MICROBIOLOGICAL QUALITY AND HEAVY METAL LEVELS IN WELLS LOCATED IN THE VICINITY OF MECHANIC WORKSHOPS IN MINNA, NIGERIA

By

YUSUFU HAUWA NUHU (MRS.)

(M.TECH./SSSE/2000/296)

B.TECH. MICROBIOLOGY (1997)

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, FEDERAL UNIVERSITY OF TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY IN ENVIRONMENTAL MICROBIOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES

SCHOOL OF SCIENCE AND SCIENCE EDUCATION,

FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA,

NIGER STATE

SEPTEMBER 2003

DECLARATION

I, **Yusufu, Hauwa Nuhu (Mrs.)**, do hereby declare that this project work "***Microbiological Quality and Heavy Metal Levels in Wells Located in the Vicinity of Mechanic Workshops in Minna, Nigeria***" is an original work of mine and has never been presented anywhere for the award of any degree. Information derived from published and unpublished work of other sources have been acknowledged and referenced accordingly in this text.

STUDENT

CERTIFICATION

This project entitled ***"Microbiological Quality and Heavy Metal Level in Well Located in the Vicinity of Mechanic Workshops in Minna, Nigeria"*** was carried out by **YUSUFU HAUWA NUHU** under my supervision and has been read, examined and found to meet the regulations governing the award of the degree of Masters of Technology in Microbiology of Federal University of Technology Minna, and is approved for its contribution to knowledge literary presentation.

U.E.E. Ijah — 29/12/2003

DR. U.J.J. IJAH
(SUPERVISOR) — **DATE**

H.O. Akanya — 29/12/2003

(HEAD OF DEP., BIOL., SCIENCES) — **DATE**

H.O. Akanya — 29/12/2003

PROF. (MRS) H.O. AKANYA
(DEAN, SCH. OF SCI. AND SCI. EDUC) — **DATE**

J.A. Abalaka 15/6/04 — ---

PROF. J.A. ABALAKA
(DEAN, P.G. SCHOOL) — **DATE**

K.B. Tanyigna — 18-12-03

DR. K.B. TANYIGNA
EXTERNAL EXAMINER — **DATE**

ACKNOWLEDGEMENT

I wish to acknowledge with deep gratitude the untiring efforts and patience of DR. U. J. J. IJAH for his useful advice, encouragement and support as my supervisor in the course of this project.

My appreciation also goes to all my lecturers past and present most especially Prof. (Mrs.) H. O. AKANYA, Dean SSSE and DR. S. B. OYELEKE. I also thank Mallam Abdullahi Hamidu and Mallam Mohammed Kudu of the Microbiology lab for the technical support. Equally, I am grateful to Mallam Dauda Ahmed of the M.I.S for all the graphs and statistical analyses; staff of the UNDP for heavy metal analyses and all the mechanics who permitted me to collect water samples. I fondly remember all my classmates and say "thank you".

My family members have just been wonderful: Husband ALHAJI ALI JIGAM, who gave me the chance and encouragement to improve myself. I deeply cherish the memories created through this work by my daughter AMIRAH. I continue to appreciate my DAD, ALHAJI NUHU YUSUFU, brothers and sisters, and above all **ALHAMDULILLAHI**.

DEDICATION

Dedicated to the people in my life: **AMIRAH**, **ALI JIGAM AND NUHU YUSUFU** for every inspiration they gave me in their very special ways.

ABSTRACT

Water samples from wells in the vicinity of mechanic workshops in Bosso, Chanchaga, Keteren-Gwari and Maitumbi, Niger state, Nigeria were analysed to ascertain their degree of microbial and heavy metal pollution. Generally, the population of viable bacteria was higher in the rainy than the dry seasons in the four locations. Chanchaga mechanic wells gave the highest mean monthly viable counts of $2.20x10^4$CFU/ml while Bosso had the lowest counts ($3.0x10^3$Cfu/ml). Coliform counts were also high and they peaked in August. Maitumbi site had the highest counts of $8.0x10^3$CFU/ml while the lowest counts ($1.45x10^3$CFU/ml) was recorded in Chanchaga. The highest faecal *Streptococcal* count ($3.6x10^2$CFU/ml) was recorded in Bosso wells while the lowest count ($2.0x10^1$CFU/ml) was recorded in Maitumbi. The control wells had counts ranging from $1.8x10^1$CFU/ml to $2.0x10^2$CFU/ml. *Staphylococci* counts in Bosso mechanic wells ($2.2x10^2$CFU/ml) far exceeded those of Keteren-Gwari and Maitumbi. Chanchaga wells gave consistently low counts ($1.1x10^1$CFU/ml). Control values were between $1.0x10^1$-$3.0x10^1$ CFU/ml. *Salmonella* species were isolated in most wells in the mechanic sites but were not detected in many control wells especially in the dry season. Mean counts of spent lubricating oil-utilizing bacteria were exceptionally high ($7.1x10^1$CFU/ml) in the rainy season in Bosso. This dropped in October to the generally low levels encountered in Chanchaga, Keteren-Gwari and Maitumbi wells. The oil utilizers were identified as species of *Bacillus*, *Micrococcus* and *Pseudomonas*. *Micrococcus sp.* (B3 and M3) degraded the oil at considerably high rates. The mean pH values of the water; 8.46 (highest) and 6. 33 (Lowest) (mechanic wells) and 6.60 – 7.80 (controls) were within the WHO maximum permissible level (6.5 – 9.2) for drinking water (WHO, 1974). Heavy metals (Hg, Pb, Co, Cu, Cd, Fe) analysed generally gave higher values in the rainy than the dry seasons because of the surface run off: among mechanic site wells than controls.

Most wells had heavy metal levels above the WHO maximum permissible level for drinking water. The results obtained suggest that most of the wells studied are polluted with bacterial pathogens and heavy metals. Therefore, such wells are unfit for domestic use.

TABLE OF CONTENTS

LISTS OF TABLES

LIST OF FIGURES

FIGURES **PAGE**

LISTS OF APPENDICES

CHAPTER ONE

1.0 INTRODUCTION

The acute shortage of portable water for domestic consumption in developing countries is a reality of the day. It has been reported that over one and a half billion people across the globe drink contaminated water and about 3.4 million people, majority of them children die annually of water contracted diseases (*Jones, 2001*). It has also been asserted that many of the communicable diseases having the greatest impact on mankind are waterborne, and a permanent reduction in morbidity and mortality can most effectively be achieved by providing safe drinking water and satisfactory sanitation (*Junaidu et al; 2000*).

In addition to the problem of microbiological pollution, the levels of inorganic contaminants especially in natural waters continue to increase. Among the causes are the large volumes of wastewater often subject to little or no control originating from highly populated cities, the discharge of untreated effluents by industrial complexes, and the use of a wide variety of fertilizers and pesticides in agriculture. Its results include harm to humans and to animals and plant life, unpleasant odours, reduced water clarity, damage to property, and a reduction in the recreational quality of coastal and inland waters (*Ayalogu et al; 2001*).

The need for bacteriological and chemical analyses of water cannot therefore be overemphasized. Such examination is carried out to ascertain the sanitary quality and suitability of water for general use (*Itah et al; 1996*). The sanitary quality of water is described as the relative extent of the absence of suspended matter, colour, taste, unwanted dissolved chemicals, bacteria indicative of faecal pollution and other aesthetically offensive objects (*Suess, 1982*). Water supplies liable to contamination with sewage or other matter are

the vehicles of waterborne diseases such as typhoid fever, cholera, amoebic and bacillary dysentry, infectious hepatitis, giardiasis, etc. (Prescott *et al.,* 1990).

In some developing countries like Nigeria where toxic industrial and domestic wastes are disposed off by dumping into the earth, waters, rivers and streams with total disregard for aquatic life and urban dwellers, water becomes a critical determinant in the transmission of enteric diseases in most communities (Haroun *et al.,* 2002). Tube and open wells are a major source of water supply to most inhabitants of developing nations (Adesiyun *et al.,* 1998; Abubakar and Ibrahim, 2002). Well water is subterranean water that occurs where all pores in the soil or rock containing materials have been saturated. As a result bacteria as well as suspended particles are removed by filtration in varying degrees depending on the permeability characteristics of the soil and depth to which the water penetrates (Adesuyi and Alabi, 1986; Agbu *et al.,* 1988; Junaidu *et al.,* 2000). Wells not less than 30M deep are not shallow and may contain very few or no bacteria compared to shallow wells which may contain high numbers of bacteria (Itah *et al.,* 1996). However, the sanitary quality of drinking water is determined not by the numbers but by the kinds of microorganisms (Itah *et al.,* 1996).

In bacteriological water analysis, the recovery of conventional indicator bacteria such as the coliforms particularly *E. coli*, faecal *Streptococcus* and anaerobic spore forming *Clostridium perfringens* provide a reliable means of assessing the extent of pollution (Standard Methods for The Examination of Water and Wastewater, SMEWW, 1989) as their presence is indicative of a possible presence of enteric pathogens in such waters. Other pathogens often encountered in polluted water are *Shigella spp.* (bacillary dysentry), *Salmonella spp.* (typhoid fever and food poisoning), *Vibrio cholerae* (cholera), a large number of other bacteria and viruses (Reed and Singh, 2001). So also are water borne parasitic microorganisms such as *Proteus* and *Pseudomonas* species (Prescott *et al.*, 1990). *Pseudomonas* is a common cause of pneumonia, urinary

tract infection, abscesses, otitis, and corneal diseases, etc (*Talaro and Talaro 1996*). Most of these organisms are transmitted to man through the drinking or use of contaminated water.

WHO (1974) stated that the coliform index of untreated water should be less than 10 and no sample should show most probable number (MPN) index greater than 20, in the case of treated water, the allowed MPN index is 1 – 10.

Pollutants gain entrance to well water through a number of routes, most important of all is sewage (*Okafor; 1985*). Untreated sewage contains pathogens, which may contaminate drinking water. Human, animal and agricultural wastes could gain entry in the process of digging the well or when the well is not properly constructed (*Adesiyun et al; 1983*). Besides supplying organic nutrients, the wastes could contain microorganisms responsible for enteric infections diseases.

Wells and underground waters are also polluted by inorganic wastes emanating from domestic and industrial effluents that contain soaps, detergents, pesticides, herbicides, fertilizers etc as they are applied by farmers in agricultural practices (*Amadi, 1991; Haroun et al; 2000*). Poisonous chemicals (organic and inorganic in origin) are known to percolate the layers of the earth and terminate in underground water thereby constituting public health hazards (*Abubakar and Ibrahim, 2000*). Fertilizers contaminate well water through infiltration and surface run-offs and are known to increase nitrate content of water (*Fagbemi and Ijah, 1998*). Nitrate is harmful to infants but not to adults and growing children (*Gbodi,et al; 2001*). High levels in infants can be reduced to nitrites leading to infantile metheglobinemia (infant cyanosis or blue baby disease) as a result of the reduction in oxygen transport in the blood. This condition is precipitated by intake of water nitrate level of between 10 – 45ppm (*Ojiegbe, 1990*).

The present study has targeted wells located in the vicinity of motor mechanic workshops. The demand and usage of lubricating oils have increased

considerably in recent years. Spent lubricants from industries and crankcase of automobile engines together with solvents, metals and additives are being indiscriminately dumped from the mechanic workshops and garages. These materials constitute serious environmental pollutants (*Amund et al; 1993*). Currently there is an influx of used automobiles into Nigeria, which have an attendantly high inefficient utilization of fuels. Such wastes inevitably contaminate sources of water supplies e.g. well. The motor mechanics and people around drink from these wells and also use the water to perform other domestic chores including the preparation of foodstuff for sale to the wider public causing health hazards.

Hydrocarbon contamination of water bodies encourages the proliferation of hydrocarbon utilizing bacteria, some of which may be pathogenic. Generally, when natural ecosystems are contaminated with petroleum hydrocarbons, the indigenous microbial communities are likely to contain microbial populations of different taxonomic characteristics (*Bartha and Atlas, 1977; Amund et al; 1993*). Several workers have identified such microorganism especially in the soil ecosystem (*Raymond et al; 1976; Amund et al; 1987; Amund et al; 1993; Ijah and Antai, 2002*). Infact *Mulkins Phillips and Stewart (1974)* have asserted that the measurement of hydrocarbon utilizing bacteria should indicate the degree of oil pollution, as well as the microbial capability of the location to handle oil pollutants. Microorganisms with the capability to utilize petroleum hydrocarbons as sole sources of carbon and energy are not restricted to a few genera. They include bacteria, fungi and algae (*Bello, 1995*).

This study also focused on the presence and levels of some heavy metals (Lead, Cadmium and Mercury) and other biologically important elements (Iron, Cobalt and copper) in well waters in the vicinity of mechanic workshops. These metals apart from amounts from natural sources, a greater portion are derived from pollutants (*Haroun et al; 2002*). Heavy metal intoxication has a profound pharmacological and medical significance to man (*Nordberg, 1990*). Elemental

cramps (Kendler, 1993). Cadmium is equally toxic to man. It adversely affects kidneys, reproductive organs and has been implicated in human prostrate cancer and the dreaded Japanese "Itai-Itai" disease (Klein and Snodgrass, 1997). Mercury poisoning is manifested by neurological, reproductive, respiratory and other disorders (Clarkson, 1990).

Although studies have been conducted elsewhere on the quality of drinking water from different sources, literature on the contamination by microbial pathogens, lubricating oil and heavy metals of wells located in the vicinity of motor mechanic workshops appear to be very scarce. This is why the present study has become necessary. The main aims of this work are:

a. To enumerate bacterial pathogens in water from wells located in the vicinity of mechanic workshops in Minna.
b. To isolate and identify the bacterial pathogens.
c. To determine the presence and levels of heavy metals Lead (Pb), Mercury (Hg), Cadmium (Cd), Iron (Fe), Cobalt (Co), Copper (Cu), in the well water.
d. To determine the load of spent lubricating oil utilizing bacteria in the well water.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 INDICATOR BACTERIA OF POLLUTED WATER

Routine bacteriological analyses of domestic water have become important because safe drinking water and adequate sanitation are basic human needs. Water is essential not only for drinking but also for recreation, transportation, manufacturing, laundering, production of food, etc (*Sangodoyin and Osuji, 1990*).

The greatest dangers associated with water are due to direct and indirect contamination by the excrements of warm-blooded animals, including man (*Suess, 1982*). Human faeces contain 20-30% of undigested food residues, the remaining consisting of water and bacteria. In the healthy individual these bacteria consist of the normal flora of the gut of which ***Escherichia coli*** is one. The number of these organisms is of the order of 10^9 per gram of faeces (*McCoy, 1981*). In acute intestinal diseases and in the carrier state, the normal flora may be replaced by Pathogenic Organisms. These pathogens are often present in the order of many millions per milliliter in the effluents or sewage of septic tanks. This is because excreta in these places are often suspended in a very small volume of water (*Suess, 1982*). *The World Health Organisation (1974)* International Standards for drinking water listed the indicator organisms to be examined in the analysis of water to include: Coliform bacteria, ***Faecal streptococci, E. coli*** and anaerobic spore forming organisms. These are discussed below:

2.1.1 Coliforms:

Coliforms are Gram-negative, oxidase negative, non-sporing rods capable of growing aerobically on agar medium containing bile salts, and capable of fermenting lactose within 48hours at a temperature of 37°C with the production of both acid and gas. These organisms belong to the family Enterobacteriaceae

(*Prescott et al; 1990*). Routine bacteriological analysis of water is aimed mainly at detecting and enumerating coliform organisms (*Suess, 1982*). Their presence in drinking water is an indication of a potential public health hazard because of the possible presence of pathogenic enteric organisms responsible for human diseases (*Itah et al; 1996*).

The coliform bacteria include ***E. coli, Enterobacter aerogenes, Klebsiella pneumonia*** etc. (*Prescott et al; 1990*). ***E. coli*** inhabits primarily the gastro intestinal tract of man and animals. It produces indole in pepton water containing tryptophan and can use Sodium citrate as sole carbon source (*Okafor, 1985*). ***Enterobacter aerogenes*** occasionally are found in man but are more associated with vegetation (*SMEWW, 1989; Itah et al; 1996*). Coliforms are the most sensitive faecal indicators at our disposal and number about 10^6-10^9/g in human faeces (*Meesters, 1983*).

There are two main groups of coliforms. These are faecal and non-faecal coliforms (together forming total coliforms). The former are exclusively faecal in origin; the later can often be found in faeces but are also naturally occurring in faecally unpolluted waters and soil. Hence the presence of the later is regarded, as presumptive evidence for faecal pollution and they should as well as faecal coliforms be absent from treated water supplies (*Okafor, 1985*). In the bacteriology of surface and wastewater, they are of much less importance as under suitable conditions (e.g. in the presence of decaying vegetation and especially in hot climates, multiplication of non faecal coliforms in the environment can take place and occurrence is hence not necessarily related to faecal pollution or to the degree of the later. Therefore, in hot climates only faecal coliforms should be used as faecal tracers in the examination of surface and wastewater and treatment and reuse process of the later (*Meesters, 1983; Schlegel, 1985*).

Two methods are used in the test for coliforms, these are, Most Probable Number (MPN) and Membrane Filtration Methods described by *Okafor (1985)*,

and *Prescott et al (1990)*. In the membrane filtration techniques, the water sample is passed through a membrane filter, and the filter with its trapped bacteria is transferred to the surface of a solid medium or to an absorptive pad containing the desired liquid medium. The membrane filtration method is preferred over the MPN because

(i) It saves labour, media and glasswares.

(ii) Neither spore-bearing anaerobes, nor mixture of organism, which may give false positive results on membrane are allowed to be trapped and possibly overgrown on the filter.

(iii) It could be done rapidly, thus obtaining direct counts of coliforms and ***E. coli*** in 18hours without the use of probability tables.

(iv) A sample may be filtered on the spot with limited facilities instead of taking the liquid to the laboratory.

The membrane filtration method, however, has certain disadvantages. These are as follows (*Suess, 1982*).

(i) Unlike the MPN method where there are replicates, it does not hold in membrane filtration method; therefore, the results may not be confirmed.

(ii) When non-coliforms predominate over coliforms, the former may overgrow the membrane. Thus making counting of coliforms difficult and laborious.

(iii) Membrane filters are unsuitable for waters of high turbidities in which the required indicator organisms are also few in numbers, since they are blocked before enough organisms have been collected.

(iv) If non-gas producing lactose-fomenters predominate in the water, false results will be obtained.

2.1.2 *Faecal streptococci*

The presence of these organisms in water shows a fairly recent faecal pollution (*Cheesbrough, 1991; Itah et al; 1996*). The ***Faecal streptococci*** group consists of a number of the species of the genus ***Streptococcus***. These include ***S. faecalis, S. avium, S. bovis*** and ***S. equinus***. The members all react positively with Lance fields' group D antisera and have been isolated from the faeces of warm-blooded animals (*SMEWW, 1989*). *Phirke and Verma (1972)* suggested that faecal streptococci provide valuable additional data in determining faecal water contamination if conducted with faecal coliform test.

Similarly, *Meesters (1983)* stated that the main value of ***Faecal streptococcus*** test helps in assessing the significance of doubtful results from the coliform tests, particularly when large members of coliforms occur in the absence of ***E. coli***. Detection of ***Faecal streptococci*** would then confirm the ***faecal*** origin of pollution. Hence ***faecal streptococci*** tests are mostly used supplementary to coliform tests and are especially of value in national water and samples from repaired mains.

Faecal streptococci have the ability to grow at 45°C, 10% bile and in concentrations of sodium azide, which are inhibitory to coliform organisms and most other Gram-negative bacteria (*SMEWW, 1989; Cheesbrough, 1991*).

The number of ***Faecal streptococci*** in human faeces is $10^5 - 10^8$/g, hence in general, smaller than that of total coliforms, and faecal coliforms may vary considerably between different communities due to dietary differences (*Meesters, 1983*). According to *Prescott et al (1990)*, the ratio of faecal coliforms to faecal streptococci is useful in providing information on the source of pollution (human versus animal) (*Junaidu et al; 2000*).

2.1.3 *Clostridium perfringens*

The genus Clostridium consists of gram-positive, spore-forming rods that are widely distributed in nature. It is differentiated from Bacillus on the basis of

being anaerobic and catalase negative. The large genus with nearly a 100 species is extremely varied in its habitats. Saprobic members reside in soil, sewage, vegetation and organic debris, and commensals inhabit the bodies of humans and other animals. Infections caused by pathogenic species are not normally communicable, but occur when spores are introduced into injured skin (*Talaro and Talaro, 1996*). The majority of clostridial soft tissue and wound infections are caused by ***Clostridium perfringens*** and the disease is called gas gangrene in reference to the gas produced by the bacteria growing in the tissue (*Prescott et al; 1990*). ***Cl. perfringens*** produces several physiologically active toxins; the most potent one, alpha toxin (lecithinase C), causes red blood cell rapture, edema, and tissue destruction. Additional enzymes that enhance tissue destruction are collagenase, hyalurimidase, and Dnase and the gas formed in tissues is due to fermentation of muscle carbohydrates (*Talaro and Talaro, 1996*).

Bonde (1977) and Cheesbrough (1991) advocated the use of this bacterium as an indicator organism of faecal pollution. It is regularly found in faeces, though its numbers are much smaller than ***E. coli*** in the faecal matter. Clostridial spores are capable of surviving in water for a longer time than organisms of faecal origin, and usually resist chlorination at doses normally used in water (*Akinluyi, 1981; Junaidu et al; 2000*). Its presence in a natural contamination in the absence of organisms of the coliform group, suggest that the contamination occurred long ago (*Agbu et al; 1988*).

2.1.4 *Bifidobacterium species* and *Pseudomonas aeruginosa*

Bifidobacterium species are non-sporulating anaerobic bacteria, normally occurring in human and animal faeces. In human faeces, they are present in large numbers $10^8 - 10^{11}$/g, exclusively faecal in origin and not growing outside the intestine. The most common species are ***B. adolescence*** and ***B. longum***. They have been proposed for use as faecal indicators in tropical waters (*Evison*

and James, 1984) as they overcome the principal disadvantage of faecal coliform counts in tropical samples possibly containing a significant proportion of coliforms strain able to ferment lactose and produce indole at 44°C, but of non-faecal origin. However, information on survival in extra-intestinal environments other than river waters is still insufficient, and additional studies need to be carried out.

Pseudomonas aeruginosa

The ***pseudomonas*** is a large group of free-living bacteria, which live primarily in soil, seawater, and fresh water. They are small, gram negative rods with single polar flagella and produce oxidase and catalase but do not ferment carbohydrates (*Talaro and Talaro, 1996*).

Pseudomonas has extensive impact on ecology, agriculture and commerce. Those species that can grow in fossil fuels create a problem in manufacturing petroleum products. This very same characteristic makes them useful in cleaning up oil spills (*Gilbert and Higgins, 1987*).

Pseudomonas aeruginosa is an intestinal resident in about 10% of normal people. On occasion, it can be isolated from saliva or even the armpit or groin. Because the species is resistant to soaps, dyes quaternary ammonium disinfectants, drugs, drying and temperature extremes, it is a chromic nosocomial pathogen that is difficult to control. It is a frequent contaminant of humidifiers, ventilators, intravenous solutions, and anesthaesia and resuscitation equipments. Even disinfected instruments, utensils, bathroom fixtures and mops have been incriminated in hospital outbreaks (*Talaro and Talaro, 1996*). In a pattern similar to that of the enteric bacteria, ***Ps. aeruginosa*** is a typical opportunist. Healthy people are subject to outbreaks of skin rashes, urinary tract infections and external ear infections from community hot tubs and swimming pools. Sponges and washcloths serve as a common reservoir for this species which when rubbed into the skin can cause a rash. Unusual characteristics of

Ps. aeruginosa infections are a grapelike odour and the noticeable colour that appears in tissue, pus, or other exudates ("blue-pus"). The colour is due to the bacterium's production of a blue-green or greenish-yellow pigment (pyocyanin) that can fluoresce. Laboratory identification relies on a battery of tests similar to those for ***Enterobacteriaceae***, ***Ps. Aeruginosa*** has a very doubtful status as intestinal indicator organism (*Okuofu et al; 1990*). Other non-sporing anaerobes, normally occurring in faeces have been proposed as faecal indicators e.g. ***Bacteroids species*** (especially ***B. fragilis***), ***Peptococcus*** and ***Peptostreptococcus*** species and ***Eubacterium*** species (*Suess, 1982*).

2.2 LOGICAL BASIS FOR BACTERIOLOGICAL EXAMINATION OF WATER

Water is one of the most abundant commodities in the planet earth since it occupies about 70% of the earth's surface (*Okafor, 1985*). For water to be safe for consumption, it must be free from pathogenic organisms or other biological forms, which may be harmful to health. Besides, it should not contain chemicals, which may be physiologically harmful. Bacteriology offers the most delicate test for the detection of recent and potentially dangerous faecal pollution (*Volks and Wheeler, 1988*). It is necessary for the day-to-day assessment of the bacterial purity and safety of water supplies. It is mainly concerned with:

(a) Detection and assessment of the degree of faecal pollution in a potential source of water supply in order to design a suitable method of treatment.

(b) Demonstration by regular bacteriological surveillance that the quality of water is maintained throughout distribution.

(c) Confirmation of hygienic safety of the final water entering the water supply system to the public.

Contamination by sewage or human excrement is the greatest danger associated with drinking water. Control of enteric diseases transmitted through water is accomplished through purification of water supplies and proper sewage disposal (*Talaro and Talaro, 1996*). The purification methods may include filtration, boiling and addition of alum (alluminium sulphate). Alum forms a gelatinous floc that gradually settles out, carrying along particulate matter that includes a large number of microorganisms (*Prescott et al; 1990*). Even after filtration and coagulation, the possibility still exists that water may contain some bacteria hence the need for disinfection (*Talaro and Talaro, 1996*). This is accompanied by adding chlorine to the water. Chlorine is a very effective bactericidal compound even when used in a concentration of 1 or 2 parts per million (ppm). In addition, it is fairly stable (in the absence of excess organic matter) and reasonably inexpensive (*Bello et al; 1996*).

2.3 BACTERIOLOGICAL STANDARDS OF WATER QUALITY.

The quality of water and its suitability for particular purposes are assessed greatly by carrying out bacteriological examination. The quality of drinking water should be determined by the types of microbes rather than number (*Itah et al; 1996*). *Holderness and Lambert (1982)* stated that pure water does not exist in natural state but supplies of water are obtainable over the world in degrees of purity from rainwater (which contains 0.005% of solid impurities) to seawater (in which impurities reach 3.6%).

It is for this, reason that the governments of various countries set standards to be met for drinking water. For instance, the United States Environmental Protection Agency in 1977 produced sets of standards for that country while *World Health Organisation (1971)* produced original standards for drinking water. *WHO (1974)* recommended that, for untreated water such as well water, the coliform index should be less than 10, and no sample should be greater than 20 in the MPN index. The standards however, may not be

obtainable in all countries, particularly in the third world countries like Nigeria where 75% of her populace live in rural areas.

2.4 WATER BORNE DISEASES

INTRODUCTION

Where potable water is not available and people either consciously or unconsciously drink contaminated water, transmission of water born diseases becomes eminent. Some of these diseases are discussed below.

2.4.1 TYPHOID FEVER

The ***Salmonellae*** and ***Shigellae*** are distinguished from the coliforms and the proteus group by having well developed virulence factors, being primarily pathogens, and not being normal flora of humans (*Talaro and Talaro, 1996*). The illnesses they cause-called ***Salmonelloses*** and ***Shigelloses***-show some gastrointestinal involvement and diarrhoea but also often affect other systems as well.

The most serious pathogens in the genus ***salmonella*** is ***S. typhi***, which causes typhoid fever. The other members of the genus are divided into two subgroups: ***S. cholerae-suis***, a zoonosis of swine and ***S. enteridis***, a large super species that includes around 1700 different sero types, based on the major O, H and Vi antigens.

Salmonellae are motile, they ferment glucose with acid and sometimes gas, and most of them produce H_2S but not Urease. They are not fastidious and grow rapidly on most laboratory media and can survive outside the host in inhospitable environments such as freshwater and freezing temperatures. These pathogens are resistant to chemicals such as bile and dyes (The basis for isolation on selective media) and do not loose virulence on long term artificial cultivation (*Bonde, 1977*).

Typhoid fever is so named because it bears a superficial resemblance to typhus, a rickettsial disease, even though the two diseases are otherwise very different (*Talaro and Talaro, 1996*). While the incidence of typhoid fever has been on the decline in the United States, it is still a serious health problem in

other parts of the world, responsible for 25,000 deaths each year and probably millions of cases (Center for Disease Control – CDC, 1999). In poor developing nations, where the availability of potable water and sanitary conditions are inadequate, typhoid fever has become endemic and fatality is quite high (*Itah et al; 1996*). In Nigeria, inadequate data has made it impossible to have specific figures but the disease is quite alarming among the populace (*Hutley et al; 1990; Inabo, 1996*).

The typhoid bacillus usually enters the alimentary canal along with water or food contaminated by faeces, although it is occasionally spread by close personal contact (*Prescott et al; 1990*). Since humans are the exclusive hosts for ***S. typhi*** a symptomatic carriers are important in perpetuating and spreading typhoid. Even six weeks after convalescence, the bacillus is still shed by about half of recovered patients. A small number of people chronically carry the bacilli for longer periods in the gallbladder; from this site the bacilli are constantly released into the intestine and faeces (*Jawetz et al; 1987*). This carrier state, combined with unclean personal habits provides a recipe for disaster.

The size of the ***S. typhi*** dose that must be swallowed to initiate infections is between 1,000 and 10,000 bacilli. At the mucosa of the small intestine, the bacilli adhere and initiate a progressive, invasive infection that leads eventually to septicemia. The typhoid bacillus infiltrates the mesentric lymph nodes and the phagocytes of the liver and spleen. The periodic escape of bacilli from infected lymphoid tissue gives rise to bacteremia and establishment in organs. The disease has an insidious onset and is characterized by a headache and sustained fever as high as $103^{o} - 104^{o}C$ ($39 - 40^{o}C$) malaise, stomach pains, loss of appetite, chills, constipation and myalgia. Diarrhoea is uncommon. As the enteric progresses, the lymphoid tissue of the small intestine develops areas of ulcerations that are vulnerable to haemorrhage, and in a few patients, deep erosion, perforation, and peritonitis occur. Untreated cases last a month, with

about a 10% - 15% mortality rate (*Levy et al; 1998*). Prompt treatment greatly reduces deaths.

A preliminary diagnosis of typhoid fever can be based upon the patient's history and presenting symptoms, supported by a rising antibody titre. However, definitive diagnosis requires isolation of the typhoid bacillus (*Cheesbrough, 1991*). Treatment is commonly through the administration of antibiotics and chloramphenicol and ampicillin are the drugs of choice, though some resistant strains occur. Chronic carriers are also similarly treated but surgical removal of the gallbladder may be necessary in individuals with chronic gallbladder inflammation. Many vaccines against typhoid fever exists but could loose effectiveness after sometime hence the need for booster doses (*CDC, 1999*) some of these vaccines include TY21a (Vivotif Berna) VICPS (Typhim Vi) and inactivated typhoid vaccine (Wyeth Ayerst) etc. In Nigeria, a group at the Federal University of Technology Minna has undertaken Phase I trial of a typhoid fever vaccine but work is still in progress. The disease can best be avoided though proper hygiene, purification of drinking water, pasteurization of milk, prevention of food handling by carriers and complete isolation of patients if possible.

2.4.2 SHIGELLOSIS

Shigella causes a common but often incapacitating dysentery called shigellosis, which is marked by crippling abdominal cramps and frequent defection of watery stool filled with mucous and blood. The aetiologic agents (***Shigella dysenteriae, Sh. sonnei, Sh. flexneri*** and ***Sh. boydii***) are primarily human parasites though they can infect apes (*Talaro and Talaro, 1996*). All produce similar disease that can vary in intensity. They are non-mobile, non-encapsulated, and not fastidious, and do not produce H_2S or Urease (*Geldreich, 1992*). The ***shigellae*** resemble some types of pathogenic ***E. coli*** so closely that they are placed in the same subgroup.

In the early 1990's a dramatic increase in the incidence of shigellosis was reported in the USA with about 32,000 cases reported per year, which is the highest incidence since 1955 (*Levy et al; 1998*). Children between one and 10 accounted for more than half of the cases. This statistic is expectedly much higher in the poor developing countries of Asia and Africa (*Galinas, et al; 1996*). In addition to the usual oral route, shigellosis is also acquired through direct person-to-person contact, largely because of the small infectious dose required (200 cells). The disease is mostly associated with lax sanitation, malnutrition and crowding, and is spread epidemically in day care centers, prisons, mental institutions, nursing homes and military camps. As in other enteric infections, a chronic carrier period of weeks to months occurs in some people (*Chessbrough, 1991*).

Shigellosis is different from Salmonellosis in that ***Shigella*** invades the villi cells of the large intestine, rather than the small intestine. In addition it is not as aggressive as ***Salmonella*** and does not perforate the intestine or invade the blood. The symptoms are caused by the release of toxins at the site of multiplication. Endotoxin causes fever, while enterotoxins brings on inflammation of the underlying gut wall layer, degeneration of the villi, and local erosion that causes bleeding and heavy mucous secretion. Abdominal cramps and pains are caused by the disruption of the muscular function of the intestine. ***Shigella dysenteriae*** produces a heat-labile exotoxin (Shigatoxin) that has a number of effects, including injury to nerve cells and nerves, and damage to the intestine.

Diagnosis is complicated by the coexistence of several alternative candidates for bloody diarrhoeae such as ***E. coli*** and the protozoans ***Entamoeba histolytica*** and ***Giardia lamblia*** (*Cheesbrough 1991*). Isolation and identification follow the usual protocols for enterics. Infection is treated by fluid replacement and oral drugs such as ampicillin and Sulfatrimethoprim (SXT) unless drug resistance is detected, in which case nalidixic acid or cephalosporins

are prescribed (*Talaro and Talaro, 1996*). Prevention follows the same steps as for Salmonellosis and there is no vaccine.

2.4.3 CHOLERA

Cholera is caused by ***Vibrio cholerae*** a comma shaped organism that shares many, cultural and physiological characteristics with members of the Enterobacteriaceae, a closely related family (*Talaro and Talaro, 1996*). They are fermentative, grow on ordinary or selective media containing bile at 37°C and are oxidase positive. They possess unique O(somatic) antigens, H(flagella) antigens and membrane receptor antigens that provide some basis for classifying members of the family.

Epidemic cholera or Asiatic cholera has been a devastating disease for centuries. Although the human intestinal tract was once thought to be the primary reservoir, it is now known that the parasite is free-living in certain endemic regions (Levy et al; 1998). The microbe is spread by water and food contaminated by asymptomatic carries in non endemic areas and additionally by poor sanitary conditions in endemic areas of the world. *Umoh et al; (1983)*, described the epidemiological features of an outbreak of gastroenteritis and cholera in Katsina, Northern Nigeria. Over 5000 people were officially treated of a cholera outbreak epidemic that ravaged Kano, Nigeria in which more than 1,000 deaths occurred (*Ogunleye, 2001*). A re-occurrence is a possibility anywhere due to the poor sanitary conditions and lack of potable water supplies.

Following ingestion with food or water, ***V. cholerae*** encounters the potentially destructive acidity of the stomach, which influences the size of infectious dose (10^8 cells) (*Cheesbrough, 1991*), though certain types of food also shelter the pathogen more readily than others. The ***Vibrios*** penetrate the mucosa and adhere to the outside of the epithelium, and multiply there. The cells are strictly epipathogens that do not enter the cells or invade the mucosa. The virulence of ***V. cholerae*** is due entirely to an enterotoxin called cholera

toxin (CT) that disrupts the normal physiology of intestinal cells. When this toxin binds to specific intestinal receptors, a secondary signaling system is activated. Under the influence of this system, the cells shed large amounts of electrolytes into the intestine, an event that is accompanied by profuse water loss (*Stryer, 2000*). Most cases of cholera are mild or self-limited, but in children and weakened individuals, the disease can explode with tremendous force.

Incubation period ranges between a few hours to a few days and the symptoms begin abruptly with vomiting, followed by copious watery faeces called secretory diarrhoeae. This voided fluid is odourless and contains flecks of mucous, hence the description "rice water stool". Fluid losses of nearly one liter per hour have been reported in severe cases, and untreated patients can loss up to 50% of body weight during the cause of the disease. The diarrhoeae causes loss of blood volume, acidosis from bicarbonate loss, and potassium depletion that predispose the patient to muscle cramps, severe thirst, flaccid skin, and sunken eyes, and in young children, coma, convulsions and fever. Secondary circulatory consequences can include hypertension, tachycardia, cyanosis and collapse from shock within 18 – 24 hours. If cholera is left untreated death can occur in less than 48 hours and the mortality rate approaches 55% (*Jones, 2001*).

During epidemics, clinical evidence is usually sufficient to diagnose cholera. But confirmation for the disease is often required for epidemiological studies and detection of sporadic cases. ***V. cholerae*** can be readily isolated and identified in the laboratory from stool samples. Direct dark field microscopic observation reveals characteristics curved cells with brisk, darting motility as confirmatory evidence. Immobilization or fluorescent staining of faeces with group specific antisera is supportive as well. Difficult or elusive cases can be treated by detecting a rising antitoxin titre in the serum (*Levy et al; 1998*).

The key to cholera therapy is prompt replacement of water and electrolytes, since their loss accounts for the severe morbidity and mortality and cases in which the patient is unconscious or has complications from severe dehydration require intravenous replenishment as well. Only after the patient has been treated for physiologic disruption can antibiotics to control the growth of the microbes be employed. Oral antibiotics such as tetracycline and drugs such as trimethoprium-sulfa can terminate the diarrhoeae in 48hours, and they also promote recovery and diminish the period of ***vibrio*** excretion.

Prompt and aggressive detection and treatment alone are insufficient to control cholera. Effective prevention is contingent upon proper sewage disposal and water purification. Detecting and treating carriers with mild or asymptomatic cholera is a serious goal, but one that is frequently difficult to attain because of inadequate medical provisions in those countries where cholera is endemic (*Briscoe, 1978*). Results of vaccination have been luck luster. The principal vaccine containing killed cholera ***vibrios*** protects for only six months or less and does not stop the continued shedding of ***vibrios*** by infected individuals. An oral vaccine using live attenuated ***vibrios*** is 85% effective but does not have lasting effects (*Talaro and Talaro, 1996*).

2.4.4 INFECTIOUS HEPATITIS

Any infection that results in inflammation of the liver is called hepatitis. Three closely related viruses and one agent are responsible. Hepatitis A (infectious hepatitis) is transmitted by faecal-oral contamination. The other types include hepatitis B (serum hepatitis); non-A, non-B hepatitis and hepatitis associated with delta agent.

Hepatitis A is usually transmitted by faecal-oral contamination of food, drink or infected shellfish that live in contaminated water (*Prescott et al; 1990*). The disease is caused by the hepatitis A virus. The hepatitis A virus is

icosahedral, single-stranded RNA virus that lacks an envelope and is quite, different from the hepatitis B virus.

Once in the digestive system, the viruses multiply within the internal epithelium. In most cases only mild intestinal symptoms result. Occasionally viremia (the presence of viruses in the blood) occurs and the viruses may spread to the liver, kidneys and spleen.

Symptoms last from 2 to 20 days and include anorexia, general malaise nausea, diarrhoea, fever and chills. If the liver becomes infected, jaundice ensues.

In general, the disease is associated with insufficient personal hygiene and lack of public health measures. In countries with inadequate sewage control, most outbreaks are associated with faecally contaminated water and food. The United States has a yearly incidence of 20, 000-25000 cases (*Talaro and Talaro, 1996*). Most of these results from close institutional contact, unhygienic food handling, eating shellfish, sexual transmission, or travel to other countries. Occasionally, blood or blood products can spread hepatitis A. In developing countries, children are the most common targets, because exposure to the virus tends to occur early in life, while in North America and Europe, more cases appear in adults. The virus is not carried chronically hence the principal reservoirs are asymptomatic, short-term carries or people with clinical disease.

A patient's history, liver and blood tests, and viral identification all play a role in diagnosing hepatitis A and differentiating if from the other forms of hepatitis. There is no specific treatment for hepatitis A once the symptoms begin. Patients receiving immune serum globulin early in the disease usually experience milder symptoms. Prevention of hepatitis A is based primarily on prophylactic immunization with pooled immune serum of globulin. An inactivated viral vaccine has been approved and an oral vaccine, based on an attenuated strain of the virus is also available (*Talaro and Talaro, 1996*).

Control of this disease can be improved by sewage treatment, hygienic food handling and preparation, and adequate cooking of shellfish (*Bryan, 1977*).

2.4.5 GIARDIASIS

Giardia lamblia is a pathogenic flagellate first observed by Antonie Van Leeuwenhoek in his own faeces. For 200 years it was considered a harmless or weak intestinal pathogen and only in the last 40years has its prominence as a cause of diarrhoea been recognized. Infact it is the most common flagellate isolated in clinical specimen (*Talaro and Talaro, 1996*). This trophozoite has a unique, symmetrical heart shape with organelles positioned in such a way that they resemble a face. Four pairs of flagella emerge from the ventral surface, which is concave and acts like a suction cup for attachment to a substrate. ***Giardia*** cysts are small, compact and multinucleate.

Giardiasis has a general epidemiologic pattern similar to other protozoan intestinal infections. The protozoan has been isolated from beavers, cattle, coyotes, cats and human carriers but the reservoir is still unclear (*Graczyk et al; 1998*) unlike other pathogenic flagellates, ***Giardia*** is very hardy, and its cysts can survive for two months out of the host. Cysts are taken in with water and food, or swallowed after close contact with infected people or contaminated objects. In a study with prison volunteers, the cysts were shown to be highly infectious only 10-100 were required (*Hopkins and Juranek, 1991*). In tropical climates giardiasis is most common in children; in industrialized countries, adults are infected as frequently as children.

Instances of outbreaks of giardiasis have been so many and varied that a partial list can only hint at the possible modes of transmission. Community water supplies in areas throughout the United States have been implicated as common vehicles of infection. Giardia epidemics have broken out in resorts with pristine mountain streams and have even been traced to chlorinated municipal water supplies (*Levy et al; 1998*) G. Lamblia is worldwide in

distribution. As many as 200 million humans may be infected worldwide (*Isaac – Renton, 1987*). Cases of food-borne illness have been traced to carriers who contaminate food through unclean personal habits. Day Care Centers have reported outbreaks associated with diaper changing and contact with other types of fomites.

Ingested ***Giardia*** cysts exist in the duodenum and travel to the jejunum to feed and multiply. Some trophozoites remain on the surface, while others invade the glandular crypts to varying degrees. The outcome of infection ranges from asymptomatic to severe, chronic giardiasis superficial invasion by trophozoites causes damage to the epithelia cells, oedema and infiltration by white blood cells, but these effects are reversible. Typical symptoms include diarrhoea, abdominal pain and flatulence. Stools containing large amounts of unabsorbed fat are probably the result of impaired absorption (*Cheessbrough, 1991*). Although both trophozoites and cysts escape in the stool, the cysts play a greater role in transmission. The infection is eradicated with quinacrine or metronidazole.

Now that this parasite is apparently on the increase (*Ugbagwu, 2002*), many water agencies have to rethink their policies on water maintenance and testing. The agent is killed by boiling, ozone, and iodine but unfortunately, the amount of chlorine used in municipal water supplies does not destroy the cysts. People who must use water from remote sources should assume that it is contaminated and boil or filter it.

2.5 SOME PHYSICOCHEMICAL PARAMETERS OF WATER

2.5.1 HYDROGEN ION CONCENTRATION (pH)

The pH of a solution is the measure of its hydrogen ion concentration $[H^+]$ expressed mathematically as the negative logarithm of base 10 $-\log_{10}[H^+]$. The actual values range from 0-14 (The pH scale) (*Vesiland, 1975*).

The determination of pH in water is vital since the alkalinity and acidity of the medium do affect the growth of microorganisms and the taste of water (*World Health Organization, 1971; Vesiland, 1975; Abubakar and Ibrahim, 2002*). *Okafor (1985)* also reported that pH is one of the factors affecting the efficacy of chlorine as disinfectant in water. Chlorination is more effective at pH 7.2 and below than above 9.2. Fortunately the pH of most water ranges from 6.0 – 7.5 (*SMEWW, 1989; Odokuma and Okpokwasili, 1993; Itah et al, 1996*), hence 50 – 95% of the free chlorine will be present as hypochlorous acid (HOCl). The later is germicidal (*Talaro and Talaro, 1996*). At neutral pH, bacteria predominate over fungi but at pH below 6.5 fungi take over (*Okafor, 1985*).

pH is among the important physicochemical parameters routinely taken in water analyses. *Abubakar and Ibrahim (2002)* found mean pH values for tube well water to be 7.2; *Itah et al (1996)* gave a mean pH range of between 6.8-7.8 for various water sources in Calabar, Nigeria.

2.5.2 HEAVY METALS

There are approximately 65 elements that exhibit properties, which may be termed "heavy metals" (*Duxbury, 1986*): In the biological context, the principal chemical species are cations and the many definitions based on for example density, reactivity or position in the periodic table include a wide variety of elements with diverse chemical and biological properties (*Gadd, 1986*). A general feature of the heavy metals is their well-known potential toxicity towards microbial and other life forms, which is the basis of many

biocidal preparations (*Genarro, 1995*). However, many "trace elements" are essential for growth and metabolism at low concentrations (ppm) e.g. Cu, Zn, Fe, Ni, Mn, Co and micro organisms posses mechanisms of varying specificity for their intracellular accumulations from the external environment. In contrast, many others have no biological functions e.g. Pb, Sn, Cd, Al, Hg etc yet may be accumulated (*Klein* and *Snodgrass, 1997*) and be highly toxic.

(i) Lead (Pb)

Lead is unique among metals because of its softness, malleability, and low melting point. Lead has been used by people for several thousands of years. The oldest known decorative lead object has been dated at about 4000BC (*Kendler, 1993*). Because of its durability and resistance to both corrosion and the freeze-thaw cycle, lead has been used for centuries in plumbing. Lead tends to expand with water, and even if a pipe bursts the fracture is small and easily repaired. Currently, the primary use of lead is in storage batteries. Another major use is in solder, as lead readily forms alloys with other metals. Dozens of other contemporary use of lead include those in radiation shielding, ammunition, cables, gasoline, photography, dyeing and printing etc. until recently Pb colour paints were used in the USA but have now been banned (*United States Public Health Service, 1998*).

The primary source of lead in the environment is from emissions into the atmosphere, mainly from the combustion of leaded gasoline, lead smelting and municipal waste incineration. Roughly, 3million tons of lead are released into the atmosphere, worldwide each year (*Kendler, 1993*). Of this less than 5% originates from natural sources such as weathering of the earth's crust and volcanic activity (*United State Public Health Service, 1998*).

Blood levels of lead in human populations from industrialized societies far exceed those found in non-industrialized populations. Up to half of the body burden of lead has been traced to atmospheric lead (*EPA, 1986*). Accordingly,

significant decreases in atmospheric lead levels would be expected to result in widespread reductions in blood lead levels. This outcome has been confirmed in the United States following the reduced use of leaded gasoline (*Macrae, 1997*).

Solid wastes constitute the next largest source of lead released into the environment. This is derived mostly from domestic Ore production and from the use of solder and ammunition. Lead released to the aquatic environment primarily originates from the Urban run off and atmospheric deposition (*Egereonu and Onuchukwu, 2000*). Extremely high concentrations of lead have been detected in freshwater sediments receiving industrial or municipal wastes (*Ayalogu et al; 2001; Haroun et al; 2002*). Up to 2g/kg have been reported in sediments from mine discharges (*Kendler, 1993*).

The total amount of lead discharge into freshwater, worldwide exceeds 100,000 metric tons annually, most of which originates from atmospheric fall out (*United States Public Health Service, 1998*). Lead tends to form compounds of low solubility, which precipitate out of solution (The solubility of lead depends upon the pH of water, acidic range being most favourable). The presence of sulphate ions in soft water, however leads to the formation of $PbSO_4$, an insoluble salt. Most of the lead is tightly bound to sediments and is thus unavailable to most aquatic organisms (*Kendler, 1993*). Studies have shown that uncontaminated seawater contains only about 0.03micrograms of lead per liter but it increases to about 10times the value of shorelines (*World Health Organisation, 1989*). Lead concentrations in fresh water are generally much higher. In a survey of 727 samples of surface water, lead levels were in the range of 50ppb (50µg/L) (*Durum et al; 1996*). Similar studies with rain water and well waters have given close values (*Ayodele and Abubakar, 1998; Abubakar and Ibrahim 2002; Garba, 2000*).

The *WHO (1989)* has stipulated 0.1mg/L as the maximum permissible level of lead in drinking water. The increasing concern about the consequences of low level lead exposure prompted the United States Environmental

Protection Agency to reduce the standard for lead in drinking water at the tap from 50 to 15μg/L (ppb) (*EPA; 1986*).

Elemental lead and both inorganic and organic lead compounds are toxic. Alkyl lead compounds still widely used in some countries including Nigeria as gasoline additives, are 100times more toxic than inorganic lead (*Kendler, 1993*). The effects of severe lead poisoning, which have been known for centuries, include paralysis, brain damage, visual disturbances and severe intestinal cramps.

Reports of clinical lead poisoning are on the decline in developed nations due to strict enforcement of regulations in industrial lead production and use but reports of toxicity do appear periodically, and occupational exposure to lead has been cited as a cause of kidney disease (*Vanderveen and Vanderveen 2000*). *Wallace and Cooper (1986)* complied a list of 120 occupations (e.g. auto mechanic, painter, printer and welder) that may involve exposure to lead. Non-occupational lead poisoning has occurred among hobbyists, home renovators and users of ceramic dinnerwares.

Low-level lead exposure, especially among young children and pregnant women is a major concern of public health authorities. Adverse effects among children include learning deficits, reading disabilities behavioral disorders, growth retardation and hearing loss (*Gennaro, 1995*).

Among the deleterious effects of even small amounts of lead in the body are the blockage of calcium channels and neurotransmitter-inhibition. Lead also interferes with the conversion of vitamin D into its active form (1,25 $(OH)_2$ cholecalciferol). An important effect of lead is interference with the enzymes used to synthesize heme (*Kendler, 1993*). Since heme is a constituent of hemoglobin and cytochrome enzymes in the mitochondria electron transport system, impaired heme synthesis can have marker effects on a variety of physiological processes (*United States Public Health Service 1989*). The heme system is affected by lead levels of only 10 - 15μg/dl of blood with major

impact occurring above 30μg/L. Blood lead levels of less than 100μg/dl can be fatal, especially in children (*Wallace and Cooper, 1986*).

(ii) Mercury (Hg)

Mercury is probably the most ubiquitous heavy metal in the environment, resulting from natural geological activities and industrial pollution (*Macrae et al; 1997*) despite being present in the earths crust at very low concentrations, about 80μg/kg (*Suess, 1982*). Although it is extremely useful, it is also highly toxic, depending on its specific chemical form.

Mercury input into the environment can be natural or man made of which the later is the most significant. Hg from natural activities can enter air, soil, and water via weathering, dissolution, vaporization and biological processes. Three oxidation states are possible in nature: metallic (Hg^{o}), mercurous (Hg^{2+}) and mercuric (Hg^{2+}). The two main categories of human activities that release Hg are:

(a) Agriculture – runoff of excess pesticide application and absorption by vegetation, roots and foliage (*Clarkson, 1990; Haroun et al; 2002*). Use of fungicides and seed preservatives also contribute Hg to the environment.

(b) Industry – Waste water discharge, disposal of solid wastes containing Hg residues (thermometers, fluorescent lamps, lab ware, paints), burning of coal and petroleum based materials (*Macrae et al; 1997; Ayalogu et al; 2001*). Other industrial contributors of Hg to the environment include chlorine and caustic soda production (electrolytic process), amalgams, as well as pulp and paper preservatives, as catalyst for plastic production in pharmaceuticals (drugs antiseptics) etc.

Natural geological activities e.g. weathering of rocks and volcanic processes also contribute to a small extent to Hg pollution in the environment.

The *WHO (1971)* maximum permissible level of Hg in drinking water is put at 0.001mg/L (1ppb) although its absence is the preferable alternative.

The hazards of Hg and its compounds have been long known and of much concern. It has historically been documented as an occupational hazard. However, it was only well into this century that specific outbreaks of environmental Hg poisoning demonstrated that specific Hg species can exert serious health effects on exposed human populations. Examples of major out breaks are:

(i) Japan (Minamata, Niigata) from the mid 1950s to the early 1960s from ingestion of contaminated fish and shellfish from waters polluted by industrial discharges.

(ii) Sweden and Canada in the mid to late 1960's from similar but milder cases of industrial pollution.

(iii) Iraq in the early 1970s, from ingestion of grain and grain products originating from seed treated with a Hg fungicide.

In each case (methyl mercury) was the causative agent (*Krenkel, 1994*). It has been predicted that environmental Hg whether from natural or human food chain and that presently, the aquatic food chain is more susceptible than the terrestrial food chain (*Cappon, 2001*).

Methyl mercury is approximately 100% absorbed in the intestine of humans and mice, while inorganic Hg salts (Mercurous and/or mercuric) are only about 15% absorbed in human volunteers (*Klein and Snodgrass, 1997*). Once absorbed from the digestive or respiratory tracts or absorbed transcutaneously, Hg produces a variety of symptoms affecting the marrow, kidneys, skin and blood cells. In children, it produces a symptom complex called acrodynia or pink disease. Acrodynia is characterized by redness of the lips and pharynx, loss of teeth, a strawberry tongue, sweating, redness desquamation of the skin, with pink or red fingertips, palms and soles (*Cappon, 2001*). Occupational exposure to Hg vapour has led to acute cases of respiratory

distress, renal failure requiring dialysis, and severe Oropharyngeal inflammation and flu-like syndrome (*Macrae et al; 1997*).

(iii) Cadmium (Cd)

Cadmium is one of the rarer elements in the earth crust, but it is fairly widely distributed and is found in shale's and igneous rocks, coal, sand stones, limestone lake and oceanic sediments, soils etc (*Zurera-Cosano, 1998*). The chemistry of Cd is similar to that of lead and zinc and is found largely in the form of the sulphides.

After Hg and Pb, Cd can probably be considered next in importance as an environmental pollutant. It may enter surface waters as a consequence of mining and smelting operations. Cd levels in water in the absence of contamination are seldom above 1µg/L (1ppb) (*Durum et al; 1996*). Its occurrence in drinking water has been of special interest since this directly affects human intake (*Foulkes, 1998*).

Contamination may occur as a result of the use of galvanized pipes and cisterns. Cd containing solders in water heaters and other fittings can be another cause. Pollution of water due to the use of Cd-rich sewage sludge for agricultural purposes has been reported (*Foulkes, 1998*).

The mean concentration of Cd in rainwater is 0.001mg/L in clean areas and 0.0037mg/L in contaminated areas (*Durum et al; 1996*).

Egereonu and Onuchukwu, (2000) analysed rainwater in Port Harcourt, Nigeria and the Cd content were discovered to be high (0.02mg/L). Groundwater Cd concentration as high as 3.2mg/L has resulted from the seepage of Cd from electroplating plants (*Durum et al; 1996*). *Kopp and Kroner (2001)* detected dissolved Cd in less than 3% of 1577 water samples collected in the United States of America but reported that one sample contained 0.12mg/L. The World Health Organization permissible level of Cd in drinking water is 0.01mg/L (*WHO, 1974*). Initial acute toxicity by Cd includes nausea and

vomiting which is usually so violent that little of the Cd is absorbed and fatal poisoning does not occur. Additional important symptoms may include salivation, abdominal pain, diarrhoea and headaches (*Durum et al; 1996*). The kidney is the critical organ in poisoning resulting from long term (Chronic) excessive environmental and occupational exposure to Cd. Cancinogenicity are other reported possible outcomes of Cd intoxication (*Foulkes, 1998*).

The sources of environmental pollution by Cd are many and include smelting and plating operations, Lithography, engraving soldering and welding. Others include dust and fumes from the mining and refining of metals, miscellaneous industrial sources, as an impurity in fertilizers, petrol, oil, coal, organic waste products and even cigarette smoke (*Friberg et al; 1994*).

Cadmium is toxic to man; the hazardous nature of Cd to human health is as a result of its long-term persistence in the environment. Its rapid uptake and accumulation by food crops, its inherent, high toxicity and its efficient retention and accumulation in the body throughout life (*Foulkes, 1998*).

(iv) Cobalt (Co)

Cobalt is widely distributed in the earth's crust but only 30th in order of abundance and thus less common at 25mg/kg than all other elements in the first transition series with the exception of scandium (22mg/kg) (*Smith and Carson, 1990*). Cobalt is found only in trace amounts because it is adsorbed to sediments. *Durum et al; (1996)* found that 63% of over 720 surface water samples analysed in the USA contained less than 1µg Co/L (1ppb).

Cyanocobalamine is synthesized in nature only by microorganism e.g. those found in the rumen of ruminants. Human like non-ruminants must have their requirement for Co by consuming foods rich in cyanocobalamine (*Macrae, 1997*).

(v) Copper (Cu)

Copper occurs in nature as ores, less commonly, as metal deposits. The most common ores of Cu are sulphide, oxide and Carbonate salts. All soils and plants and animal tissues contain at least trace amounts of Cu (*Durum et al; 1996*). Most Cu minerals are relatively insoluble and Cu is always sorbed to solid phases hence only low concentrations are normally present in natural waters (*Suess, 1982*). Equilibrium with CuO or hydroxycarbonate minerals would limit the concentrations of free Cu in aerated H_2O to about 64µg/L (64ppb) at pH 7.0 and about 6.4ppb at pH 8.0. Because of the presence of sulphides, Cu would be expected to be even less soluble in anoxic systems (*Smith, 1987*). A mean concentration of 15µg/L (15ppb) free copper have been found for surface waters in the United States of America by *Kopp and Kroner (2001)*, and *Smith and Carson (1990)* reported similar values in a survey of rivers. A range of 0.03 and 1.53mg/L Cu was found in some rural water supplies in Calabar, Nigeria (*Itah et al; 1996*). Data from rainwater in Port Harcourt Nigeria gave 0.011mg/L Cu (*Egereonu and Onuchukwu, 2001*).

The presence of higher concentrations of Cu can usually be attributed to corrosion of Cu pipes, industrial wastes or particularly in reservoirs, the use of $CuSO_4$ as an algicide. Because of the astringent taste caused by the presence of Cu in drinking water and its discoloration and corrosion of pipes, fittings and utensils, the *WHO (1974)* gave 0.05mg/L and 1.5mg/L as the maximum desirable and permissible levels respectively in drinking water.

10 – 30mg of orally ingested Cu from various ionic salts or from foods stored in Cu vessels may cause intestinal discomfort, dizziness and headaches. Ingestion of Cu salts in excess of 500 – 1000mg has caused acute poisoning in humans and has been fatal (*Macrae et al; 1997*). Acute copper poisoning is very similar to poisoning by other heavy metals and causes vomiting, diarrhoea with bleeding, circulatory collapse, failure of liver and kidneys and severe haemolysis (*Fewtrel, et al; 1996*).

(vi) Iron (Fe)

Iron is an abundant element in the earths crust (1.5%, the 8th most abundant). It however generally exists in only minor concentrations in natural water systems. The form and solubility of Fe in natural waters are strongly dependent upon the pH and the redox potential of the water (*Suess, 1982*). Surface waters in a normal pH range of about 6 – 9 rarely carry more than 1mg of dissolved Fe per liter. However in ground water affected by mining, the quantities of Fe routinely measured may be several hundreds of milligrams per litre (*Macrae et al; 1997*). Seawater contains low concentrations of Fe (0.002 – 0.02 ppm) complexed by chloride ions. *Itah et al; (1996)* found Fe levels of 0.05 – 0.36mg/L in some rural water supplies in Calabar, Nigeria, while *Egereonu and Onuchukwu (2000)* found a level of 0.046mg/L in rain waters in Port Harcourt, Nigeria. The *WHO (1974)* established 0.1mg/L and 1.0mg/L as the highest desirable and maximum permissible levels of iron respectively in water intended for consumption and other domestic use.

Iron plays a central role in metabolic processes involving O_2 transport and storage as well as oxidative metabolism and cellular growth. The fact that it readily serves as an electron donor or acceptor accounts both for its potential toxicity. The normal human body contains 3.4g of iron (40 – 50mg per kg body weight). 75% (approximately 36mg/kg) of which is present in metabolically active compounds. The remainder is contained in a storage pool (approximately 10mg/kg in men and 5mg/kg in menstruating women), which is readily available if metabolically active Iron is depleted for any reason (*Macrae et al; 1997*).

More importantly from the point of toxicity, is the gradual accumulation, which occurs when the quantity of Fe entering the body exceeds requirements by even a small margin. The body has no means of increasing Fe excretion significantly. The outcome is the pathophysiological presentation observed for Chronic Fe toxicity (*Durum et al; 1996*).

2.6 CONTAMINATION OF THE ENVIRONMENT BY HYDROCARBONS

In addition to the long-standing problem of microbiological pollution, industrialization and modernization in general have introduced a wide range of chemicals e.g. fertilizers and other agrochemicals and hydrocarbons into the list of pollutants into the environment. Hydrocarbons are components of petroleum (crude oil). Crude oil is a naturally occurring and extremely complex mixture of hydrocarbons including small quantities of compounds that contain oxygen (alkylthiol, thiophene) and nitrogen (pyridine, pyrrole, indole) as well as trace amounts of metallic constituents (*Bossert and Bartha, 1984*).

The demand for petroleum has been on the increase not only as an energy source, but also as raw materials for the production of pharmaceuticals, agricultural chemicals and feeds, plastics, cosmetics, detergents, fibers and other polymers like lubricating agents, stabilizing agents etc (*Bello, 1995*). The proportional increase in the production, refining and distribution of petroleum in order to fulfill the on going demand has brought an ever-increasing problem of environmental pollution. According to *Bartha (1986)*, World wide, over 2Billion metric tons of petroleum are produced annually, and it has been estimated that 1.7-8.8 metric tones end up polluting the oceans. No comparable estimates have been worked out for terrestrial petroleum hydrocarbon (PHC) pollution but considering that the greater portion of petroleum is produced, refined and utilized on land the resulting routine, accidental and illegal discharges are likely to equal if not exceed the figure cited for the marine environment *(Bello, 1995)*.

Both in the marine and the terrestrial environments, the low level routine discharges (effluents, urban run – offs, cleaning operations, oil treatment of roads for dust control, etc), account for probably over, 90% of the total PHC pollution. Production and transportation accidents such as well blow outs, tanker disasters and pipeline breaks accounts for only 5 – 10% of total PHC

discharge but because of their drastic and highly visible local effects, press coverage and public attention is disproportionately focused on these types of incidents.

PHC and other poisonous chemicals are known to percolate the layers of the earth and terminate in underground waters thereby constituting public health hazards (*Itah et al, 1996*). Accidental and deliberate blowouts of pipes located in the ground used for the distribution of PHC, as is the common practice in Nigeria and other parts of the world contribute immensely to ground water pollution. In the case of wells, surface run offs especially in the rainy season, constitute an additional source of PHC in such water bodies (*Agbu et al; 1988, Adesiyun et al; 1998).*

Amund et al. (1993) reported that an important source of petroleum contamination of the Nigeria environment that has attracted little public awareness is the indiscriminate discharge of used crankcase oil (lubricants) on the soil. This could percolate the soil or be carried by runoffs into wells and ground water especially in the vicinity of such dumpsites.

When natural ecosystems are contaminated with PHC s, the indigenous microbial communities are likely to contain microbial populations of different taxonomic characteristics, which are capable of degrading the contaminating hydrocarbons (*Amund et al, 1993*). Several workers have identified such microorganisms especially in the soil ecosystem (*Ijah et al, 2000, Ijah and Antai, 2003*). Infact, *Mulkins–Phillips and Stewart (1974)* have asserted that the measurement of hydrocarbon utilizing bacteria should indicate the microbiological capability of the location to handle oil pollutants. Microorganisms with capability to utilize PHCs as sole sources of carbon and energy are not restricted to a few genera. They include bacteria; yeasts, fungi and even alga have been implicated in PHC biodegradation in both aquatic and terrestrial habitats. *Bossert and Bartha (1984)* have found 22 and 31 genera of bacteria and fungi respectively. Some examples include ***Nocardia spp,***

Pseudomonas,Flavobacterium, Vibrio, Achronobacter, Arthrobacter, Bacillus, Corynebacterium, Acinetobacter, Aspergillus, Penicillium, Candida and Hansenulla (*Ijah and Antani, 2003*).

The consumption of water polluted with hydrocarbons is injurious to health. Petroleum contains mutagens, carcinogenic and growth inhibitory substances (*Gutnick and Rosenberg, 1977, Bossert and Bartha, 1984*). Industrial hydrocarbon wastes e.g. polychlorinated benzenes and hydrocarbon solvents such as benzene and toluene are often hard to detect and remove and hence persist in aquatic environment (*Talaro and Talaro, 1996*). Pb and other additives are also highly toxic. These material act in many ways to effect toxicity such as the blocking of oxygen penetration of tissues (*Cooney, 1984*). Others poison the haemoglobin and also block mitochondrial electron transport, hence resulting into acute toxicity, etc (*Stryer, 2000*).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 DESCRIPTION OF STUDY SITES

Wells sited near mechanic workshops in Bosso, Chanchaga, Keteren-Gwari and Maitumbi, which are suburbs of Minna, Niger State, Nigeria were studied (Fig1). Bosso village is located 6km from Minna on the Kontagora-Sokoto route. The Federal and State low cost houses, secondary and primary schools are situated in this satellite village. The operating headquarters of Julius Berger Nigeria Plc is also sited at Bosso. There is a high population pressure in this area and the supply of pipe borne water is epileptic. Inhabitants rely on streams and wells including those located in the vicinity of mechanic workshops for their supply of water.

Chanchaga is situated about 7 km from Minna along the Abuja route. It has a fair supply of pipe borne water but inhabitants occasionally rely on well water and streams when there are power outages or other contingencies. Roads Nigeria, Plc, a construction outfit has its headquarters at Chanchaga. Keteren-Gwari is part of Minna municipality and is located along the Bida-Lagos route. Maitumbi is also part of Minna and is on the route to Shiroro Dam site.

With the increasing cosmopolitan nature of Minna, its close proximity to Abuja, the Federal Capital Territory, the 4 mechanic sites chosen are the major ones which receive many vehicles for repairs all year round with the attendant discharge of automobile related waste and spent lubricants into the environment.

3.2 COLLECTION OF WATER SAMPLES

A total of fourteen wells were sampled over 6 months (July – December) comprising the rainy season (July, August, September) and dry season (October, November, December). These included 2 wells each located in Bosso and Maitumbi, 3 each located in Keteren-Gwari and Chanchaga. These ten wells were located within the vicinity of mechanic workshop sites. The other 4 wells

served as a control and were located in the respective areas studied but far away (about 1 km) from the mechanic workshop sites.

Water samples were colleted using sterile sampling bottles of 25ml capacity. The sampling bottle was lowered into the water in the well and its neck held below the surface by means of a string. The water sample in the bottle after being drawn out of the well was immediately covered tightly with screw caps. The samples were kept in an iced box, transported to the laboratory and analyzed within 2 hours (Itah *et al.*, 1996). An important feature of the wells sampled was their shallow nature, ranging from 1.8m to 4.2m deep (Table1).

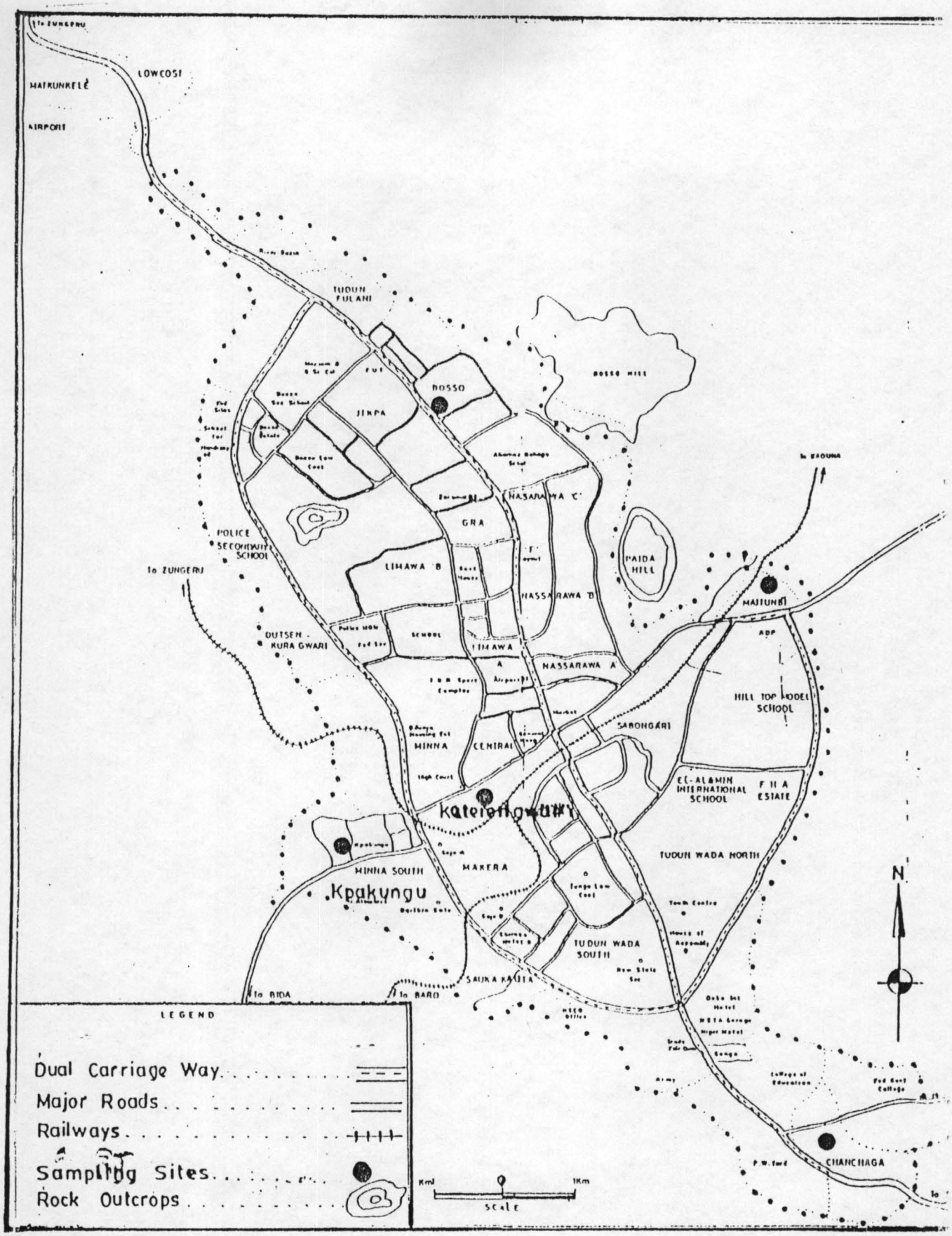


Fig.1 : *Map of Minna and its environs showing the sampling sites.*

Table 1:Depths (M) and description of wells sampled

Localities	Coded wells	Rainy season	Dry season	Comments
Bosso	B1	2.0	2.3	Elevated but not covered.
	B2	3.0	3.4	Not elevated, not covered.
	BN3	4.0	4.2	Elevated and well covered
Chanchaga	C4	3.2	3.4	Ordinary opening in the ground
	C5	3.6	4.2	Not elevated well covered.
	C6	3.0	3.1	Not elevated, well covered.
	CN7	2.8	3.1	Elevated and well covered.
Keteren-Gwari	K8	2.5	2.9	Not elevated, covered with metal sheet.
	K9	1.8	2.3	Elevated and covered.
	K10	3.6	4.1	Not elevated, not covered
	KN11	3.8	3.9	Elevated, not covered.
Maitumbi	M12	3.5	3.6	Not elevated, not covered.
	M13	2.9	3.2	Not elevated, not covered.
	MN14	3.7	3.9	Not elevated but covered.

BN3, CN7, KN11 and MN14: Control wells

B1, B2, C4, C5, C6, K8, K9, K10; M12 and M13: wells located within the vicinity of mechanic workshops.

3.3 BACTERIOLOGICAL ANALYSES

3.3.1 ENUMERATION OF BACTERIA (VIABLE COUNTS) IN WATER

A serial dilution from 10^{-1} to 10^{-4} was carried out. Using sterile graduated pipettes, a 0.5ml of 10^{-3} and 10^{-4} dilutions were pipetted into sterile petri dishes, then 10 to 12ml liquefied media maintained at 36°C was poured into each dish. These were thoroughly mixed by rotating the petri dish first in one direction and then in the opposite direction. Care was taken to avoid splashing. Plates were allowed to solidify on a leveled surface and inverted upside down, then placed in an incubator at 37°C for 24 hours. This entire exercise was carried out in a safety cabinet. Petri dishes containing media without test samples were momentarily exposed to the air of the safety and incubated as controls to determine the aseptic condition of the safety cabinet. Two replicate plates were made for each sample dilution (10^{-3}, 10^{-4}). The colonies, which developed were counted and expressed as colony forming units per millilitre of water sample (CFU/ml).

3.3.2 ENUMERATION OF FAECAL COLIFORM BACTERIA

To enumerate faecal coliforms in the water samples, 1ml of serially diluted water sample was aseptically introduced into sterile petri dishes and 20ml of McConkey agar was poured. The plates were allowed to solidify after which they were incubated at 44°C – 45°C for 24hours. Colonies, which developed in the plates were counted and expressed as colony forming units per millilitre (CFU/ml) of water sample.

3.3.3 ENUMERATION OF FAECAL *STREPTOCOCCI* IN WATER.

The enumeration of faecal *Streptococci* was carried out by inoculating glucose-azide agar with the serially diluted water samples and incubating initially at 37°C for 4 hours, then at 44°C for 24 hours (Okafor, 1985). Red and maroon coloured colonies were indicative of faecal *streptococci*. These were

counted and recorded as colony forming units per milliliter (CFU/ml) of water sample.

3.3.4 ENUMERATION OF *STAPHYLOCOCCUS AUREUS* IN WATER

Staph. aureus were enumerated using a selective medium, Mannitol Salt Agar (MSA). Serially diluted water samples were in aseptically innoculated in MSA by the spread plate method. The plates were incubated at 37°C for 24 hours after which the counts were recorded as colony forming units per millilitre (CFU/ml) of water.

3.3.5 ENUMERATION OF *SALMONELLA* SPECIES IN WATER .

Salmonella species were enumerated using a selective medium, Brilliant Green Agar (BGA). Serially diluted water samples were aseptically inoculated in the BGA plates and incubated at 37°C for 24 hours. The bacterial colonies, which developed on the plates (amber coloured) were counted and recorded as CFU/ml of water.

3.3.6 ENUMERATION OF SPENT LUBRICATING OIL UTILIZING BACTERIA

Serially diluted water samples were inoculated in oil agar and incubated at room temperature (28±2°C) for 5 days. At the end of the incubation, colonies, which developed in the plates were counted and recorded as colony forming units per millilitre (CFU/ml) of water sample.

3.4 CHARACTERIZATION AND IDENTIFICATION OF BACTERIAL ISOLATES

The bacterial isolates were gram stained and characterized based on morphology and biochemical tests as described in Appendix II. The isolates were identified by comparing them with those of known taxa (Cowan, 1974).

3.5 UTILIZATION OF SPENT LUBRICATING OIL BY BACTERIAL ISOLATES

The extent of utilization by spent lubricating oil utilizing bacterial isolates was investigated. MSM was dispensed in test tubes containing various concentrations (0.1, 0.5, 1.0% v/v) of spent lubricating oil. To each tube, 0.1ml of nutrient broth grown culture of oil degrading bacteria was added and incubated at room temperature for 5 days without shaking. Turbidity produced as a result of bacterial growth was visually monitored at the end of the incubation period and assigned (+) to (+++) depending on the intensity of growth.

3.6 DETERMINATION OF pH

The pH of water was measured by the use of an electronic pH meter (Model 7010, Kent, England). This was carried out as soon as the water was brought to the laboratory. The pH meter was calibrated with standard buffer solutions (pH 4.0, pH 7.0, 12.0). The electrode was rinsed in the sample whose pH was then determined. The pH was read to the nearest tenth of a pH unit. The readings of three samples were taken and the mean recorded.

3.7 ESTIMATION OF HEAVY METALS (Pb, Hg, Cd, Co, Fe, Cu) IN WATER

This was done using Atomic Absorption Spectrophotometer (Model 9190 Pye Unicam,). The hollow cathode lamp for the specific element to be quantified was inserted into the lamp holder inside the A.A.S and switched on. The required wavelength was then set (Pb: 283.3nm, Hg: 253.7nm, Cd: 228.8nm, Co: 240.7nm, Fe: 248.3nm and Cu: 324.7nm). The gas and air supplies were turned on and the flame ignited. Air pressure was regulated to the required level. The A.A.S. was allowed to warm up for about 10 minutes, set on absorbance mode and distilled deionized water containing 1.5ml conc. HNO_3

per litre was used to calibrate it, after which the sample concentrations were read at specific wavelengths of the metals. Standards were used to check the calibration after 4 readings each. The actual concentration for each element was obtained by extrapolation from the prepared calibration curves (Appendix XB)

3.8 STATISTICAL ANALYSIS

Microbial counts and PH were analyzed by taking ranges and means and plotting the latter against the months under study. pH and heavy metal values were statistically evaluated using t-tests for independent samples of groups and Levene's Test for the equality of variances (Gennaro,1995.)

CHAPTER FOUR

4.0 RESULTS

4.1 VIABLE COUNTS OF BACTERIA (CFU/ml) IN WELL WATER.

The results (Table 2) reveal that Chanchaga wells in the vicinity of mechanic workshops had the highest counts followed by Keteren-Gwari while Maitumbi and Bosso wells had the lowest counts. A plot of the mean viable bacterial counts (Fig. 2) indicate that the microbial loads of wells in Chanchaga, Keteren-Gwari and Maitumbi peaked between August and September after which values dropped reaching their lowest in December. Appendix III shows that the mean viable counts of mechanic wells were generally higher than those for the specific controls

4.2 COLIFORM COUNTS (CFU/ml) OF WELL WATER

The range of coliform counts (Table 3) indicates that the mechanic wells in Maitumbi had the highest values followed by Keteren-Gwari wells. Coliform counts peaked in August (Fig. 3) with the lowest figures in the dry season (October-December). Mechanic wells consistently had higher mean coliform counts than specific control wells (Appendix IV).

4.3 FAECAL *STREPTOCOCCI* (CFU/ml) COUNTS OF WELL WATER

The ranges of Faecal *Streptococci* counts are given on table 4 and this shows that Bosso mechanic wells had the highest figures followed by Chanchaga. The lowest were encountered in Keteren-Gwari and Maitumbi wells. The counts exhibited a seasonal variation with the highest in August and the lowest for most sites in December (Fig. 4). Appendix V shows a trend in which wells in the vicinity of mechanic workshops generally had higher Faecal *Streptococci* counts than their controls.

Table 2: Range of viable counts (CFU/ml) of well water

Month	Bosso		Chanchaga		Keteren-Gwari		Maitumbi	
	Range	Control	Range	Control	Range	Control	Range	Control
July	$6.1x10^3$ – $8.5x10^3$	$3.6x10^3$	$4.2x10^3$ – $8.8x10^3$	$3.2x10^3$	$9.8x10^3$ – $2.2x10^4$	$3.8x10^3$	$5.1x10^3$ – $7.6x10^3$	$4.5x10^3$
August	$3.8x10^3$ – $8.8x10^3$	$5.2x10^3$	$7.5x10^3$ – $2.5x10^4$	$4.0x10^3$	$9.9x10^3$ – $2.4x10^4$	$5.5x10^3$	$1.0x10^4$ – $1.2x10^4$	$6.2x10^3$
September	$4.1x10^3$ – $7.5x10^3$	$3.2x10^3$	$4.3x10^3$ – $5.5x10^4$	$3.5x10^3$	$7.4x10^3$ – $1.35x10^4$	$4.2x10^3$	$6.1x10^3$ – $8.8x10^3$	$3.1x10^3$
October	$3.5x10^3$ – $5.1x10^3$	$2.8x10^3$	$4.2x10^3$ – $6.0x10^3$	$2.8x10^3$	$4.9x10^3$ – $9.9x10^3$	$3.8x10^3$	$5.6x10^3$ – $6.0x10^3$	$3.0x10^3$
November	$3.0x10^3$ – $4.8x10^3$	$3.5x10^3$	$3.0x10^3$ – $5.6x10^3$	$1.4x10^3$	$3.0x10^3$ – $4.5x10^3$	$3.3x10^3$	$4.8x10^3$ – $5.5x10^3$	$2.8x10^3$
December	$2.5x10^3$ – $3.5x10^3$	$2.2x10^3$	$2.9x10^3$ – $4.3x10^3$	$4.8x10^2$	$2.8x10^3$ – $4.8x10^3$	$3.8x10^3$	$4.2x10^3$ – $5.2x10^3$	$2.6x10^3$

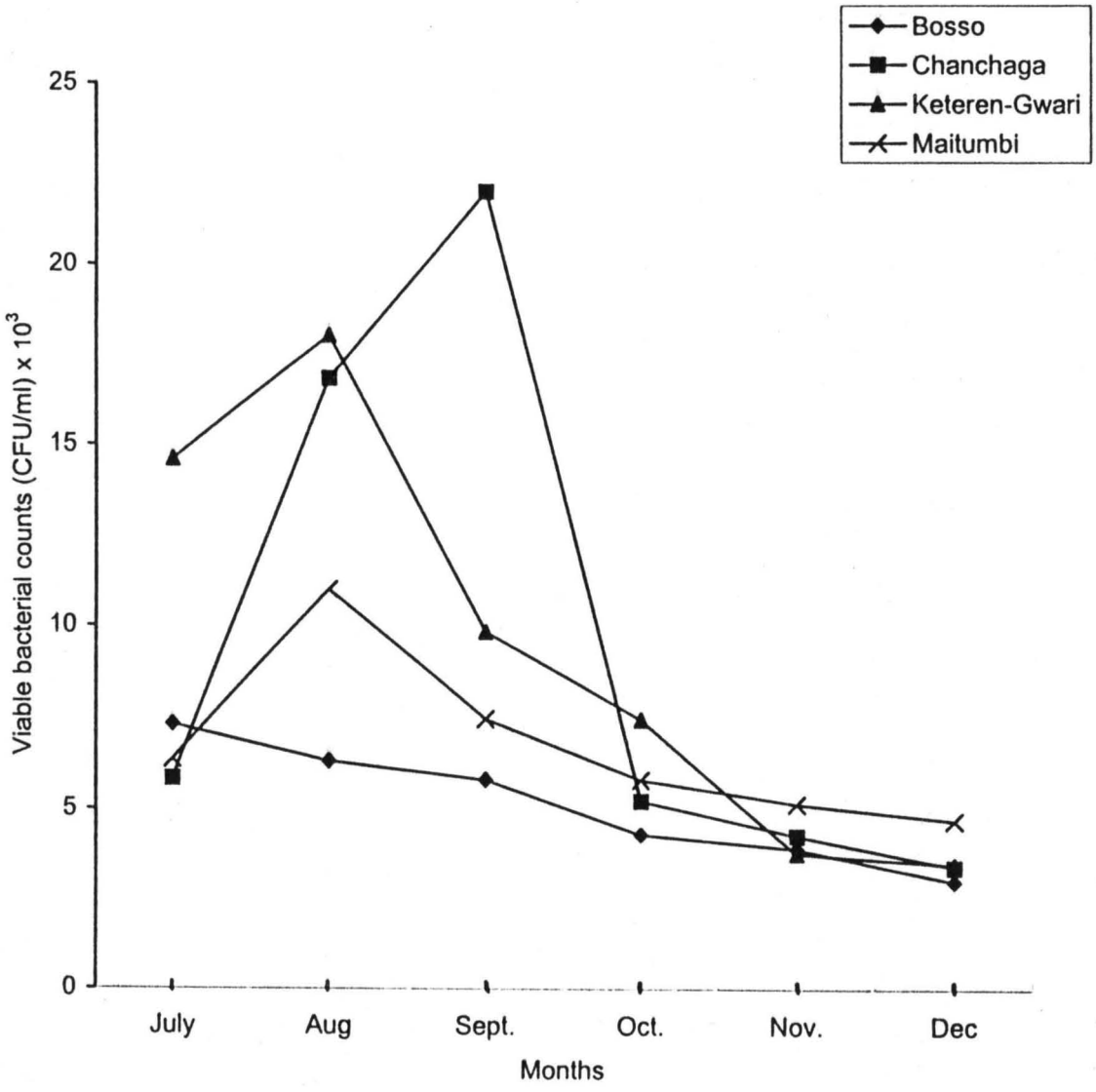


Fig.2: Mean viable bacterial counts (CFU/ml) in well water

Table 3: Range of Coliform counts (CFU/ml) of well water

Month	Bosso		Chanchaga		Keteren-Gwari		Maitumbi	
	Range	Control	Range	Control	Range	Control	Range	Control
July	$5.6x10^3$ – $5.8x10^3$	$1.8x10^3$	$1.9x10^3$ – $6.5x10^3$	$1.8x10^3$	$4.0x10^3$ – $6.8x10^4$	$1.8x10^3$	$2.1x10^3$ – $4.5x10^3$	$1.5x10^3$
August	$2.0x10^3$ – $7.5x10^3$	$2.5x10^3$	$6.7x10^3$ – $8.6x10^3$	$2.1x10^3$	$6.2x10^3$ – $7.7x10^3$	$2.9x10^3$	$7.5x10^3$ – $8.5x10^3$	$2.3x10^3$
September	$3.0x10^3$ – $6.2x10^3$	$1.8x10^3$	$2.5x10^3$ – $6.2x10^3$	$1.0x10^3$	$3.6x10^3$ – $4.8x10^3$	$1.6x10^3$	$3.5x10^3$ – $4.4x10^3$	$1.1x10^3$
October	$3.3x10^3$ – $3.5x10^3$	$1.0x10^3$	$3.0x10^3$ – $3.8x10^3$	$1.1x10^3$	$3.1x10^3$ – $4.8x10^3$	$1.1x10^3$	$2.0x10^3$ – $2.9x10^3$	$1.3x10^3$
November	$2.0x10^3$ – $2.8x10^3$	$4.5x10^2$	$2.2x10^3$ – $2.8x10^3$	$1.0x10^3$	$2.0x10^3$ – $3.0x10^3$	$1.5x10^3$	$2.5x10^3$ – $3.0x10^3$	$1.0x10^3$
December	$2.0x10^3$ – $3.3x10^3$	$6.6x10^2$	$5.6x10^2$ – $2.0x10^3$	$2.6x10^2$	$1.5x10^3$ – $2.8x10^3$	$1.0x10^3$	$2.1x10^3$ – $2.2x10^3$	$5.4x10^2$

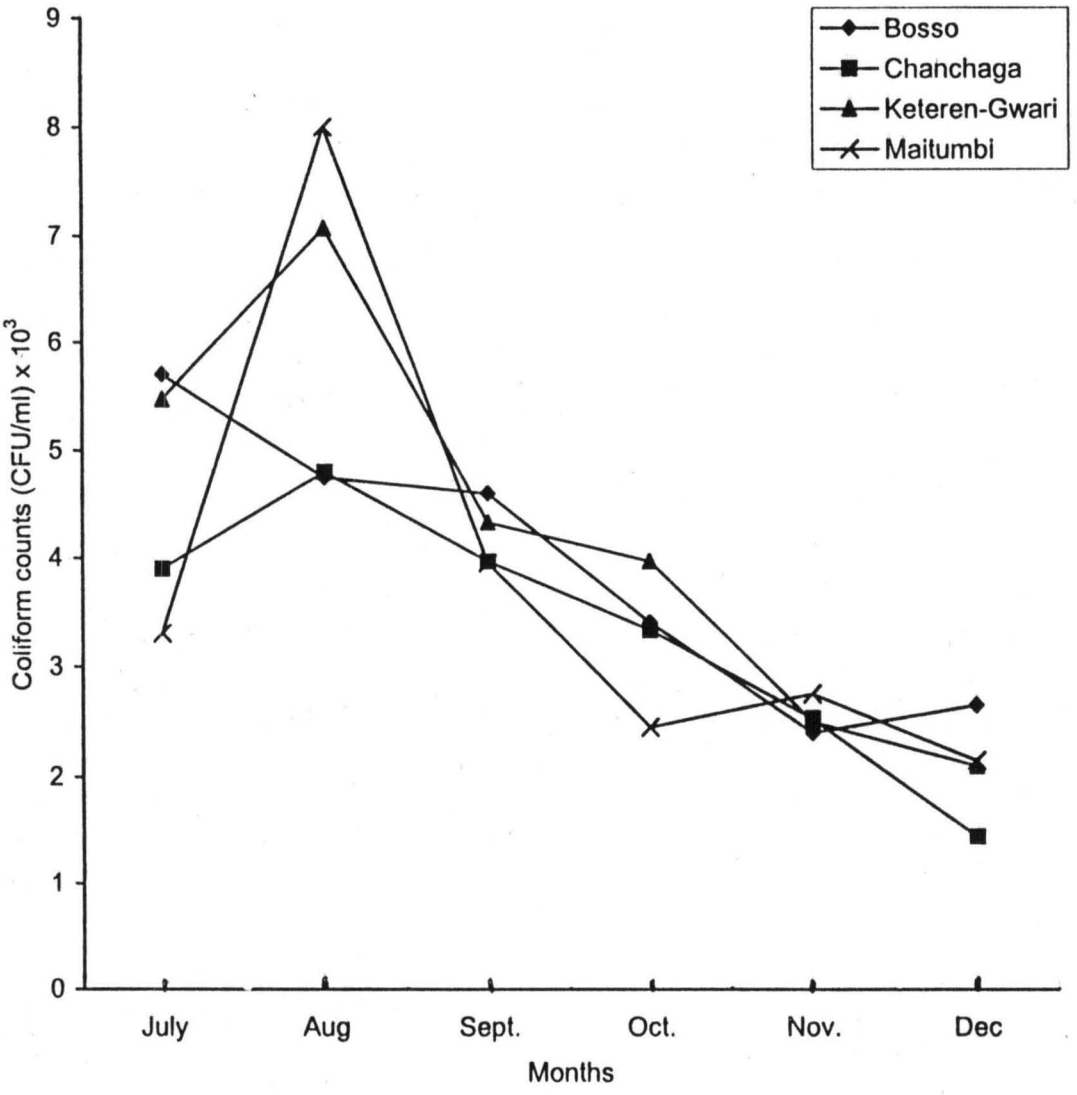


Fig.3: Mean Coliform counts (CFU/ml) in well water

Table 4: Range of *Streptococci* counts (CFU/ml) of water

Month	Bosso		Chanchaga		Keteren-Gwari		Maitumbi	
	Range	Control	Range	Control	Range	Control	Range	Control
July	$2.5x10^2$ – $3.0x10^2$	$1.3x10^2$	$1.8x10^2$ – $3.0x10^2$	$1.6x10^2$	$1.8x10^2$ – $3.0x10^2$	$1.5x10^2$	$5.2x10^1$ – $2.1x10^2$	$1.6x10^2$
August	$3.2x10^2$ – $4.0x10^2$	$1.6x10^2$	$2.6x10^2$ – $4.1x10^2$	$2.0x10^2$	$2.2x10^2$ – $3.5x10^2$	$1.6x10^2$	$2.4x10^2$ – $3.0x10^2$	$1.8x10^2$
September	$1.5x10^2$ – $3.0x10^3$	$1.1x10^2$	$4.0x10^1$ – $2.3x10^2$	$1.3x10^2$	$1.6x10^2$ – $2.0x10^2$	$1.3x10^2$	$1.8x10^2$ – $2.1x10^2$	$1.1x10^2$
October	$1.8x10^2$ – $2.3x10^2$	$1.4x10^2$	$1.2x10^2$ – $2.1x10^2$	$1.1x10^2$	$1.2x10^2$ – $1.5x10^2$	$2.5x10^1$	$3.3x10^1$ – $3.4x10^1$	$4.1x10^1$
November	$1.0x10^2$ – $1.4x10^2$	$1.1x10^2$	$1.2x10^2$ – $1.6x10^2$	$1.3x10^2$	$1.3x10^2$ – $1.4x10^2$	$2.0x10^1$	$3.0x10^1$ – $3.5x10^1$	$3.5x10^1$
December	$1.1x10^2$ – $1.3x10^2$	$1.0x10^2$	$1.0x10^2$ – $1.4x10^2$	$1.1x10^2$	$1.1x10^2$ – $1.4x10^2$	$1.8x10^1$	$1.9x10^1$ – $2.1x10^1$	$2.8x10^1$

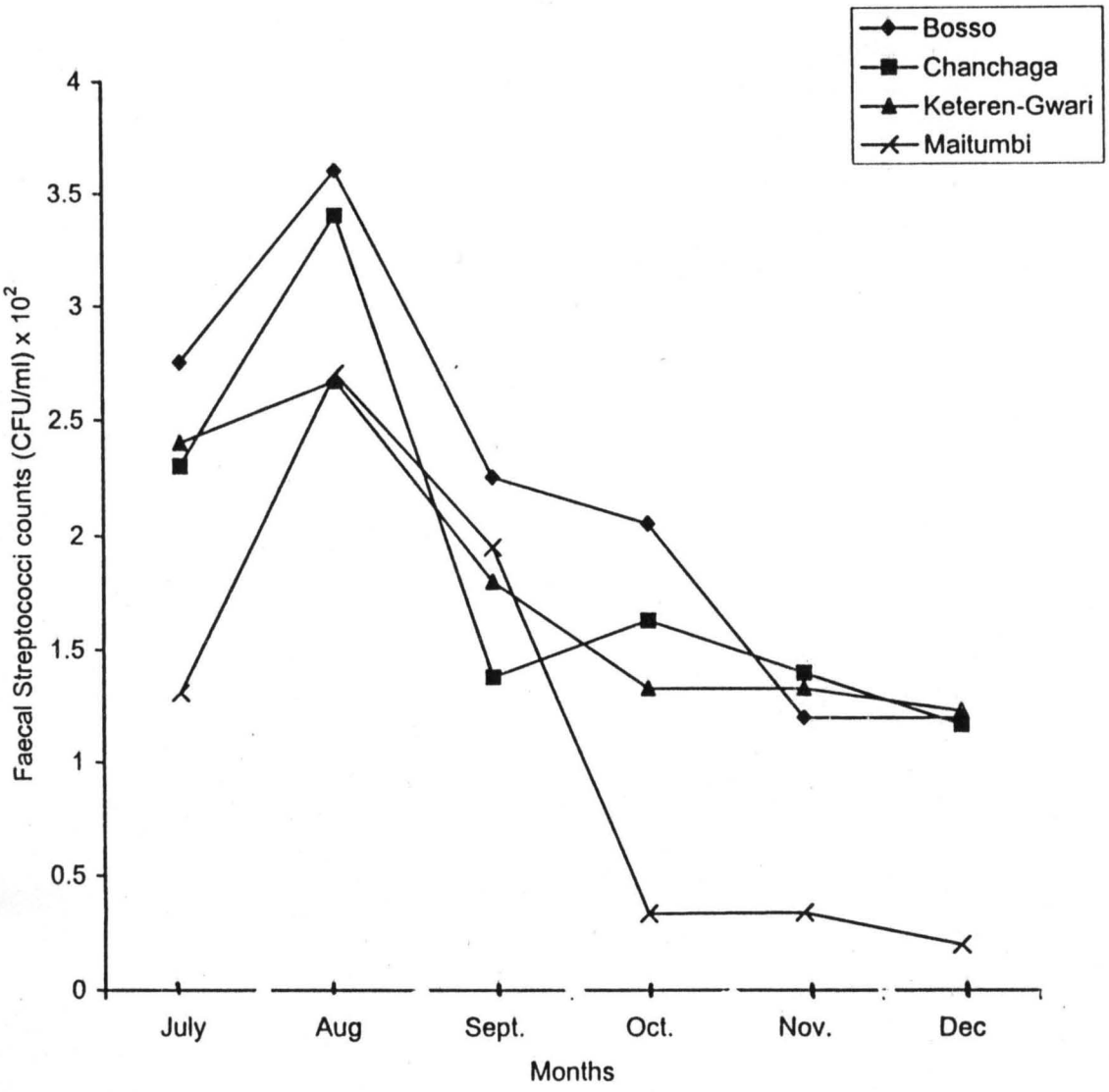


Fig.4: Mean faecal *Streptococci* counts (CFU/ml) in well water

4.4 *STAPHYLOCOCCI* COUNTS (CFU/ml) OF WELL WATER

The results of *Staphylococci* counts are given on Table 5 and it indicates that Bosso mechanic wells had the highest range followed by Maitumbi and Keteren-Gwari wells. The wells in Chanchaga had the lowest values. Fig. 5 illustrates the mean monthly counts with peak values in August and September and a general decline in the dry season (October-December). It also shows the consistently low counts in Chanchaga wells. Appendix VI indicates that not all mechanic wells had higher *Staphylococci* counts than their controls.

4.5 *SALMONELLA* COUNTS (CFU/ml) OF WELL WATER

The range of *Salmonella* counts (CFU/ml) are presented on Table 6 and it shows that Bosso had the highest values followed by Chanchaga. A plot of the mean monthly counts (fig. 6) illustrates that the highest *Salmonella* counts were in August for the 4 sites under study, which dropped by the dry season (October-December). Appendix VII indicates the generally higher counts obtained from wells in the vicinity of mechanic workshops in comparison to the respective controls, most of which had undetectable levels in the dry season months.

4.6 SPENT LUBRICATING OIL UTILIZING BACTERIAL (LOUB) COUNTS (CFUV/ml) OF WELL WATER

The LOUB counts (CFU/ml) on Table 7 indicate that Bosso mechanic wells had the highest range. Fig 7 illustrates that Chanchaga, Keteren-Gwari and Maitumbi had generally low counts with minimal seasonal variation. Bosso had very high counts in the rainy season, which dropped abruptly with the onset of the dry season. Controls had lower counts (Appendix viii) than wells located in the vicinity of mechanic workshops. The organisms were not detected in most of the controls especially in the dry season.

Table 5: Range of *Staphylococci* counts (CFU/ml) of well water

Month	Bosso		Chanchaga		Keteren-Gwari		Maitumbi	
	Range	Control	Range	Control	Range	Control	Range	Control
July	$2.1x10^2$ – $2.3x10^2$	$1.5x10^1$	$1.8x10^1$ – $2.1x10^2$	$1.3x10^1$	$1.6x10^1$ – $2.4x10^2$	$1.2x10^1$	$3.2x10^1$ – $2.2x10^2$	$1.8x10^1$
August	$1.2x10^2$ – $2.1x10^2$	$2.1x10^1$	$1.2x10^1$ – $2.1x10^2$	$1.2x10^1$	$1.4x10^1$ – $2.1x10^2$	$1.4x10^1$	$4.6x10^1$ – $2.4x10^2$	$1.4x10^1$
September	$1.1x10^2$ – $3.1x10^3$	$3.0x10^1$	$1.4x10^1$ – $3.1x10^1$	$1.0x10^1$	$1.0x10^1$ – $3.2x10^1$	$2.3x10^1$	$2.8x10^1$ – $3.5x10^1$	$2.2x10^1$
October	$1.0x10^2$ – $1.2x10^2$	$1.5x10^1$	$1.0x10^1$ – $2.5x10^1$	$1.0x10^1$	$1.2x10^1$ – $1.8x10^1$	$2.2x10^1$	$1.0x10^1$ – $1.8x10^1$	$2.0x10^1$
November	$1.6x10^1$ – $1.1x10^2$	$1.0x10^1$	$1.2x10^1$ – $1.6x10^1$	$1.1x10^1$	$1.0x10^1$ – $1.4x10^1$	$1.8x10^1$	$1.2x10^1$ – $1.6x10^1$	$1.6x10^1$
December	$1.2x10^1$ – $1.2x10^2$	$1.1x10^1$	$1.1x10^1$ – $1.4x10^1$	$1.1x10^1$	$1.0x10^1$ – $1.2x10^1$	$1.0x10^1$	$1.4x10^1$ – $2.0x10^1$	$1.2x10^1$

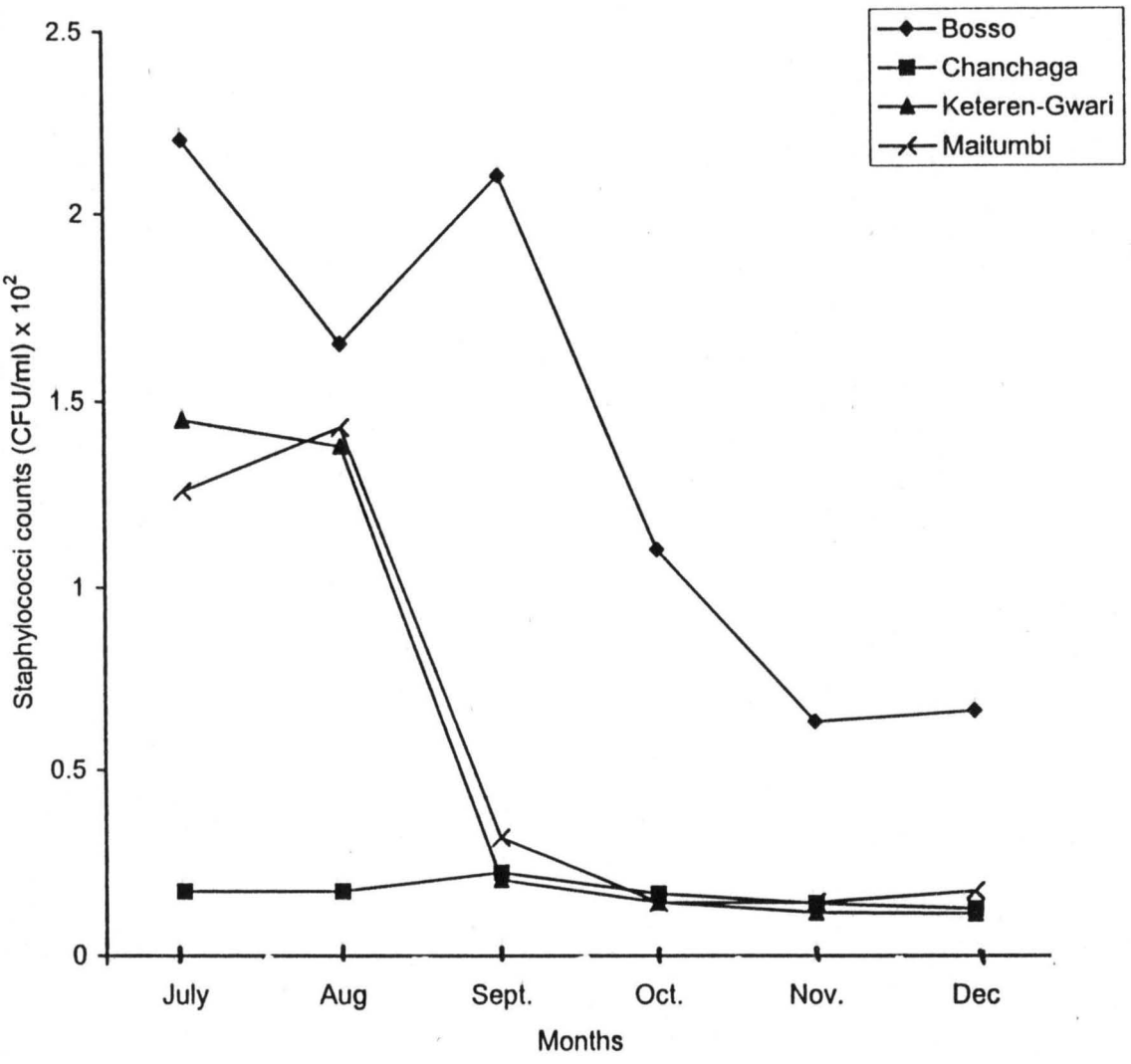


Fig.5: Mean *Staphylococci* counts (CFU/ml) in well water

Table 6: Range of *Salmonella* counts (CFU/ml) of well water

Month	Bosso		Chanchaga		Keteren-Gwari		Maitumbi	
	Range	Control	Range	Control	Range	Control	Range	Control
July	$0.6x10^1$ – $1.8x10^1$	$1.0x10^1$	$0.5x10^1$ – $1.1x10^1$	$0.4x10^1$	$0.2x10^1$ – $1.0x10^1$	$0.2x10^1$	$0.4x10^1$ – $0.8x10^1$	ND
August	$1.0x10^1$ – $2.0x10^1$	$0.8x10^1$	$0.8x10^1$ – $1.2x10^1$	$0.6x10^1$	$0.8x10^1$ – $1.1x10^1$	$0.3x10^1$	$0.6x10^1$ – $1.0x10^1$	$0.6x10^1$
September	$0.5x10^1$ – $1.3x10^1$	$0.2x10^1$	$0.3x10^1$ – $1.1x10^1$	$0.2x10^1$	$0.5x10^1$ – $0.6x10^1$	$0.2x10^1$	$0.5x10^1$ – $0.8x10^1$	$0.5x10^1$
October	$0.8x10^1$ – $1.5x10^1$	$0.4x10^1$	$0.3x10^1$ – $0.5x10^1$	$0.2x10^1$	$0.3x10^1$ – $0.5x10^1$	ND	$0.4x10^1$ – $0.6x10^1$	ND
November	$0.5x10^1$ – $1.3x10^1$	$0.2x10^1$	$0.3x10^1$ – $0.4x10^1$	ND	$0.2x10^1$ – $0.4x10^1$	ND	$0.6x10^1$ – $0.6x10^1$	ND
December	$0.7x10^1$ – $1.1x10^1$	ND	ND	ND	$0.2x10^1$	ND	$0.4x10^1$	ND

ND: Not detected.

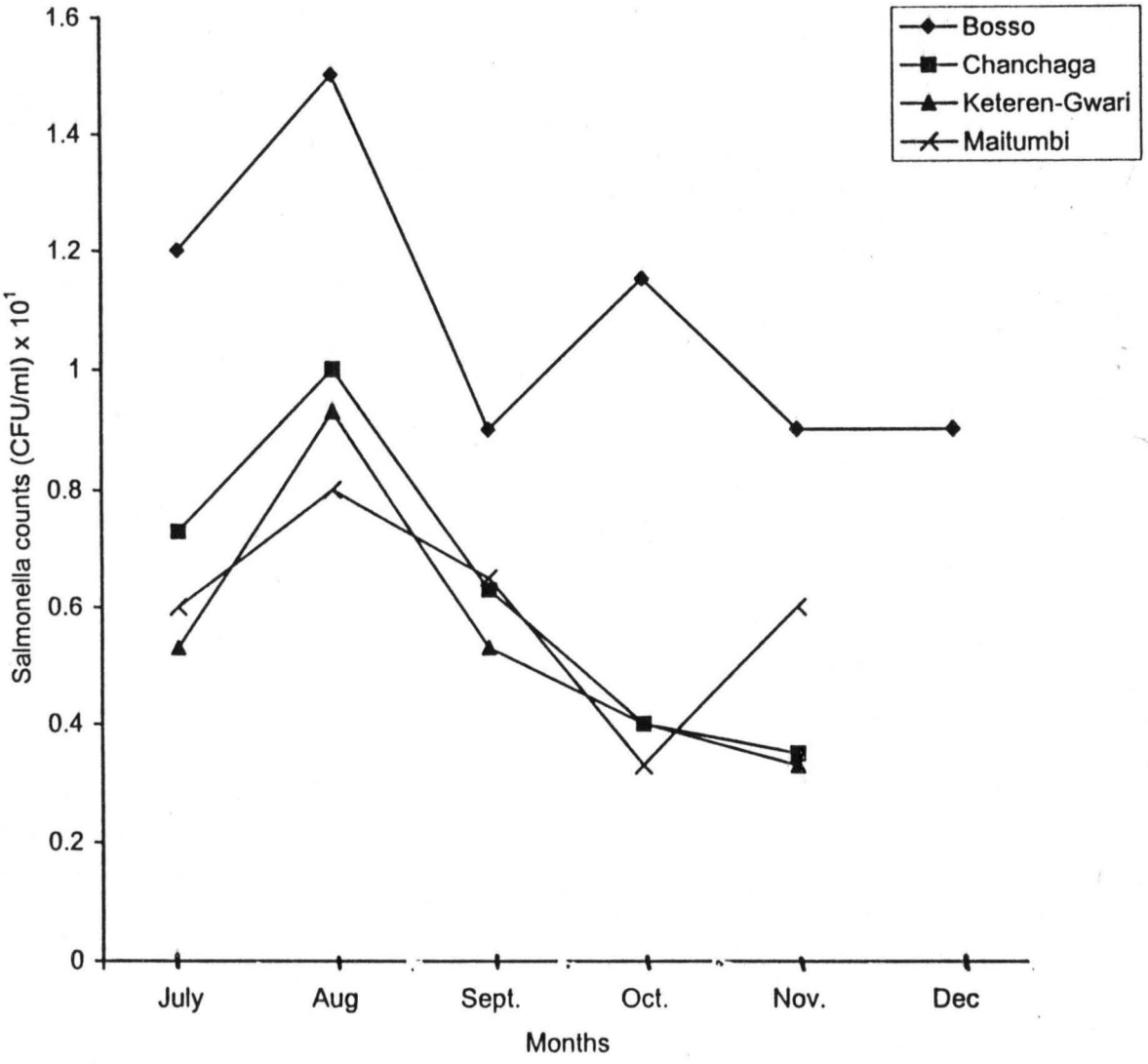


Fig.6: Mean *Salmonella* counts (CFU/ml) in well water

Table 7: Range of spent lubricating oil utilizing bacterial counts (CFU/ml) of well water

Months	Bosso		Chanchaga		Keteren-Gwari		Maitumbi	
	Range	Control	Range	Control	Range	Control	Range	Control
July	$1.2x10^1$ – $1.3x10^2$	$0.2x10^1$	$0.2x10^1$ – $0.4x10^1$	$0.6x10^1$	$0.6x10^1$ – $1.2x10^1$	$0.4x10^1$	$0.6x10^1$ – $1.0x10^1$	$0.4x10^1$
August	$1.0x10^1$ – $1.0x10^2$	$0.3x10^1$	$0.6x10^1$ – $1.2x10^1$	$0.8x10^1$	$0.4x10^1$ – $1.5x10^1$	$0.6x10^1$	$0.4x10^1$ – $1.2x10^1$	$0.3x10^1$
September	$0.8x10^1$ – $0.9x10^2$	ND	$0.8x10^1$ – $1.4x10^1$	$0.4x10^1$	$0.3x10^1$ – $1.6x10^1$	$0.7x10^1$	$0.4x10^1$ – $0.6x10^1$	$0.6x10^1$
October	$0.8x10^1$ – $1.0x10^1$	ND	$0.6x10^1$ – $0.7x10^1$	$0.2x10^1$	$0.4x10^1$ – $1.4x10^1$	$0.4x10^1$	$0.2x10^1$ – $0.6x10^1$	ND
November	$0.6x10^1$ – $0.8x10^1$	ND	$0.4x10^1$ – $0.8x10^1$	ND	$0.3x10^1$ – $0.8x10^1$	ND	$0.4x10^1$	ND
December	$0.4x10^1$ – $0.5x10^1$	ND	$0.5x10^1$ – $0.6x10^1$	ND	$0.6x10^1$	ND	ND	ND

ND: Not detected.

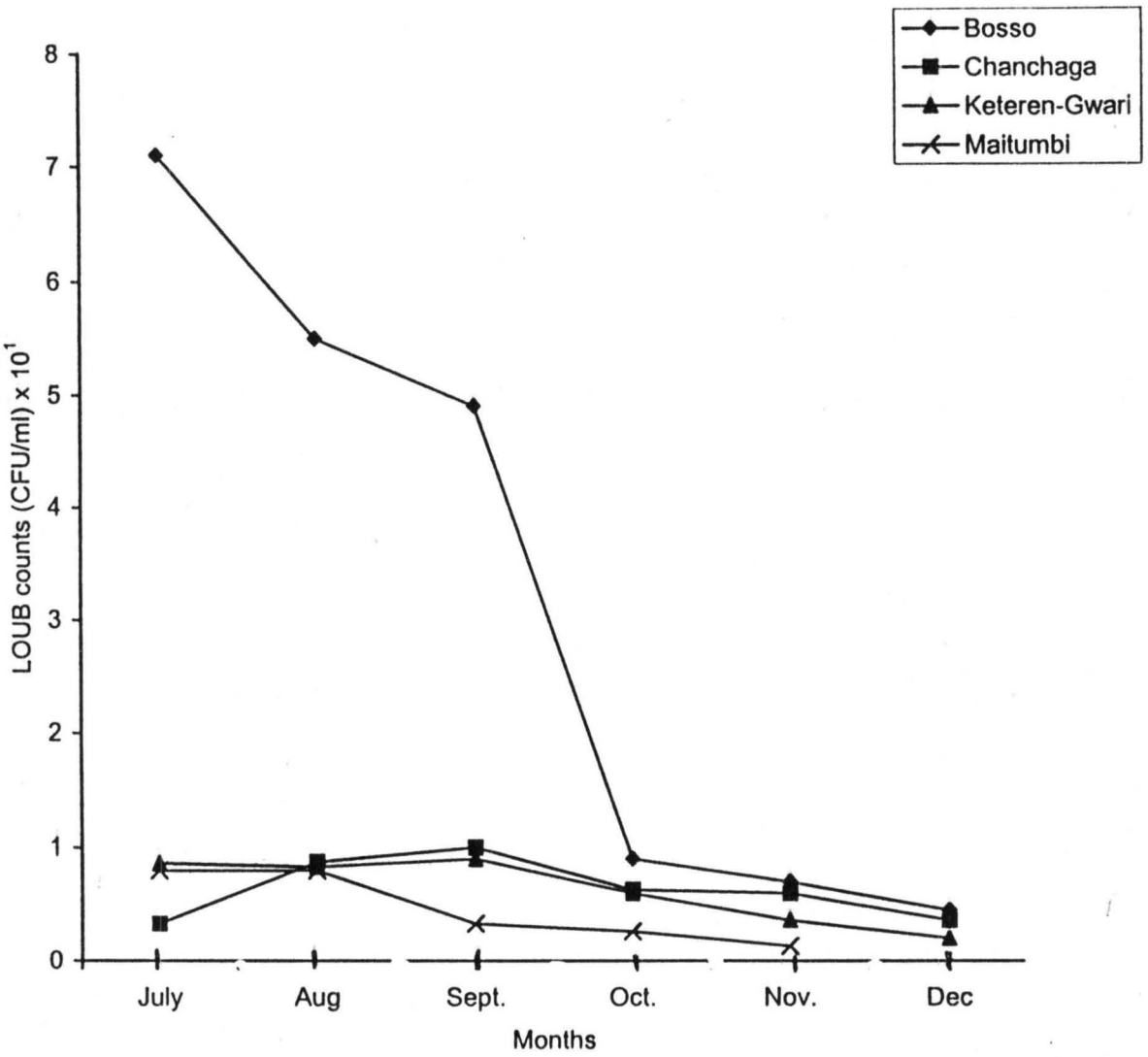


Fig.7: Mean lubricating oil utilizing bacterial (LOUB) counts (CFU/ml) in well water

4.7 CHARACTERIZATIONS AND IDENTIFICATION OF ISOLATES.

A variety of organisms were identified based on their morphology and biochemical tests (Table 8) from a total of 31 isolates obtained from the wells analysed in the 4 localities. These include *Bacillus* sp., *E. coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Staphylococcus aureus*, *Streptococcus faecalis*, *Klebsiella* sp. and *Micrococcus* sp.

4.8 FREQUENCY OF OCCURRENCE OF ISOLATES.

The frequency of occurrence of bacterial isolates (Fig 8) indicated that *Bacillus* sp. had the highest percentage from Keteren-Gwari wells. Klebsiella was the least in all the sites.

4.9 RATES OF UTILIZATION OF SPENT LUBRICATING OIL BY BACTERIAL ISOLATES.

Table 9 represents the level of bacterial utilization of spent lubricating oils. These were isolates from the different wells analysed A total of 12 were obtained. The most intense growth was recorded with 0.1% oil, while 1% oil concentration had minimal growth. *Micrococcus* sp. (M 31, B3) exhibited the highest oil utilizing potentials. The *Bacillus* sp. (B5, M29, K 19), did averagely well. A minimum growth was shown by the *Bacillus sp.* C13 and K17 with the *Pseudomonas* (K12) as the least

4.10 pH VALUES OF WELL WATER

The range of pH values (Table 10) shows that the highest figures were obtained from Keteren-Gwari wells followed by Maitumbi and the lowest was in Chanchaga. Fig 9 indicates that dry season ph values were higher than in the rainy season. The pH of controls was also generally lower than those of wells in the vicinity of mechanic workshops (Appendix ix).

Table 8: Characterization and identification of bacterial isolates

Isolates code	Gram reaction	Shape	Spore	Motility	Catalase	Indole	Citrate	Methyl red	V.P	H_2S on TSI	Coagulase	Ureare	Oxidase	Starch hydrolysis	Sugar fermentation				Bacteria
															Glucose	Sucrose	Manniol	Lactose	
B1	-	R	-	-	+	-	+	-	+	-	-	+	-	-	+	+	+	+	*Klebsiella sp.*
B2	-	R	-	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	*E. coli*
B3	+	C	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	*Micrococcus sp.*
B4	-	R	-	+	-	-	-	+	-	+	-	-	-	-	+	-	+	-	*Salmonella sp.*
B5	+	R	+	+	+	-	-	-	+	-	-	-	-	+	+	+	+	+	*Bacillus sp.*
B6	-	R	-	+	+	-	-	-	+	-	-	-	+	-	+	+	+	+	*Enterobacter aerogenes*
C7	-	R	-	+	+	-	+	+	-	-	-	+	+	-	-	-	-	-	*Pseudomonas aeruginosa*
C8	-	R	-	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	*E. coli*
C9	+	C	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	*Micrococcus sp.*
C10	-	R	-	+	-	-	-	+	-	+	-	-	-	-	+	-	+	-	*Salmonella sp.*
C11	+	C	-	-	+	+	-	-	-	-	+	+	-	-	+	+	+	-	*Staph. aureus.*
C12	-	R	-	-	+	-	+	-	+	-	-	+	-	-	+	+	+	+	*Klebsiella sp.*
C13	+	R	+	+	+	-	-	-	+	-	-	-	-	+	+	+	+	+	*Bacillus sp.*
C14	-	R	-	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	*E. coli*
K15	+	C	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	*Strept. faecalis*
K16	-	R	-	+	-	-	-	+	-	+	-	-	-	-	+	-	+	-	*Salmonella sp.*
K17	+	R	+	+	+	-	-	-	+	-	-	-	-	+	+	+	+	+	*Bacillus subtilis*
K18	+	R	+	+	-	-	-	-	+	-	-	-	-	+	+	+	-	+	*Bacillus megaterium*
K19	+	R	+	+	+	-	-	-	+	-	-	-	-	+	+	+	+	+	*Bacillus sp.*
K20	-	R	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	*Shigella sp.*
K21	-	R	-	+	+	-	+	-	-	-	-	+	+	-	-	-	-	-	*Pseudomonas aeruginosa*
K22	+	C	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	*Strept. faecalis*
K23	-	R	-	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	*E. coli*
K24	-	R	-	+	+	-	-	-	+	-	-	-	+	-	+	+	+	+	*Enterobacter aerogenes*
M25	+	C	-	-	+	+	-	-	-	-	+	+	-	-	+	+	+	-	*Staph. aureus.*
M26	-	R	-	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	*E. coli*
M27	+	C	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	*Strept faecalis*
M28	-	R	-	+	+	-	+	-	-	-	-	+	+	-	-	-	-	-	*Pseudomonas aeruginosa*
M29	+	R	+	+	+	-	-	-	+	-	-	-	-	+	+	+	+	+	*Bacillus sp.*
M30	-	R	-	+	+	-	+	+	-	-	-	-	+	-	+	+	+	+	*Enterobacter aerogenes*
M31	+	C	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	*Micrococcus sp.*

+: Positive result, -: Negative result, C: Cocci, R: Rod

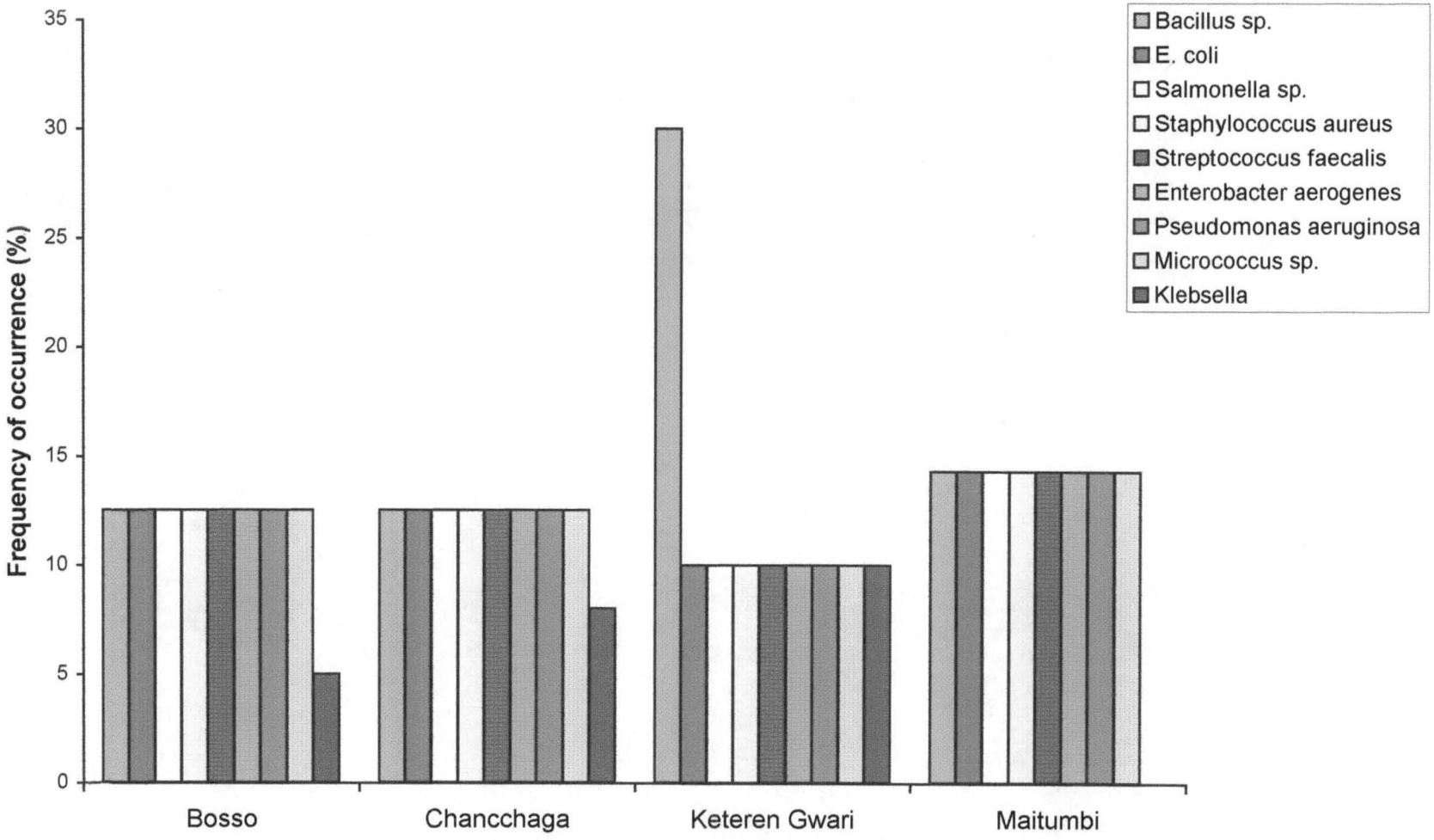


Fig. 8.0 Frequency of occurrence of bacterial isolates

Table 9: Rates of utilization of spent lubricating oil by bacterial isolates

Isolate No.	Coded Bacteria	Conc. (%) Lubricating Oil		
		0.1	0.5	1.0
B3	*Micrococcus sp.*	+++	++	+
B5	*Bacillus sp.*	+++	++	-
C7	*Ps. aeruginosa*	++	+	-
C9	*Micrococcus sp.*	++	++	-
C13	*Bacillus sp.*	++	-	-
K17	*Bacillus subtilis*	++	-	-
K18	*Bacillus megaterium*	++	-	-
K19	*Bacillus sp.*	+++	+	+
K21	*Ps. aeruginosa*	+	-	-
M28	*Ps. aeruginosa*	++	++	-
M29	*Bacillus sp.*	++	++	+
M31	*Micrococcus sp.*	+++	+++	+

+++ Heavy growth

++ Moderate growth

+ Scanty growth

- No growth

Table 10: Range pH values of well water

Month	Bosso		Chanchaga		Keteren-Gwari		Maitumbi	
	Range	Control	Range	Control	Range	Control	Range	Control
July	6.5 – 7.00	6.60	6.00 – 6.80	6.80	6.20 – 7.50	7.40	6.00 – 7.20	7.60
August	6.8 – 7.5	7.20	7.40 – 8.20	7.60	6.00 – 8.20	7.00	6.50 – 8.00	6.90
September	7.4 – 7.80	7.40	7.20 – 7.60	7.60	7.86 – 8.40	7.20	7.60 – 8.00	6.80
October	7.80 – 8.40	7.20	7.80 – 8.20	7.60	7.40 – 8.40	6.80	8.20 – 8.60	7.40
November	6.90 – 8.40	7.60	7.20 – 7.80	7.20	8.20 – 8.80	7.80	7.40 – 7.60	7.40
December	7.80 – 8.00	7.40	7.40 – 7.80	6.80	7.60 – 8.40	7.60	7.40 – 8.60	7.60

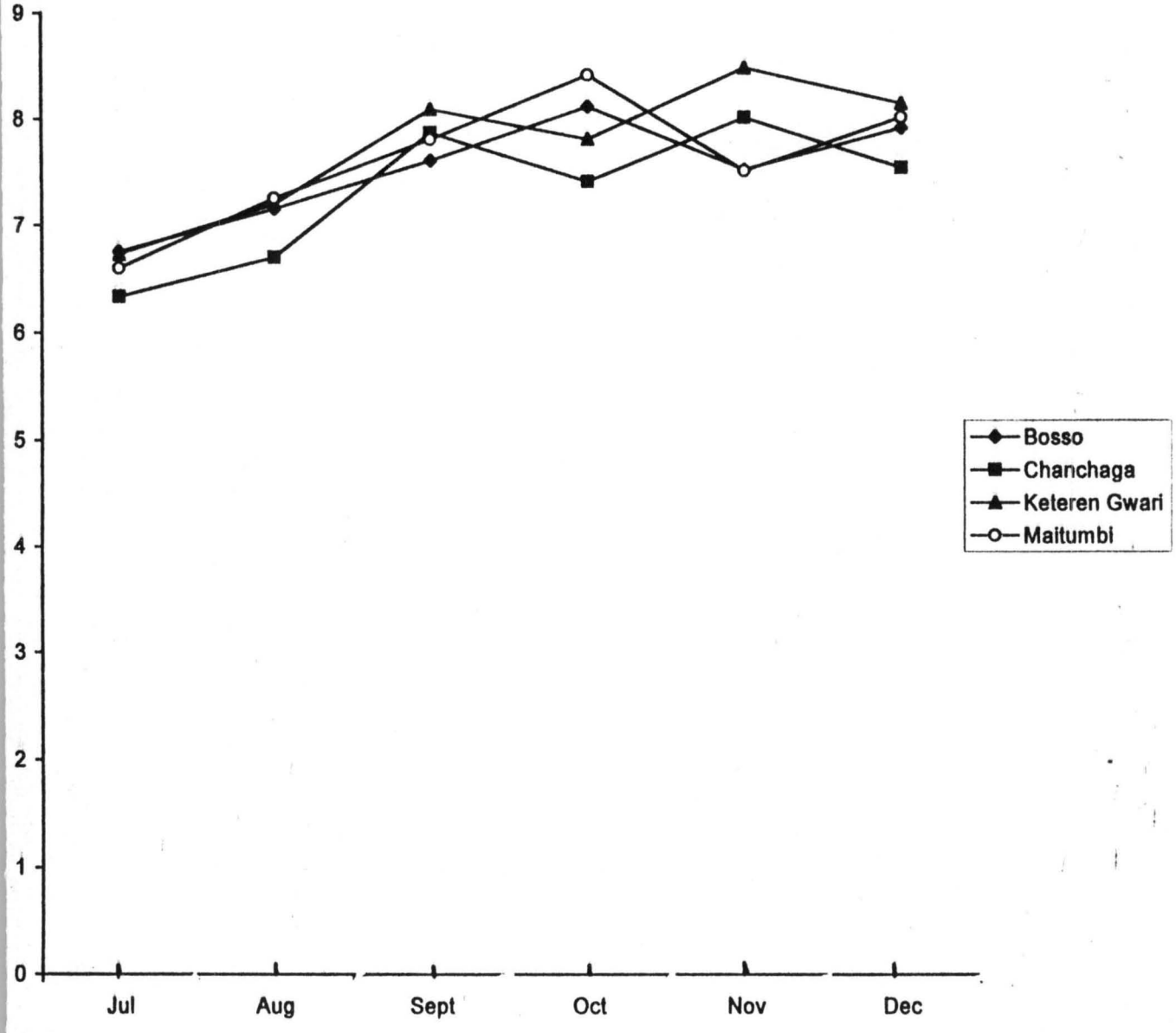


Fig. 9: Mean pH values of well water

4.11 HEAVY METAL CONTENT OF WELL WATER.

Table 11 shows the mean heavy metal (Co, Cu, Fe) concentrations (mg/L) of wells located in the vicinity of mechanic workshops and controls. Maitumbi had the highest figure for cobalt while Bosso had the highest levels of copper and iron. Control wells generally had lower values than the mechanic site wells.

On table 12 are the means for Cd, Hg and Pb (mg / L) and it shows that Maitumbi had the highest levels of Cd, Hg and Pb respectively. A few of the control wells had no detectable levels of Pb and, most had no Hg.

Table13 is a comparison of the seasonal (rainy vs dry) values of individual heavy metals of wells in the vicinity of mechanic workshops and it indicates a significant difference ($p<0.05$) in the cases of Hg Pb, Cu and Cd. The table also contains comparisons between individual heavy metals for a particular season and their controls, indicating significant differences ($p< 0.05$) in all the metals analysed.

Table 11: Mean monthly heavy metal concentrations (mg/L) in well water (Cobalt, Copper, Iron)

Cobalt

Location	July		August		September		October		November		December	
	a	b	a	b	a	b	a	b	a	b	a	b
Bosso	0.330	0.165	0.310	0.160	0.285	0.160	0.293	0.145	0.275	0.140	0.205	0.150
Chanchaga	0.185	0.044	0.174	0.042	0.185	0.040	0.145	0.050	0.127	0.020	0.010	0.139
Keteren-Gwari	0.172	0.095	0.170	0.093	0.160	0.067	0.160	0.07	0.160	0.060	0.180	0.002
Maitumbi	0.400	0.173	0.390	0.168	0.380	0.095	0.340	0.099	0.350	0.095	0.200	0.162

Copper

Location	July		August		September		October		November		December	
	a	b	a	b	a	b	a	b	a	b	a	b
Bosso	0.905	0.900	0.900	0.853	0.900	0.825	1.450	0.925	1.400	0.775	1.500	1.185
Chanchaga	1.060	0.200	1.041	0.650	1.003	0.750	0.905	0.500	0.847	0.450	1.140	0.500
Keteren-Gwari	0.963	0.950	0.960	0.900	0.957	0.900	0.900	0.893	0.887	0.850	0.950	0.620
Maitumbi	1.400	1.350	1.350	1.300	1.300	1.252	1.280	0.900	1.250	0.800	1.200	0.675

Iron

Location	July		August		September		October		November		December	
	a	b	a	b	a	b	a	b	a	b	a	b
Bosso	1.650	1.050	1.600	1.015	1.500	1.015	1.400	1.200	1.400	0.975	1.500	1.05
Chanchaga	1.420	1.168	1.400	0.235	1.300	1.092	1.350	1.100	1.200	0.850	1.250	1.140
Keteren-Gwari	1.440	0.998	1.420	0.960	1.350	0.963	1.300	1.150	1.300	0.933	1.320	0.960
Maitumbi	1.330	0.480	0.870	0.460	1.373	0.460	1.475	0.620	1.395	0.450	1.450	0.580

a: Wells located in the vicinity of mechanic workshops.

b: Control wells.

Table 12: Mean monthly heavy metal concentrations (mg/L) in well water (Cadmium, Lead and Mercury)

Cadmium

Location	July		August		September		October		November		December	
	a	b	a	b	a	b	a	b	a	b	a	b
Bosso	0.030	0.025	0.020	0.015	0.015	0.010	0.010	0.008	0.010	0.010	0.010	0.005
Chanchaga	0.060	0.021	0.060	0.014	0.060	0.014	0.050	0.005	0.050	0.005	0.003	ND
Keteren-Gwari	0.019	0.001	0.019	0.001	0.012	ND	0.007	0.001	0.0003	ND	0.010	ND
Maitumbi	0.020	0.015	0.020	0.010	0.010	0.010	0.005	0.001	0.005	ND	0.005	ND

Mercury

Location	July		August		September		October		November		December	
	b	b	a	b	a	b	a	b	a	b	a	b
Bosso	0.001	ND	0.001	ND	0.001	ND	0.001	ND	0.0005	ND	0.0005	ND
Chanchaga	0.002	ND	0.001	ND	0.001	ND	0.001	ND	0.0003	ND	0.0002	ND
Keteren-Gwari	0.0003	ND	0.003	0.003	0.001	0.001	0.003	ND	0.0003	ND	0.0005	0.0005
Maitumbi	0.0001	ND	0.001	ND	0.001	ND	0.001	0.0003	0.0001	ND	0.003	0.001

Lead

Location	July		August		September		October		November		December	
	a	b	a	b	a	b	a	b	a	b	a	b
Bosso	0.083	0.005	0.082	0.004	0.080	0.004	0.078	0.003	0.075	0.002	0.0005	0.0001
Chanchaga	0.077	0.003	0.017	0.002	0.069	0.002	0.060	0.001	0.058	ND	ND	ND
Keteren-Gwari	0.054	0.004	0.075	0.003	0.053	0.002	0.031	0.001	0.030	ND	0.0005	0.0001
Maitumbi	0.095	ND	0.084	ND	0.080	ND	0.075	ND	0.068	ND	0.001	0.0001

a: Wells located in the vicinity of mechanic workshops.

b: Control wells

ND: Not detected

Table 13: A comparison of the concentration (mg/L) of heavy metals in well water

Heavy Metal	Season	$\bar{\chi}$	SD	$S\bar{E}\chi$	t.values
Hg	R/D	0.0007 0.005	0.00	0.00	1.76
	R/C	0.0007 0.0002	0.00	0.00	3.19
	D/C	0.005 0.0002	0.00	0.00	1.84
Pb	R/D	0.071 0.038	0.025 0.036	0.005 0.007	4.17
	R/C	0.071 0.024	0.025 0.002	0.005 0.000	9.48
	D/C	0.038 0.0007	0.036 0.001	0.007 0.000	3.58
Co	R/D	0.171 0.134	0.096 0.082	0.018 0.015	1.59
	R/C	0.171 0.094	0.096 0.067	0.018 0.019	2.52
	D/C	0.134 0.021	0.082 0.036	0.015 0.009	4.60
Cu	R/D	1.031 0.898	0.246 0.276	0.045 0.050	1.96
	R/C	1.031 0.243	0.246 0.174	0.045 0.050	10.10
	D/C	0.898 0.326	0.276 0.240	0.050 0.069	6.29
Cd	R/D	0.018 0.007	0.009 0.005	0.002 0.001	5.67
	R/C	0.018 0.008	0.009 0.009	0.002 0.003	2.99
	D/C	0.007 0.004	0.005 0.005	0.001 0.001	1.45
Fe	R/D	1.063 1.187	0.403 0.330	0.074 0.060	- 1.30
	R/C	1.063 0.133	0.403 0.157	0.074 0.045	6.23
	D/C	1.187 0.259	0.330 0.185	0.060 0.053	9.13

n

R: Rainy season = 30
D: Dry season= 30
C: Control = 12
R/D: Rainy vs. Dry season
R/C: Rainy vs. controls
D/C: Dry vs. control

P0.05 = 1.6

CHAPTER FIVE

5.0 DISCUSSION

The mean monthly viable bacterial counts in the well water show a sharp increase from July through September (rainy season) for most sites and an abrupt drop from the onset of the dry season (October) through December. This trend could be attributed to the effect of rainfall in the rainy season in transporting bacteria into these wells. As the rain falls, soil microbiota and microorganisms adhering to vegetation, municipal sewage, garbage, domestic and industrial wastes are washed into the wells thereby polluting them. Most of the wells studied had no lids and are at ground level or mere openings in the ground. Even the wells that were protected received a fair share of such microorganisms through underground seepage. These constitute serious public health hazard. However, between October and December, there was a general decline in the viable counts as the rainy season gave way to the dry season. This seasonal trend is in agreement with the findings of Itah *et al* (1996) and Ugbagwu (2002).

The particularly high viable counts from wells in Chanchaga ($2.20x10^4$CFU/ml) and Keteren-Gwari ($1.80x10^4$CFU/ml) may be due to the combined effects of run-offs, seepages and topography. This is because a river that drains most of the settlement is located at Chanchaga. The Keteren-Gwari site receives effluents from the Minna Central market via the municipal drainage system. The control wells had lower counts than wells located in the vicinity of mechanic workshops probably because they are better protected. The sanitary quality of water is based on the quantities of indicator bacteria it contains (Suess, 1982). The bacteriological quality of drinking water must meet the World Health Organization (WHO) standard of less than ten coliforms per 100ml for the water to be acceptable. Other criteria are that coliform organisms should not be detectable in 100ml of any two consecutive samples and in 95% of samples examined throughout a year; coliform bacteria should be absent in 100ml (WHO, 1974). In the present study, none of the wells sampled irrespective of season met these

standards. The mean monthly coliform counts from mechanic site wells were between $1.45x10^3$CFU/ml-$8.3x10^3$CFU/ml while controls gave $2.6x10^2$CFU/ml – $2.9x10^3$CFU/ml. The coliform counts in wells located at Maitumbi and Keteren-Gwari peaked in August probably due to the effects of rainfall and consequent pollution by run-off. Besides, in these areas shallow pit latrines abound and the residents defecate indiscriminately. Coliform counts from control wells were relatively low but unacceptable. Run-off contributes minimally in polluting the control wells because they are mostly located in residential quarters. Most have raised concrete casings, secured lids and clean environment. Ugbagwu (2002) reported an overall mean coliform count of $2.3x10^3$CFU/ml with the peak value of $2.9x10^3$CFU/ml in September and the lowest ($1.7x10^3$CFU/ml) in December. The results obtained in the present study compare with these values. The recovery of coliforms from wells and other sources of water supply in intolerable numbers constitute a serious public health hazard, as their presence is indicative of a possible presence of microorganisms associated with water borne diseases (Junaidu *et al.*, 2000).

Faecal *Streptococci* as in the case of coliforms in general had a higher magnitude of occurrence in the rainy season compared to the dry season. The levels in wells from the four mechanic sites peaked in August. Values from mechanic site wells ($2.0x10^1$ – $3.6x10^2$CFU/ml) were much higher than control values ($1.8x10^1$ – $2.0x10^2$CFU/ml). These values are within the ranges reported by Okuofu *et al.* (1990) ($2.0x10^1$–$7.0x10^2$CFU/ml) but much higher than the $0.6x10^1$–$1.0x10^2$CFU/ml obtained by Itah *et al.*, (1996) from wells. The distribution of *S. faecalis* can be attributed to seasonal influences, shallow nature of these wells, indiscriminate discharge of excrement, as well as animal and poultry droppings (SMEWW, 1989). This organism like the coliforms is an indicator of fairly recent faecal pollution (Okuofu *et al.*, 1990).

The mean monthly counts of *Staphylococci* were quite high in Bosso compared with Keteren-Gwari and Maitumbi. There was a sharp drop in figures

with the commencement of the dry season. The levels in Chanchaga were quite low and they exhibited apparently no seasonal variation. The controls gave relatively low counts compared with the mechanic site wells. *Staphylococci* are widely distributed in the environment. They form part of the normal microbial flora of the skin, upper respiratory tract and intestinal tract. *S. aureus* is carried in the nose of 40% or more of healthy people (Cheesebrough, 1991). Despite these, the organism is an opportunistic pathogen, which can cause abscesses, boils, impetigo, pneumonia, empyema and even toxic shock syndrome due to colonization especially of the vagina, etc. The high count encountered especially with Bosso mechanic site wells (2.2x102CFU/ml) is disturbing because such water sources can precipitate an outbreak of some of the ailments mentioned above.

The isolation of *Salmonella* sp. from the water samples analyzed is of serious public health concern as these organisms are intolerable under any circumstance (Itah *et al.*, 1996). Although all the controls except Bosso ($1.0x10^{1}$CFU/ml) had very low counts, the picture remains alarming in respect of most of the wells in the mechanic site wells. There was a seasonal variation with the peak counts at all sites in August while in December most wells had no detectable levels. The Bosso site recorded the highest counts ($1.5x10^{1}$CFU/ml). The local market is within close proximity, shallow pit latrines abound and the residents defecate indiscriminately. All these drain in the direction of the Bosso mechanic workshops. This contrasts sharply with the non-detection of these organisms in wells in Calabar by Itah *et al,* (1996). Similarly, Inabo (1996) and Ugbagwu (2002) reported counts of less than $1.0x10^{1}$CFU/ml from wells in Northern Nigeria.

In addition to the problem of microbiological pollution, industrialization has introduced a wide range of chemicals including hydrocarbons into the list of pollutants into the environment (Atlas and Bartha 1981). Amund *et al* (1993) have reported that an important source of petroleum contamination of the Nigeria environment that has attracted little public awareness is the indiscriminate

discharge of used crankcase oil (lubricants) on the soil. This could percolate the soil or be carried by runoff into wells and ground water especially in the vicinity of such dumpsites as the mechanic workshops under study. When natural ecosystems are contaminated with petroleum hydrocarbons, microbial populations do appear to degrade such contaminants (Amund *et al.*, 1993; Ijah *et al.*, 2000). In the present study, the mean oil-utilizing bacteria counts were generally low and exhibited minimal seasonal variation in the case of Chanchaga, Keteren-Gwari and Maitumbi mechanic site wells. The highest count ($7.1x10^1$CFU/ml) was recorded in Bosso in July probably due to high rates of spills of spent lubricating oil in their vicinity and consequent run-off by rainwater into the wells. The control wells recorded very low counts suggesting minimal oil pollution.

The lubricating oil-utilizing bacteria isolated were species of *Micrococcus, Bacillus* and *Pseudomonas*. These organisms have earlier been reported by other investigators (Antai and Mgbomo, 1989; Amund and Adebiyi, 1991; Ijah and Antai, 2003) as hydrocarbon utilizers. The organisms thrived 0.1% and 0.5% of lubricating oil than 1% oil. *Micrococcus* sp. (M31 and B3) exhibited the highest oil utilizing potentials. This may imply that these organisms have very active degradative enzymes and hence could be useful in clearing lubricating oil-polluted environment (Ijah *et al.*, 2000).

pH is among the important physicochemical parameters routinely considered in water analyses. The highest mean pH obtained for wells located near mechanic workshops was 8.46 while the lowest was 6.33. Minimal seasonal variations among the 4 sites were observed although the trend tended towards an increase from rainy to dry seasons. The control wells recorded a peak pH value of 7.80 while the lowest was 6.60. All pH values in this study were within the WHO (1974) maximum permissible level of 6.5–9.2 but above the highest desirable level of 7-8. Apart from other contaminants, the soil pH might have influenced to a great deal the results so obtained (Okuofu *et al.*, 1990). Itah *et al.*, (1996) reported a mean pH of 7.6 for wells in Calabar municipality while Abubakar and Ibrahim

(2000) report pH 7.2 as the value for Kaduna well water. Similarly, Ugbagwu (2002) reported 7.6 as the mean pH value for well water in Zaria, Northern Nigeria and its environs.

Wells in Keteren-Gwari had the highest (0.003mg/L) mercury level. The mean concentration of mercury in wells located near mechanic workshops for the rainy months was $7.0x10^{-4}$mg/L compared to $5.0x10^{-4}$mg/L in the dry season. There was a significant difference ($P<0.05$) between the two means although both values are below the WHO (1974) recommended maximum permissible level of $1.0x10^{-3}$mg/L. There existed significant differences ($P<0.05$) between the mechanic wells and the controls. These confirm the unacceptable levels of mercury (Hg) pollution in wells located in the mechanic sites studied. Ideally, water for consumption should be Hg free (Agbu, *et al.*, 1988). Hg can be accumulated by the kidney, brain and bone marrow and pathophysiological presentations can occur (Ayalogu, *et al.*, 2001). The particularly higher concentration of the metal in wells in the rainy season highlights the important effect of runoff in its deposition.

The highest Pb concentration (0.095mg/L) was obtained in Maitumbi wells. Lead (Pb) levels in the rainy season (0.071mg/L) were significantly different ($P<0.05$) from the dry season (0.038mg/L) values. The same is true when seasonal values were compared with their controls. All the concentrations, mechanic wells and controls were beyond the WHO (1974) maximum permissible levels (0.01mg/L) of the metal in drinking water. Itah, *et al.*, (1996) reported mean Pb concentrations of 0.02mg/L for wells in Calabar, Nigeria. Ugbagwu (2002) obtained 0.03mg/L in Zaria. The rainy season control value (0.024mg/L) obtained in the present study approximates their values but the dry season controls ($7.0x10^{-4}$mg/L) was much lower than the values reported by Itah *et al.*, (1996) and Ugbagwu (2002)

Other values cited for this important metal are $7.0x10^{-3}$mg/L (Davies, 1996) and $2x10^{-3}$mg/L (Nordberg, 1990) for natural waters in Kenya and Sweden

respectively. The very high mean values obtained from the wells in the mechanic sites are therefore quite alarming considering the fact that lead is a potent neurotoxicant.

Cobalt (Co) has a high safety margin (250 – 300ppm) (Smith, 1987). This notwithstanding, high Co levels has been implicated as a triggering factor in cases of severe cardiac failure in heavy beer drinkers (Brown and Savory, 1984). Wells located in the vicinity of mechanic workshops in Maitumbi had the highest (0.40mg/L) Co levels. Mean rainy season values (0.17mg/L) were higher than dry season concentrations (0.134mg/L). When seasonal values were compared with their respective controls, there also existed significant differences in the mean concentrations. Although the concentrations reported in this work fall within acceptable limits, the chance of toxicity is enhanced if large volumes of water from these sources are taken daily.

The concentrations of copper in wells located in the vicinity of mechanic workshops in the rainy (1.031mg/L) and dry (0.898mg/L) seasons were below the WHO (1974) recommended maximum permissible level of 1.5mg/L. Similarly the controls for the two seasons ($2.34x10^{-1}$mg/L and $3.26x10^{-1}$mg/L) were also within the WHO (1974) limit. However all the mean Cu levels obtained for all the wells were above the highest desirable level ($5.0x10^{-2}$mg/L) enumerated by WHO (1974) for drinking water. Bosso wells had the highest (1.500mg/L) Cu levels. When seasonal copper concentrations were compared, significant differences ($P<0.05$) existed. The same was also true in the case of the polluted samples and their controls. Copper affects many biological processes through its cofactor role in specific Cu proenzymes (Prohaska, *et al.*, 1985). Excessive levels of the metal are hazardous to health. High levels of copper have been implicated in necrosis of hepatic parenchymal cells (Schroeder *et al.*, 1996). Itah, *et al.*, (1996) did not detect copper in wells and boreholes in Calabar Nigeria but the streams in the same municipality contained free, total and chelated copper in the range of

0.03mg/L–1.58mg/L. Nordberg (1990) reported that copper concentrations in ground water depends on soil pH and usually does not exceed a few µg/L.

With the exception of some specific conditions, Cadmium (Cd) concentrations in drinking water are generally low (Nordberg, 1990). The WHO (1974) specified a maximum permissible Cd level in drinking water as $1x10^{-2}$ mg/L but its total absence is most desirable. This is due to its high toxicity. In the present study, Chanchaga wells had the highest (0.060mg/L) mean Cd level and the mechanic site wells generally had mean cadmium levels of $1.8x10^{-2}$mg/L (rainy season) and $7.0x10^{-3}$mg/L (dry season). The latter is below the WHO (1971) maximum permissible level.

Iron (Fe) concentrations determined in well water samples from mechanic sites in the rainy season was 1.063mg/L and the dry season figure was 1.187mg/L. Statistically, there was no significant difference ($P>0.05$) between these two. The values were above the highest desirable level (0.1mg/L) and maximum permissible level (1mg/L) given by WHO (1971) for drinking water. The controls for rainy season ($3.13x10^{-1}$mg/L) and dry season ($2.59x10^{-1}$mg/L) were above the highest desirable level ($1.0x10^{-1}$mg/L) but below the maximum permissible level (1.0mg/L). Each control when tested against their respective seasonal means of mechanic well samples was significantly lower. Wells located in the vicinity of mechanic workshops in Bosso had the highest (1.65mg/L) levels of iron. An important contributory factor to the high Fe levels in such wells could be due to the corrosion and subsequent deposition from metal scraps. Iron is important in the synthesis of haemoglobin during haemopoiesis. The high concentrations obtained in this work may be injurious to health because iron is a potent dietary antagonist of copper metabolism (Humphries *et al.*, 1985). Long-term consumption of such water can lead to iron overload resulting in hypertension and hepatic diseases (Finch and Huebers, 1982).

5.1 CONCLUSION

Coliform, Faecal *Streptococci, Staphylococci, Salmonella* and lubricating oil utilizing bacteria were detected in the well water samples analyzed. Most of the wells in the mechanic sites were characteristically shallow, poorly constructed and in unhygienic environments. These factors probably accounted for the high microbial load encountered with the wells. The study has revealed higher mean viable bacterial counts in wells in mechanic sites than the control wells and the organisms were more prevalent in the rainy than the dry seasons. Wells in mechanic sites from Chanchaga gave the highest mean counts ($2.20x10^4$CFU/ml) while the lowest mean viable count ($3.0x10^3$CFU/ml) was obtained from Bosso. Generally low counts were obtained from control wells than wells located in the vicinity of mechanic workshops. Coliform counts were also generally higher in the rainy than the dry seasons, in the wells from mechanic sites than the controls. The highest mean monthly coliform count ($8.0x10^3$CFU/ml) was obtained from Maitumbi wells in the rainy season while the lowest ($1.45x10^3$CFU/ml) was obtained from Chanchaga in the dry season. Faecal *Streptococci* which are a more definite index of recent faecal contamination of a given water source were monitored and their relative abundance was seasonal (more in the rainy season) and also correlated well with the sanitary condition of the sites. *Staphylococci* count of the water sources were also taken. Values were higher in the rainy than dry season, higher also in mechanic than control sites. Bosso mechanic well *Staphylococci* counts were exceptionally high while Chanchaga counts were very low and varied little with season. The pathogens, *Salmonella* were encountered in much higher levels in Bosso mechanic site wells. The mean monthly counts were unacceptably high in all the localities but most of the controls had no counts especially in the dry season. Hydrocarbon degraders were sought for and the Bosso wells gave the highest mean monthly count of $7.1x10^1$CFU/ml. This dropped by October to the lower counts encountered in the other sites. The Bosso value was attributed most likely to higher oil pollution at the study period. The

controls had low or no counts especially in the dry season. Lubricating oil degrading bacterial isolates incubated with 0.1%, 0.5% and 1.0% oil concentrations respectively, demonstrated that the organisms thrived best in 0.1% oil concentration. The most promising oil utilizers were found to be *Micrococcus* and *Bacillus* sp. The highest mean monthly pH value (8.46) was obtained in Keteren-Gwari while the lowest (6.33) was from Chanchaga mechanic wells. The controls gave 7.80 (highest – Keteren-Gwari) and 6.60 (lowest – Bosso). Keteren-Gwari sites consistently gave the highest mean pH values. Heavy metal levels (mg/L) were very high especially in the rainy season (Hg = 0.0007; Pb = 0.071; Co = 0.171; Cu = 1.031; Cd = 0.018 and Fe = 1.063). Well water samples from the mechanic sites had significantly ($P<0.05$) higher metal levels than the control sites. Most of the metals exceeded the WHO (1974) maximum permissible level. The results of the bacteriological and heavy metal analyses of water samples from wells located in the vicinity of mechanic workshops in Minna have demonstrated microbial loads and pollutant levels above the International Standards recommended by the World Heath Organisation (1974) for untreated water. These wells are hence unfit for use in domestic chores most especially in the rainy season.

5.2 RECOMMENDATIONS

The following recommendations are made:

1. Wastes generated within mechanic sites must be disposed of properly.
2. Mechanic and automobile workshops should be sited away from residential areas to avoid contamination of wells in their vicinity.
3. Motor mechanics and the general populace should be educated on the risks posed by industrial pollutants, most especially the wastes generated from automobile work.

4. Wells within mechanic sites should be deep enough, well constructed, paved with concrete and provided with good lids and locks. Pipe borne water should be supplied for use within such sites.
5. Pollution within mechanic sites should be monitored regularly and regulations should be set and enforced.
6. The microbial isolates that thrived best in the oil media should be studied further with the hope of obtaining strains that can be used to clear oil pollutants in the environment.

REFERENCES

Abubakar, N. and Ibrahim, A. A. (2002). Physicochemical analysis of tube – well water from Anguwar – Shanu and Anguwar – Kanawa, Kaduna, Nigeria. *Nigerian Journal of Biochemistry and Molecular Biology* ***20****:11-22.*

Adesiyun, A.A., Adekeye, J. O., Umoh, J. U. and Nadarajah, M. (1988). Studies on well water and possible health risks in Katsina Nigeria. *Journal of Hygiene* ***90****: 199 – 201*

Agbu, A. A., Alaribe, H. C., Singh, K. and Adesiyun, A. A. (1988). Bacteriological studies and chemical analyses of public well water in Samaru and Zaria city, Northern Nigeria. *Nigerian Journal of Microbiology* ***8*** (1 – 2): 88 – 89

Akinluyi, T. O. (1981). An investigation of extent of faecal pollution of the Niger surface waters. *MSc. Thesis University of Benin, Nigeria*

Adesuyi, A. A. and Alabi, D.A. (1986). A study on the microbiological quality of filtered water in house holds of a University community. *Journal of Hygiene* ***96***:2239 – 248

Amadi, A. (1991). The effect of nitrogen fertilizer plant effluent discharges in the soil. *Journal of Chemistry, Agriculture and the Environment* ***13***:221 – 231

Amund, O.O. and Adebiyi, A. G. (1991). Effect of viscosity on the biodegradability of automotive lubricating oils. *Tribology International* ***3***: 235–7

Amund, O.O., Omale, A. C., Esiobu, N. and Ugoji, O. E. (1993). The effect of waste engine oil spillage on soil physical chemical and microbiological properties. *Journal of Science, Research and Development* ***1*** (1): 61 –64.

Antai, S.P. and Mgbomo, E (1989). Distribution of hydrocarbon utilizing bacteria in oil spill areas. *Microbios letters* ***40***: 137 – 185.

Ayalogu, E.O., Onyeike, E.N. and Ugochukwu, C.N.C. (2001). Physico chemical characteristics of effluents from some industrial establishments in Rivers State, Nigeria. *Proceedings of the 21st Annual Conference of Nigerian Society of Biochemistry and Molecular Biology* ***21***: 71 – 76.

Ayodele, J. T. and Abubakar, M. B. (1998). Trace element contamination of rainwater in the semi-arid region of Kano, Nigeria. *Journal of Environmental Management and Health* ***9***: 180 –188

Bartha, R. and atlas, R.M. (1977). The Microbiology of aquatic oil spills. *Advances in Applied Microbiology* ***22***:225.

Bartha, R. (1986). Biotechnology of petroleum pollutants and biodegradation. *Microbial Ecology* ***12***:155-172

Bello, Y.M (1995). Oil utilizing capabilities of microorganisms isolated from waste oil contaminated soil. *M. Tech. Thesis, Federal University of Technology, Minna, Nigeria.*

Bonde, G.S. (1977). Bacteriological method for the estimation of water pollution. *Journal of Health Laboratory Science* ***3***: 1240 – 1280

Bossert, I. and Bartha, R. (1984). The fate of petroleum in soil ecosystems. *In: Petroleum Microbiology.* Atlas, R. M. (ed.) Macmillan, New York pp. 435 – 473.

Briscoe, A. (1978). Role of water supply in improving health in poor countries with special reference to Bangladesh. *American Journal of Clinical Nutrition* ***31***: 2100 – 2143

Brown, S. and Savory, J. (1984). *Chemical Toxicology and Clinical Chemistry of Metals*. Academic Press, New York, pp. 58 – 60

Bryan, F. L. (1977). Diseases transmitted by foods contaminated by wastewater. *Journal of Food Protection* ***40***: 36 – 45

Cappon, C. J. (2001). Mercury: proportions and determination. *Encyclopedia of Food Science Food Technology and Nutrition* ***4***:315 – 320

Centre for Disease Control and Prevention, CDC, (1999). Atlanta, USA.

Cheesebrough, M. (1991). *Medical Laboratory Manual for Tropical Countries* (1st edn). Butter worth and Co. Ltd., England, pp 60-120

Clarkson, T.W. (1990). Mercury: An element of mystery. *New England Journal of Medicine* ***323***:1137–1139

Davies, G.K. and Mertz, W. (1996). Copper. In: Mertz, W. (Ed). *Trace Elements in Humans and Animal Nutrition*. 5th edn. Academic Press, New York, pp. 301 – 364.

Durum, W. Hem, T.D. and Heidel, S. G. (1996). Reconnaissance of selected minor elements in surface waters of United States. *Geological Survey Circular* ***643***

Duxbury, T. (1986). Microbial metabolism of heavy metals. *Microbiological Science* ***3***:330 – 333

Egereonu, U.U. and Onuchukwu, A.I. (2000). Assessment of atmospheric residual aerosol from different satellite stations 11: Heavy elemental pollutants. *Journal of the Chemical Society of Nigeria* ***25***:23 – 40

Environmental Protection Agency, EPA (1979). National Interim Drinking Water Regulations in USA. *Environmental Reporter* ***132:*** 1-107

Environmental Protection Agency, EPA (1986). Air quality criteria for lead. *EPA Research*, Triangle Park, New York.

Evison, L. M. and James, A. (1984). *Befidobacterium*, an indicator of faecal pollution in water. *Proceedings of 7th International Conference on Water Pollution Research, Paris* ***2***: 16-28

Fagbemi, A. O. and Ijah, U. J. J. (1998). Effect of indiscriminate use of fertilizer and pesticides on rural water sources. In: *Proceedings of 11th Annual National Association of Teachers of Technology (NATT).* Pp. 220-224, July 12-17, Minna, Nigeria.

Fewtrel, L., Kays, D., Jones, F. Baker, A. and Mowat, A. (1996). Copper in drinking water, an investigation into possible health effects. *Journal of Public Health* ***45***: 175 – 177

Finch, C.A. and Heuebers, H. (1982). Perspectives in iron metabolism. *New England Journal of Medicine* ***306***:1520 – 1528

Foulkes, E. C. (Ed.) (1998). *Handbook of Experimental Cadmium Pharmacology*, Springer – Verlag, Berlin, pp. 28 – 36.

Friberg, L. Piscator, M. Nordberg, G.R. and Kjellstrom, T. (1994). *Cadmium in the Environment*. CRC Press, Cleveland, pp. 42-59.

Gadd, G.M. (1986). *Microbes in Extreme Environments.* Academic Press, London, pp. 83-110.

Galinas, Y., Ranjdall, N., Robioloux, L. and Schmidt, J.P. (1996). Well water survey in two districts of Conakry (Republic of Guinea) and comparison with the piped city water. *Journal of Water Quality and Management* ***121 (2)****:* 66- 9.

Garba, M. L. (2000). Lead concentrations in the water of Kubani River Dam, Zaria, Nigeria. MSc Thesis, Ahmadu Bello University, Zaria, Nigeria.

Gbodi, T.A., Makun,H.A., Kabiru,Y.A. and Mudashiru,M.D. (2001) Seasonal nitrate contents of tap and well water in Bosso Niger State, Nigeria. *Journal of Agricultural Science* ***26***(6): 4049-4054.

Geldreich, E. E. (1992). *Water borne Pathogens In Water Pollution Microbiology*. R. M. Williams (ed.) Wiley International London. Pp 5-82.

Gennaro, A. R. (Ed.) (1995). *Remingtons Pharmaceutical Sciences*. (18^{th} edn.) Mack Publishing Co., Pennsylvania, pp. 1029- 1034

Goldberg, A. (1974). Drinking water as a source of lead pollution. *Environmental Health Perspectives* ***7***:103 – 107

Graczyk, T. K., Fayer, R., Trout, J. M. and Suleiman, I. (1998), *Giardia sp.* cysts and infections *Cryptospordium parvum* oocysts in the faeces of migratory Canada geese *(Banta canadenis). Applied and Environmental Microbiology* ***64***:28 – 50.

Gutnick,D. C. and Rosenberg, E. (1977). Oil pollution, a microbiological approach. *Annual Review of Microbiology,* ***31***:3379-396

Haroun, A., Husseini, A. G. and Namadi, M. M. (2002). Effects of industrial effluents (heavy metals) on irrigated crops along Makera stream in Kaduna metropolis, Nigeria.*Proceedings of the Annual Conference of the Botanical Society of Nigeria* ***11***:23-26

Holderness A. and Lambert J. A. (1982). *A New Certificate Chemistry(* 6th edn.) Heinemann Publishers, Ibadan, Nigeria. pp 271.

Hopkins, R. S. and Juranek, D.D. (1991). Acute giardiasis, an improved clinical case definition for epidemiological studies. *American Journal of Epidemiology****133 (4)***: 402-407

Humphries, W. R., Bremuer, I. and Phillippo, M. (1983). The influence of dietary iron on copper metabolism in the calf. In: *Trace Elements in Man and Animals*. Mills, C.F. (ed.). Commonwealth Agricultural Bureaux, U. K., pp. 371-373.

Hutley, S.R.A., Blum, D., Kirkwood, B.R., Dosumuogunbi, O. and Feaachem, R. G. (1990). The Imo State (Nigeria) drinking water supply and sanitation project 2. Impact on drancunculiasis, diarrhea and nutritional status. *Transactions of the Royal Society Of Tropical Medicine and Hygiene* ***84***:316 – 321

Ijah, U.J.J. and Antai, S.P. (2003). Removal of Nigerian light crude oil in the soil over a 12-month period. *International Biodeterioration and Biodegradation* ***51***: 93-99

Inabo, H.I. (1996). Bacteriological quality of water supplies and the prevalence of *Salmonella* and parasitic infections in some rural communities of Kaduna State, Nigeria. PhD. Thesis Ahmadu Bello University, Zaria Nigeria.

Isaac-Renton, J.L. (1987). Giardiasis: A Review. *British Columbia Medical Journal* ***29***:341-344.

Itah, A.Y., Etukudo, S. M. and Akpan, E. J. (1996). Bacteriological and chemical analysis of some rural water supplies in Calabar, Nigeria. *West African Journal of Biology and Applied Chemistry* ***41****:*1-10.

Jawetz, E., Melnick, J.L. And Adelberg, E.A. (1987). *Revisions in Medical Microbiology* (17th edn). Appleton and Lange Publishers, Los Angeles California, pp. 28-38.

Jones, I. (Ed.)(2001). Welcome News Research and Funding. The Welcome Trust (London), Issue 27, pp. 15.

Junaidu, A. U., Adamu, Y.A. and Ibrahim, M.T.O. (2000). Bacteriological analysis of pipe-borne water in Sokoto metropolis, Nigeria. *Proceedings of the Annual Conference of the Nigerian Society of Biology* ***20****:* 11-18

Kendler, B.S. (1993). Lead: An element of danger. *Carolina Tips* ***56*** (1): 1-3.

Klein, I. A. and Snodgrass, W.R. (1997). Heavy metal toxicology. *Encyclopedia of Food Science Food Technology and Nutrition* ***1****:*2319-2325.

Kopp, E.C. and Kroner, B. (2001). Trace element levels in surface waters-a survey. *American Journal of Toxicology* ***23 (2)****:* 1125.

Krenkel, P.A. (1994). Mercury: environmental considerations. Part II. *Critical Reviews in Environmental Control* ***4****:* 252-314.

Levy, D. A., Bens, M.S., Craun, G.F. and Calderon, R. L. (1998). Surveillance for water borne disease out breaks – United States, 1995 – 1996. Morbidity and Mortality Weekly Report. *CDC Surveillance Summary* ***47***(5): 1 – 34

Macrae, R., Robinson, R.K. and Sadler, M. J. (1997). Heavy metals. *Encyclopedia Of Food Science, Food Technology And Nutrition* ***1***: 301-10

McCoy, G.J.W (1981). Sewage pollution of water. In: *Microbial Aspect of Pollution*. Academic Press Incorporated, London, pp 25 – 28

Meesters H.W.G (1983). Bacterial indicators of faecal pollution. *Fellowship programme on environmental science*, Banjul, The Gambia.

Mulkins-Phillps, G.J. and Stewart, J.E. (1974). Distribution of hydrocarbon – utilizing bacteria in Northwestern Atlantic waters and coastal sediments. *Canadian Journal of Microbiology* ***20***: 955-962.

Nordberg, F.G. (1990). Human health effects of metals in drinking water: relationship to cultural acidification. *Environmental Toxicology and Chemistry* ***9***: 887-894.

Odokuma, L.O. and Okpokwasili, G. C. (1993). Seasonal influences on inorganic anion monitoring of the new Calabar River, Nigeria. *Environmental Management* ***17(4)***: 491-496.

Ogunleye, G. (2001). Cholera out breaks in the north of Nigeria. *Journal of hygiene* ***118***: 20-25.

Ojiegbe, R.U. (1990). Rural water pollution and fertilizer application. *Journal of Water Resources* ***2***(1): 116-124.

Okafor, N. (1985). *Aquatic and Waste Water Microbiology*. Fourth Dimension Publishers, Enugu, Nigeria. pp. 51-58.

Okuofu, A. C., Echafona, N.O. and Ayeni, O. G. (1990). Bacteriological and physicochemical examination of well waters in Ahmadu Bello University (main campus) Zaria, Nigeria. *Journal of Water Resources* ***2*** (1): 111-115

Phirke, P. M. and Verma, S. R (1972). Significance of Enterococci as indicators of stream pollution. *Indian Environmental Health* ***14***: 328-334

Prescott, L.M., Harley, J.P. and Klein, C.A (1990). *Microbiology*. Wm. C. Brown publishers, Dubuque pp. 250-325

Prohaska, J. R., Bailey W.F and Cox, D.A. (1985). Failure of iron to reverse copper-dependent anemia in mice. In: *Trace Elements in Man and Animals*, Mills, C.F. (Ed.) Commonwealth Agricultural Bureaux, U.K., pp.27-31

Reed, R. and Singh, B. (2001). Bright idea: Could sunlight provide a cheap and effective means of purifying drinking water in India? *Welcome News (London) Issue **27**:*26-27.

Reed, P.S., Phillips M. and Gashwund D. M. (1993). *Environmental Organic Chemistry*. John Wiley and Sons Inc. New York, U.S.A. pp 50 - 51

Sangodoyin, A. I and Osuji, G. B. (1990). A short fermentation storage effect on water quality. *The Polytechnic Journal of Science and Technology **1(1)***: 14 –16

Schlegel, H. G. (1985). *General Microbiology*. University Press, Cambridge, England, pp. 54-59.

Schroeder, H.A., Nelson, C. P., Tipton, I.A. and Balasa, J.J. (1996). Essential trace elements in man: Copper. *Journal of Chronic Diseases **19**:*1007-34

Smith, R.M. (1987). *Trace Elements in Human and Animal Nutrition*, (5th edn). Academic Press, San Diego, pp.143-183.

Standard Methods for the Examination of Water and Wastewater, SMEWW (1989). American Public Health Association, Washington, D. C

Stryer, L. (2000). *Biochemistry*. Freeman Press, New York, pp. 420.

Sues, M. (Ed.) (1982). *Examination of water for pollution control* (vol. 3). World Health Organization Regional Office for Europe. Pergamon Press, Frankfurt, pp. 205-210.

Talaro, K. and Talaro, A. (1996). *Foundations in Microbiology* (2nd edn). WCB McGraw-Hill Publishers, Boston, pp.610-623.

Ugbagwu, A. A. (2002). Bacteriological, parasitological and physicochemical quality of well water in Zaria, Nigeria. MSc Thesis, Ahmadu Bello University, Zaria, Nigeria, pp.16-20

Umoh, J.U., Adesiyun, A. A., Adekeye, J.O. and Nadarajah, M. (1983). Epidemiological features of an break of gastroenteritis and cholera in Katsina, Nigeria. *Journal of Hygiene* ***101***:3-20.

United States Public Health Service (US PHS) (1998). Toxicological profile for lead. U.S. Government Printing Office, Washington, D.C

Vanderveen, E. and Vanderveen, E.J. (2000). Survey of mercury, lead and cadmium intoxication among exposed workers. *American Journal of Toxicology* ***281*** (1): 1456-1459

Vesiland, P.A. (1975). Environmental Pollution and Control. Ann Abor Science Publishers, Inc.Michigan, pp. 54-59

Volks, W.A. and Wheeler, M.F. (1988). *Basic Microbiology* (6th edn.). Harper and Row Publishers, New York. Pp. 587-589.

Wallace, B. and Cooper, K. (1986). *The Citizens Guide to Lead: Uncovering a Hidden Health Hazard.* New Canadian Press Ltd., Toronto. pp.28-32.

World Health Organization (WHO) (1971). International Standard for Drinking Water. (3rd edn), WHO, Geneva.

World Health Organization (WHO.) (1974). International Standard for Drinking Water (3rd edn), WHO, Geneva.

World Health Organization (WHO) (1989). Lead–Environmental aspects. WHO Publication Centre, Albany, New York.

Zurera-Cosano, G. (1998). Cadmium chemistry and toxicology. *Encyclopedia of Food Science, Food Technology and Nutrition* ***1***:557-61.

APPENDIX I

MEDIA USED:

The media used are listed below.

A. Lactose broth (Oxoid)

This was used for the detection of coliform bacteria. Lactose broth consists of:

Beef extract	3.0g
Lactose	5.0g
Gelysate peptone	5.0g
Distilled water	1 litre
The final pH was adjusted to	6.9

B. Levine's eosin methylene blue agar (EMB) (Oxoid)

This medium was used to confirm the presence of coliform bacteria. It inhibits the growth of Gram-positive bacteria such as *Clostridium perfringens*. Constituents:

Peptone	10.0g
Dipotassium hydrogen phosphate	2.0g
Eosin. Y	0.4g
Methylene blue	0.065g
Agar	15g
Distilled water	1 litre
pH	6.8

C. *Enterococcus* confirmatory broth (Oxoid)

This medium was used to confirm the presence of *Streptococcus faecalis*.

Constituents:

Yeast extract	5.0g
Tryptone	5.0g
Dextrose	5.0g
Sodium azide	0.40g
Sodium chloride	65.0g
Methylene blue	0.001g
Distilled water	1 litre
pH was adjusted to	8.0

D. *Salmonella – Shigella* **(SS) Agar (Oxoid)**

Lab-lemco powder	5.0g
Peptone	5.0g
Lactose	10.0g
Bile salts	8.5g
Sodium citrate	1.0g
Sodium thiosulphate	8.5g
Ferric citrate	10.0g
Neutral red	0.025g
Agar	15.0g
Distilled water	1 litre
pH	7.0

E. Triple sugar iron (T. S. I) agar (Oxoid)

Colonies of *Salmonella* and *Shigella sp.* were streaked on this medium to differentiate between the two organisms.

T. S. I agar has the following composition:

Yeast extract	3.0g
Lab-lemco powder	3.0g
Peptone	20.0g
Lactose	10.0g
Sucrose	10.0g
Dextrose	1.0g
Ferric citrate	0.3g
Sodium thiosulphate	0.3g
Phenol red	0.025g
Agar	15.0g
Sodium chloride	5.0g
Distilled water	1 litre
pH	7.4

F. Mineral salts medium (MSM)

Stock solution A:

NH_4NO_3	4.0g
KH_2PO_4	0.53g
Na_2HPO_4	2.0g
K_2SO_4	0.17g
$MgSO_4.7H_2O$	0.1g
Distilled water	1 litre
pH	7.4

Stock Solution B (Trace Element)

The solution was composed of the following salts:

$CaCl_2.2H_2O$	1.50g
NaCl	0.01g
KI	0.83g
$COCl_2.6H_2O$	0.048g
$NaMoO_4.2H_2O$	0.048g
$CuSO_4.5H_2O$	0.125g
H_3BO_3	0.01g
$MnSO_4.4H_2O$	0.223g
$ZnCl_2$	0.287g
Distilled water	1litre
pH	7.4

G. Oil agar

This was composed of:

Mineral salt medium	100ml
Nutrient agar	2g
Lubricating oil	0.2ml

APPENDIX II

BIOCHEMICAL TEST FOR THE IDENTIFICATION OF MICROBIAL ISOLATES

(i) Gram's staining

The bacterial cultures, which were usually about 24 hours old, were used. Using a sterile wire loop, a discrete colony was picked and a smear was made, allowed to air dry and heat fixed. Crystal violet was used to flood the slide for 1minute, and then washed with Gram's iodine for 1minute and 95% ethanol was used to decolourise the smear. It was counter stained with safranin for 30seconds and washed with water, blotted dry and observed under the oil immersion objective lens of the microscope. The structures and morphology of all isolates were recorded.

(ii) Indole test

Indole test is normally carried out so as to determine the ability of an isolate to break down the amino acid, tryptophan with the enzyme tryptophanase. To carry out this test, peptone water culture of the organism was prepared, kept for 2days after which 0.5ml of Kovac's reagent was added. Positive indole test showed development of a pink rose colour while negative result showed no colony change.

(iii) Methyl red test

Some organisms have the ability to produce acid in dextrose phosphate broth. To carry out this test 5 drops of 0.004% methyl red was added to a 3 day old dextrose phosphate broth culture of the organism under test. Positive result gave red colour while yellow colour indicated negative result.

(iv) Coagulase test

The test was carried out by making a smear of the test organism on a clean slide. Sterile wire loop was used to carry a loopful of undiluted human plasma and added to the smear. A positive test showed immediate clumping, while no clumping indicated negative result.

(v) Sugar fermentation

Phenol red fermentation medium which, contained glucose, fructose, mannitol, sucrose and arabinose were used. The test organism was aseptically inoculated into these tests media. Durham's tubes were introduced into each test tube in an inverted position. A positive change in colour was from red to yellow. This signified acid production showing that fermentation has taken place. This was in addition to gas production, which was noticed at the top part of the Durham tubes. The tubes, which did not show these changes, indicated negative result (i.e. no fermentation).

(vi) Voges-Proskaur test

This test was employed to show the ability of the organism to produce a neutral end product (acetoin = acetylmethylcarbinol) from glucose fermentation. This test was carried out by introducing 1ml of 10% potassium hydroxide to a 2-day old dextrose broth culture of the organism under investigation. This was left at room temperature for 1hour. Appearance of red colour within the first 5 minutes showed positive result while no change in colour indicated negative reaction.

(vii) Catalase test

This test was performed in order to identify the ability of aerobic bacteria to produce varying level of catalase enzymes when they break down hydrogen peroxide to water and oxygen. This was performed by removing a good growth

of the organism with sterile wooden stick or glass rod and immersing it in 3ml of 3% hydrogen peroxide in a test tube. The production of effervescence or gas bubbles indicated that the organism was a catalase producer while negative results showed no change.

(vii) Motility test

The organism under investigation was aseptically inoculated into sterile nutrient broth and incubated at 35°C for 6hours. A loopful of the young broth culture was placed on the centre of a clean cover slip. A small amount of plasticine was placed round the spot where the organism was placed. A clean microscopic slide was inverted with the cover slip on top and the culture hanging underneath. This was observed under the microscope for motility.

(ix) Spore staining

The smear of the organism was flooded with 5% malachite green solution. This was placed over a beaker of boiling water for 5minutes and 0.5% of safranin solution was added, and allowed to stand for 30seconds. The slide was afterwards rinsed in water and examined using the oil immersion objective lens. Spores stained green whereas vegetative cells stained red.

(x) Preparation of MSM stock solutions

In part A, the phosphates were prepared separately and adjusted to pH 7.6 and autoclaved at 1.0kg/cm^2 at 121°C for 15minutes. This solution was added to the bulk medium before cooling. In the case of stock solution B, the salts were dissolved in concentrated Hydrochloric acid (51.3ml/L) and made up to 1litre with distilled water. The pH was also adjusted to 7.6. Solution B was added at 1.0%v/v before autoclaving as in the case of A. Similarly spent motor oil was sterilized separately by autoclaving before being added to the MSM at 0.1%v/v. 1.5%w/v nutrient agar was then added to the minerals.

APPENDIX III

VIABLE COUNTS OF BACTERIA (CFU/ml) IN WELL WATER

Location	Coded wells	Rainy season			Dry season		
		July	Aug.	Sept.	Oct.	Nov.	Dec.
Bosso	B1	$8.5x10^3$	$8.8x10^3$	$7.5x10^3$	$5.1x10^3$	$4.8x10^3$	$3.5x10^3$
	B2	$6.1x10^3$	$3.8x10^3$	$4.1x10^3$	$3.5x10^3$	$3.0x10^3$	$2.5x10^3$
	$\bar{\chi}$	$7.3x10^3$	$6.3x10^3$	$5.8x10^3$	$4.3x10^3$	$3.9x10^3$	$3.0x10^3$
	BN3	$3.6x10^3$	$5.2x10^3$	$3.2x10^3$	$2.8x10^3$	$2.5x10^3$	$2.2x10^3$
Chanchaga	C4	$8.8x10^3$	$2.5x10^4$	$5.5x10^4$	$5.5x10^3$	$4.2x10^3$	$4.3x10^3$
	C5	$4.2x10^3$	$1.8x10^4$	$4.3x10^3$	$4.2x10^3$	$3.0x10^3$	$2.9x10^3$
	C6	$4.5x10^3$	$7.5x10^3$	$6.6x10^3$	$6.0x10^3$	$5.6x10^3$	$3.0x10^3$
	$\bar{\chi}$	$5.83x10^3$	$1.68x10^4$	$2.20x10^4$	$5.23x10^3$	$4.27x10^3$	$3.40x10^3$
	CN7	$3.2x10^3$	$4.0x10^3$	$3.5x10^3$	$2.8x10^3$	$1.4x10^3$	$4.8x10^2$
Keteren-Gwari	K8	$1.2x10^4$	$2.0x10^4$	$1.35x10^4$	$9.9x10^3$	$3.8x10^3$	$4.8x10^3$
	K9	$9.8x10^3$	$9.9x10^3$	$7.4x10^3$	$4.9x10^3$	$3.0x10^3$	$2.8x10^3$
	K10	$2.2x10^4$	$2.4x10^4$	$8.6x10^3$	$1.13x10^4$	$4.5x10^3$	$2.8x10^3$
	$\bar{\chi}$	$1.46x10^4$	$1.80x10^4$	$9.83x10^3$	$7.43x10^3$	$3.77x10^3$	$3.47x10^3$
	KN11	$3.8x10^3$	$5.5x10^3$	$4.2x10^3$	$3.8x10^3$	$3.3x10^3$	$3.8x10^3$
Maitumbi	M12	$7.6x10^3$	$1.2x10^4$	$8.8x10^3$	$5.6x10^3$	$4.8x10^3$	$4.2x10^3$
	M13	$5.1x10^3$	$1.0x10^4$	$6.1x10^3$	$6.0x10^3$	$5.5x10^3$	$5.2x10^3$
	$\bar{\chi}$	$6.35x10^3$	$1.1x10^4$	$7.45x10^3$	$5.8x10^3$	$5.15x10^3$	$4.70x10^3$
	MN14	$4.5x10^3$	$6.2x10^3$	$3.1x10^3$	$3.0x10^3$	$2.8x10^3$	$2.6x10^3$

BN3, CN7, KN11, MN14: Control wells

B1, B2. C4. C5, C6, K8, K9, K10, K12 AND M13: Wells located in the vicinity of mechanic workshops.

APPENDIX IV

COLIFORM COUNTS (CFU/ml) IN WELL WATER

Location	Coded wells	Rainy season			Dry season		
		July	Aug.	Sept.	Oct.	Nov.	Dec.
Bosso	B1	$5.6x10^3$	$7.5x10^3$	$6.2x10^3$	$3.5x10^3$	$2.8x10^3$	$3.3x10^3$
	B2	$5.8x10^3$	$2.0x10^3$	$3.0x10^3$	$3.3x10^3$	$2.0x10^3$	$2.0x10^3$
	$\overline{\chi}$	$5.7x10^3$	$4.75x10^3$	$4.6x10^3$	$3.4x10^3$	$2.4x10^3$	$2.65x10^3$
	BN3	$1.8x10^3$	$2.5x10^3$	$1.8x10^3$	$1.0x10^3$	$4.5x10^2$	$6.6x10^2$
Chanchaga	C4	$6.5x10^3$	$7.6x10^3$	$6.2x10^3$	$3.8x10^3$	$2.2x10^3$	$1.8x10^3$
	C5	$1.9x10^3$	$8.6x10^3$	$2.5x10^3$	$3.2x10^3$	$2.8x10^3$	$5.6x10^2$
	C6	$3.3x10^3$	$6.7x10^3$	$3.2x10^3$	$3.0x10^3$	$2.6x10^3$	$2.0x10^3$
	$\overline{\chi}$	$3.9x10^3$	$4.8x10^3$	$3.97x10^3$	$3.33x10^3$	$2.53x10^3$	$1.45x10^3$
	CN7	$1.8x10^3$	$2.1x10^3$	$1.0x10^3$	$1.1x10^3$	$1.0x10^3$	$2.6x10^2$
Keteren-Gwari	K8	$5.6x10^3$	$7.3x10^3$	$4.6x10^3$	$4.8x10^3$	$3.0x10^3$	$2.8x10^3$
	K9	$4.0x10^3$	$6.2x10^3$	$4.8x10^3$	$4.0x10^3$	$2.0x10^3$	$1.5x10^3$
	K10	$6.8x10^3$	$7.7x10^3$	$3.6x10^3$	$3.1x10^3$	$2.5x10^3$	$2.0x10^3$
	$\overline{\chi}$	$5.47x10^3$	$7.07x10^3$	$4.33x10^3$	$3.97x10^3$	$2.5x10^3$	$2.1x10^3$
	KN11	$1.8x10^3$	$2.9x10^3$	$1.6x10^3$	$1.1x10^3$	$1.5x10^3$	$1.0x10^3$
Maitumbi	M12	$4.5x10^3$	$8.5x10^4$	$3.5x10^3$	$2.9x10^3$	$3.0x10^3$	$2.1x10^3$
	M13	$2.1x10^3$	$7.5x10^4$	$4.4x10^3$	$2.0x10^3$	$2.5x10^3$	$2.2x10^3$
	$\overline{\chi}$	$3.3x10^3$	$8.0x10^3$	$3.95x10^3$	$2.45x10^3$	$2.75x10^3$	$2.15x10^3$
	MN14	$1.5x10^3$	$2.3x10^3$	$1.1x10^3$	$1.3x10^3$	$1.0x10^3$	$5.4x10^2$

BN3, CN7, KN11, MN14: Control wells

B1, B2. C4. C5, C6, K8, K9, K10, K12 AND M13: Wells located in the vicinity of mechanic workshops.

APPENDIX V

FAECAL *STREPTOCOCCI* COUNTS (CFU/ml) IN WELL WATER

Location	Coded wells	Rainy season			Dry season		
		July	Aug.	Sept.	Oct.	Nov.	Dec.
Bosso	B1	$2.5x10^2$	$1.0x10^2$	$3.0x10^2$	$1.8x10^2$	$1.0x10^2$	$1.1x10^2$
	B2	$3.0x10^2$	$3.2x10^2$	$1.5x10^2$	$1.3x10^2$	$1.4x10^2$	$1.3x10^2$
	$\bar{\chi}$	$2.75x10^2$	$3.6x10^2$	$2.25x10^2$	$2.05x10^2$	$1.2x10^2$	$1.2x10^2$
	BN3	$1.3x10^2$	$1.6x10^2$	$1.1x10^2$	$1.4x10^2$	$1.1x10^2$	$1.0x10^2$
Chanchaga	C4	$3.0x10^2$	$4.1x10^2$	$2.3x10^2$	$1.6x10^2$	$1.4x10^2$	$1.0x10^2$
	C5	$2.1x10^2$	$2.6x10^2$	$1.8x10^2$	$1.2x10^2$	$1.2x10^2$	$1.1x10^2$
	C6	$1.8x10^2$	$3.5x10^2$	$4.0x10^1$	$2.1x10^2$	$1.6x10^2$	$1.4x10^2$
	$\bar{\chi}$	$2.3x10^2$	$3.40x10^2$	$1.38x10^2$	$1.63x10^2$	$1.40x10^2$	$1.17x10^2$
	CN7	$1.6x10^2$	$2.0x10^2$	$1.3x10^2$	$1.1x10^2$	$1.3x10^2$	$1.1x10^2$
Keteren-Gwari	K8	$2.4x10^2$	$2.2x10^2$	$1.8x10^2$	$1.2x10^2$	$1.4x10^2$	$1.4x10^2$
	K9	$1.8x10^2$	$2.3x10^2$	$1.6x10^2$	$1.3x10^2$	$1.3x10^2$	$1.2x10^2$
	K10	$3.0x10^2$	$3.5x10^2$	$2.0x10^2$	$1.5x10^2$	$1.3x10^2$	$1.1x10^2$
	$\bar{\chi}$	$2.40x10^2$	$2.67x10^2$	$1.80x10^2$	$1.33x10^2$	$1.33x10^2$	$1.23x10^2$
	KN11	$1.5x10^2$	$1.6x10^2$	$1.3x10^2$	$2.5x10^1$	$2.0x10^1$	$1.8x10^1$
Maitumbi	M12	$5.2x10^1$	$3.0x10^2$	$2.1x10^2$	$3.4x10^1$	$3.5x10^1$	$2.1x10^1$
	M13	$2.1x10^2$	$2.4x10^2$	$1.8x10^2$	$3.3x10^1$	$3.0x10^1$	$1.9x10^1$
	$\bar{\chi}$	$1.31x10^2$	$2.70x10^2$	$1.95x10^2$	$3.35x10^2$	$3.40x10^2$	$2.0x10^2$
	MN14	$1.6x10^2$	$1.8x10^2$	$1.1x10^2$	$4.1x10^1$	$3.5x10^1$	$2.8x10^1$

BN3, CN7, KN11, MN14: Control wells

B1, B2. C4. C5, C6, K8, K9, K10, K12 AND M13: Wells located in the vicinity of mechanic workshops.

APPENDIX VI

STAPHYLOCOCCI COUNTS (CFU/ml) IN WELL WATER

Location	Coded wells	Rainy season			Dry season		
		July	Aug.	Sept.	Oct.	Nov.	Dec.
Bosso	B1	$2.3x10^2$	$2.1x10^2$	$3.1x10^2$	$1.2x10^2$	$1.1x10^2$	$1.2x10^2$
	B2	$2.1x10^2$	$1.2x10^2$	$1.1x10^2$	$1.0x10^2$	$1.6x10^1$	$1.2x10^1$
	$\bar{\chi}$	$2.2x10^2$	$1.65x10^2$	$2.1x10^2$	$1.10x10^2$	$6.3x10^1$	$6.6x10^1$
	BN3	$1.5x10^1$	$2.1x10^1$	$3.0x10^1$	$1.5x10^1$	$1.0x10^1$	$1.1x10^1$
Chanchaga	C4	$1.8x10^1$	$2.1x10^2$	$1.4x10^1$	$1.0x10^1$	$1.2x10^1$	$1.2x10^1$
	C5	$1.1x10^2$	$1.2x10^1$	$2.1x10^1$	$1.4x10^1$	$1.3x10^1$	$1.1x10^1$
	C6	$2.1x10^2$	$1.8x10^2$	$3.1x10^1$	$2.5x10^1$	$1.6x10^1$	$1.4x10^1$
	$\bar{\chi}$	$1.7x10^1$	$1.7x10^1$	$2.2x10^1$	$1.63x10^1$	$1.37x10^1$	$1.23x10^1$
	CN7	$1.3x10^1$	$1.2x10^1$	$1.0x10^1$	$1.0x10^1$	$1.1x10^1$	$1.1x10^1$
Keteren-Gwari	K8	$2.4x10^2$	$2.1x10^2$	$1.8x10^1$	$1.2x10^1$	$1.0x10^1$	$1.0x10^1$
	K9	$1.6x10^1$	$1.4x10^1$	$1.0x10^1$	$1.2x10^1$	$1.0x10^1$	$1.1x10^1$
	K10	$1.8x10^2$	$1.9x10^2$	$3.2x10^1$	$1.8x10^1$	$1.4x10^1$	$1.2x10^1$
	$\bar{\chi}$	$1.45x10^2$	$1.38x10^2$	$2.0x10^1$	$1.4x10^1$	$1.13x10^1$	$1.1x10^1$
	KN11	$1.2x10^1$	$1.4x10^1$	$2.3x10^1$	$2.2x10^1$	$1.8x10^1$	$1.0x10^1$
Maitumbi	M12	$2.2x10^2$	$2.4x10^2$	$3.5x10^1$	$1.8x10^1$	$1.6x10^1$	$2.0x10^1$
	M13	$3.2x10^1$	$4.6x10^1$	$2.8x10^1$	$1.0x10^1$	$1.2x10^1$	$1.4x10^1$
	$\bar{\chi}$	$1.26x10^2$	$1.43x10^2$	$3.15x10^1$	$1.4x10^1$	$1.4x10^1$	$1.7x10^1$
	MN14	$1.8x10^1$	$1.4x10^1$	$2.2x10^1$	$2.0x10^1$	$1.6x10^1$	$1.2x10^1$

BN3, CN7, KN11, MN14: Control wells

B1, B2. C4. C5, C6, K8, K9, K10, K12 AND M13: Wells located in the vicinity of mechanic workshops.

APPENDIX VII

COUNT OF *SALMONELLA* SPECIES (CFU/ml) IN WELL WATER

Location	Coded wells	Rainy season			Dry season		
		July	Aug.	SEPT.	OCT.	NOV.	DEC.
Bosso	B1	$1.8x10^1$	$2.0x10^1$	$1.6x10^1$	$1.5x10^1$	$1.3x10^1$	$1.1x10^1$
	B2	$0.6x10^1$	$1.0x10^1$	$0.5x10^1$	$0.8x10^1$	$0.5x10^1$	$0.7x10^1$
	$\bar{\chi}$	$1.2x10^1$	$1.5x10^1$	$0.9x10^1$	$1.15x10^1$	$0.9x10^1$	$0.9x10^1$
	BN3	$1.0x10^1$	$0.8x10^1$	$0.2x10^1$	$0.4x10^1$	$0.2x10^1$	ND
Chanchaga	C4	$1.1x10^1$	$1.2x10^1$	$0.5x10^1$	$0.5x10^1$	$0.3x10^1$	ND
	C5	$0.5x10^1$	$1.0x10^1$	$1.1x10^1$	$0.4x10^1$	ND	ND
	C6	$0.6x10^1$	$0.8x10^1$	$0.3x10^1$	$0.3x10^1$	$0.4x10^1$	ND
	$\bar{\chi}$	$0.73x10^1$	$1.0x10^1$	$0.63x10^1$	$0.4x10^1$	$0.35x10^1$	ND
	CN7	$0.4x10^1$	$0.6x10^1$	$0.2x10^1$	$0.2x10^1$	ND	ND
Keteren-Gwari	K8	$0.2x10^1$	$0.8x10^1$	$0.5x10^1$	$0.3x10^1$	$0.2x10^1$	ND
	K9	$1.0x10^1$	$0.9x10^1$	$0.5x10^1$	$0.4x10^1$	$0.4x10^1$	$0.2x10^1$
	K10	$0.4x10^1$	$1.1x10^1$	$0.6x10^1$	$0.5x10^1$	$0.4x10^1$	ND
	$\bar{\chi}$	$0.53x10^1$	$0.93x10^1$	$0.53x10^1$	$0.40x10^1$	$0.33x10^1$	ND
	KN11	$0.2x10^1$	$0.3x10^1$	$0.2x10^1$	ND	ND	ND
Maitumbi	M12	$0.8x10^1$	$1.0x10^1$	$0.5x10^1$	$0.6x10^1$	$0.6x10^1$	$0.4x10^1$
	M13	$0.4x10^1$	$0.6x10^1$	$0.8x10^1$	$0.4x10^1$	$0.6x10^1$	ND
	$\bar{\chi}$	$0.6x10^1$	$0.8x10^1$	$0.65x10^1$	$0.33x10^1$	$0.6x10^1$	ND
	MN14	ND	$0.6x10^1$	$0.5x10^1$	ND	ND	ND

BN3, CN7, KN11, MN14: Control wells

B1, B2. C4. C5, C6, K8, K9, K10, K12 AND M13: Wells located in the vicinity of mechanic workshops.

ND: Not detected

APPENDIX VIII

LUBRICATING OIL UTILIZING BACTERIAL COUNTS (CFU/ml) IN WELL WATER

Location	Coded wells	Rainy season			Dry season		
		July	Aug.	Sept.	Oct.	Nov.	Dec.
Bosso	B1	$1.2x10^{1}$	$1.0x10^{1}$	$0.8x10^{1}$	$0.8x10^{1}$	$0.6x10^{1}$	$0.4x10^{1}$
	B2	$1.3x10^{2}$	$1.0x10^{2}$	$0.9x10^{2}$	$1.0x10^{1}$	$0.8x10^{1}$	$0.5x10^{1}$
	$\bar{\chi}$	$1.2x10^{1}$	$1.5x10^{1}$	$0.9x10^{1}$	$1.15x10^{1}$	$0.9x10^{1}$	$0.9x10^{1}$
	BN3	$0.2x10^{1}$	$0.3x10^{1}$	ND	ND	ND	ND
Chanchaga	C4	$0.4x10^{1}$	$0.6x10^{1}$	$0.8x10^{1}$	$0.6x10^{1}$	$0.4x10^{1}$	ND
	C5	$0.2x10^{1}$	$0.8x10^{1}$	$1.0x10^{1}$	$0.7x10^{1}$	$0.8x10^{1}$	$0.6x10^{1}$
	C6	$0.4x10^{1}$	$1.2x10^{1}$	$1.4x10^{1}$	$0.6x10^{1}$	$0.6x10^{1}$	$0.5x10^{1}$
	$\bar{\chi}$	$0.73x10^{1}$	$1.0x10^{1}$	$0.63x10^{1}$	$0.2x10^{1}$	$0.35x10^{1}$	ND
	CN7	$0.6x10^{1}$	$0.8x10^{1}$	$0.4x10^{1}$	$0.2x10^{1}$	ND	ND
Keteren-Gwari	K8	$0.8x10^{1}$	$0.6x10^{1}$	$0.3x10^{1}$	$0.4x10^{1}$	$0.3x10^{1}$	ND
	K9	$1.2x10^{1}$	$1.5x10^{1}$	$1.6x10^{1}$	$1.4x10^{1}$	$0.8x10^{1}$	$0.6x10^{1}$
	K10	$0.6x10^{1}$	$0.4x10^{1}$	$0.8x10^{1}$	ND	ND	ND
	$\bar{\chi}$	$0.53x10^{1}$	$0.93x10^{1}$	$0.53x10^{1}$	$0.40x10^{1}$	$0.33x10^{1}$	ND
	KN11	$0.4x10^{1}$	$0.6x10^{1}$	$0.7x10^{1}$	$0.4x10^{1}$	ND	ND
Maitumbi	M12	$1.0x10^{1}$	$1.2x10^{1}$	$0.6x10^{1}$	$0.6x10^{1}$	$0.4x10^{1}$	ND
	M13	$0.6x10^{1}$	$0.4x10^{1}$	$0.4x10^{1}$	$0.2x10^{1}$	ND	ND
	$\bar{\chi}$	$0.6x10^{1}$	$0.8x10^{1}$	$0.65x10^{1}$	$0.33x10^{1}$	$0.6x10^{1}$	ND
	MN14	$0.4x10^{1}$	$0.3x10^{1}$	$0.6x10^{1}$	ND	ND	ND

BN3, CN7, KN11, MN14: Control wells

B1, B2, C4, C5, C6, K8, K9, K10, K12 AND M13: Wells located in the vicinity of mechanic workshops.

ND: Not detected

APPENDIX IX

pH VALUES OF WELL WATER

Location	Coded wells	Rainy season			Dry season		
		July	Aug.	Sept.	Oct.	Nov.	Dec.
Bosso	B1	7.00	6.80	7.80	8.40	8.40	8.00
	B2	6.50	7.50	7.40	7.80	6.90	7.80
	$\bar{\chi}$	6.75	7.15	7.60	8.10	7.51	7.90
	BN3	6.60	7.20	7.40	7.20	7.60	7.40
Chanchaga	C4	6.80	7.60	7.40	7.20	7.80	7.20
	C5	6.00	6.50	8.20	7.60	8.20	7.60
	C6	6.20	6.00	8.00	7.40	8.00	7.80
	$\bar{\chi}$	6.33	6.70	7.86	7.40	8.00	7.53
	CN7	6.80	7.60	7.60	7.60	7.20	6.80
Keteren-	K8	6.50	8.20	8.00	7.60	8.80	7.60
Gwari	K9	7.50	6.00	7.86	7.40	8.40	8.40
	K10	6.20	7.40	8.40	8.40	8.20	8.40
	$\bar{\chi}$	6.73	7.20	8.08	7.80	8.46	8.13
	KN11	7.40	7.00	7.20	6.80	7.80	7.60
Maitumbi	M12	6.00	6.50	8.00	8.60	7.40	8.60
	M13	7.20	8.00	7.60	8.20	7.60	7.40
	$\bar{\chi}$	6.60	7.25	7.80	8.40	7.50	8.00
	MN14	7.60	6.90	6.80	7.40	7.40	7.60

BN3, CN7, KN11, MN14: Control wells

B1, B2. C4. C5, C6, K8, K9, K10, K12 AND M13: Wells located in mechanic sites.

APPENDIX XA

PREPARATION OF STOCK SOLUTIONS (SUES, 1982)

Cadmium (Cd) standard solution was prepared by dissolving 0.1g cadmium metal in 20ml of distilled water plus 5ml concentrated HCl. Heat was used to aid dissolution. The solution was transferred to a 1 litre volumetric flask and diluted to volume with distilled water (1 ml = 100 ug Cd). This standard was also added 1.5 ml of conc., HNO_3 per litre. The calibration curve was obtained by appropriate dilutions of the stock (0, 0.2, 0.4 0.8, 1.0ml) which were read at 228.8nm with a Cadmium lamp (App.XB).

A standard solution of Co was prepared by dissolving 4.037g cobaltous chloride ($COCl_2.6H_2O$) in distilled water. 10ml of Conc. HNO_3 was added and diluted to 1 litre with distilled water (1.0ml = 1.0mg Co). Appropriate dilutions to yield 0,2,4,8 and 10ppm were read at 240.7nm using a Co cathode lamp. The absorbances were used to plot a calibration curve for Co. (App XB.).

The standard solution for Cu was prepared by dissolving 1.00g of Copper metal in 10ml 1+1 HNO_3 and diluted to 1 litre with distilled water (1.0ml= 1.0mg Cu). The standard also contained 1.5 ml conc. HNO_3 per litre; a calibration curve was plotted with dilutions corresponding to 0, 2, 4, 8, 16 ppm, after reading the absorbances at 324.7 nm with a copper cathode lamp (App XB .).

Iron (Fe) standard solution was prepared by dissolving 1.0g of iron wire in 50ml of HNO_3 (1+1). It was then diluted to 1litre with deionized water (1ml= 1mg Fe). Calibration dilutions of 0,2,4,8,16ppm were prepared from the stock solution and absorbance for each was taken at 248.3 nm using an Iron hollow cathode lamp and used for plotting the calibration curve (App XB).

Lead (Pb) standard solution was prepared by dissolving 1.599g of lead nitrate ($Pb(NO_3)_2$) in distilled water,10ml concentrated nitric acid (HNO_3) was added and the solution diluted to one litre with distilled water (1.0 ml = 1.0mg Pb).

Into five 100ml volumetric flasks, 0, 0.2, 0.4, 0.8 and 1.0 ml each of the Pb standard solution was pippeted and made up to the mark with distilled water. These gave 0,2,4 ,8,10 ppm of Pb respectively. Their absorbances were taken at a wavelength of 283.3 nm using Pb cathode lamp and used to plot the calibration curve (App XB).

Standard mercury (Hg), solution was prepared by dissolving 1.354g of mercuric chloride (HgCl) in 7000ml of deionized water.1.5ml of conc. nitric acid was added and diluted to one litre with water (1.0 ml = 1. 0 mgHg). Absorbances of 0,2,4, 8,10 ppm dilutions were taken at 253.7nm using a mercury hollow cathode lamp and used for the construction of the calibration curve (App XB).

APPENDIX XB
STANDARD CURVES

(a) Cadmium (Cd) 228.8nm

Conc. (ppm)	0.00	2.0	4.0	8.0	10.0
Abs.	0.0001	0.0006	0.0011	0.0021	0.0028

(b) Cobalt (Co) 240.7nm

Conc. (ppm)	0.00	2.0	4.0	8.0	10.0
Abs.	0.000	0.0012	0.0023	0.0048	0.0057

(c) Copper (Cu) 324.7nm

Conc. (ppm)	0.00	2.0	4.0	8.0	16.0
Abs.	0.0002	0.0016	0.0035	0.0069	0.0140

(d) Iron (Fe) 248.3nm

Conc. (ppm)	0.00	2.0	4.0	8.0	16.0
Abs.	0.000	0.0014	0.0029	0.0054	0.0109

(e) Lead (Pb) 283.3nm

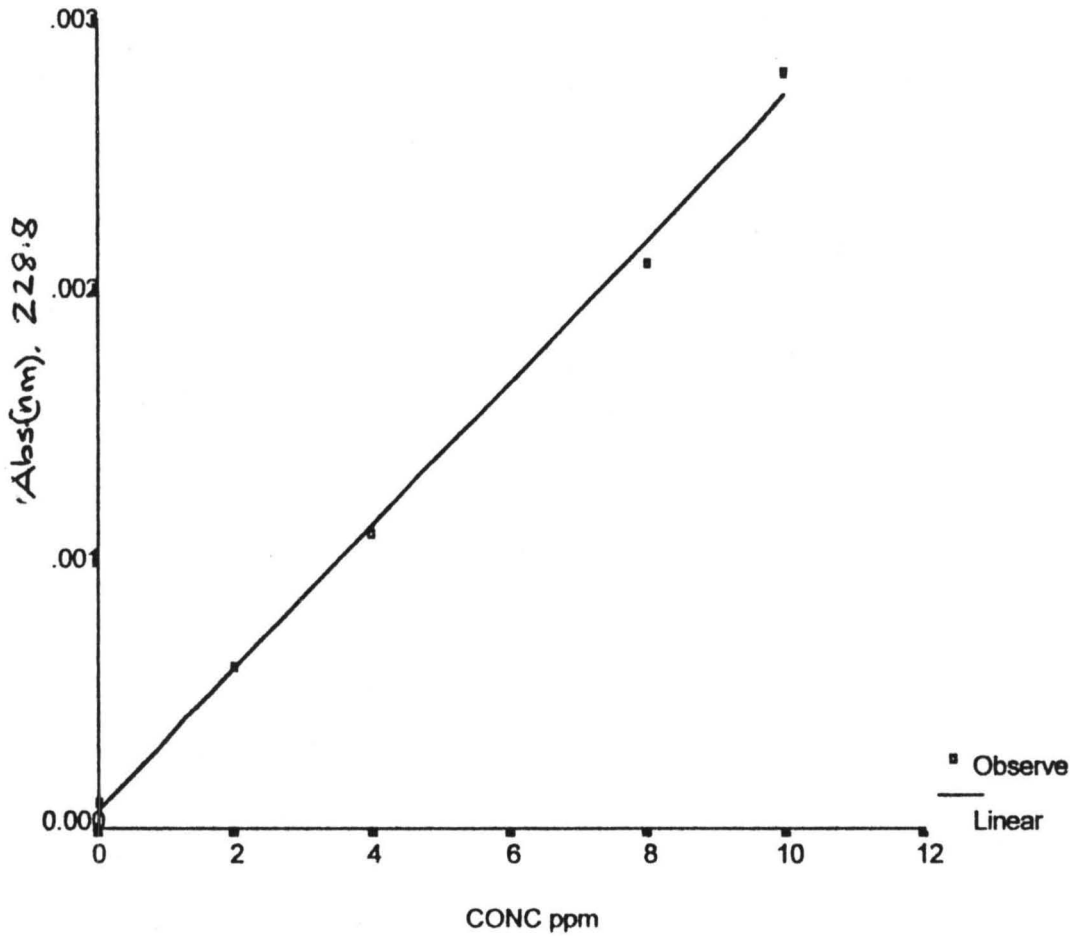
Conc. (ppm)	0.00	2.0	4.0	8.0	10.0
Abs.	0.00002	0.0010	0.0018	0.0040	0.0050

(f) Mercury (Hg) 253.7nm

Conc. (ppm)	0.00	2.0	4.0	8.0	10.0
Abs.	0.000	0.0009	0.0014	0.0029	0.0044

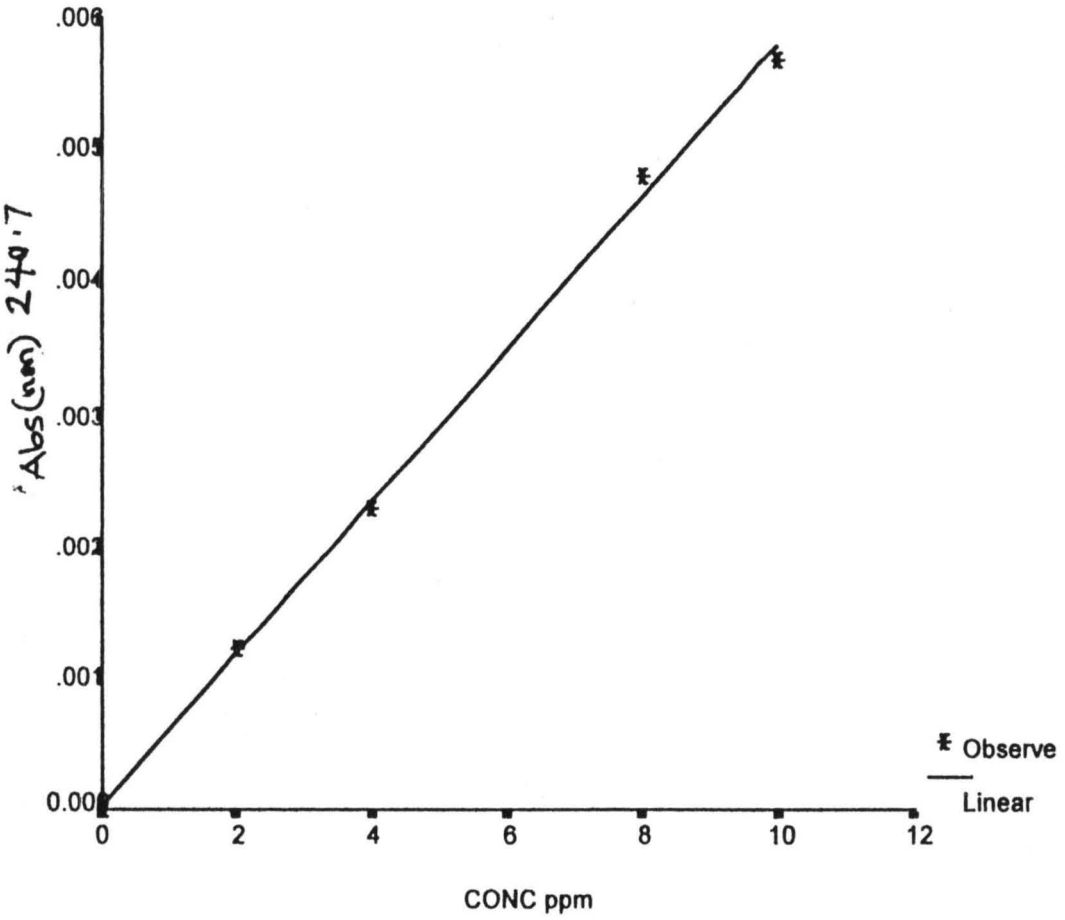
Cadmium = 0.000067 +0.000265 Conc

R^2 = 99.7%



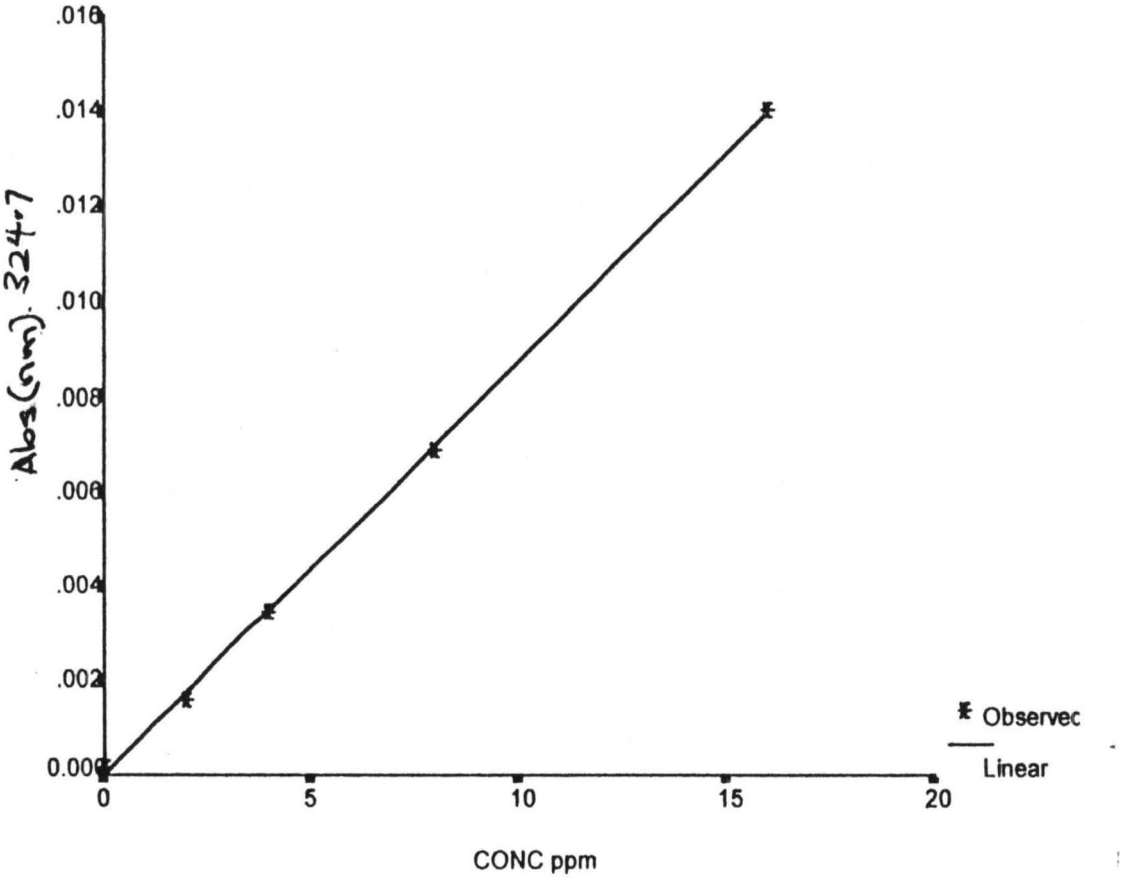
a. Calibration curve for Cadmium

Cobalt =0.000031 + 0.000578 Conc
R^2 = 99.8%



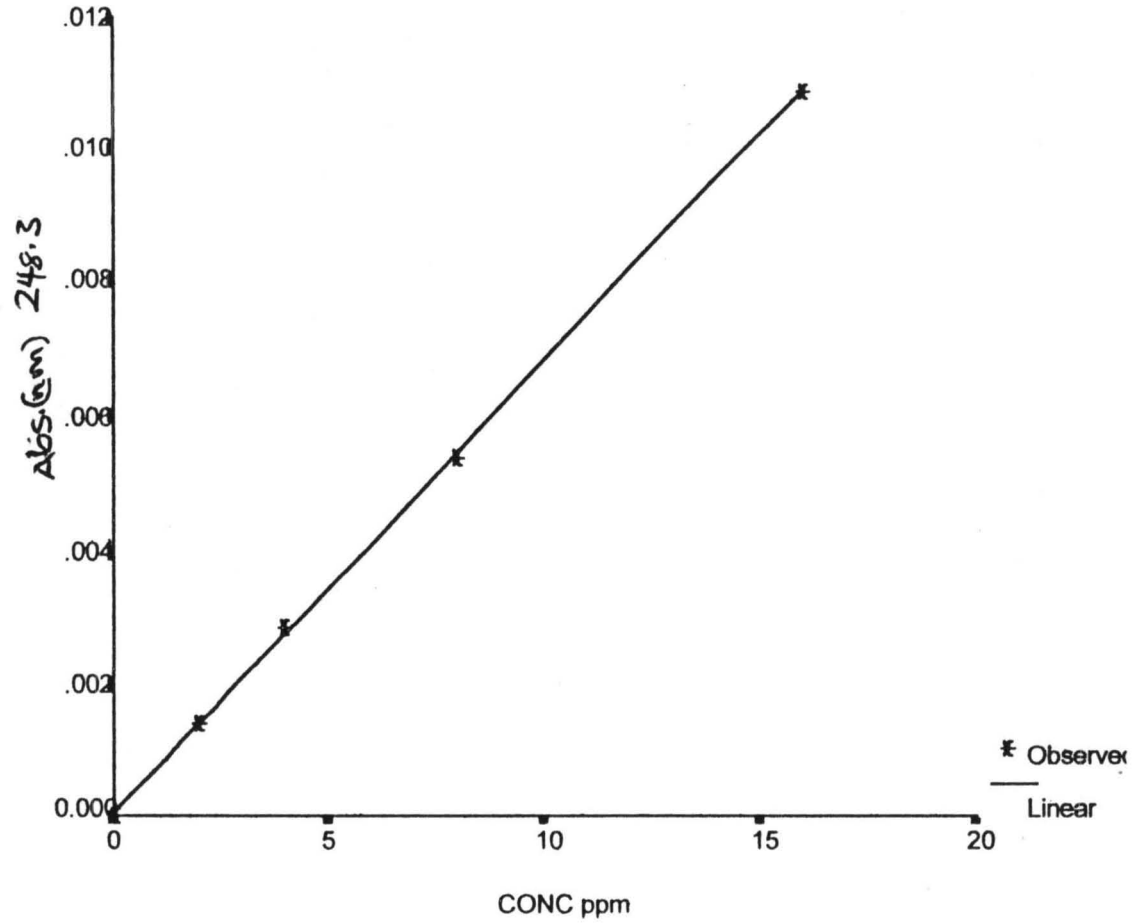
b. Calibration cuve for Cabalt

Copper = 0.000020 +0.000870 Conc
R^2 = 99.2%



c. *Calibration curve for Copper*

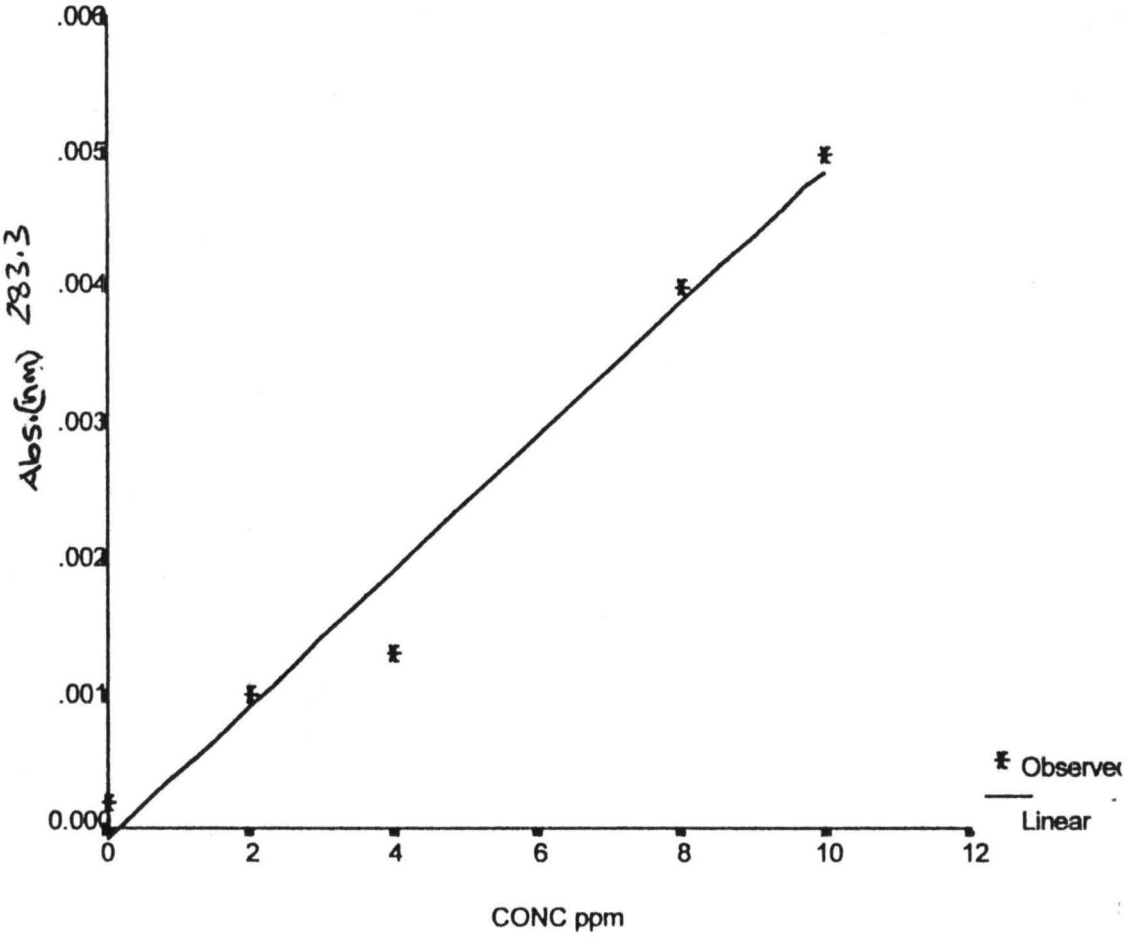
Iron = 0.000055 +0.000678 Conc
R^2 = 100%



d. Calibration curve for Iron

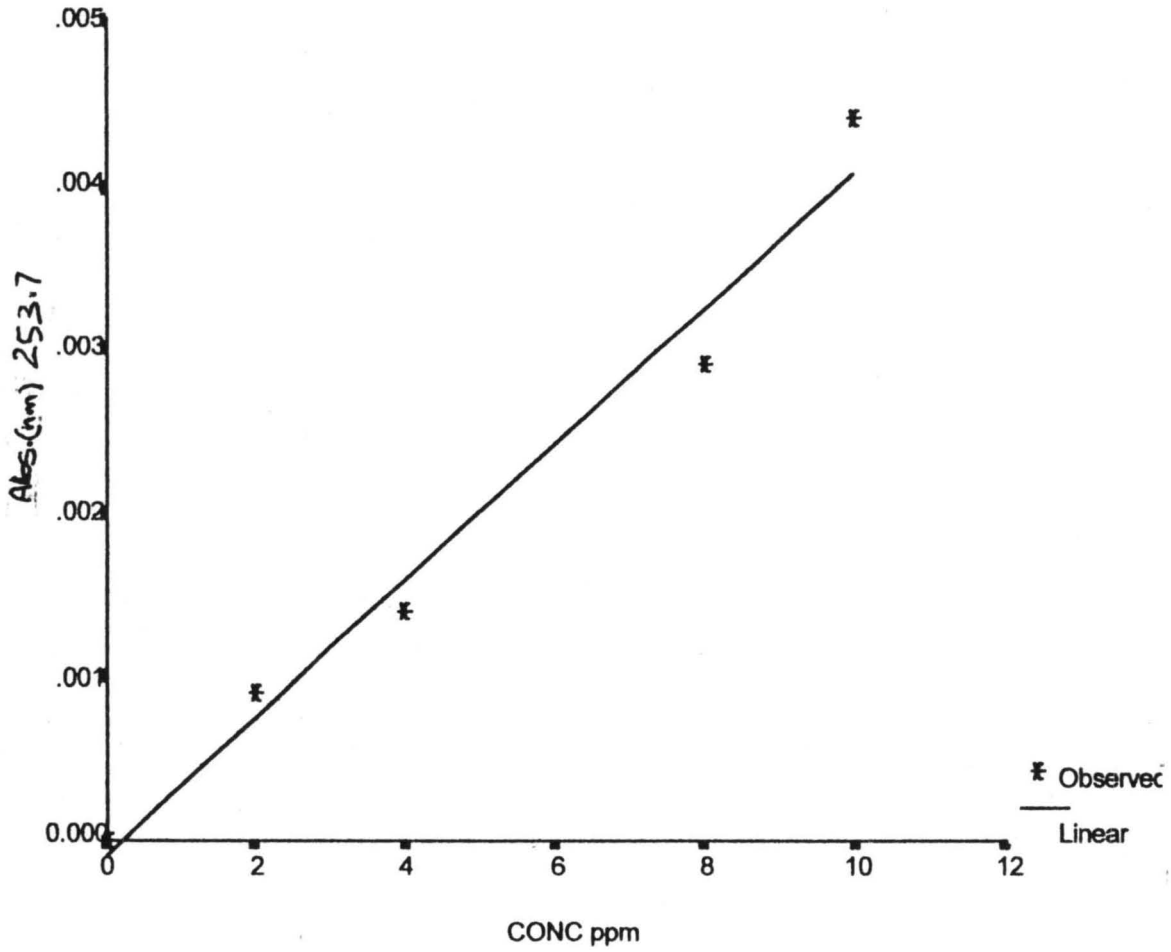
Lead = -0.000072 +0.000494 Conc

R^2 = 97.2%



e. *Calibration curve for head*

Mercury = -0.000070 +0.000415 Conc
R^2 = 97.6%



f. Calibration curve for Mercury

APPENDIX XI

WORLD HEALTH ORGANIZATION DRINKING WATER STANDARDS

CHARACTERISTIC	HIGHEST DESIRABLE LEVEL	MAXIMUM PERMISSIBLE LEVEL
Total solids (mgk)	500	1500
Colour ('H)	5	50
Tast odour	Unobjectionable	-
Odour	Unobjectionable	-
Turbidity (FTU)	5	25
Chloride (mg/L)	200	600
Iron "	0.1	1
Manganese "	0.05	0.5
Copper "	0.05	1.5
Zinc "	5	15
Calcium "	75	200
Magnesium "	30	150
Sulpahte "	200	400
Total hardness (as $CaCO_3$) (mg/L)	100	500
Nitrates (as NO_3) mg/L	45	-
Phenol (mg/L)	0.001	0.002
Anionic detergent	0.02 0.9-1.7 (mean temp 12^0C) 0.6-0.8 (mean temp 32^oC)	1.0
Fluoride (mg/L)		
pH (units)	7-8	Mean 6.5 Max. 9.2
Arsenic (mg/L)	-	0.05
Cadmium (mg/L)	-	0.01
Chromium (6+1) "	-	0.05
Cyanide "	-	0.05
Mercury "	-	0.001
Lead "	-	0.10
Selenium "	-	0.10
Polynuclear Aromatic Hydrocarbons (mg/l)	-	0.0002
Gross alpha radioactivity (pc/L)	-	3
Gross beta radioactivity (pc/L)	-	30

Bacteriological standards for water in distribution system

(i) In 95% of samples examined throughout a year coliform bacteria should be absent in 100ml
(ii) No sample should contain *E. coli* in 100ml
(iii) No sample should contain 10 coliform organisms for 100ml
(iv) Coliform organisms should not be detectable in 100ml of any two consecutive samples.