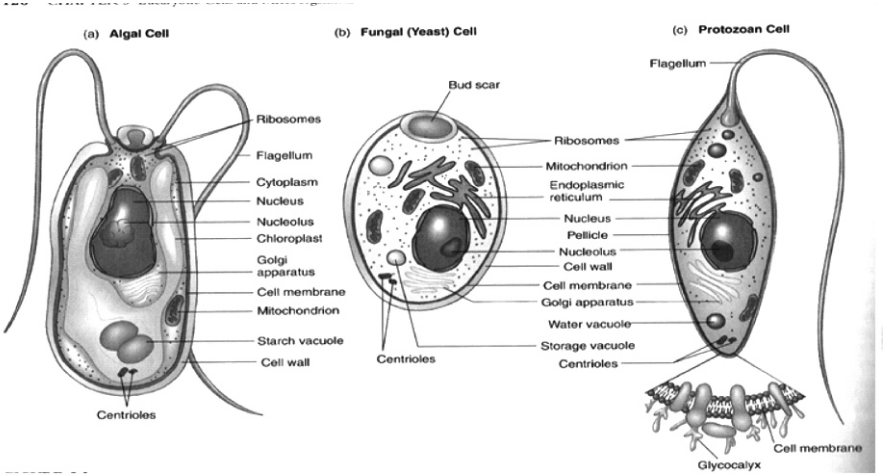


Fig. 9: Structure of three representative of eucaryotic cells groups: *Chlamydomonas*, yeast and *Paranema*

Source: Talaro and Talaro (2002)

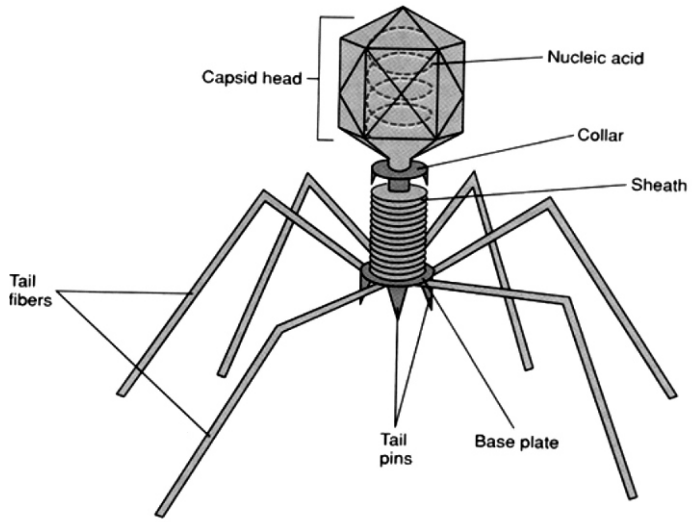


Fig. 10: Diagram of T-even Bacteriophage
Source: Talaro and Talaro (1999)

Microbial dimensions: How small is small?

When we say that microbes are too small to be seen with the unaided eye, what sorts of dimensions are we talking about? This concept is best visualized by comparing microbial groups with the larger organisms of the macroscopic world and also with the molecules and atoms of the molecular world. Whereas the dimensions of macroscopic organisms are usually given in centimeters (cm) and meters (m), those of most microorganisms fall within the range of micrometers (μm) and sometimes, nanometers (nm) and millimeters (mm). The size range of most microbes extends from the smallest viruses, measuring around 20 nm and actually not much bigger than a large molecule, to protozoan measuring 3 to 4 mm and visible with the unaided eye. Most microbes encountered in microbiology studies fall between 100 μm and 10 μm in overall dimensions (Talaro, 2005).

Counts of petroleum utilizing bacteria ranged from 7×10^5 to 2.3×10^6 cfu/gm soil in the natural soil, from 7×10^5 to 1.39×10^7 cfu/gm soil in polluted soil without nutrient, from 1.0×10^6 to 1.31×10^7 cfu/gm soil in polluted soil treated with cow dung, and from 4.0×10^5 to 1.38×10^7 cfu/gm soil in polluted soil treated with poultry manure.

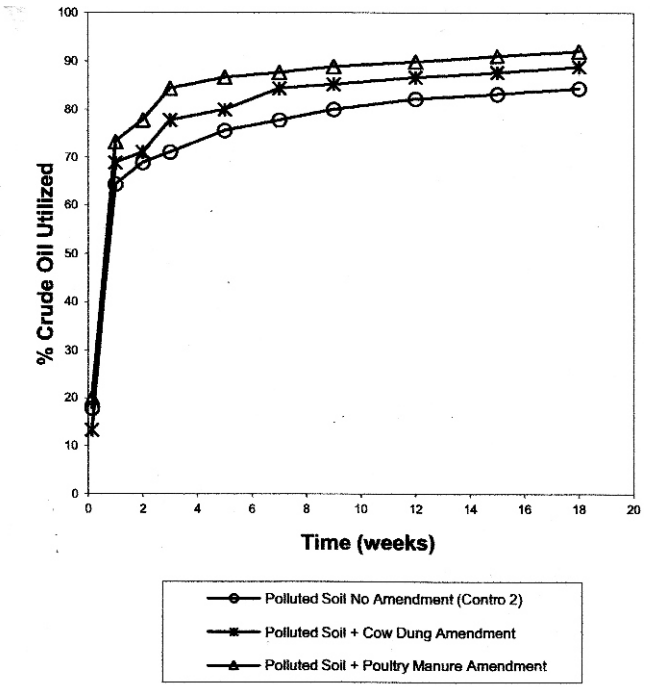


Figure 33: Percentage of oil degraded in polluted control and polluted bioremediated soils
Source: (Obire and Akinde, 2006)

Our research on the populations and types (diversity) of saprophytic and crude oil degrading fungi from cow dung and poultry droppings revealed that addition of cow dung or poultry dropping to polluted soil was beneficial because they could enhance the proliferation of mycoflora that might be suppressed by the addition of crude oil to the soil (Obire and Akinde, 2004; Obire and Anyanwu, 2008). The counts of petroleum utilizing bacterial in the controls and oil polluted bioremediated soils are as shown on Figure 32.

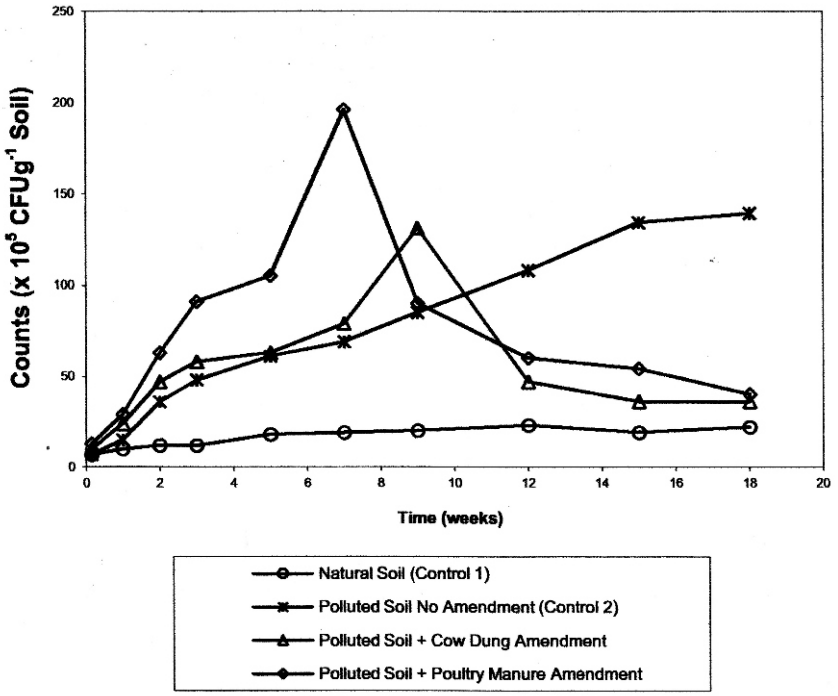


Fig. 32: Counts of petroleum utilizing bacteria in oil polluted bioremediated soils
Source: Obire and Akinde (2006).

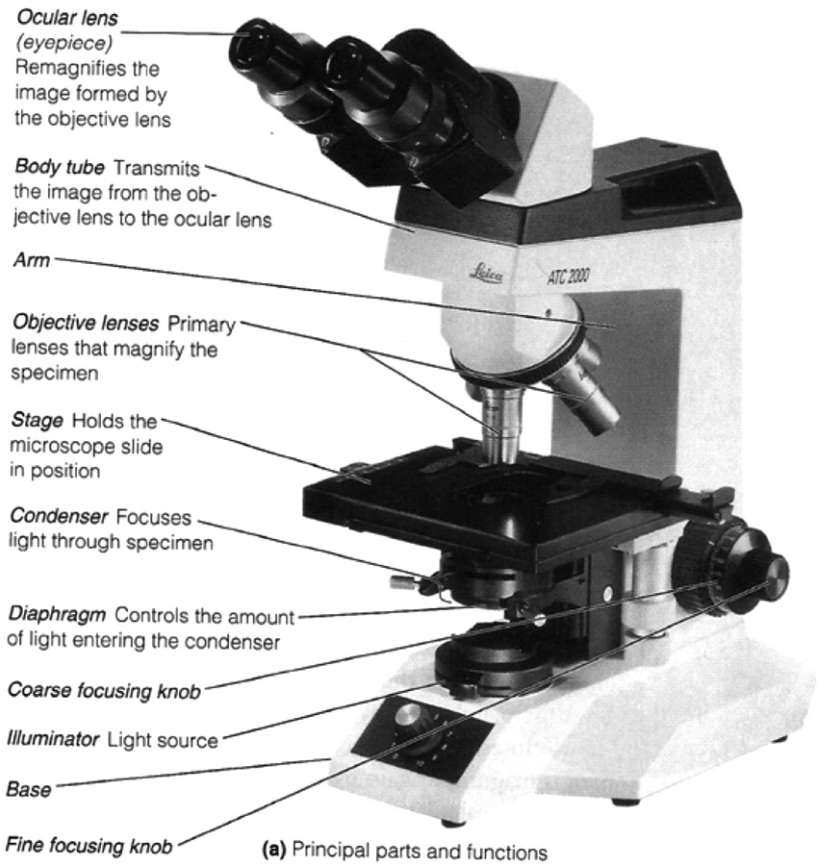
Methods for Studying Microorganisms

The study of microbiology requires appropriate methods for observing microorganisms. The nature of microorganisms makes them both very easy and very difficult to study. Easy, because they reproduce so rapidly and we can quickly grow large populations in the laboratory. Difficult, because we cannot see them directly. We rely on indirect means of analyzing them in addition to using microscopes.

Apart from the microscopic protozoans that may be up to 3-4mm, all other microorganisms can only be seen with the aid of microscopes. Microscopy and the ability to culture microorganisms are fundamental methodologies that enable microbiologists to study every aspect of microbes:- their genetics, their physiology, their characteristics that may lead to disease or to benefits, the way they interact with the environment, with mammalian hosts, and their uses in industry and agriculture.

Microscopy is the use of a microscope (an instrument that magnifies the size of the image of an object) to view objects too small to be visible with the unaided eye. There are many types of microscope which are the basic tools employed by microbiologists for the observation of microorganisms. The various parts of a compound light microscope are shown in Figure 11.

With the microscope, numerous microorganisms can be observed from many sources, including soil and water.



.Fig. 11: The compound light microscope
Source: Tortora *et al.*, (1998)

The size of a microorganism or a microbial structure determines the degree of magnification needed to see it. While the resolution is the degree to which the detail in the specimen is retained in the magnified image. The ability to see detail is essential lest everything appear as an unresolved blur. Table 1 shows variations of microscopy for the study of microorganisms.

recolonization pattern in triplicate randomized complete block for 18-week period revealed that poultry dropping application was effective for soil treatment which would improve various biodegradation processes as well as increase crop production (Obire and Akinde, 2004). Our studies also showed that poultry manure was a better alternative to cow dung in bio-remediation of oil polluted soils (Obire and Akinde, 2006). The total aerobic heterotrophic bacteria counts of the controls and oil polluted bioremediated soils of some of our studies are shown in the Figures below. Counts of total aerobic heterotrophic bacteria ranged from 5.1×10^6 to 7.4×10^6 cfu/gm soil in the natural soil, from 4.5×10^6 to 2.20×10^7 cfu/gm soil in polluted soil without nutrient, from 5.0×10^6 to 2.45×10^7 cfu/gm soil in polluted soil treated with cow dung, and from 6.1×10^6 to 2.55×10^7 cfu/gm soil in polluted soil treated with poultry manure.

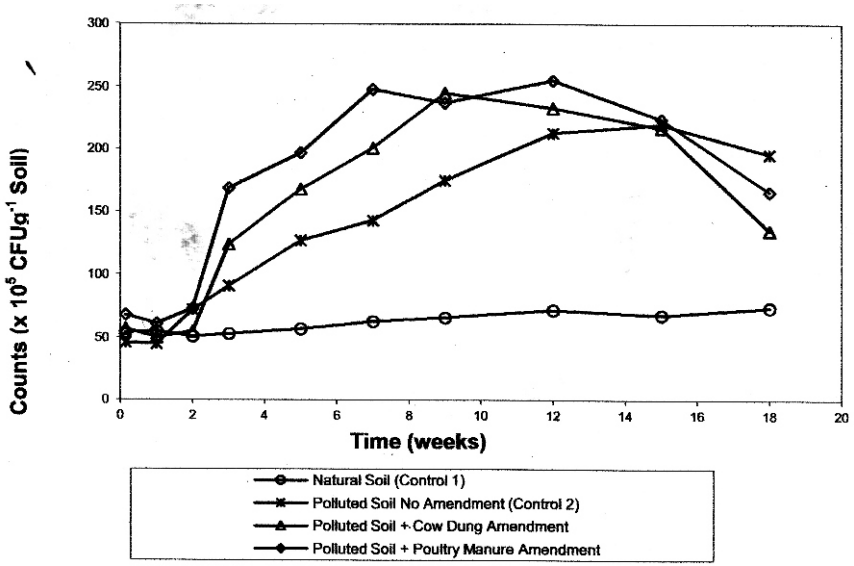


Fig. 31: Heterotrophic bacterial count in oil polluted bioremediated soils
Source: Obire and Akinde (2006).

The suitability of various Nigerian petroleum fractions as substrate for bacterial growth revealed that the heavier petroleum fractions kero and automotive gas oil (AGO) showed higher bacterial growth and conversion into biomass (Obire, 1993). Using Gas Liquid Chromatographic (GLC) techniques, I was able to establish the utilization of particular crude oil by microorganisms based on bacterial growth and demonstrated the concentration of components utilized (Obire, 1990). A comparison of the chemical components of the degraded crude oils by GLC showed that the lower molecular weight (n-saturates) fractions were preferentially utilized. Short chain alkanes (less than C_9) are toxic to many microorganisms but they generally evaporate rapidly from oil slicks; *n*-Alkanes of intermediate chain length (C_{10}-C_{24}) are degraded most rapidly. As alkane chain length increases, so does resistance to biodegradation.

Our studies on the biological activities (carbon-dioxide production and hydrocarbon degradation) in soils treated with 5% of refined hydrocarbons (gasoline, kerosene and diesel oil) revealed that carbon-dioxide production was generally higher in the hydrocarbon contaminated soils than in the control soil in the order of gasoline soils > kerosene soils > diesel oil soils > control soils (Obire and Nwaubeta, 2001a; Obire and Nwaubeta, 2001b).

Our studies on the biostimulation and bioaugmentation revealed that specific bacterial isolates with petroleum degrading potential were present in cow dung and poultry manure. *Pseudomonas* spp occurred as petroleum utilizer in cow dung and poultry manure. *Bacillus* spp occurred only in cow dung as petroleum utilizer while *Actinobacter* spp and *Micrococcus* spp occurred only in poultry manure as petroleum utilizers (Akinde and Obire, 2008). The impact of 2% poultry droppings on soil physico-chemical properties, bacterial

Table 1: Comparison of various types of microscopes

Type	Maximum useful magnification	Resolution	Description
Bright-Field	1,500×	100 - 200 nm	Extensively used for the visualization of microorganisms. Usually necessary to stain specimen for viewing
Dark-Field	1,500×	100 - 200 nm	Used for viewing live microorganisms, particularly those with characteristic morphology. Staining not required. Specimen appears bright on a dark background
Ultraviolet	2,500×	100 nm	Improved resolution over normal light microscope. Largely replaced by electron microscopes
Fluorescence	1,500×	100 - 200 nm	Uses fluorescence staining. Useful in many diagnostic procedures for identifying microorganisms
Phase-contrast	1,500×	100 - 200 nm	Used to examine structures of living microorganisms; does not require staining specimens
Nomarski differential interference	1,500×	100 - 200 nm	Used to examine structures of micro-organisms; produces sharp, multicolored image with three-dimensional (3D) appearance
Confocal	1,500×	100 - 200 nm	Used to examine structures of microorganisms and individual microorganisms within mixtures of various types of microorganisms; uses fluorescence staining; produces blur-free image; used to produce 3D images.
Transmission electron (TEM)	500,000 - 1,000,000×	1 - 2 nm	Used to view thin sections of cells or ultrastructure of microorganisms, including viruses; much greater resolving power and useful magnification than can be achieved with light microscopy. Can only be used to view preserved organisms
Scanning electron (SEM)	10,000 - 1,000,000×	1 - 10 nm	Used for showing detailed surface structures of microorganisms; produces a 3D image

Source: Atlas (1997)

Growth and Cell Division (Reproduction)

In everyday language, growth refers to an increase in size. We are accustomed to seeing children, other animals, and plants grow. Numerically, microorganisms are the most successful living organisms on earth. "Microbes have dominated life on earth for most of its 4.5 billion year history. Unicellular organisms also grow, but as soon as a cell, called the **mother** (or *parent*) **cell**, has approximately doubled in size and duplicated its contents, it divides into two **daughter cells**. Then the daughter cells grow, and subsequently they also divide. Because individual cells grow larger only to divide into two new individuals, **microbial growth** is defined not in terms of cell size but as the increase in the number of cells, which occurs by cell division; a process known as binary fission. Binary fission involves three processes: increase in cell size (cell elongation), DNA replication, and cell division.

In **binary fission**, a cell duplicates its components and divides into two cells (Figure 12). The daughter cells become independent when a *septum* (partition) grows between them and they separate. Unlike eukaryotic cells, prokaryotic cells do not have a cell cycle with a specific period of DNA synthesis. Instead, in continuously dividing cells, DNA synthesis also is continuous and replicates the single bacterial chromosome shortly before the cell membrane, which grows and separates the replicated chromosomes.

of bioremediation of environment impacted by PAHs. The order of decreasing rate of biodegradation of PAHs by microorganisms was *Aspergillus* sp > *Penicillium* sp > *Pseudomonas* sp > *Klebsiella* sp > mixed culture of fungi > mixed culture of bacteria > mixed cultures of bacteria and fungi.

Microorganisms Associated with Remediation of Petroleum Polluted Soils and Water

Oil-degrading microorganisms are abundant and are not limited to oil producing areas, but are present in any conceivable environment. Filamentous fungi, yeasts, actinomycetes and bacteria all have the ability to utilize hydrocarbon substrates—though their ability to do so varies among individual strains and, in some cases, depends on hydrocarbon chain length (Rowell, 1977; Walker *et al*., 1973). For instance, bacteria and yeasts showed decreasing abilities to degrade alkanes with increasing chain length. While filamentous fungi did not exhibit preferential degradation for particular chain lengths. Specific fungal genera and species with potential for bioremediation have been detailed by Obire and Putheti (2009).

My studies on the ability of microorganisms isolated from two petroleum producing areas (Chanomi and Forcados creeks in Delta State) to degrade Bonny Light and Forcados blend crude oils showed that the best oil degrading bacterium, yeast and mould was *Pseudomonas fluorescens*, *Candida lypolytica* and *Trichoderma harzianum* respectively (Obire, 1988). Our studies on Obagi soils also showed that bacteria utilizers of hydrocarbon are more abundant among the bacillus group than the cocci group of bacteria (Wemedo and Obire, 1998).

We also investigated the biodegradation of polycyclic aromatic hydrocarbons in soil, wastewater, surface water and sediment samples from adjoining creeks to the abattoirs in Rivers State and Imo State by associated microorganisms. Levels of polycyclic aromatic hydrocarbons (PAHs) left after incubation were determined using Gas chromatographic method. Our studies revealed that soil samples from various abattoirs had high concentration of PAHs which ranged from 0.176 mg/kg from Ahoada abattoir to 2.44 mg/kg from Egbu abattoir. Biodegradation test revealed that there was a decrease in the initial concentration of PAHs from 0.03 mg/l to 0.024 mg/l and a decrease in the concentration of PAHs with increase in exposure time when compared to controls. There was observable loss of low molecular weight PAHs than the high molecular weight components. It is evident from the study that both mixed cultures of bacteria and fungi can biodegrade polycyclic aromatic hydrocarbons. This study showed that abattoir wastes have high pollution strength and thus should be treated before discharge into the environment (Nwachukwu, Obire *et al*., 2015).

The polycyclic aromatic hydrocarbons (PAHs) biodegradation potential of some bacteria and fungi isolated from Forcados Terminal effluents in Delta State was investigated. Gas chromatographic technique was used to determine the levels of PAHs left after the incubation period. The bacterial isolates were *Pseudomonas* sp, *Klebsiella pneumoniae*, *Escherichia coli*, *Micrococcus* sp and *Staphylococcus* sp. while the fungi isolates were *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus* and *Candida* species. *Pseudomonas* sp and *Aspergillus* sp were the predominant bacterium and fungus respectively. The biodegradation results showed decrease in concentration of PAHs with increase in exposure time. Both pure and mixed cultures of bacteria and fungi were able to biodegrade the recalcitrant PAHs and are found to be potential agents

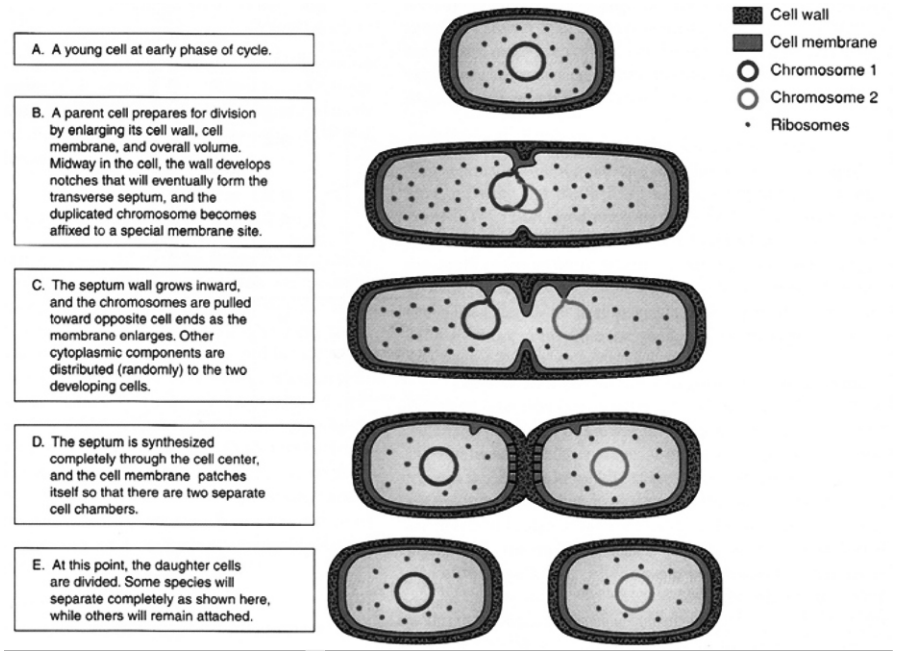


Fig. 12: Binary fission: Cell division in a prokaryote (rod-shaped bacterium)
Source: Talaro (2005)

Replication of the chromosome is completed before cell division, when the cell may temporarily contain two or more nucleoids. Not all bacteria reproduce by binary fission. Yeast cells and some bacteria divide by **budding** in which a small, new cell develops from the surface of an existing cell and subsequently separates from the parent cell.

When the arithmetic number of cells in each generation is expressed as a power of 2, the exponent tells the number of doublings (generations that have occurred). The arithmetic number of cells in each generation is represented in Table 2 below while the Generation number and Log_{10} of Arithmetic numbers of cells are represented in Table 3:

Table 2: Arithmetic number of cells in each generation

Arithmetic of cells	Numbers expressed as a power of 2	
1	2^0	•
2	2^1	••
4	2^2	••••
8	2^3	••••••••
16	2^4	••••••••••••••••
32	2^5	••••••••••••••••••••••••••••••••

Source: Tortora *et al*., (1998)

Table 3: Generation number and Log_{10} of Arithmetic numbers of cells

Generation number	Arithmetic numbers of cells	Log_{10} of Arithmetic numbers of cells
0	1	0
5 (2^5) =	32	1.51
10 (2^{10}) =	1,024	3.01
15 (2^{15}) =	32,768	4.52
16 (2^{16}) =	65,536	4.82
17 (2^{17}) =	131,072	5.12
18 (2^{18}) =	262,144	5.42
19 (2^{19}) =	524,288	5.72
20 (2^{20}) =	1,048,576	6.02

Source: Tortora *et al*., (1998)

Biodegradation of abattoir wastes and oilfield wastewater

The continuous discharge of pollutants such as PAHs into the abattoir environment has resulted in a proliferation of a microbial community capable of utilizing such toxicant. Because microbes are sensitive to changes in the environment there is a continuous experience of microbial succession to give room to organisms that can survive the changes. Consequently, information on the composition of microorganisms in a polluted site is of valuable importance in order to estimate the self-purification capability of the ecosystem and the feasibility of biological decontamination if engineered bioremediation should be considered (Allen *et al*., 2007; Said *et al*., 2008).

Our studies on abattoir soils and wastewater and oilfield wastewater from a crude oil producing terminal revealed that, bacteria and fungi isolated from and within these environments are potential agents of remedying environments impacted by polycyclic aromatic hydrocarbons (PAHs). Abattoir soils are rich in hydrocarbon utilizing microbes that can be harnessed for the clean-up of hydrocarbon contaminated soils. The wide ranges of population of hydrocarbon degraders thus indicate proliferation due to pollution by Polycyclic Aromatic Hydrocarbons (PAHs) being emitted through the burning of tyres used for the roasting processes in the abattoirs (Nwachukwu, Obire *et al*., 2015). The hydrocarbon utilizing bacteria isolated from abattoir soils were *Alcaligenes*, *Bacillus*, *Escherichia*, *Enterobacter*, *Proteus*, *Pseudomonas*, *Staphylococcus* and *Micrococcus*. Both *Bacillus* and *Pseudomonas* species were the most occurring bacteria while *Micrococcus* sp. *and Alcaligenes* were the least occurring bacteria. On the other hand, the fungi which demonstrated hydrocarbon utilizing potentials from abattoir soils were *Aspergillus*, *Fusarium*, *Geotrichum*, *Mucor* and *Penicillium* (Ariyo and Obire, 2016).

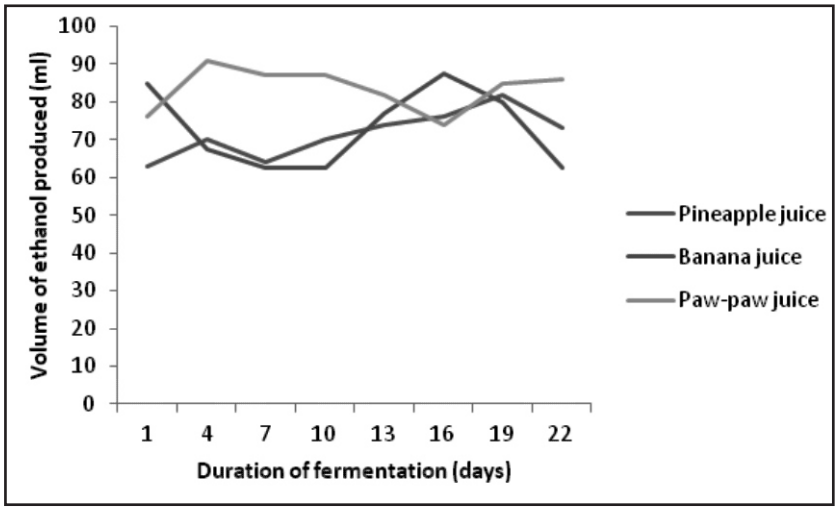


Fig. 30: Ethanol production from fermented (100ml) waste fruit juices
Source: Obire et al., (2008h)

Uses of Ethanol:

Commercial and medicinal purposes, As fuel for automobiles either alone (E100) in a special engine, As an oxygenate additive for standard gasoline as a replacement for methyl t-butyl ether (MTBE),To power fuel cells, As a potentially sustainable energy resource that may offer environmental and long-term economic advantage over fossil fuel (gasoline) because it burns more slowly, coolly and completely, Automakers rolled out flex-fuel cars able to run on ethanol gasoline or any mixture of the two in 2003, Today, 70% of new car sales in Brazil are "flex", Fuel ethanol, besides its environmental value, is and will remain first and foremost, an instrument to support farmers, as they will profit from fuel ethanol programmes.

The time required for a cell to divide (and its population to double) is called the generation time. It varies considerably among organisms and with environmental conditions such as temperature. Most bacteria have a generation time of 1 – 3 hours, others require more than 24 hours per generation. If binary fission continues unchecked, an enormous number of cells will be produced. If a doubling occurred every 20 minutes ---which is the case with *E. coli* under favorable conditions – after 20 generations a single initial cell would increase to over 1 million cells. This would require a little less than 7 hours. In 30 generations or 10 hours, the population would be 1 billion, and in 24 hours it would be a number trailed by 21 zeros.

The population size of bacteria doubles with each cycle of reproduction and cell division. As long as the nutrient status and environmental conditions favour the growth of microorganisms, a few hours is enough to give rise to several million cells. The impact of such a fast generation time is most evident in food spoilage.

The Holy Bible acknowledges the activities of microbes in this regard. Exodus 16 v 15 – 16, 19 – 20

> *Moses said to them, "This is the food the Lord has given you to eat. The Lord has commanded that each of you is to gather as much as he needs for his household". "No one is to keep any of it for tomorrow. But some of them did not listen to Moses and saved part of it. The next morning, it was full of worms and smelt rotten, and Moses was angry with them*".

The growth rate of some representative bacteria under optimal conditions is shown in Table 4

Table 4: Growth rate of some representative bacteria under optimal conditions

Organism	Temperature	Generation Time (min)
Bacillus stearothermophilus	60	11
Escherichia coli	37	20*
Bacillus subtilis	37	27
Bacillus mycoides	37	28
Staphylococcus aureus	37	28*
Streptococcus lactis	37	30
Pseudomonas putida	30	45
Lactobacillus acidophilus	37	75
Vibrio marinus	15	80
Mycobacterium tuberculosis	37	360*
Bradyrhizobium japonicum	25	400
Nostoc japonicum	25	570
Anabaena cylindrica	25	840
Treponema pallidum	37	1980

Source: Atlas (1997)

Bacterial Shapes and Arrangements

Bacteria exhibit considerable variety in shape, size and colonial arrangement. It is convenient to describe most bacteria by one of three general shapes: spherical, rod, and spiral as dictated by the configuration of the cell wall (Figure 13). If the cell is spherical or ball-shaped, the bacterium is described as a coccus. Cocci can be

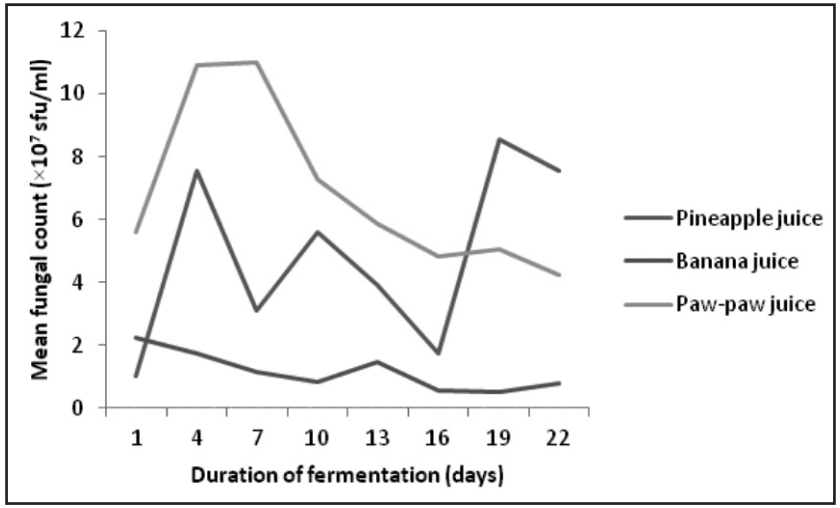


Fig. 28: Mean values of fungal counts of fermented waste fruit juices
Source: Obire *et al.*, (2008h)

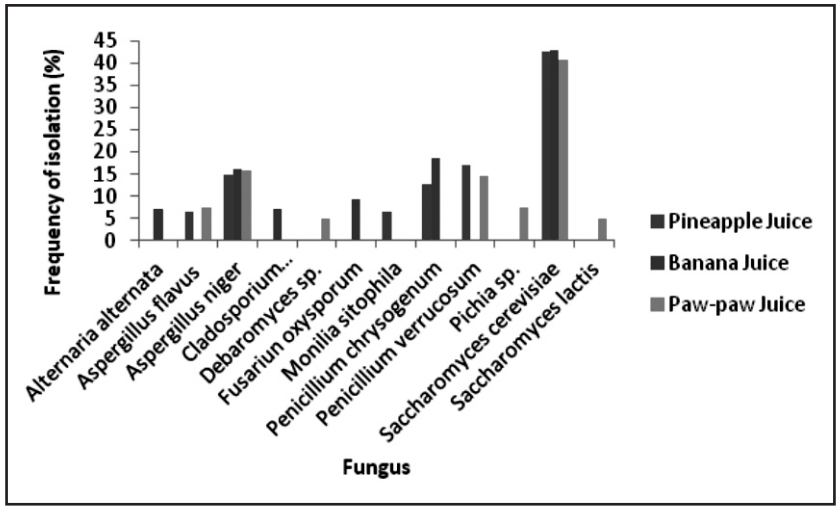
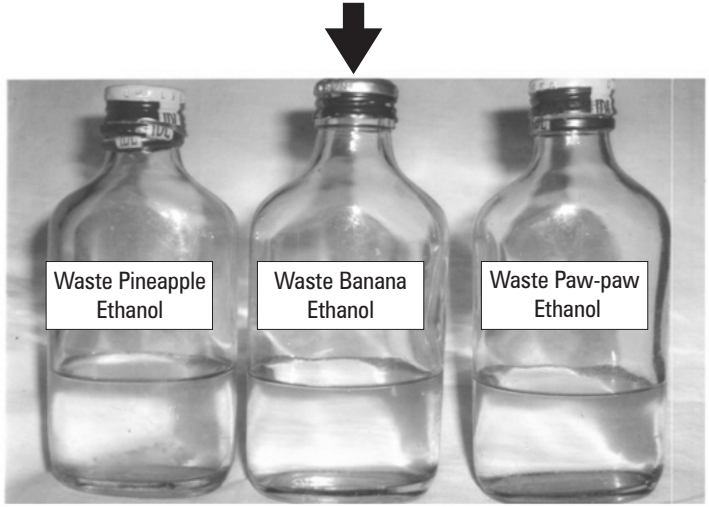


Fig 29: Frequency of isolation of fungi from the waste fruit juices
Source: Obire et al., (2008h)

Recovery of Ethanol by Distillation process



Ethanol distillate from spoilt/waste fruits (Source: Obire *et al.*, 2008h)

Plate 10: Flow process of bio-ethanol production

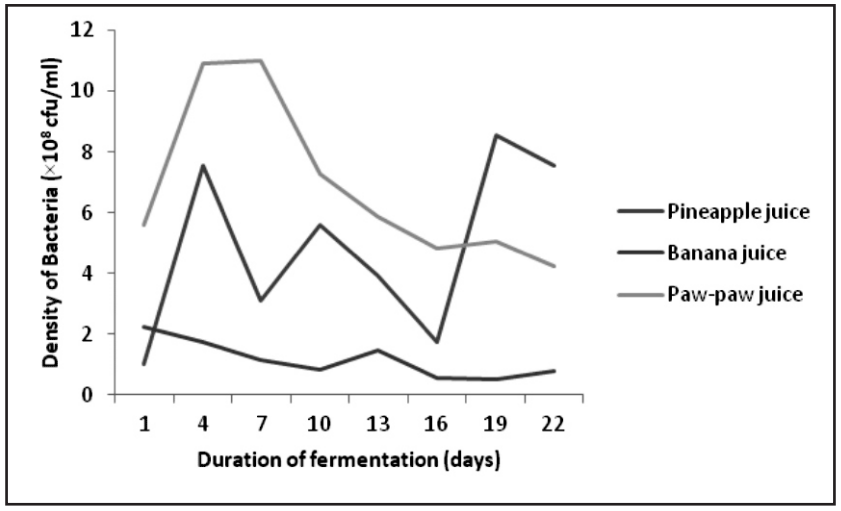


Fig. 27: Mean density of bacteria of the fermented waste fruit juices
Source: Obire *et al.*, (2008h)

perfect spheres, but they also can exist as oval, bean-shaped, or even pointed variants. A cell that is cylindrical is termed a rod, or bacillus. As might be expected, rods are also quite varied in their actual form. Depending on the bacterial species, they can be blocky, spindle-shaped, round ended, long and thread-like (filamentous), or even clubbed or drumstick-shaped. When a rod is short and plump, it is called a coccobacillus, if it is gently curved, it is a vibrio. A bacterium having the shape of a curviform or spiral-shaped cylinder is called a spirillum, a rigid helix, twisted twice or more along its axis (like a corkscrew). Another spiral cell mentioned earlier in conjunction with periplasmic flagella is the spirochete, a more flexible form that resembles a spring.

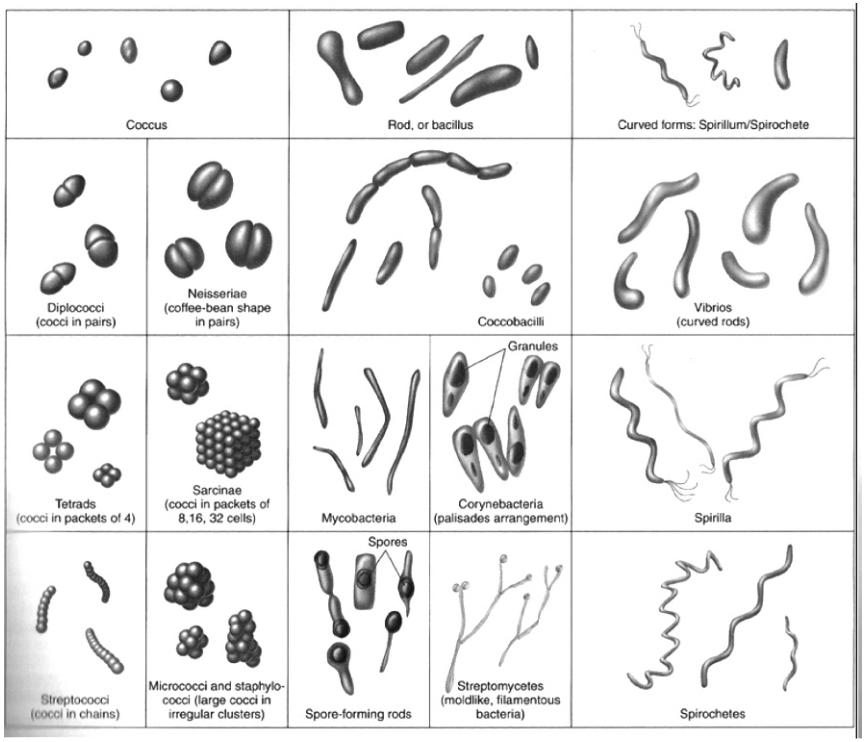


Fig. 13: Bacterial shapes and arrangements (Source: Talaro and Talaro, 2002)

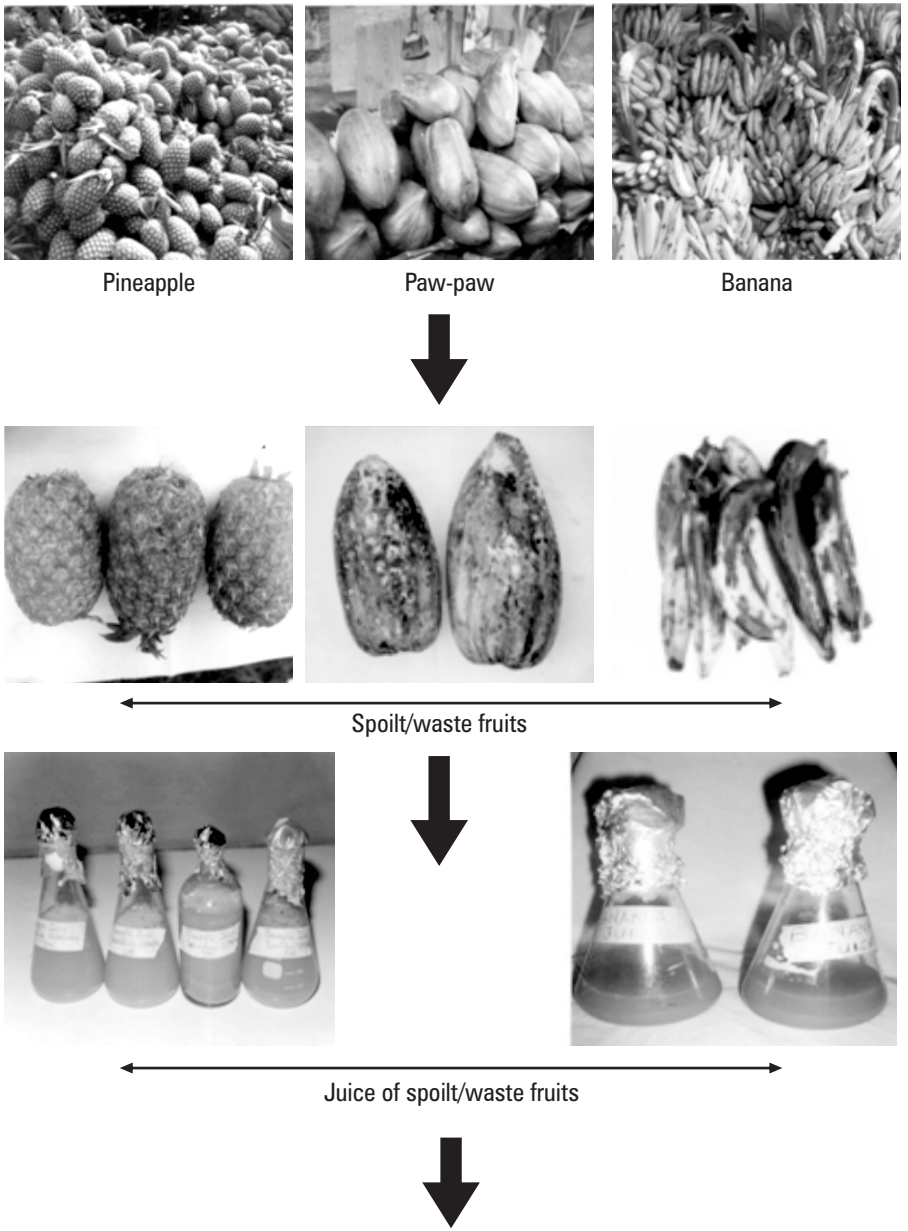
It is rather common for cells of the same species to vary to some extent in shape and size. This phenomenon called pleomorphism is due to individual variations in cell wall structure caused by nutritional or slight hereditary differences.

The cells of bacteria can also be categorized according to arrangement or style of grouping. The main factors influencing the arrangement of a particular cell type are its pattern of cell division and how the cells remain attached afterward. The greatest variety in arrangement occurs in cocci because they can divide in many planes. They can be single, in pairs (diplococcic), in tetrads (groups of four cocci), in irregular or grapelike clusters (both staphylococci and micrococci), or in chains of a few hundreds of cells (streptococci). An even more complex grouping is a cubical packet of 8, 16, or more cells or cocci called sarcina.

Bacilli are less varied in arrangement because they divide only in the transverse plane (perpendicular to the axis). They occur either as single cells, as a pair of cells with their ends attached (diplobacilli), or as a chain of several cells (streptobacilli). Spirilla are occasionally found in short chains, but spirochetes rarely remain attached after division.

Culture media: A nutrient material prepared for the growth of microorganisms in the laboratory is called a culture medium. Some bacteria can grow well in just about any culture medium; others require special media, and still others cannot grow on any nonliving medium yet developed. The microbes that grow and multiply in or on a culture medium are referred to as a culture. Suppose we want to grow a culture of a certain microorganism, perhaps the microbes from a particular soil sample. What criteria must the culture medium meet?

Flow process of bio-ethanol production



Our studies therefore investigated microorganisms associated with the natural fermentation of waste fruit juices and therefore in the production of ethanol (Obire *et al*., 2008h). Samples of waste fruit juices of pineapple (*Ananas comosus*), banana (*Musa acuminata*) and pawpaw (*Carica papaya L.*) were collected for the analyses using standard microbiological techniques at regular intervals. While the fermented fruit juices of the waste fruits were also collected at regular intervals and distilled into ethyl alcohol (ethanol) using the Soxhlet distillation unit. After twenty-two days of fermentation, the total mean density of fungal count of the juice of waste pineapple, banana and pawpaw fruit was 6.01×10^7 sfu/ml, 1.17×10^6 sfu/ml, and 6.84×10^7sfu/ml respectively. The species of fungi and frequency of isolation (%) from the waste fruit juices is shown in Figure below. Our results showed that *Saccharomyces cerevisiae* had the highest frequency of occurrence in all the fruit juices, recording 42.55%, 41.86% and 40.9% in pineapple, banana and pawpaw juice respectively. *The total volume of ethanol (ethyl alcohol) distilled from 800ml of the waste fruit juice of* pineapple, banana, and paw-paw after 22 days of *fermentation* was 572ml, 585ml, and 668ml respectively. This showed that the fermented pawpaw waste fruit produced the greatest volume of ethanol distillate. This is not unconnected with the fact that, in addition to *Saccharomyces cerevisiae, there are other yeast species such as Debaromyces* sp, *Pichia* sp, and *Saccharomyces lactis* occurring in the paw-paw fruit juice which facilitated the fermentation and transformation of the sugars present to ethanol.

1. It must contain the right nutrients for the specific microorganism we want to grow
2. It should also contain sufficient moisture
3. A properly adjusted pH and a
4. Suitable level of oxygen, perhaps none at all
5. The medium must initially be sterile--- That is it must initially contain no living microorganisms----So that the culture will contain only the microbes (and their offspring) we add to the medium
6. Finally, the growing culture should be incubated at the proper temperature

A wide variety of media are available for the growth of microorganisms in the laboratory. Most of these media, which are available from commercial sources, have pre-mixed components and require only the addition of water and sterilization.

When it is desirable to grow bacteria on a solid medium, a solidifying agent such as agar is added to the medium. Table 5 summarizes the purposes of the main types of culture media.

General considerations in microbial control

The methods of microbial control belong to the general category of decontamination procedures, in that they destroy or remove contaminants. Contaminants are microbes present at a given place and time that are undesirable or unwanted. A flowchart which summarizes the major applications and aims in microbial control is shown in Fig. 14.

Table 5: Main Types Culture Media

Types	Purpose
Chemically defined	Growth of chemoautotrophs and photoautotrophs, and for microbiological assays
Complex	Growth of most chemoheterotrophs
Reducing	Growth of obligate anaerobes
Selective	Suppression of unwanted microbes, encouraging desired microbes
Differential	Distinguishing colonies of desired microbes from others
Enrichment	Similar to selective media but designed to increase numbers of desired microbes to detectable levels

Source: Tortora *et al.*, (1998)

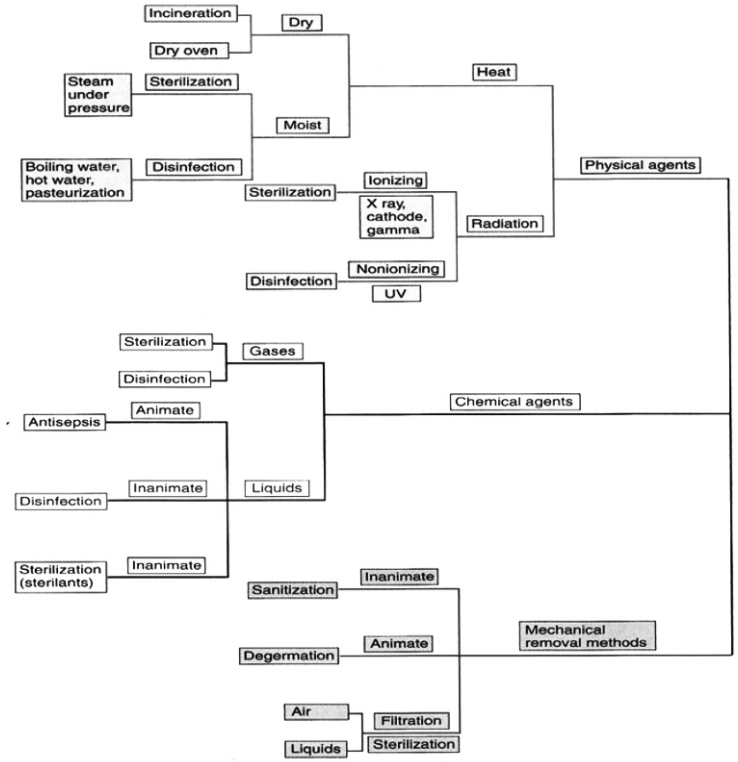


Fig. 14: Flowchart of microbial control measures (Source: Talaro and Talaro, 2002)

portion of the dissolved organic substrates is mineralized and another portion is converted to microbial biomass. In the advanced stage of aeration, most of the microbial biomass becomes associated with flocs that can be removed from suspension by settling.

Combined with primary settling, the activated sludge process reduces the **BOD** of the effluent to 10% to 15% of that of the raw sewage. The treatment also drastically reduces the number of intestinal pathogens in the sewage. This reduction is the result of the combined effects of competition, adsorption, predation, and settling. Predation by ciliates, rotifers, and *Bdellovibrio* is probably indiscriminate and affects pathogens and nonpathogenic heterotrophs. Nonpathogenic heterotrophs reproduce to compensate for their predatory removal while the pathogens are continuously decimated. Settling of the flocs removes additional pathogens and the number of *Salmonella*. *Shigella* and *Escherichia coli* typically are 90% to 99% lower in the effluent of the activated sludge treatment process than in the incoming raw sewage. Enteroviruses are removed to a similar degree. The principal removal mechanism appears to be adsorption of the virus particles onto the settling sewage sludge floc (Obire *et al*., 2008f).

Production of Biofuel from Waste Fruits

Tropical fruits indigenous to Nigeria such as pineapple (*Ananas comosus L. Merr.*), banana (*Musa acuminata Colla*) and pawpaw (*Carica papaya L.*) have been fermented for wine production. Interestingly, these fruits are very much abundant in Nigeria. The problem is lack of distribution of the available fruits after harvesting, lack of processing methods, preservation, and storage facilities after low sales. Hence, large quantities of these naturally fermenting fruits are discarded as "spoilt or rotten", as observed in our various markets. This has caused great economic loss to the trader as well as inestimable food wastage.

The trickling filter system is a simple and relatively inexpensive film-flow type of aerobic sewage treatment method. The porous material of the filter bed in sewage treatment becomes coated with a dense, slimly bacterial growth, principally composed of *Zooglea ramigera* and similar slime-forming bacteria. The slime matrix thus generated accommodates a heterogeneous microbial community, including bacteria, fungi, protozoa, nematodes, and rotifers. The most frequently found bacteria are *Beggiatoa alba, Sphaerotilus natans*, *Achromobacter spp, Alcaligenes spp., Flavobacterium spp*., *Pseudomonas* spp., and *Zooglea* spp. This microbial community absorbs and mineralizes dissolved organic nutrients in the sewage, thereby reducing the **BOD** of the effluent.

The **activated sludge process**: During the holding period in the **activated sludge process**, growth of heterotrophic microorganisms occurs. The heterotrogeneous nature of the organic substrates in sewage allows the development of diverse heterotrophic bacterial populations, including gram-negative rods, predominantly *Escherichia, Enterobacter, Pseudomonas, Achromobacter*, *Flavobacterium, and Zooglea spp.; other bacteria, including Micrococcus, Arthrobacter,* various *Coryneforms* and *Mycobacteria, Sphaerotilus,* and other large filamentous bacteria; and low numbers of filamentous fungi, yeasts, and protozoa, mainly ciliates.

The protozoa are important predators of the bacteria, along with rotifers. The bacteria in the activated sludge tank occur in free suspension and as aggregates or flocs. The flocs are composed of microbial biomass held together by bacterial slimes, produced by *Zooglea ramigera* and similar organisms. Most of the ciliate protozoa, such as *Vorticella*, are of the attached filter-feeding type and adhere to the flocs, while feeding predominantly on the suspended bacteria. As a consequence of extensive microbial metabolism of the organic compounds in sewage, a significant

Most decontamination methods employ either physical agents, such as heat or radiation, or chemical agents such as disinfectants and antiseptics. This separation is convenient, even though the categories overlap in some cases; for instance, radiation can cause damaging chemicals to form, or chemicals can generate heat.

Table 6: Quality control surveillance procedures of commonly used microbiology equipment

Equipment	Procedure	Schedule	Tolerant limits
Refrigerators	Recording of temperature	Daily	2°C to 8°C
Freezers	Recording of temperature	Daily	-8°C to -20°C -60 °C to -75°C
Incubators	Recording of temperature	Daily	35.5°C ± 1°C
Water baths	Recording of temperature	Daily	36°C to 38°C 55°C to 57°C
Autoclaves	Test with spore stripe *(Bacillus stearothermoplilus)*	At least weekly	No growth of spores in subcultures indicate sterile run
Anaerobic jars	Methylene blue indicator stripe	With each use	Conversion of stripe from blue to white indicate low O_2 tension
Serology rotators	Count revolution per minute	With each use	180rpm ± 10rpm
Centrifuges	Check revolutions with tachometer	Monthly	Within 5% of dial indicator setting
Safety hoods	Measure air velocity across face opening	Semiannually or quarterly	50 feet airflow per minute ± 5 feet per minute

Source: Arora (2004)

What is Environmental pollution?

Environmental pollution is the introduction of any substance which could be in form of liquid, solid or gas into the atmospheric, aquatic or terrestrial environment. Such a substance is called a pollutant.

Consequences of Environmental Pollution

On the aquatic and terrestrial environments, pollution causes extensive damages ranging from the destruction of plants and animals to biomagnifications of the toxic components of the pollutant, conversion of arable land to barren land and the destruction of the aesthetic quality of both aquatic and terrestrial environments. Direct discharge of untreated or raw municipal and or industrial effluent into rivers and lakes contribute to microbial pollution, have negative effect such as nutrient enrichment, deterioration of the water quality, destruction of spawning grounds for aquatic and marine life and general fish kill and drinking use (Ntiba *et al*., 2001). Other environmental consequences of pollution include ground water contamination (Odu, 1972; Atlas *et al.,* 1976).

In addition to its effects on visible plants and animals, pollutants impacts microbial populations (Ahearn and Meyers, 1976). The effect of a pollutant on microbial populations depends upon the chemical composition of the pollutant and on the species of microorganisms present. Populations of some microbes increase (enrichment effect); typically, such microbes are able to utilize the pollutant as nutrients. On the other hand, some microbial populations decrease (suppressive effect), show a neutral response to the pollutant (static effect) or are completely eliminated (microcidal effect). As with crude oil, in heavily polluted areas, there are immediate detrimental effects on plant and animal life, including agriculture (Steinhart and Steinhart, 1972; Rowell). The scale of

reactor by the biodegradation of the organic matter. Compost is a good soil conditioner/fertilizer and supplies some plant nutrients. The composting process prevents groundwater contamination as opposed to landfill operations.

Treatment of Liquid Wastes (Sewage)

The treatment of liquid wastes (domestic sewage) is aimed at removing organic matter, human pathogens, and toxic chemicals so that the discharged sewage effluent will not cause unacceptable deterioration of environmental quality. Sewage is subjected to different treatments, depending on the quality of the effluent deemed necessary to be achieved to permit the maintenance of acceptable water quality. **Primary sewage treatments** rely on physical methods while secondary sewage treatment rely on microbial biodegradation to further reduce the concentration of organic compounds in the effluent; and **tertiary treatments** use additional methods (often chemical treatments) to remove inorganic compounds and pathogenic microorganisms.

Naturally, in the self-purification inherent capability of natural waters, populations of enteric and other pathogens that enter aquatic ecosystems are maintained at low levels or eliminated by the pressures of competition and predation of the aquatic populations. The secondary sewage treatment step that relies on microbial activity may be aerobic or anaerobic. Some newly developed wastewater treatment systems are designed so that microorganisms can remove nitrates from the wastewater by denitrification (an anaerobic respiratory process) and thereby prevent eutrophication due to algal blooms when the wastewater is released into the wastewater to favor the growth of denitrifying methanol – utilizing bacteria; the methanol is subsequently removed using activated charcoal or an aerobic bioreactor treatment.

hydrogen peroxide, which rapidly decompose to liberate O_2 and water, or pumping air into soil, **bioventing**. Off-site processes may be performed using a **bioreactor,** a large tank designed to accelerate microbial processes. Both nutrients and oxygen may be added to facilitate microbial growth and metabolism. Presently, a wide range of microorganisms namely: bacteria and fungi (yeasts and moulds) are widely used in the process of environmental clean-up.

Whether *in situ* or *ex situ*, of particular concern is the carrying capacity of the microbial population that is the maximum toxic load that the environmental factors and microbial population is able to withstand. The main advantages of bioremediation over conventional treatment methods include: low cost, high efficiency, minimization of chemical and biological sludge, selectivity to specific metals, regeneration of biosorbent and the possibility of metal recovery.

Treatment of solid waste - Composting

Composting is a microbial process that converts organic waste materials into a stable, sanitary, humus-like product. Reduced in bulk, it can be used for soil improvement. The organic portion of solid waste can be biodegraded by composting, the process by which solid heterogeneous organic matter is degraded by aerobic, mesophilic and thermophilic microorganisms.

Thermophilic microorganisms prominent in the composting process include the bacteria *Bacillus stearothermophilus*, *Thermomonospora* spp; *Thermoactinomyces* spp; and *Clostridium thermocellum* and the fungi *Geotrichum candidum*, *Aspergillus fumigatus*, *Mucor pusillus*, *Chaetomium thermophile*, *Thermoascus auranticus*, and *Torula thermophila*. In the continuous-reactor composting process, the reactor is maintained continuously at thermophilic temperatures by using the heat produced within the

pollution depends on the quantity of oil and the damage done to the environment (Colwell and Walker, 1977).

In polluted water, microorganisms contribute to a chain of events that drastically alters the ecology of the environment. When phosphates accumulate in water, algae bloom. The algae supply nutrients to microorganisms which multiply rapidly and use up the available oxygen (dissolved oxygen). Soon other protozoa, small fish, crustaceans, and plants die and accumulate on the bottom. Anaerobic bacteria such as *Desulfovibrio* and *Clostridium* then thrive in the mud and produce gases that give the water a stench reminiscent of rotten eggs. The organisms in the mud may also pose a hazard to health (Alcamo, 2001).

What is Environmental Microbiology?

Environmental microbiology is the scientific study of the activities such as physiology, metabolism, composition and distribution of microbial communities in the environment. The environment in this case means the soil, water, air, and sediments covering the planet and can also include the plants and animals that inhabit these areas. An **ecosystem (soil, air, or water)** comprises all the organisms in a given area together with the surrounding abiotic (non-living) and biotic (living) factors. The organisms within an ecosystem live in communities. An ecological **community** consists of all the kinds of organisms that are present in a given environment. Communities are made up of *populations*, groups of organisms of the same species. In general, communities composed of many or diverse populations of organisms are more stable than those composed of only a few populations that is, of only a few different species. The various species create a system of "checks and balances" such that the numbers of each species remain relatively constant.

At the 1995 annual meeting of the North American Benthological Society, researchers discussed new techniques for determining the integrity and stability of ecological environments. Many of these researchers use bacterial diversity as a measure of the health of an ecosystem.

Bacteria and fungi regulate most ecosystem dynamics---- such as the biogeochemical cycles---- by producing materials that are necessary for the growth of other organisms in a specific environment. Therefore if a habitat is found to contain a healthy and diverse community of microbes, researchers can say that it is, in fact, a healthy environment. If there is instead a lack of microbial diversity, then nutrients and other abiotic factors are insufficient, and the ecosystem can be said to have limited functions. In such cases, waste products are not broken down or recycled efficiently. Such an environment is unhealthy (polluted).

A relatively uncontaminated ecosystem has the highest diversity of microorganisms and could be home to a number of organisms found nowhere else and to many that are rare. In the same area, microbial species diversity and productivity are significantly higher than in contaminated ecosystems. Because the uncontaminated ecosystem has more oxygen gas and water than does the contaminated ecosystem, decomposition and recycling by microorganisms produces more organic matter. With more nutrients available, microbial populations are larger and more diverse there. Bottom line: The more ecologists understand about ecosystem dynamics, the more we come to appreciate the essential role of microbes in our environment.

Fungi and bacteria are important agents in biogeochemical cycling and mineralization. Any factor therefore whose presence in the environment affects these microbial activities, also adversely

organisms already present, possibly adding nutrients to encourage their growth.

Many factors influence the degradation rate of a pollutant. As a general rule, any practice that favors multiplication of microorganisms will increase the rate of biodegradation. Thus, providing adequate nutrients, maintaining the pH near neutrality, raising the temperature and providing an optimal amount of moisture and oxygen are likely to promote pollutant degradation.

Bioremediation can occur on its own nature (natural attenuation or intrinsic bioremediation) or can be spurred by addition of fertilizers for the enhancement of bioavailability within the medium (biostimulation). Some of the various techniques that can enhance the natural process of bioremediation are biostimulation, bioaugumentation, bioleaching, bioreactors, bioventing, land farming and composting.

There are two general bioremediation strategies- bio-stimulation and bioaugumentation. **Bio-stimulation** enhances growth of indigenous microbes in a contaminated site by providing additional nutrients such as nitrogen and phosphorous. When fertilizer is applied to an oil spill, microbial growth is stimulated, leading to at least a threefold increase in the speed of degradation by the bacteria. **Bioaugumentation** exploits the activities of microorganisms that are added to the contaminated material, complementing the resident population.

Bioremediation may be done either *in situ* (on site of contamination) or *ex situ* (off-site). *In situ* bioremediation generally relies on bio-stimulation and is less disruptive. Among the most important microbes in bioremediation are species of *Pseudomonas* and *Bacillus* and various "toxin-eating" fungi. Oxygen (O_2) can be added to contaminated ground water and soil either by injecting

MICROBES AS REMEDIATORS OF POLLUTED ENVIRONMENTS

Pollutants from domestic, agricultural and industrial wastes have often been dumped into the environment as a matter of convenience. Fortunately, most organic compounds of natural origin can be degraded by one or more species of indigenous soil or aquatic microorganisms. As oil spills dramatically demonstrate, however, some natural materials can cause devastating effects before they are degraded. Synthetic compounds are more likely to be degradable if they have a chemical composition similar to that of naturally occurring substance (Obire and Eli, 2014).

Methods for restoring oil-polluted sites vary from complete removal of the affected soil to doing nothing at all and "letting nature take its course" (McGill and Nyborg, 1975). The remediation of polluted or contaminated soils could be achieved by physical, chemical or biological methods. However, owing to the problems associated with physical, mechanical and chemical methods, there is a need for a safer and less expensive approach to remediation of polluted environments which make bioremediation more attractive.

Bioremediation is a means of cleaning up contaminated environments by exploiting the diverse metabolic abilities of microorganisms to convert contaminants to harmless products by mineralization, generation of carbon (IV) oxide and water, or by conversion into microbial biomass and useful compounds (Obire and Nwaubeta, 2001; Obire and Akinde, 2006).

Bioremediation is a necessary and cost-effective means of removing certain environmental pollutants that adversely affect human health or environmental quality. Bioremediation may involve the use of specific organisms introduced into the polluted environment or, more commonly; it may take advantage of the

affects plant growth as well as detoxification of organic pollutants (Jaja and Obire, 2015).

Microorganisms readily reveal the effects of a pollutant in their habitat because they possess large surface area to volume ratio inherent in their minute size and are in direct contact with their environment. They are useful in predicting the impact of a particular stress on the environment by their ability to respond to these adverse conditions through a shift in their numbers (Mckinley *et al.*, 1982; Clark and Patrick, 1987; Obire, 1988) and the elimination of some certain types. Microbes are particularly important because of their unique role of trophic dynamics in aquatic and terrestrial ecosystems. Microbes are easy to standardize for toxicity in comparison to many other organisms. They provide a source of enriched particulate organic carbon by utilization of both dissolved and particulate organic carbon.

MICROORGANISMS AS INDICATORS OF WATER QUALITY

What is an indicator Microorganism?

The term *indicator microorganism* refers to a kind of microorganism whose presence in water is evidence that the water is polluted with faecal material from humans or other warm-blooded animals. A major type of bacteria in polluted water is coliform bacteria, a group of Gram-negative non-spore forming bacilli usually found in the human intestine. This kind of pollution means that any pathogenic microorganisms that occur in the intestinal tract of these animals may also be present.

Some of the important characteristics of an indicator organism are:

1. It is present in polluted water and absent from unpolluted (potable) water.

2. It is present in water when pathogens are present.
3. The quantity of indicator organism correlates with the amount of pollution.
4. It survives better and longer than the pathogens.
5. It has uniform and stable properties.
6. It is generally harmless to humans and other animals.
7. It is present in greater numbers than those of pathogens (detection relatively easy).
8. It is easily detected by standard laboratory techniques.

Escherichia coli most closely satisfy the requirements of an ideal indicator of pollution and is the organism used. Other bacteria have been suggested and sometimes used as pollution indicators. These include *Streptococcus faecalis and Clostridium perfringens;* both are normal inhabitants of the large intestine of humans and other animals.

E. coli is a normal inhabitant of the intestinal tract of humans and other warm-blooded animals and is thus regarded as a ***faecal type*** of coliform. Other members of the coliform group, for example, *Enterobacter aerogenes*, are widely distributed in nature and found in soil, water, grain, and also the intestinal tract of humans and other animals and are regarded as ***non-faecal*** coliforms. Non-coliform bacteria also common in polluted water include *Streptococcus*, *Proteus* and *Pseudomonas* species. The coliforms have several characteristics in common with species of the genera *Salmonella and Shigella,* all of which are pathogenic.

showed that there is the necessity of oilfield wastewater treatment prior to discharge into the recipient water body or soil (Obire and Amusan, 2003; Obire *et al*., 2008a; Jaja and Obire, 2015a; Jaja and Obire, 2015b; Wemedo *et al*., 2012).

Surfactants are synthetic organic chemicals used in high volumes in detergents, personal care and household cleaning products. Surfactant detergents are in widespread usage worldwide. There are two types of detergents with different characteristics: phosphate detergents and surfactant detergents. Detergents that contains phosphate are highly caustic, and surfactant detergents are very toxic (Lenntech, 2008) and the greatest impact is on the aquatic environment (NEST, 1991). In Nigeria, large volumes of industrial surfactant detergents are discharge on daily basis from petroleum companies into the environment. Detergents have poisonous effects in all types of aquatic life. All detergents destroy the external mucus layer that protects the fish from bacteria and parasites; plus they can cause severe damage to gills. Most fish will die when detergent concentrations approach 15 parts per million (15ppm) while concentration as low as 5ppm will kill fish eggs. Surfactant detergents are implicated in increasing the breeding inability of aquatic organisms. Detergents also add another problem to aquatic life by lowering the surface tension of the water.

Our studies using the median lethal concentration (LC_{50}) to compute the toxicities of different concentrations of different detergents (powdered and liquid) and of chlorine (washing bleach) on aquatic microflora (bacteria and fungi) revealed that the detergents were toxic to the microorganisms. The chlorine (washing bleach) exhibited the highest toxicity strength (Mean LC_{50} = 27.67ppm or mg/l); while evaluation of different groups of microorganisms showed that bacteria were comparatively more sensitive to the toxicants than fungi Obire and Nrior, 2014; Nrior and Obire, 2015).

Our studies on the effect of crude oil pollution and of refined petroleum hydrocarbons on physiochemical and bacteriological characteristics of freshwater streams and on soils in Nigeria showed that; the relative abundance of hydrocarbon utilizing bacteria to other heterotrophic bacteria increased after the pollution as compared to controls. The results implied the microbial community composition and the physiochemical constituents of the stream and soils were altered by crude oil refined petroleum hydrocarbons pollution (Obire and Okudo, 1997; Obire and Nwaubeta, 2001a; Obire and Nwaubeta, 2002). Our results also showed that crude oil and refined petroleum hydrocarbons such as kerosene have toxic effect on microbial populations and growth rate (Wemedo *et al*., 2005). Our results of the effect of different concentrations (0% - control 0.5%, 1%, 3% and 5%) of crude oil on the physicochemical characteristics of soils showed that there was a reduction of total nitrogen in all the polluted soils as compared to the control soil after 18 weeks (Obire *et al*., 2008c).

Impact of Oilfield Wastewater and detergents on Soil and Water

Our studies on the effect of oilfield waste water on the microbial population of a soil in Nigeria showed that there was a numerical effect on the population and diversity of bacteria (Obire and Wemedo, 1996) and that different seasons selectively favour the growth of certain microbial types, but that seasonal influence was more pronounced on the fungi than on the bacteria (Obire and Wemedo, 2002). The study on the impact of oilfield 'formation water' (oilfield wastewater) on a freshwater stream in Nigeria showed that there was reduction in the biological activities by the "treated" oil field formation water (Obire and Amusan, 2003). The results of physicochemical constituents including heavy metals and total viable bacterial count, total coliform count and coliform types

There also is considerable interest in the development of a routine procedure for the detection of a virus as an indicator of pollution. Like the coliform bacteria, enteric viruses can be carried by human wastes into water (Obire and Lockhart, 2003). Analysis of a water sample for presence of viruses requires more elaborate procedures than those used for isolation of bacteria.

The routine microbiological examination of water to determine its potability is not and should not be based on the isolation and identification of pathogenic microorganisms, for the following reasons:

1. Pathogens are likely to enter a water supply sporadically, and since they may not survive for long periods of time, they could be missed in a sample submitted to the laboratory.
2. If they are present in very small numbers, pathogens are likely to escape detection by laboratory procedures.
3. It takes 24 h or longer to obtain results from a routine laboratory examination for pathogenic microorganisms. By the time pathogens are found, many people would have consumed the water and would be exposed to these pathogenic microbes before action could be taken to correct the situation.

For these reasons, microbiologists have developed water testing procedures that do not rely on the isolation and identification of pathogens. Instead, tests are based upon finding a microorganism whose presence indicates the possibility of the presence of pathogenic microorganisms. The indicator organism serves as an "alarm" system.

MY CONTRIBUTIONS TO THE FIELD OF MICROBIOLOGY THROUGH RESEARCH AND HUMAN RESOURCE DEVELOPMENT

Mr. Vice Chancellor Sir, I have always been fascinated by the activities of microorganisms in the environment. The environment in microbiology is anywhere microorganisms can be found. That anywhere could be the medium may be our body surfaces, orifices, gut, food, drugs, or just anything. Because of the link between the environment and our health, I have had to work with environment pathogens (organisms that are found in the environment and capable of causing disease) and organisms that are capable of cleaning the environment of pollutants and those associated with food either as fermentative or spoilage organisms. The soil has been described as a sink for wastewater (domestics, industrial and otherwise), pathogenic organisms discharged by indiscriminate passage of faeces, urine and clinical wastes. The soil is also home to a wide range of other organisms especially those that assist man in the transformation and degradation of recalcitrant chemicals and pesticides that enter the soil through man's activities. Organisms that increase soil fertility are also resident in soils. I have had to handle a wide range of organisms i.e

1. Microorganisms as indicators of pollution or contamination of soil, water, crops and aquatic life

(Obire and Wemedo, 1996; Obire and Okudo, 1997; Obire and Nwaubeta, 2001; Obire and Nwaubeta, 2002; Obire Nwaubeta and Adue, 2002; Obire and Wemedo, 2002; Obire and Aguda, 2002; Obire, Tamuno and Wemedo, 2003; Obire and Amusan, 2003; Wemedo, Obire and Obara, 2005; Obire, Ogan and Okigbo, 2008; Obire, Anyanwu and Okigbo, 2008; Obire *et al*., 2008a; Obire *et al*., 2008b; Obire, Aguda and Putheti, 2008; Obire and Anyanwu, 2009;

organisms are wiped out in favour of oil utilizers. Since microorganisms play an essential role in biogeochemical cycling, interference with microbial metabolic activities by pollutants in the environment can have far reaching ecological consequences.

Table 13: Impact of various concentrations of crude-oil on soil fungi

Concentration of crude oil (%)				
Control (0%)	**0.5**	**1**	**3**	**5**
Moulds	**Moulds**	**Moulds**	**Moulds**	**Moulds**
Alternaria	*Alternaria*	*Alternaria*	****	****
Aspergillus	*Aspergillus*	*Aspergillus*	*Aspergillus*	*Aspergillus*
Cephalosporium	*Cephalosporium*	*Cephalosporium*	*Cephalosporium*	****
Cladosporium	*Cladosporium*	*Cladosporium*	*Cladosporium*	*Cladosporium*
Fusarium	*Fusarium*	*Fusarium*	*Fusarium*	*Fusarium*
Geotrichum	*Geotrichum*	*Geotrichum*	*Geotrichum*	*Geotrichum*
Mucor	*Mucor*	*Mucor*	*Mucor*	*Mucor*
Penicillium	*Penicillium*	*Penicillium*	*Penicillium*	*Penicillium*
Rhizopus	*Rhizopus*	*Rhizopus*	****	****
Trichoderma•	*Trichoderma*•	*Trichoderma*•	Trichoderma•	****
Yeasts	**Yeasts**	**Yeasts**	**Yeasts**	**Yeasts**
Candida	*Candida*	*Candida*	*Candida*	*Candida*
Rhodotolura	*Rhodotolura*	*Rhodotolura*	*Rhodotolura*	****
Saccharomyces	****	****	****	****
Torulopsis	****	****	****	****

Obire and Anyanwu (2009)

There was an initial reduction in population of fungi after 24 hrs of pollution with crude oil after which there was a continuous increase while fungal population of the control soil was virtually stable throughout the duration of the study. This subsequent increase in population was due to the proliferation of hydrocarbon utilizing fungi but not of the original population (Obire and Anyanwu, 2009). The order of decreasing average counts of fungi in the soils treated with various concentrations of crude oil is 5% > 3% > 1% > 0.5% > 0%. Statistical analysis using analysis of variance (ANOVA) and the randomized complete block design (RCBD) on the counts of the total heterotrophic fungi and the counts of petroleum-utilizing fungi in the control and oil-polluted soils showed that there is a high significant difference between the control and the oil treated soils at $p \leqslant 0.05$ level of significance.

The petroleum-utilizing fungal counts expressed as percentage (%) of the corresponding total fungal counts in the control and oil-polluted soils at various concentrations ranged from 4.7 to 12.5% for 0% oil treatment, 10.7 to 35% for 0.5% oil treatment, 21.2 to 53.7% for 1% oil treatment, 15.6 to 58.8% for 3% oil treatment, and 11.6 to 48% for 5% oil treatment. Fungal species belonging to a total of fourteen (14) known fungal genera and one unidentified fungus were isolated from the control and oil treated soils.

The decreasing order of isolation or occurrence of a variety of fungal genera (i.e., fungal diversity) of both heterotrophic fungi and petroleum degrading fungi in the oil treated soils is 0% (control) > 0.5% > 1% > 3% > 5%. This decreasing order of fungal diversity is a direct opposite or reverse of the decreasing order of fungal counts of these same soils. The study revealed that the addition of crude oil to soils resulted in a selective increase in fungal populations and a reduction of species diversity by the total elimination of certain species. This is dangerous to the ecosystem because the beneficial

Obire and Barade, 2009; Obire, **Aguda and Putheti, 2010;** Wemedo and Obire, 2012; Wemedo, Obire and Akani, 2012; Wemedo, Obire and Orubite, 2012; **Akani and** Obire**, 2014;** Obire and Eli, 2014; Obire and Nrior, 2014; Akani and Obire, 2015; Obire and Aguda, 2015; Nrior and Obire, 2015; Jaja and Obire, 2015; Jaja and Obire, 2015; Obire **and Ariyo, 2015;** Nwachukwu, Obire and Ogbonna, 2015; Nwankwo and Obire, 2016; Obire and Nwankwo, 2016; Obianime and Obire, 2017, Obire and Vincent, 2017).

2. Microorganisms as remediators of contaminated soils and water

(Ekundayo and Obire, 1987; Obire, 1988; Obire, 1990; Obire, 1993; Wemedo and Obire, 1998; Obire and Nwaubeta, 2001; Obire and Nwaubeta, 2001; Obire and Akinde, 2004; Obire and Akinde 2006; Akinde and Obire, 2008; Obire, Anyanwu and Okigbo, 2008; Obire and Ojim, 2008; Obire and Putheti, 2009; **Akinde, Iwuozor and** Obire, **2012;** Amaku and Obire, 2014; Amaku and Obire, 2015; Obire and Ariyo, 2016; Obianime and Obire, 2017, Obire and Vincent, 2017).

3. Microorganisms and their activities in water and sewage

(Obire and Aguda, 2002; Obire and **Lockhart,** 2003; Obire, Tamuno and Wemedo, 2005; Obire *et al*., 2008a; Obire *et al*., 2008b; Obire, Aguda and Putheti, 2008; Obire, Barade and Putheti, 2009; Obire, Putheti and Igoni, 2009; *Akinde and* Obire, *2011;* Obire and Alali, 2015; Onwusah, Amafina, and Obire, 2015; Obire and Osigwe, 2016; Obianime and Obire, 2017).

4. Environmental pathogens & microbes in soil fertility

(Obire *et al*., 2002; Obire and Aguda, 2002; Obire and Akinde, 2005; Obire and Ojim, 2008; Obire*, Nwankwo and Putheti, 2009;* Obire and Abuba, 2010; Obire**, Putheti and Otomba, 2010;** Obire and Amafina, 2014; **Ikeokwu, and** Obire, **2016; Otuokwun and** Obire, **2016).**

5. Spoilage and food associated microorganisms

(Obire *et al*., 2008; Obire, 2005; Obire, *et al*., 2008c; Okigbo and Obire, 2009; Obire **and Minimah,** 2013; Obire and Berembo, 2014; Obire and Amadi, 2015; Obire **and Hakam, 2015;** Obire, Wali and Azaiki, 2016; Obire, Wekhe and Azaiki, 2016).

6. Resistance and susceptibility of microorganisms to antimicrobial agents

(Obire, 2003; Obire, Davis and Putheti, 2009; Obire, Gbarawin and Puteti, 2009; Obire, Putheti and Nzor, 2009; *Won the Best Article Award,* Obire and Amadi, 2015; Obire and Dollah, 2017; Obire and Obianime, 2017).

7. Microorganisms and alcoholic fermentation and biofuel production

(Obire, 2005; Obire, *et al*., 2008c; Okigbo and Obire, 2009).

8. Edible microorganisms

(Obire and Amadi, 2013).

Mr. Vice Chancellor Sir, My contribution towards man-power or human resource development which is also part of my primary responsibilities in the university is the successful supervision of both undergraduate and post graduate students of which I was the major or chief supervisor.

Plate 9: Crude oil spills in Nigeria (Source: https://www.google.com.ng. Accessed, 2018)

Our studies of the effect of the addition of various concentrations (0% - control, 0.5%, 1%, 3% and 5%) of crude oil (Brass blend, black colour 'API 41.7 – 43.0') on the fungal populations of soils Obire and Anyanwu (2009) revealed that total heterotrophic fungal counts of soils ranged from 2.6×10^4 to 1.43×10^4cfu/g soil while counts of petroleum-utilizing fungi ranged from 2.0×10^2 to 1.02×10^4cfu/g soil with the average total counts being lowest in the control soil.

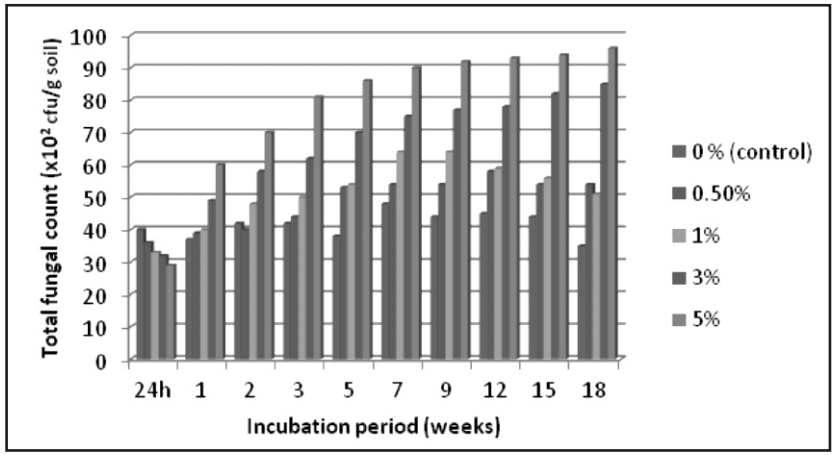


Figure 26: Impact of various concentrations of crude-oil on fungal populations of soil
Source: Obire and Anyanwu (2009)

Effect of oil pollution on environment, vegetation, fisheries and wildlife in Nigeria

Plate 9: Crude oil spills in Nigeria (Source: https://www.google.com.ng. Accessed, 2018)

I have supervised over one hundred undergraduate students since my appointment in this university. At the postgraduate level and of special mention amongst my Masters (***M.Sc***) **degree students are** Mr. **Wemedo** Samuel Amadi, Miss **Nwaubeta** Ogochukwu, Mr. **Tamuno** Deinma Charles, Mr. **Ogan** Abiye (Senior Lecturer), Mr. **Akinde** Sunday Babatunde, Mr. **Dollah** Samuel, Miss **Anyanwu** Elizabeth Chianakwalam, Mr. **Ukaji** Damian Chima, Mr. **Barade** Wisdom Ndagborme, Mr. **Aguda** Monday, Mr. **Dick**, Asiton-A Asifamabia, Mrs. **Oyibo** Ntongha, Mr. **Nrior**, Renner Renner, Miss **Nwogu** Chinelo Peace, Miss **Nwankwo** Christiana Chika, Miss **Nwosu** Onyinyechi Rosemary, and Mr. **Ogbonna** Solomon Ikechi.

My Doctoral (***Ph.D***) **degree students are** Dr. **Wemedo** Samuel Amadi (Now Associate Professor and immediate past Acting Head of Department of Microbiology, Rivers State University), Dr. **Akinde** Sunday Babatunde (Now Associate Professor and Acting Head of Department of Biological Sciences, Osun State University, Dr. **Dollah** Samuel Azubuike (Proprietor of his group of schools), Dr. **Barade** Wisdom Ndagborme (Senior Lecturer, was Head of Department of Science Laboratory Technology in Saro-Wiwa Polytechnic, Bori and he is now the Director, Industrial Services Centre), Dr. **Nwachukwu, Michael Ikechukwu** (Now Associate Professor and Acting Head of Department of Microbiology, Imo State University), **Dr. (Mrs) Jaja, Emylia Tamunodiari (Now** Associate Professor and Associate **Dean, students' affairs in RSU and ASUU Zonal Chairperson), Dr. (Mrs) Amaku** Ebele Grace (Senior Lecturer), **Dr. (Mrs) Akani, Nedie Patience** (Senior Lecturer and Postgraduate Co-ordinator of Microbiology Department), **and Dr. (Mrs) Ariyo**, Adenike Bosede (Senior Lecturer).

Professor Teddy Adias (present Deputy Vice Chancellor, Federal University, Otuoke), Professor Osaro Erhabor, Dr Moslen Miebaka and Dr Obioma Azuonwu were also some my undergraduate students just to mention but a few. Many of my students are consultants with NAFDAC, UNICEF, multinational companies and other foreign organizations at home and abroad. A lot of my students are also in the Breweries, Pharmaceutical and Food processing companies, Armed forces, Secret Service, Prison service, etc.

I will like to inform this distinguished audience that many of the researches in the various aspects of my contribution to knowledge do over-lap. However, I will try as much as possible for the purpose of this inaugural lecture, to limit my coverage to Microbes as Indicators and Remediators of Polluted Environments.

Vice Chancellor Sir, I have earlier mentioned that, Microorganisms act as early alarm system to warn us of impending danger.

Obire and her colleagues used techniques of microbiology to elucidate more about the diversity of microbial communities that inhabit the soils (Obire and Wemedo,1996; Obire and Anyanwu, 2009), fish ponds (Obire and Obianime, 2017; Obianime and Obire, 2017; Obire and Vincent, 2017), creeks (Obire *et al*., 2008b; Obire *et al*., 2008g), streams (Obire *et al*., 2008e), rivers (Obire *et al*., 2008a; Obire *et al*., 2008e; Obire *et al*., 2009a), and the Atlantic Ocean (Akinde and Obire, 2011; Akinde *et al*., 2012).

Our studies determined for example, the distribution and abundance of bacteria and fungi in these ecosystems and the distribution and abundance of viruses in creek waters and domestic sewage (Obire and Lockhart, 2003).

consequences of pollution include ground water contamination (Odu, 1972; Atlas *et al.,* 1976).

Crude oil is not the only pollutant of the environment from petroleum exploration and productive activities. "Produced water (also called formation water, oilfield brine, oilfield wastewater or connate water) is the water that occurs in association with oil and gas in reservoir rocks. When oil is pumped out of the ground, a mixture of oil, gas and water emerges. (Obire and Amusan, 2003). It is by far the largest volume of by-product or waste stream associated with oil and gas production and is one of the major pollutants of both terrestrial and aquatic environments (Obire and Wemedo, 1996).

The numerous inorganic and organic constituents including several potentially toxic metals and radionuclides dissolved in formation water can be potentially or actually far more hazardous than the crude oil itself. Following treatment – and in some cases without any treatment – much of this wastewater is discharged into pits, rivers and the sea. Experts (Obire and Amusan, 2003 cited by Amnesty International, 2009) have queried the quality of the treatment in some cases.

Produced water constituents have shown the ability of to alter habitat integrity of the natural bodies. Since there is often no petroleum-related sheen associated with spills of these highly saline fluids, they can go unnoticed initially, becoming evident much later when overlying vegetation begins to show signs of stress or dies (Jaja and Obire, 2015b).

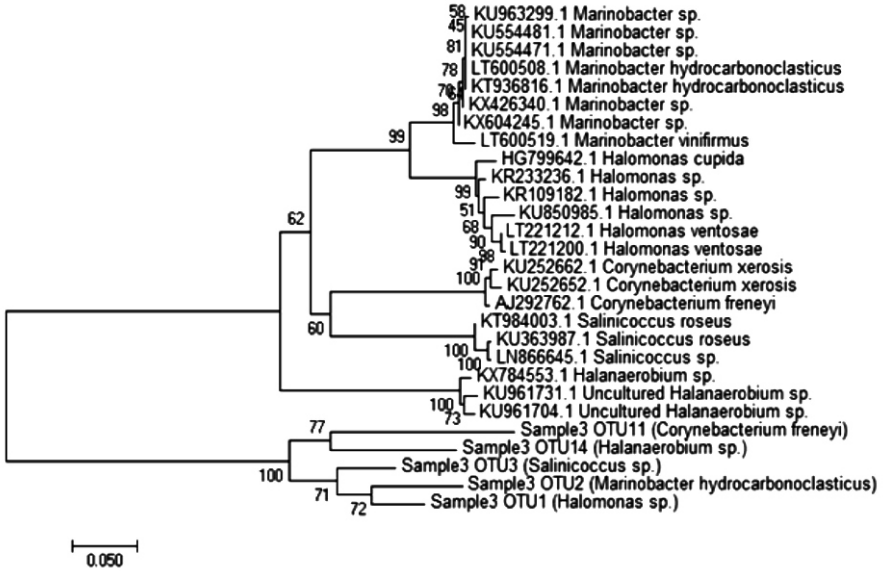


Fig 25: Neighbour joining phylogenetic tree of 5 most abundant OTUs from Rumuokoro abattoir soil sample with their genBank closest relative (Source: Ariyo and Obire, 2018).

Impact of Crude Oil Pollution on Soil and Water Quality

Crude-oil is a naturally occurring complex mixture of hydrocarbon and non-hydrocarbon compounds which at appropriate concentration, possesses a measurable toxicity towards living systems (Oppenheimer, 1980).

Crude oil pollution on the aquatic and terrestrial environments, causes extensive damages ranging from the destruction of plants and animals to biomagnifications of the toxic components of the pollutant, conversion of arable land to barren land, deterioration of the water quality, destruction of spawning grounds for aquatic and marine life and general fish kill and drinking use and the destruction of the aesthetic quality of both aquatic and terrestrial environments. (Ntiba *et al*., 2001). Other environmental

With new molecular techniques, we have been able to make observations on individual microbes and species associations, to judge what microbes are present, and to understand the functions within microbial communities using Operational Taxonomic Units (OTUs) (Obire and Lockhart, 2003; Ariyo and Obire, 2018).

Impact of Municipal Solid Wastes on Soil and Water Quality

Wastes are substances, solutions, mixtures or articles for which no direct use is envisaged but which are transported for reprocessing, dumping, elimination by incineration or other methods of disposal. In time past, wastes and their disposal did not pose a significant problem, for the population was small, the amount of land available for the assimilation of wastes was large and people ate directly from nature so that processing and packaging were little or non-existent.

The dense human and animal population as well as urbanization in developing countries have resulted in the generation of wastes often beyond the local ecosystem's biodegradative threshold. Ecological imbalances have occurred where the natural assimilative capacity has been exceeded (Yao *et al*., 2016). The inability of most administrations to manage the wastes has resulted in serious environmental pollution and epidemic outbreaks of diseases (*Amoah and Kosoe, 2014*).

Public waste receptacles are seen at various points in our cities which retain waste for days and sometimes weeks before they are cleared. Recently, these waste receptacles have disappeared and wastes are dumped on the roadside, forming embarrassing mountainous heaps (UNEP, 2002). Solid waste is a major source of environmental pollution in Nigeria as is visible in the heaps of refuge which litter our towns and cities including highways.

In Nigeria, wastes are not systematically collected and disposed off. The wastes are a mix of different materials such as domestic wastes, clinical/pathological wastes from health institutions and patent medicine stores as well as agricultural and industrial wastes. These commingled wastes are transported in open trucks to open dumpsites often located near residential areas into creeks, rivers, gutters, ditches, unprotected borrow pits or simply in an open unused piece of land (Obire and Nwaubeta, 2002; Obire *et al*., 2002; Obire and Dollah, 2017).

The sequences obtained from this study generated a total of 377 OTUs which have been submitted to GenBank (National Centre for Biotechnology Information, Maryland, USA). The Accession numbers assigned are from KY385910 to KY386287

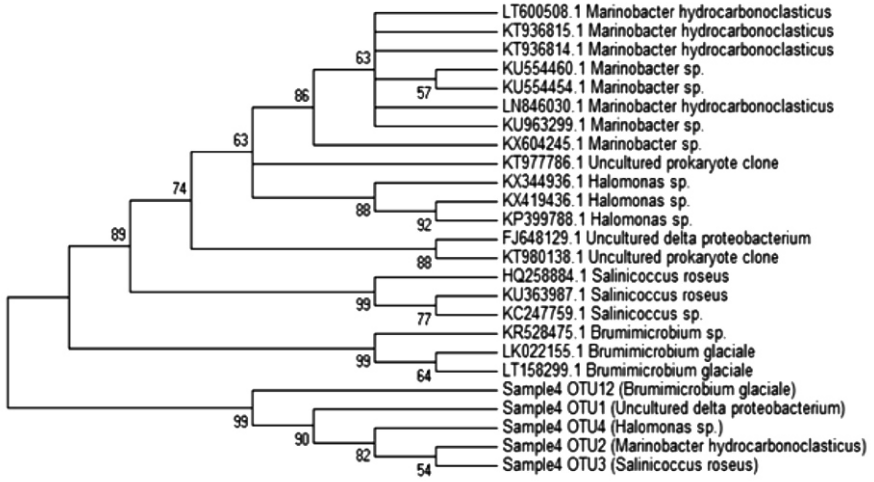


Fig. 24: Neighbour Joining Phylogenetic tree 5 most abundant OTUs from Swale abattoir soil sample with their genBank closest relative (Source: Ariyo and Obire, 2018)

Table 11: Top Kingdom distribution of abattoir soil samples'16s DNA Reads

Top Kingdom Distribution (%) of microorganisms							
Abattoir soil	**Bacteria**	**Archae**	**Protozoa**	**Virus**	**Unknown**	**Fungi**	**Total**
Opolo	98.81	1.06	0.08	0.04	Insigificant	Insigificant	100
Rukpokwu	93.31	4.38	0.17	2.03	0.11	Insigificant	100
Rumuokoro	92.99	0.45	6.36	0.18	0.01	0.01	100
Swale	98.76	0.19	0.72	0.12	Insigificant	0.21	100
Tombia	99.20	0.56	0.23	Insigificant	Insigificant	Insigificant	100
Azikoro	98.99	0.17	0.76	Insigificant	Insigificant	Insigificant	100

Source: Ariyo and Obire (2018)

Table 12: Distribution of metagenomics sequence reads in each sample into Operational Taxonomic Units (OTUs) using UCLUST

Sample	**No of Sequences**	**No of OTUs**	**MostAbundant OTU/Taxon**	**Next Most Abundant OTU/Taxon**
Opolo	25573	425	OTU 1 *Thiobacillus* sp.	OTU 3 Uncultured bacterium clone
Rukpokwu	15841	262	OTU 1 *Methylobacterium* sp.	OTU 2 *Bacillus pumilus*
Rumuokoro	7854	27	OTU 1 *Halomonas Isp.*	OTU 2 *Marinobacter hydrocarbonoclasticus*
Swale	358301	255	OTU 1 Uncultured delta proteobacterium	OTU 2 *Marinobacter hydrocarbonoclasticus*
Tombia	487566	298	OTU 1 Uncultured delta proteobacterium	OTU 2 *Marinobactersp.*
Azikoro	27051	39	OTU 2 *Marinobactersp.*	OTU 1 Uncultured bacterium clone

Source: Ariyo and Obire (2018)

Municipal Solid Waste Dump in Eagle Island Link Road and Environs, Port Harcourt

Plate 1: Solid waste dumps and leachate in Port Harcourt (photos by author, 2017)

The decomposition of the wastes and production of laechate is usually accompanied by pungent odours due to the release of some gases. Leachate is known to have contaminated soil, surface and ground water resources (Obire *et al*., 2002; Obire and Aguda, 2002; Murtaza *et al*., 2017).

Aside from environmental degradation and loss of aesthetics, the waste dumpsite can be a source of pathogenic microorganisms some of which can be resistant to commonly used antibiotics. This could dangerously threaten the health of humans and animals having direct and indirect contact with the dumpsite and its products.

We investigated the bacterial diversity and antibiotic sensitivity of bacteria (for the possible presence of antibiotic resistant organisms) of a municipal solid waste dumpsite soil, leachate and surrounding borehole water fortnightly over a period of 12 months using standard techniques (Obire and Dollah, 2017).

Our results revealed that viable heterotrophic bacterial count ranged from 4.9×10^6 to 1.93×10^7 CFU/g for dumpsite soil, 5.2×10^5 to 1.01×10^6 CFU/g for control soil, 9.0×10^6 to 1.29×10^7 CFU/ml

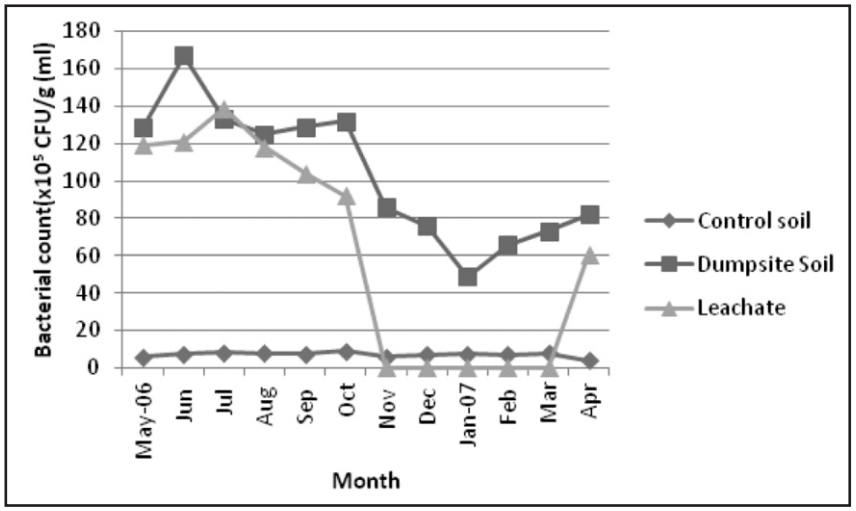


Fig. 15: Heterotrophic bacterial count of waste dump soil and leachate
Source: Obire and Dollah (2017).

Our results revealed that concentration of total PAHs in all the abattoir soil samples were higher than those of the wastewater. Next generation sequencing of DNA of bacterial community in abattoir soils revealed high diversity with a total of 1,208,403 DNA Reads belonging to 1,306 Operational Taxonomic Units (OTUs) from the six soil samples examined. BLAST result showed the most abundant OTUs are *Thiobacillus* sp, *Methylobacterium* sp, *Halomonas* sp, uncultured *Proteobacterium*, *Marinobacter* sp, *Bacillus pumillus*, *Marinobacter hydrocarbonoclasticus* and an uncultured bacterium clone (Figure 24 and Figure 25).

KEY

M - Molecular Ladder

A1 - Opolo Soil

A2 - Rupokwu Soil

A3 - Okoro Soil

A4 - Swale Soil

A5 - Tombia Soil

A6 Azikoro Soil

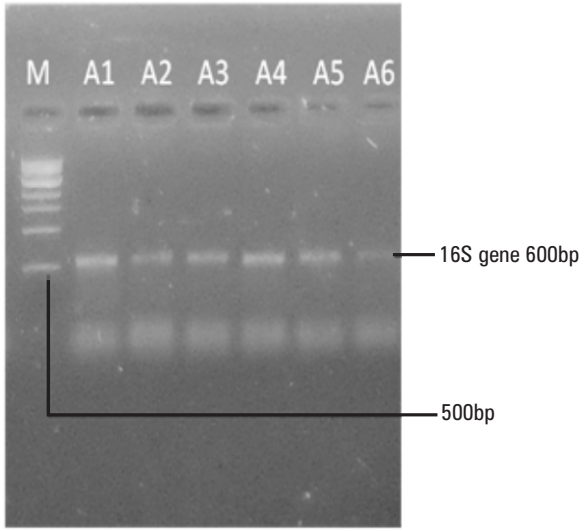


Plate: Agarose gel electrophoresis showing positive 16S gene of DNA extracted from soil smaples

Plate 8: Agarose gel electrophoresis of the amplified 16S gene of DNA fragment extracted from abattoir soils (Lanes 1 – 6 represent the soil samples while Lane M represents the Molecular ladder)
Source: Ariyo and Obire (2018)

Table 10: Bacteria and Fungi Isolated From Abattoir Samples

Abattoir soil	Abattoir Wastewater	Abattoir soil	Abattoir Wastewater
Bacteria		Fungi	
Bacillus	*Alcaligenes*	*Aspergillus*	*Aspergillus*
Enterobacter	*Bacillus*	*Fusarium*	*Fusarium*
Escherichia coli	*Escherichia coli*	*Geotrichum*	*Geotrichum*
Micrococcus	*Micrococcus*	*Mucor*	*Mucor*
Proteus	*Pseudomonas*	*Penicillium*	*Penicillium*
Pseudomonas	*Salmonella*	*Candida*	*Candida*
Salmonella	*Shigella*		
Staphylococcus	*Staphylococcus*		
	Streptococcus		
	Vibrio		

Source: Ariyo and Obire (2016).

The bacteria isolated include *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Escherichia coli*, *Staphylococcus* sp., *Proteus* sp, *Alcaligenes* sp. *Salmonella* sp. and *Enterobacter* sp. Both *Bacillus* and *Pseudomonas* species were the most occurring bacteria while *Micrococcus* sp. and *Alcaligenes* were the least occurring bacteria (Table 10). The five genera of fungi isolated were *Aspergillus*, *Fusarium*, *Geotrichum*, *Mucor* and *Penicillium*.

The abattoir soils had a higher microbial density than the control soil. All the bacterial and fungal isolates with the exception of *Salmonella* and *Mucor* demonstrated hydrocarbon utilizing potentials. This is an indication that the abattoir wastes enhanced the excessive proliferation of hydrocarbon utilizing microorganisms and an imbalance in the soil ecosystem. The presence of bacteria which are indicators of recent faecal contamination as observed in this study are pointers to the dangers associated with the discharge of untreated abattoir wastes and effluent into the soil (Ariyo and Obire, 2016).

for leachate (Figure 15) and 9.5×10^1 to 1.7×10^2 CFU/ml for borehole water (Figure 16). The order of decreasing viable heterotrophic bacterial count was dumpsite soil > leachate > control soil > borehole water. Analysis of Variance revealed significant difference between the samples and the period of study at $p \leqslant 0.05$.

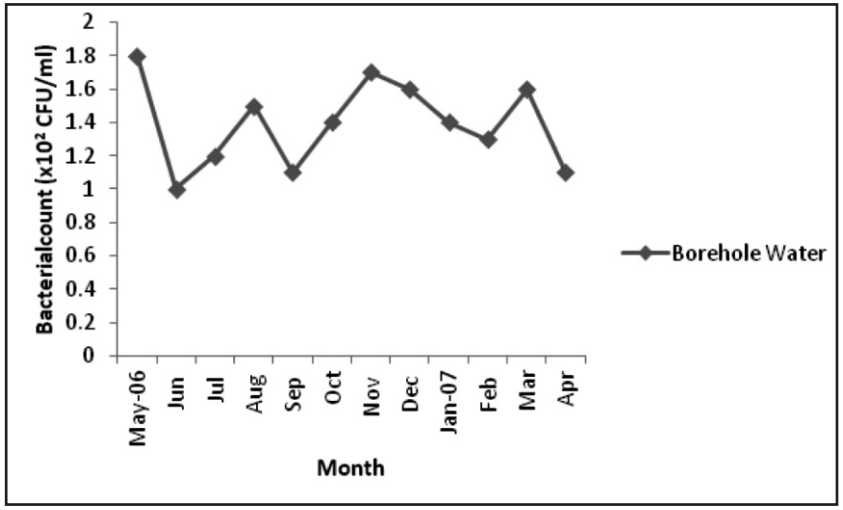


Fig. 16: Mean of heterotrophic bacterial count of borehole water
Source: Obire and Dollah (2017).

The frequency of occurrence (%) of the bacterial isolates in the various samples during the 12 months of investigation is as shown in the Figure 17 below. The bacteria isolated from the dumpsite soil and leachate were *Escherichia coli, Pseudomonas aeruginosa*, *Pseudomonas fluorescence*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Streptococcus pyogenes*, *Bacillus cereus*, and *Proteus vulgaris* while *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Micrococus luteus*, *Bacillus cereus* and *Corynebacterium xerosis* where isolated from the control soil. Bacteria isolated from the borehole water were *E. coli*, *Pseudomonas aeruginosa*, *Micrococcus luteus* and *Proteus vulgaris*.

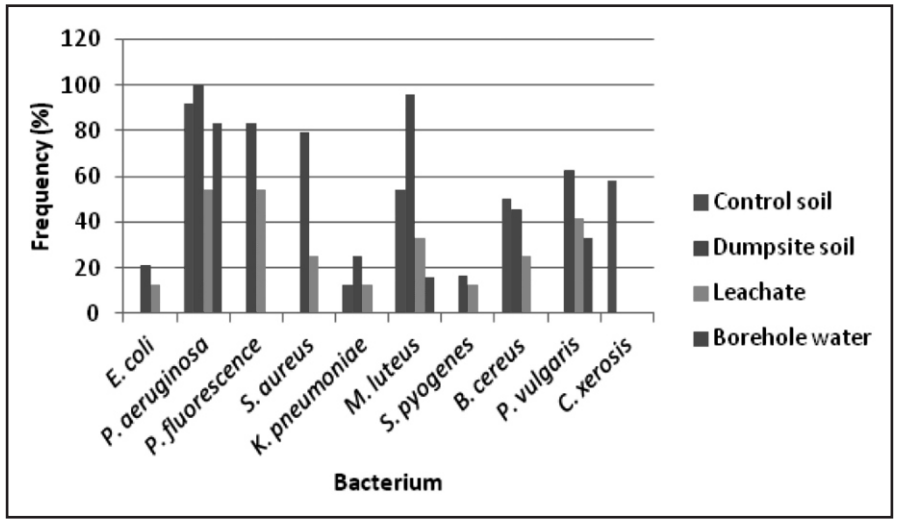


Fig. 17: Frequency of bacteria in soils, leachate and borehole water
Source: Obire and Dollah (2017).

The sensitivity (%) of bacteria isolates from waste dump soil to tested antibiotics is shown in Figure 18.

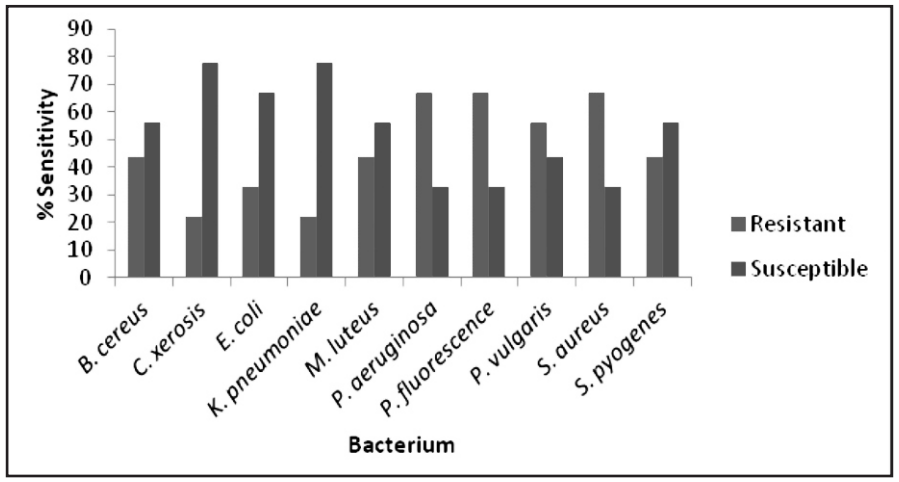


Fig. 18: Sensitivity of bacteria from waste dump soil to tested antibiotics
Source: Obire and Dollah (2017).

Our study assessed the biodiversity of organisms in the abattoir soil through comprehensive metagenomic assay. Bacterial DNA was extracted from soil samples. Pure Extracted DNA amplicons were sequenced using Next Generation Sequencing Procedure and assayed on the Illumina Miseq machine (DNA isolation, sequencing and BLASTing) as to determine the effect of these pollutants on the microbial community of the abattoir soils.

Our results showed that the mean density (population) of total heterotrophic bacteria of the abattoir soil ranged from 1.2×10^7 to 8.0×10^7CFU/g soil. While the mean density of total fungi of the abattoir soils ranged from 1.5×10^5 to 2.4×10^5 SFU/g soil (Table 9).

On the other hand, the mean density of bacteria and fungi in the control soil was 6.0×10^5 CFU/g soil and 1.3×10^3 CFU/g soil respectively. Statistical analysis showed that there was significant difference at $p \leqslant 0.05$ between the abattoir soils and the control soil for both bacterial and fungal populations (Ariyo and Obire, 2016).

Table 9: Mean density of total heterotrophic bacteria (THB) and total fungi (TF) in abattoir soil samples in Yenagoa and Port Harcourt Metropolis

Abattoir location	Total Heterotrophic bacteria (cells/g soil)	Total fungi (spores/g soil)
Opolo	1.2×10^7	2.1×10^5
Swale	8.0×10^7	2.4×10^5
Rukpoku	4.2×10^7	1.5×10^5
Tombia	4.0×10^7	2.4×10^5
Igbogene	2.1×10^7	2.14×10^5
Rumuokoro	2.2×10^7	1.8×10^5
Control	6.0×10^5	1.3×10^3

Source: Ariyo and Obire (2016).

Scrapping after roasting

Washing of Cowhide at Swale

Washing of roasted Cowhide at Tombia

Plate 7: Various activities in an Abattoir (photos by Ariyo 2016)

We investigated the impact of abattoir wastes on the both abattoir soils and wastewater quality in Yenagoa and Port Harcourt metropolis using standard procedures. This was carried out by the determination of the total count and characterization of the heterotrophic bacteria and fungi in the abattoir soil and wastewater.

Physicochemical properties of soil and wastewaters were examined using Association of Analytical Chemist standard procedures. Analyses for Heavy metals were done by extraction using mix acid digest and read on the Atomic Absorption Spectrophotometer (AAS). The concentration and composition of PAHs of all the samples were analysed using Gas chromatography.

P. aeruginosa, *P. fluorescence* and *S. aureus* were the most resistant (66.67%) to the antibiotics; being resistant to six (6) of the nine (9) antibiotics used. On the other hand, *C. xerosis* and *K. pneumoniae* were the least resistant (22.22%) being resistant to only two (2) of the antibiotics used. This showed that all the isolated bacteria exhibited multiple drug resistance. The resistance of bacterial isolates to the tested antibiotics is shown in Figure 19. All the isolates were resistant to Ampicillin. On the other hand, none of the organisms was resistant to Gentamycin and Ciprofloxacin

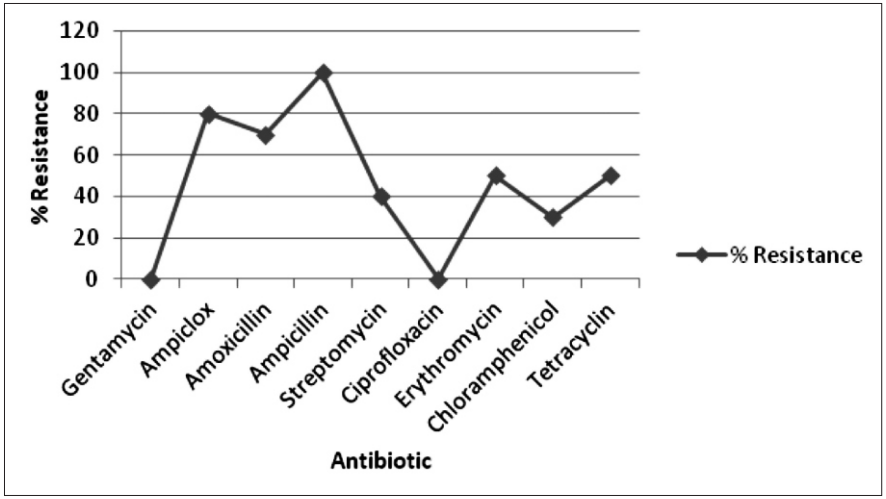


Fig. 19: Resistance of bacterial isolates to the tested antibiotics
Source: Obire and Dollah (2017).

All the bacteria were resistant to ampicillin but susceptible to gentamycin and ciprofloxacin. *Pseudomonas* species and *S. aureus* were the most resistant (66.67%) to the antibiotics while *C. xerosis* and *K. pneumoniae* were the least resistant (22.22%) being resistant to only two antibiotics. This showed that all the isolates exhibited multiple drug resistance. Bacteria isolated from the dumpsite soil and leachate were more resistant than those of control soil and

borehole water. Our results therefore revealed that the dumpsite investigated was capable of encouraging the proliferation of pathogenic microorganisms which possess multiple drug resistance. A combination therapy may be the only way to effectively eradicate the diseases caused by these organisms –a major challenge to healthcare workers.

Most of the bacterial genera isolated in this study have been reported by other workers as potential pathogens (Chesborough, 1985). In addition to the general ubiquity of microorganisms and their metabolic versatility, the presence of these potential pathogens reported in this investigation is attributed to the disposal of raw human faecal discharges and other human wastes at the dumpsite (Obire *et al*., 2002). The presence of these pathogenic forms in open dumpsite in towns and cities is a major health and environmental threat and a cause for concern considering that leachate from such dumpsite often form part of the storm water.

The bacteria isolated from the borehole water include *Pseudomonas aeruginosa* and *Micrococcus luteus*. Some of these bacterial species have been reported to be capable of growth in potable water even in the absence of coliform organisms and can be pathogenic (Pandey *et al*., 2014). Coliforms were not detected or isolated in the borehole water sample except in November when two *Escherichia coli* were isolated.

The occurrence of similar organisms such as *E. coli*, *Pseudomonas aeruginosa* and *Micrococcus luteus* in the dumpsite soil, control soil, leachate and water may be more than a coincidence. It has been shown that in some situations, biological contaminants can travel long distances underground without appreciable attenuation by aquifer material.

Impact of Abattoir activities on the environment (air soil and water)

Wastes on slaughtering slabs in Swale

Discharged of untreated waste into river

Abattoir Toilet on the river in Swale

Roasting with Firewood at Rumuokoro

Roasting with Waste Plastics at Swale

Roasting with tyres fire at Swale

Plate 7: Various activities in an Abattoir (photos by Ariyo 2016)

bacteria respectively. While a decrease in the population of soil fungi was 29.67%, 32.4%, and 39.56% respectively. This indicated that the application of pesticides inhibited the growth, decreased the microbial population and diversity of soil microorganisms and therefore creates an ecosystem imbalance in the soil.

Soil quality encompasses not only the capacity of a soil for crop productivity but also food safety for animals and humans. The persistent use of pesticides on soils should therefore be discouraged as to prevent adverse effect on beneficial soil microorganisms for the sustenance of higher soil quality and food safety for man and other animals (Obire and Eli, 2014).

Impact of Abattoir wastes on soil and water quality

In Nigeria, adequate abattoir waste management is lacking in all public abattoirs such that large solid wastes and untreated effluents are common sites which can serve as a source of disease transmission. The consumption of processed Cowhide (*Kpomo*) is also in high demand among other meat products. Processing of roasted cowhide is majorly by roasting with firewood, waste tyres and plastics. Although substances (especially the alcohols, acids and phenols) produce the characteristic desirable flavour in smoked foods they equally impart compound such as furan, dioxins and polycyclic aromatic hydrocarbons (PAHs) which are known to be carcinogenic and toxic. **The use of expired automobile tyres, waste plastic products and kerosene among other fuel sources calls for serious concern.** Anthropogenic (like in the abattoir) and industrial activities have led to increased emission of PAHs and heavy metals in the environment.

The antibiotic sensitivity response of isolated organisms indicated that none of the isolates was resistant to gentamycin and ciprofloxacin. Generally, gentamycin and ciprofloxacin are effective against most bacterial species. This effectiveness may be due to the fact that the drugs though common are not used frequently probably because they are less known, may be more expensive, or because of their nephrotoxic side effects (Fair and Tor, 2014).

In the cases where isolates exhibited multiple resistance, it is possible that the organisms have been regularly exposed to these antibiotics considering the self medication culture in Nigeria (Obire *et al*., 2009b). Thus, a selection pressure may have ensured the survival and acquisition of resistance factors against these antibiotics (Fair and Tor, 2014). This trend is disturbing considering the fact that these antibiotics are among those commonly used in Nigeria.

Impact of Human Activities on Water Quality

For purposes of simplification, scientists classify water into two major types: surface water and groundwater. Surface water is found in lakes, streams, and shallow wells. Its microbial population may reflect the air through which rain has passed, other human and industrial activities near which a stream flows, or the refuse dump located along a riverbank. Groundwater originates from deep wells and subterranean springs, and because of the filtering action of soil, deep sand and rock, it is virtually free of microorganisms. As the water flows up along channels, contaminants may enter it and alter its quality.

Water is vital to our existence in life and its importance in our daily life makes it imperative that it should have the quality characteristics of its intended use. In Nigeria, there is indiscriminate dumping of untreated waste into nearby rivers and streams, lakes, ponds, etc., that are used as sources of water supply for domestic,

agricultural and industrial purposes. The low standards of health are caused by a general lack of awareness of good hygiene practices and direct contamination of water sources through human activities - bathing and washing, and uncontrolled waste disposal around the shoreline (Obire *et al.*, 2002).

Water can be perfectly clear, odorless, and tasteless and yet be unsafe to drink. Contaminants that pollute water are classified into three categories: chemical, physical, and biological (Pelczar *et al.*, 1993). Our discussion will focus on the biological pollutants (microorganisms). Microbiologists will always detect the presence of specific indicator organisms (e.g *E. coli*) as a pointer to the fact that the water most likely contains pathogenic organisms. Thus we investigated the sanitary quality of surface waters (rivers and creeks) and ground waters (dug wells) and springs.

The New Calabar River has an array of companies sited along its banks (e.g Tidex Nigeria Limited, Horizon Fibers limited, Wilbros Nigeria Limited, West Africa oil field services (WAOS), Limited and Trans Coaster Limited) and receives contaminants and wastes which include auto fuels, heavy oils, spent lubricants and other petroleum products, untreated sewage, human and animal faeces from abattoir and various kinds of domestic, agricultural and industrial waste. The Omuihuechi stream experiences domestic activities such as bathing, washing of clothes, household utensils and motorbikes. It also serves as source of drinking water for the community (Obire *et al.*, 2008e; Obire *et al.*, 2009a; Obire and Barade, 2009).

Our studies the impact of human activities on the Mycoflora of the New Calabar River and the Omuihuechi Stream revealed that the average range of the population of fungi in the different stations of the New Calabar River and the Omuihuechi Stream ranged from 2.0×10^2CFU/ml to 3.2×10^3CFU/ml as shown in the Table 7.

1.0×10^8 to 3.5×10^8CFUml^{-1} with a mean of 1.77×10^8CFUml^{-1}. The order of decreasing bacteria count was NAFCON outfall effluent > Okrika creek > Ikpukulubie creek (control).

A total of 12 species of bacteria which include: *Aerococcus viridans*, *Alcaligenes faecalis*, *Bacillus cereus*, *Citrobacter Freundii*, *Escherichia coli*, *Flavobacterium aquatile*, *Klebsiella pneumoniae*, *Micrococcus* sp., *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Staphylococcus aureus* were isolated during our investigation. All the bacterial types were isolated from the control creek, whereas *Citrobacter freundii* did not occur in the NAFCON outfall effluent and in the Okrika creek waters. *Aerococcus viridans* was also isolated from both creek water samples, but did not occur in the NAFCON outfall effluent.

Our results therefore revealed that, though the NAFCON outfall effluent increased bacterial counts of Okrika creek by enhancing the growth of certain types of bacteria while it had a bacteriocidal effect on some bacteria and therefore adversely affected the microbial and ecosystem diversity of the Okrika creek resulting in an ecosystem imbalance (Obire *et al.*, 2008g).

The toxicity of pesticides extends well beyond insects and the accumulation of pesticide residue in soil affects soil microbial communities. We investigated the impact of various concentrations (0%, 5%, 10%, 25%, 50%, 75% and 100%) of different pesticides on soil bacteria and fungi. Our findings revealed that all the total viable count of bacteria and fungi in control soils were higher than in the pesticide treated soils. All the concentrations of the pesticides had adverse effect on the population and diversity of both bacteria and fungi in the soil (Obire and Eli, 2014). The 5% concentration of Delvap super, Karto super and rocket resulted in a decrease of 31.36%, 26.13%, and 41.46% in the population of soil

The waters of Jericho purified; 2 Kings 2: 19, 21-22:

> Some men from Jericho went to Elisha and said, *"As you know, Sir, this is a fine city, but the water is bad and causes miscarriages"…. "And he went to the spring of water and threw the salt into the water and said, "Thus says the LORD, I have purified this water; and from now on it will not cause any more deaths or miscarriages (unfruitfulness)". "**So the waters have been purified ever since, just as Elisha said it would be".***

Today, there are lots of water related deaths in our land. The Elishas of our present day (The President, the Senators, the Ministers, the Governors, the Local government Chairmen, and Councilors) should also purify our waters irrespective of our fine cities. Borehole water without treatment is not potable (drinkable) water.

Impact of fertilizer plant effluent and pesticides on water and soil quality

Our studies on the impact of National Fertilizer Company of Nigeria (NAFCON) outfall effluent on the physicochemistry and bacteriology of Okrika creek and Ikpukulubie creek (control) revealed that NAFCON effluent had adverse effect on the water quality characteristics of Okrika creek as a result of the discharge of poor quality effluent from National Fertilizer Company of Nigeria operations (Obire *et al*., 2008g).

The bacterial counts for the NAFCON outfall effluent ranged from 2.95×10^8 to 5.6×10^8 CFU ml^{-1} with a mean of 3.98×10^8 CFU ml^{-1}. The bacterial counts for all our stations of the Okrika creek ranged from 8.5×10^7 to 4.9×10^8 CFU ml^{-1} with a mean of 2.94×10^8CFUml^{-1}. The bacterial counts for the control creek ranged from

Table 7 : Population of fungi in stations of the New Calabar River and Omuihuechi Stream

Stations	A Sawmill/ Abattoir	B Oil films	C Delta Park	D Domestic waste	E Fish ponds	F Domestic washings	G (Control/ drinking)
Fungal count ($\times 10^2$CFU/ml)	2 – 7	2 – 8	2 – 8	2 – 9	2 – 9	8 – 32	7 – 14

Impact of Human Activities on the New Calabar River and Omuihuechi Stream

Horizon fibers

Abattoir close to Sawmill and H. fibers

Wilbros Nig. Ltd

Delta Park

Washing and Bathing

Plate 2: Stations along New Calabar River and Omuihuechi Stream (Obire *et al.*, 2008e)

The populations of fungi in stations of the New Calabar River (A – E) were far lower than those of the Omuihuechi Stream (F and G). This is an indication that the industrial activities along the river had adverse or inhibitory effect on the growth of fungi. On the other hand, the fungal population in station F is higher than that of station G because the food wastes washed into station F acted as nutrient which encouraged the growth and proliferation of fungi.

The frequency of isolation of fungi from stations of the New Calabar River and the Omuihuechi stream is shown in Figure 20. The isolated fungal genera during our study were ***Aspergillus, Byssochlamys, Candida, Cephalosporium, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus, Saccharomyces, Sporobolomyces and Trichoderma***. The isolation frequencies of *Cephalosporium* ***and Trichoderma*** were very highest in station A and this is attributed to the presence cow dung from the abattoir and of wood/sawdust from sawmilling activities in the vicinity of station A. The isolation frequency of *Saccharomyces* was very high in stations F and G, and this is attributed to the palm wine tapping

In another study, we investigated the bacteriological and physiochemical parameters of alum treated water, alum treated, boiled and filtered water and raw river water from the Amassoma axis of the Nun River in Bayelsa State. The study revealed that there was a decrease in the bacteria population in the order of raw river > the alum treated water > alum treated, boiled and filtered water. This showed that each treatment reduced the bacteria population of the water samples. However, the presence of *E. coli* in all water samples proved that these waters were not fit for human consumption as hygienic situations of the handlers and vessels or containers used are questionable (Obire *et al*., 2008f).

Water Purification

Water that is safe to drink is free of disease-producing microorganisms and chemical substances harmful to health, and is called *potable* water, Non-potable water, on the other hand, must be purified before it can be used for human consumption. Purification methods vary, depending on the source of water and the amount of water needed.

In order to prevent transmission of these pathogens, there must be (1) water-purification methods that provide safe drinking water, (2) treatment facilities for wastewater prior to its disposal or reuse, and (3) procedures whereby water can be examined to determine its microbiological quality.

What the Bible says about water purification: **Bitter water** Exodus 15: 23 – 25

> *"Then they came to a place called Marah, but the water there was so bitter that they could not drink it. Moses prayed earnestly to the Lord, and the Lord showed him a piece of wood, which he threw into the water; and the water became* ***fit*** *to drink"*. Good News Bible.

Our studies on the microbiological quality of spring water samples collected from three communities in Ihitte/Uboma Local Government Area of Imo State (Obire and Osigwe, 2016) revealed that the total aerobic heterotrophic bacterial counts ranged from (1.9 to 5.64) × 10^7 cfu/ml. Total Coliforms ranged from 4 to 17 (MPN index/100ml) while Thermotolerant Coliforms ranged from 6 to 20 (MPN index/100ml). The bacteria isolated were *Escherichia coli*, which occurred in the three spring water samples. *Salmonella* sp. occurred in Oturugo spring water and Inyenta spring water.

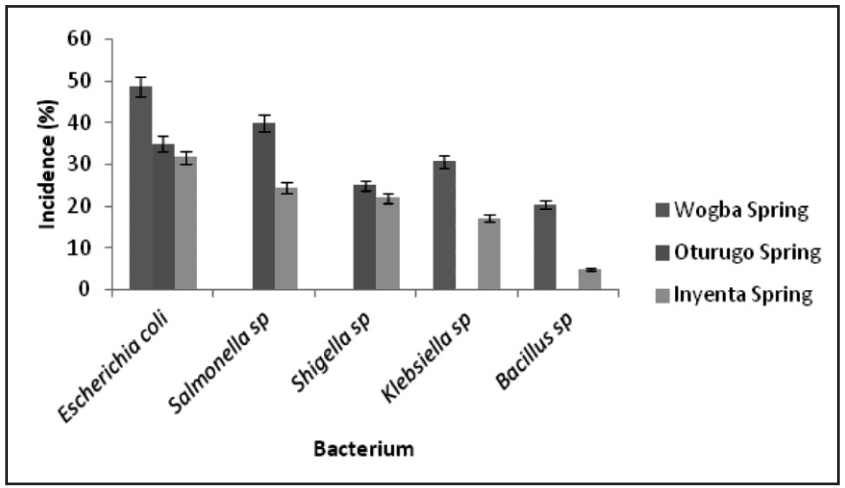


Fig. 23: Incidence (%) of bacteria in the different spring water samples

Source: Obire and Osigwe (2016)

Shigella sp occurred in Oturugo and Inyenta spring waters, *Klebsiella* sp, occurred in Wogba and Inyenta spring waters and *Bacillus* sp, occurred in Wogba and Inyenta spring waters. Analysis of variance (ANOVA) using F-Test showed that there was no significant difference at $p \leqslant 0.05$ in the incidence of bacteria between the different spring water samples. The presence of these organisms revealed microbial and faecal contamination of the spring waters. This study therefore emphasizes the need for treatment of water before human consumption.

activities along this stretch where raffia palm form the predominant tree in the swamp.

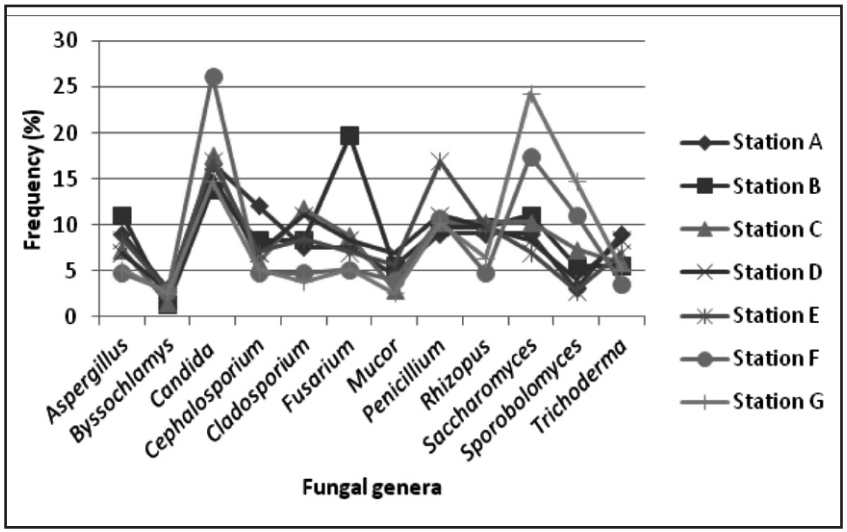


Fig. 20: Frequency of fungi in stations of New Calabar River and Stream

Source: Obire *et al.*, (2008e)

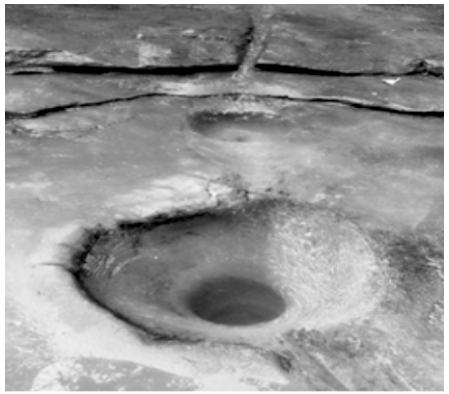
Most of the isolated fungal genera contain species that are potential pathogens or opportunistic pathogens. The main hazardous species belong to ***Aspergillus***, ***Penicillium***, ***Cladosporium***, ***Mucor***, and ***Fusarium***. Various strains of these families of molds have been implicated in being causative agents in asthma, hypersensitivity pneumonitis and pulmonary mycosis. The mean of total fungal counts were up to ×10^2cfu/ml. *Candida* was predominantly high in all the stations with an average of 17.29%. This high occurrence of *Candida* is of considerable concern as species of this genus can cause candidiasis, endocarditis, septicemia, protracted urinary tract infections, kidney and lung infections, esophagitis and other soft tissues infections.

Fusarium with a mean isolation frequency of 7.37% has species, which are common plant pathogens and causative agents of superficial and systemic infections in humans (Mayayo, 1999). The presence of these organisms is a clear indication of the need to constantly monitor the water quality of the New Calabar River.

There was a high frequency of mould (72.38%) against yeast (27.62%) along the New Calabar River. While along the Omuihuechi stream 46.23% of enumerated fungi were moulds, and 53.77% were yeasts. This showed that there is a balance in the fungal diversity of the stream than in the river. Statistical analysis of the Fungal counts using the Randomized Complete Block Design (RCBD) and Analysis of Variance (ANOVA) showed there was significant difference at $P \leqslant 0.05$ between Zones and Stations. This is an indication that the activities taking place at specific points (stations) along the river course have effect on the water quality and biological endpoint which is the fungal population and diversity.

The pathogens most frequently transmitted through water are those which cause infections of the intestinal tract. These microorganisms are present in the feces or urine of an infected person and, when discharged, may enter a body of water that ultimately serves as a source of drinking water (Pelczar *et al*., 1993). Raw excreta (faeces) may contain pathogenic microorganisms that cause many illnesses that range from typhoid and paratyphoid fevers, shigellosis, cholera, and bacillary dysentery, campylobacteriosis, viral enteritis, and amoebiasis to minor respiratory and skin diseases, etc (Ekundayo, 1977; Obire and Aguda, 2002).

of the water that emerges as springs originally fell as rain or snow on the surface of the earth (Zimmerman, 1996; Thurman *et al.,* 1998). Spring water is used for a variety of human needs primarily as a source of drinking water and is the subject of many popular misconceptions. For example, many people believe that spring water is actually "pure" water. It is common knowledge that spring water is consumed raw without any form of treatment in many communities in Nigeria.



Wogba spring of Amainyi

Collection of spring water

Oturugo spring of Umuihi

Inyenta spring of Umuoma

Plate 6: Springs of various communities in Ihitte/Uboma L.G.A of Imo state
Source: Obire and Osigwe (2016)

pollution of the drinking water. The presence of faecal indicators such as *E. coli*, *Shigella* sp and enteric pathogens such as *Chromobacterium*, *Enterobacter,* indicated that the various water sources are polluted with faecal matter. None of these well water samples use for drinking in Abua Central complied with EPA standard for coliform in water due to the presence of faecal coliform especially *E. coli* (WHO, 1971; EPA, 2003).

Diseases associated with the bacteria isolated from the well water in Abua/Odual communities are; *Bacillus spp.* Diarrhea and food poisoning, *Shigella spp.* shigellosis (bacterial dysentery), *Streptococcus spp.* streptococcal pneumonia and sore throat, *Staphylococcus spp.* various staphylococcal disease e.g. boils, *E. coli* gastroenteritis, *Enterobacter spp.* sepsis and septic shock, *Salmonella typhi* typhoid fever, salmonellosis (salmonella gastro-enteritis), *Proteus spp.* urinary tract infection. **Diseases associated with the fungi isolated** from the well water in Abua/Odual communities are; ***Aspergillus spp.*** aspergillosis and onchomycosis, ***Rhizopus spp.*** tellutis, ***Fusarium solani-*** pneumonia and onchomycosis (Singleton and Sainsbury, 2001).

Thus, the direct consumption of raw water from wells of the communities studied could contribute to the spread of many infectious diseases and the cause of serious epidemic in Abua/Odual Local Government Area. The water quality of well water in Abua Central Area violates the set standards for well water for tropical countries by WHO (WHO, 1971; 1996). This warrants treatment of the present source or the need for an alternative water supply.

Microbiological Quality of Spring Water

Spring is a water of a natural situation where water flows from an underground layer of water bearing permeable rocks and rock fractures (aquifer) to the earth's surface or emerges as a spring. Most

Our studies on the physico-chemical and bacteriological quality of Elechi Creek in Port-Harcourt revealed high values of chemical constituents, high coliform counts and presence of *E. coli* which indicated the contamination of the water body by faecal matter and pathogenic bacteria. This implied that, the human activities along the creek had adverse effect on its water quality (Obire *et al*., 2003; Obire *et al*., 2005a).

We investigated the impact of human activities on the water quality (bacteriological) of Kolo Creek in Bayelsa State for a period of six months using seven designated stations on the creek. The stations were: A - control station drinking water; B- open waste dump; C – bathing, domestic and agricultural washing; D - palm oil mill; E - cassava mill; F - "floating toilet" where raw human faeces and urine are directly discharged without treatment into the creek; G - located downstream to all other stations as to assess the physico-chemical and bacterial load of the creek away from the direct sources of pollution. Our studies revealed that total culturable heterotrophs and total coliform MPN ranged from 1.8×10^5 to 14.0×10^5 cfu/ml, and 20 cells/100ml to 180 cells/100ml respectively. The coliform MPN expressed as percentage of heterotrophs ranged from 1.91% to 3.75%. Bacterial types and their frequency of isolation were *Aeromonas* sp (2.5%), *Bacillus* sp (30%), *Citrobacter freundii* (2.5%, *Enterobacter aerogenes* (2.5%), *Enterococcus faecalis* (10%) *Escherichia coli* (15%), *Klebsiella* sp (5%), *Proteus mirabilis* (10%), *Pseudomonas* sp (7.5%), *Serratia macescens* (2.5%), *Staphylococcus aureus* (2.5%), *Streptococcus* sp (2.5%) and *Vibrio* sp (7.5%). There was significant difference between stations at $P \leqslant 0.05$ for the parameters determined. The high levels of BOD, heterotrophs, and coliforms indicate that the creek water is highly polluted.

Impact of human activities on water quality of Kolo Creek

Station A - Drinking water (control)

Station B- Open waste-dump

Station C- Bathing and washing

Station D- Oil-palm Mill

Station E- Cassava milling & processing

Station F- "Floating toilet"

Plate : Human activity stations along Kolo Creek (photos by authors, 2005)

The fungal isolates were *Aspergillus*, *Chrysosporium*, *Fusarium*, *Microsporium*, *Mucor*, *Penicillum*, *Rhizopus* and *Saccharomyces cerevisiae*. *Microsporum canis*, a dermatophyte of cats and dogs was isolated from Otari well water.

It was observed during this study that the sanitary conditions and standard of living of people in the various communities also affected the population and types (diversity) of microbes present in the well water and hence the water quality. The primary sources of these bacteria and fungi in water are animal and human wastes. These sources of bacteria and fungi contamination include surface runoff, and other land areas where animal wastes are deposited. The microbial count was higher in Amalem and Emilaghan communities because these wells were very close to toilet sites than those of Omokwa and Otari, where the microbial counts were lower.

The order of decreasing water quality in the various communities was Omokwa > Otari > Amalem > Emilagham. All the potential pathogenic bacteria isolated during this study except *Proteus* were isolated from Emilaghan and all but *Proteus*, *Staphyloccoccus* and *Streptococcus* were isolated from Amalem. *Salmonella* species occurred in well water of all the communities.

Proteus spp. is also of public health significance. *Staphylococcus aureus* is known to produce enterotoxin (Obire *et al*., 2008b). *Proteus spp.* belongs to the intestinal flora but is also widely distributed in soil and water. Environmental bacteria such as *Acinetobacter* and *Bacillus* sp., which are mostly saprophytic in origin, were isolated from well waters. It was also found that 80% of the various water samples were positive for coliform MPN showing high contamination and risk to public health. The counts of faecal coliform obtained in these drinking well waters were high and far above recommended standards of zero total coliform per 100ml of water (EPA, 2003). The detection of faecal coliform indicates feacal

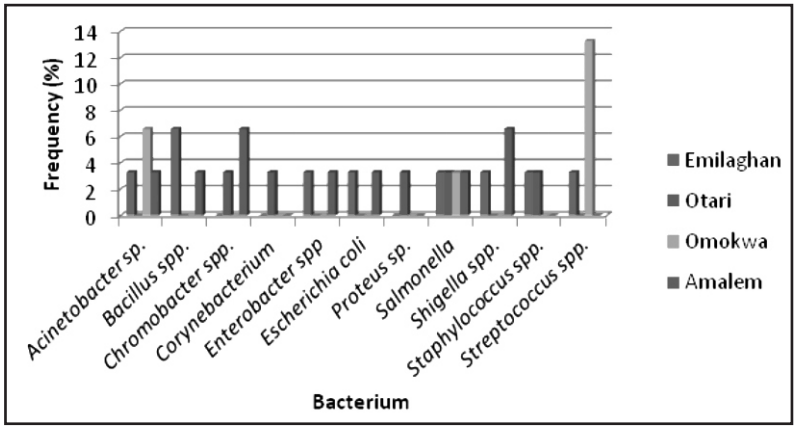


Fig. 21: Frequency (%) of bacteria in well water from the different communities
Source: Obire and Alali (2015)

Salmonella species occurred in well water of all the communities. All the potential pathogenic bacteria except *Proteus* were isolated from Emilaghan and all but *Proteus*, *Staphyloccoccus* and *Streptococcus* were isolated from Amalem.

The result of fungi isolates and frequency **in the** well waters is shown in Figure 22.

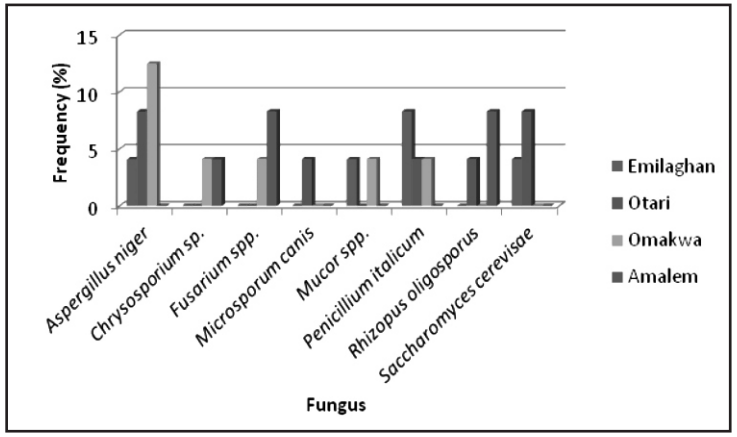


Fig. 22: Frequency (%) of fungi in well water from the different communities
Source: Obire and Alali (2015)

The presence of faecal indicator such as *E. coli* and enteric pathogens such as *Vibrio* sp indicated the contamination of the water with faecal matter implying that the creek water is not safe for drinking and for other domestic purposes that it is presently being used (WHO, 1996, FEPA, 1991). Our result of the bacteria counts and occurrence in different sampling stations of Kolo Creek during the 6 month investigation period is presented in Table 8.

Table 8: Bacterial count and occurrence in the different stations of Kolo Creek

Bacteria	**Stations**						
	A (control)	B (waste dump)	C (washing/ bathing)	D (palmoil mill)	E (cassava mill)	F (floating toilet)	G (down-stream)
Total Het. Bacteria ($\times 10^5$CFU/ml)	4.4	7.7	5.1	6.6	6.5	8.3	7.0
Total coliform (MPN/100ml)	> 165	>180	> 176.6	> 153.3	> 180	> 176.6	> 134
Aeromonas sp	-	-	+	-	-	-	-
Bacillus sp	+	+	+	+	-	+	-
Citrobacter freundii	-	-	-	-	+	-	-
Enterobacter aerogenes	-	-	-	-	-	+	-
Enteroccocus feacalis	-	-	+	-	-	+	-
E. coli	-	+	+	+	-	+	+
Klebsiella sp	-	-	+	+	-	-	-
Proteus mirabilis	+	+	-	-	-	+	-
Pseudomonas sp	-	-	+	+	-	+	-
Serratia macescences	-	+	-	-	-	-	-
Staph aureus	-	-	-	+	-	-	+
Streptoccocus sp	+	-	-	-	-	-	-
Vibrio sp	-	+	+	-	-	-	+

Source: Obire *et al.*, (2008b)

The overall mean of culturable heterotrophic counts of bacteria was highest in station F (floating toilet) and lowest in station A (control – drinking water). Mean separation using Duncan's Multiple Range Test showed that there were significant differences between the control and the other stations. This implied that, the various human activities in all the other stations had a significant effect on the bacteriological quality and hence the water quality of Kolo creek.

Station F was significantly different from all stations of Kolo creek, but not significantly different from station B (waste dump). The increased nutrient load imputed into stations through human activities supported the proliferation of bacteria resulting in the higher counts observed. Obire and Aguda (2002) reported high bacteria counts from leachate and an adjacent stream due to high content of organic matter. High bacteria counts reported in this study for the different stations indicate the high level of pollution influenced by the various human activities on the banks of Kolo creek. Hollaway *et al* (1980) indicated that the established presence of bacteria in water bodies is important as they are identified as major organisms that break down waste materials introduced into waters.

The bacterial types isolated included species known to be involved in the degradation of organic matter. These bacteria such as *Bacillus* sp, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas* sp and S*taphylococcus aureus* may have entered the water through the waste dump during run-off and leaching, at the bathing and washing site (station C) and direct discharge of faecal matter at station F (floating toilet). Most of these bacteria are potential pathogens that can be acquired through drinking of water polluted by these organisms. Most waterborne diseases that can result from drinking water polluted by these organisms ranged from gastro-intestinal tract infection that can be caused by *Aeromonas* sp., *E. coli*,

Plate 5e: Location D - Well in Amalem community collapsed after rains
(Obire and Alali, 2015)

Plate 5: Dug Wells in Abua Central Area of Rivers State

Analysis of variance (ANOVA) using F-test showed that, there is significant difference between the counts of total heterotrophic bacteria of the well waters at $\leqslant 0.05$ while the reverse is the case for counts of fungi. The total bacterial counts and total fungal count of the well water samples exceeded the International standard limits for drinking water (EPA, 2002).

Result of bacteria isolates and frequency **in** well water in the communities is shown in Figure 21.

Plate 5c: Location C - Well in Omokwa Community. This well is situated 15m from occupied houses.

Plate 5: Dug Wells in Abua Central Area of Rivers State

Plate 5d: Location D - Well in Amalem Community. This well is situated just about 3m from a plantain and coco-yam plantation where inhabitants use as an open toilet site and about 7m from occupied houses (Obire and Alali, 2015).

Vibrio sp., *Enterobacter* sp., *Enterococcus* sp., *Proteus, Pseudomonas* and *Serratia* spp are known to cause urinary tract infections in the young and elderly. Other diseases caused include wound and skin infections, respiratory infections and food poisoning.

We wish to acknowledge the then Bayelsa State Government for her prompt action in sending "Doctors without borders or frontiers" and other health professionals to Kolo 1, Kolo 2, and Kolo 3 communities and the provision of borehole water to these communities when I informed the government of the waterborne epidemic that resulted in the loss of lives in these communities.

Overhead Water tank and Bill Board of Contractor

Inhabitants of Kolo collecting water from an interim borehole provided by the BYSG

Plate 4: Mega water tank and inhabitants of Kolo collecting borehole water

Microbiological Quality of Dug Well Water

According to the World health organization (WHO, 2004), over 1.1 billion people do not have access to an improved water supply and over 2.3 billion people suffer from diseases caused by contaminated water. Each year over 1.8 million people die from diarrheal diseases, and 90% of these deaths are of children under 5 (WHO, 2004). Besides causing death, water-related diseases also prevent people from working and leading active lives.

In Nigeria, majority of the populace depend on dug well water and springs for domestic use. There are several ways a well can be contaminated. Toxic mineral spilled or dumped near a well can leach into the aquifer and contaminate the groundwater or well water. Polluted water can leak through the walls of poorly maintained or shoddily constructed wells. Wells can get contaminated from septic tanks placed too close to wells in the area. Flood events can also impact the quality of well water (Obire and Abigail, 2010; Obire and Alali, 2015).

Our studies on the water quality of drinking well water samples from Port Harcourt Metropolis (Obire and Abigail, 2010) and from four communities (Emilaghan, Otari, Omokwa, and Amalem) in Abua Central Area of Rivers State (Obire and Alali, 2015) revealed that these well waters were not suitable for consumption and other domestic purposes as specified by requirement of world Health Organization (WHO) standards for drinking water.

Our study on the wells from Abua Central Area showed that, total heterotrophic bacteria count ranged from 1.24×10^9cfuml^{-1} to 5.53×10^9cfuml^{-1}, faecal coliform bacteria ranged from 9 to 1600 MPN/100ml while total fungal count ranged from 2.3×10^6sfuml^{-1} to 4.6×10^6sfuml^{-1}.

Dug Wells in Abua Central Area of Rivers State

Front View | Inside View

Plate 5a: Location A - Well in Emilaghan Community. This well is situated in a nearby bush very close to a private Nursery and Primary school (Obire and Alali, 2015).

Plate 5: Dug Wells in Abua Central Area of Rivers State

Plate 5b: Location B - Well in Otari Community. This well is situated inside Alali's compound just 3m from the house (Obire and Alali, 2015).