BACTERIOLOGICAL ANALYSIS OF WELL WATER IN MAIKUNKELE, BOSSO AND TUNGA AREAS OF MINNA

BY

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BEING A THESIS SUBMITTED TO THE DEPARTMENT OF BIOLOGICAL SCIENCES, SCHOOL OF SCIENCE AND SCIENCE EDUCATION IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE MASTER OF TECHNOLOGY (M.TECH) DEGREE IN APPLIED MICROBIOLOGY (ENVIRONMENTAL AND PUBLIC HEALTH MICROBIOLOGY)

DEDICATION

Dedicated to the El-Shadar God, my beloved wife, Comfort Lami Kolo, my children, Elizabeth Kolo (Jnr), Joyce Kolo and Jeremiah Kolo for missing much of my presence, and my aged grand mother, Madam Elizabeth Kolo (Snr).

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APPROVAL PAGE

This Thesis entitled Bacteriological Analysis of Well Water in Maikunkele, Bosso and Tunga Areas of Minna was examined and found to meet the regulations governing the award of the degree of Master of Technology (M.TECH) of the Federal University of Technology, Minna and is approved for its contribution to knowledge and literary presentation.

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DECLARATION

I hereby declare that this work is original and has not been presented anywhere for the award of any degree. Information obtained from all literatures have been duly acknowledged in the references.

ABSTRACT

A survey was conducted on the well water samples in Maikunkele, Bosso and Tunga areas of Minna, to ascertain the degree of faecal pollution using standard microbiological techniques. Generally, the population of viable bacteria was higher in the rainy season than in the dry season in the three locations. In Maikunkele, the bacterial load range from 4.3 X 10^2cfu/ml to 2.4 X 10^6 cfu/ml and from 2.5 X 10^5 cfu/ml to 9.6 X 10^3 in the rainy and dry seasons respectively. In Bosso, the bacterial load ranged from 5.4 X 10^4cfu/ml to 9.5 X 10^5cfu/ml and from 5.1 X 10^4cfu/ml to 8.4 X 10^5cfu/ml in the rainy and dry seasons respectively. In Tunga, the bacterial counts ranged from 4.1 X 10^3cfu/ml to 9.2 X 10^3cfu/ml and from 3.2 X 10^3cfu/ml to 5.8 X 10^3cfu/ml in the rainy and dry seasons respectively. The coliform counts per 100ml of water was generally higher in the rainy season than in the dry season in the three sampling stations. Of the 30 wells sampled, 6 wells had coliform counts as high as >1100 coliforms/100ml of water while 2 wells had 210 coliforms/100ml of water each. However, the results obtained showed that 10 out of the 30 wells sampled could be said to have met the International Standard of untreated drinking water of 10 to 20 coliforms/100ml of water set by the World Health Organization (WHO). Three indicator organisms (Escherichia coli, Streptococcus faecalis and Clostridium perfringens) of faecal pollution were isolated from the well water samples. Of the three organisms, Escherichia coli was more prevalent in the

three locations. It was however observed that the frequency of occurrence of S. faecalis was higher than that of Cl. perfringens in the three locations. On the whole, with the exception of Cl. perfringens at Maikunkele which showed more frequent occurrence in the dry season, the frequency of occurrence of indicator organisms was higher in the rainy season than in the dry season in the three locations. Proteus, Shigella and Pseudomonas species were also isolated and they had higher frequency of occurrence in the rainy season than in the dry season in the three locations. For Salmonella species, the frequency of occurrence was higher in Maikunkele and Tunga, but in Bosso, it was the same in the two seasons. The pH and nitrate content of the water samples were also determined and the results revealed that the highest pH was 7.2 while the lowest 6.9 in the two seasons. These values fall within the permissible pH range (6.5 - 9.2) in potable water. Nitrate contents of the water samples in Maikunkele (2.2 to 26.6ppm), Bosso (4.4 to 22.1ppm) and Tunga (2.2 to 22.1ppm) areas of Minna were higher in the rainy season than in the dry season. These levels are higher than that allowed for drinking water by WHO.

CHAPTER ONE

1.0 INTRODUCTION:

The need for the bacteriological analysis of water in this decade cannot be over-emphasized since there are increasing incidents of water related diseases. Thus, water is a vehicle for numerous pathogens and a host of water-borne diseases. (World Health Organization, WHO; 1971; Wright and Vernom, 1976; Skirrow, 1977; Blake et al; 1980; Kim and Stone, 1980). Long before the demonstration that water was a vehicle of disease, man sometimes suspected it. For instance, following an outbreak of cholera in 1854 in London, a commission was set up under the chairmanship of John Snow, a London anaesthetist. This commission reported a year later (1855), for the first time, and established a casual relation between water and the transmission of bacterial disease (Okafor, 1985). It was found by the commission that, the epidemic was restricted to a particular area of London where the inhabitants drank from a well into which sewage entered from a nearby sewer.

Well water is subterranean water that occurs where all pores in the soil or rock containing materials are 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 the depth to which the water penetrates (Fair et al; 1968; Rheinheimen, 1973; Pelezar et al; 1977; Ajayi and Rahaman, 1986;). 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 number of bacterial load (Pelczar et al; 1977; Okafor, 1985). Salvage (1906) cited by Wilson and Miles (1975) examined fifty shallow wells and found a total of 100 to 200,000 bacterial colonies on a gelatin plate. However, the sanitary quality of drinking water (potable water) is determined by the kinds of microorganisms rather than by numbers (Clark, 1969; Moran et al; 1983; Okafor, 1985; Cheesbrough, 1985). Coliforms, faecal streptococci and Clostridium perfringens are the usual indicator organisms used in bacteriological analysis of water (Okafor, 1985; Prescott et al; 1990).

The pollution problem (of water)is of great concern, because the earth's supply of potable water is on the decline. By the standard of World Health Organization (1974), the environment (which includes soil, water and air) is polluted when it is altered in composition or condition directly or indirectly as a result of the activities of man so that it becomes less suitable for some or all of its uses for which it would be suitable in its natural state (Norman, 1975).

Pollutants enter the water through a number of routes, most important of all is sewage (Okafor, 1985). Untreated sewage is likely to contain pathogens which may contaminate drinking

water (Hemmingway, 1974; Ikporukpo, 1986). Wastes of human and animal origin, agricultural wastes, such as residues of cassava, yam, rice, guinea corn and groundnut shells could gain entry in the process of digging the well or when not properly constructed (Fair-Brother and Taylor, 1965; Kriss et al 1967; Mitchell, 1972; Wilson and Miles, 1975; Pelczar et al; 1977, Okafor, 1985). Besides supplying organic nutrients, the waste could contain microorganisms responsible for enteric infectious diseases in man.

Synthetic detergents such as soap is a group of organic pollutants. The form blowing from sewage works has been shown to contain pathogenic bacteria and ova of worms, and it causes health hazard to humans (Oladimeji and Onwumere, 1987).

Fertilizers have also been identified by many investigators (Atlas and Bartha, 1981; Okafor, 1985; Amadi, 1991) as pollutants of surface and underground water. This is particularly common in more intensive agricultural areas. Amadi (1991) reported that the significant rise in fertilizer usage in Nigeria represents a response to the demand for more food by a starving nation. Fertilizers contaminate well water through infiltration and surface run-off and are known to increase nitrate content of water (Ijah, 1994) Nitrate is harmful to infants but not to growing children and adults. Nitrates in water consumed by infants may be reduced to nitrites leading to infantile methemoglobinemia (infant cyanosis or blue baby disease), as a result of the reduction

in oxygen transport in the blood. This condition occur when nitrate content in water exceeds 45mg/L or even when it is over 10ppm (WHO, 1963; Akinluyi, 1981).

The present study therefore analysed the well water for the presence of nitrates. However, the bulk of the study was directed towards the bacteriological analysis of the water with particular attention to pathogen of faecal origin. It was also necessary to determine the pH of the well water studied, since drinking water

which has high acidic or alkaline characteristics may be dangerous to health. WHO (1963, 1971) puts the acceptable pH value for domestic water at the range of 6.5 to 9.2. The temperature of the well water was also determined since it is possible that changes in temperature could influence other physicochemical properties of water.

American Public Health Association, APHA (1976), Environmental protection Agency, E.P.A. (1976) stated that properly constructed ground waters may not be subject to any possible contamination. WHO (1970) recommended that the coliform index for the untreated water should be less than 10 and no sample should show Most Probable Number (MPN) index greater than 20; while for treated water the MPN index should be less than 1, and not higher than 10. However, (WHO, 1971) published that drinking water should be free from pathogenic forms.

Toilets, soakaways, Pit latrines and passages for the bathroom wastes are not too far from the sites of wells studied.

Therefore, the probability of urine seeping into the wells cannot be ruled out. Human excreta comprises about 135g dry weight of faeces and 1400ml of urine per day (Clark, 1969; Akinluyi, 1981). While Elliot and Rowe (1971) estimated that the daily per capital excretion of coliform in human faeces may number from 125 to 400 billion, large numbers of other types of bacteria and other micro-organisms are also present in faeces (Dukta and Tobin, 1976). Normal Urine as voided contains a few bacteria and the presence of large numbers (10 /ml) of bacteria (bacteriuria) indicates urinary tract infection. Bacteria isolated from urine samples include E. coli, Aerobacter aerogenes; Pseudomonas aeruginosa and Proteus species (Akinluyi, 1981; Nester et al; 1983).

According to Cheesbrough (1985), the World Health Organization has estimated that up to 80% of all sicknesses, and diseases in the world is caused by inadequate sanitation, polluted water, or unavailability of water. The World body also reported that approximately three out of five persons in developing country do not have access to safe or potable drinking water, and only about one in four has any kind of sanitary facility. A report (No.4) released by Water Quality International (WQI) in 1991 states that a third of the death in developing country is due to lack of potable water.

In contaminated water some of the pathogens present are Escherichia coli, Shigella (causes bacillary dysentery), Salmonella (typhoid fever and food poisoning), and a large number of other bacteria and viruses (Fair et al; 1968; Bruce,

1979; Okafor, 1985). There are other water-borne parasitic micro-orgamisms such as Proteus and Pseudomonas species which can also be encountered in contaminated water (Standard Methods for the Examination of Water and Waste water, SMEWW, 1989). Pseudomonas is a known pathogen which is a common cause of hospital acquired infections (Nester et al. 1983). It is motile and grows readily and rapidly on many media. Some strains can also thrive in aqueous solutions, even distilled water and some disinfectant solutions (Nester et al. 1983). Most of these organisms are transmitted to humans through drinking of contaminated water.

Although much work has been carried out in different parts of the world and in Nigeria, on the quality of drinking water, little or no work has been done on the bacteriological quality of water in Minna area. Based on this, the present study became important. The primary aims of this study were:

1. To determine the physicochemical quality of Well water in Maikunkele, Bosso and Tunga areas of Minna.
2. To detect the pathogens that are indicators of faecal pollution (E. coli; S. faecalis and Cl. perfringens) and other pathogenic bacteria which may pose health hazard.
3. To ascertain whether the people keep to good sanitary habits, disinfect and maintain their wells frequently.

This is necessary because a great number of inhabitants not only drink water from the wells but also use it to process food stuffs for sale as well as for other domestic purposes. It is hoped that the study will reveal the extent of pollution

of well water in Minna area due to Man's activities such as farming, improper means of sewage disposal, provision of shelter, socio-economic activities as well as the effect of animal and human wastes such as excreta and urine.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Indicator bacteria of polluted water

Bacteriological analysis of domestic water has become important because safe drinking water and adequate sanitation are basic human needs. Water is essential for a variety of human activities including drinking, recreation, transportation, manufacturing processes in industries and production of food (Diamant, 1980; Okafor, 1985; Sangodoyin and Osuji, 1990). Bacteriological analysis of water involves the examination of water for the presence of indicator organisms. These are discussed below:

2.1.1 Coliforms: These are Gram-negative, oxidase-negative, non-sporing rods capable of growing aerobically on an agar medium containing bile salts, and able to ferment lactose within 48 hours at a temperature of 37°C with the production of both acid and gas. Routine bacteriological analysis of water is aimed mainly at detecting and enumerating coliforms organisms. 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. Both coliforms and enteric pathogenic bacteria exist under like condition.

The coliform bacteria include Escherichia coli, Enterobacter aerogenes and Klebsiella pneumoniae (Jawetz et al, 1974; Prescott et al; 1990). Escherichia coli inhabits primarily the gastro-intestinal tract of man and animals (Schlegel, 1985). It is capable of fermenting lactose with the production of acid and gas at both 37°C and 44°C in less than 48 hours (Bacteriological Examination of Water Supply, BEWS, 1969). Enterobacter aerogenes occasionally are found in man, but is more associated with vegetation (Sykes and Skinner, 1971; Dutka and Tobin, 1976; Standard Methods for the Examination of Water and Waste Water, SMEWW, 1989). Coliforms are the most sensitive faecal indicators at our disposal and number about 10^6 - 10^9/g in human faeces (Meesters, 1983).

Two main groups of coliform exist: Faecal; and non-faecal; coliforms (together forming total coliforms). The former are exclusively faecal in origin; the latter can often be found in faeces, but are also naturally occurring in faecally unpolluted waters (and soil). Hence, the presence of the latter 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 waste water 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 latter. Therefore, in hot climates only faecal coliforms should be used as faecal tracers in the examination of surface and waste water and treatment and reuse processes of the latter (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). The great advantage of membrane filtration method over the M.P.N. are as follows (Okafor, 1985):

1. It could be done rapidly, thus obtaining direct counts of coliforms and E. coli in 18 hours without the use of probability tables.
2. It saves labour, media and glass wares.
3. Neither spore-bearing anaerobes nor mixtures of organisms which may give false positive results on membrane.
4. A sample may be filtered on the spot with limited facilities instead of taking the liquid to the laboratory.

The disadvantages of membrane filtration method are as follows:

1. They are unsuitable for waters of high turbidities in which the required indicator organisms are low in number, since they are blocked before enough organisms have been collected.
2. When non-coliforms predominate over coliforms, the former may over-grow the membrane thus making counting of coliforms difficult.
3. If non-gas producing lactose-fermenters predominate in the water, false results will be obtained.
4. Unlike the MPN method where there is replicates, it does not hold in membrane filtration method; therefore the result may not be confirmed. In view of the disadvantages of membrane filtration method, the MPN method was adopted in the present study.

2.1.2 Faecal Streptococci

The presence of these organisms in water shows a fairly recent faecal pollution (Galvani, 1974; Cheesbrough, 1985). The faecal Streptococcus group consists of a number of species of the genus Streptococcus. These include S. faecalis, S. avium, S. bovis and S. equinus. The members all react positively with lancefields 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 lies in assessing the significance of doubtful results from the coliform tests, particularly when large members of coliforms occur in 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 waters 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 (Cheesbrough, 1985; SMEWW, 1989).

The number of faecal streptococci in human faeces is 10^5 - 10^7/g, hence in general, smaller than that of total coliforms, although the ratio 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).

2.1.3 Clostridium perfringens

Bonde (1962) and Cheesbrough (1985) advocated the use of this bacterium as an indicator organism of faecal pollution. It is regularly found in faeces, though the number of Cl. perfringens in faeces and sewage is much smaller than E. coli and a similar relation between the numbers of Cl. perfringens is detectable in 100ml of such water. E. coli is usually present in large numbers (BEWS, 1969). 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; Moran et al; 1983). Its presence in a natural

contamination in the absence of organisms of the coliform group, suggests that the contamination occurred long ago.

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^{9} - 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 recently proposed for use as faecal indicators in tropical waters (Evison and James, 1974) as they overcome the principal disadvantage of faecal coliform counts in tropical samples, possibly containing a significant proportion of coliform strains, 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 has a very doubtful status as intestinal indicator organism and hence should not be used as much (Meesters, 1983).

Other non-sporulating anaerobes, normally occurring in faeces, have also been proposed as faecal indicators e.g. Bacteriodes species (especially B. fragilis), Peptococcus and Peptostreptococcus species and Eubacterium species. Currently, research is going on, especially on B. fragilis, but as yet there is not enough information available as to its usefulness as faecal indicator. Furthermore, the detection and enumeration techniques used are complicated for routine tests (Meester, 1983).

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 or recent and potentially dangerous faecal pollution (Volk 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 hygiene 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 (Volk and Wheeler, 1988). The purification methods may include filtration, boiling and addition of alum (aluminium sulphate). Alum forms a gelatinous floc that gradually settles out, carrying along particulate matter that includes a large number of microorganisms (Okafor, 1985; Prescott et al; 1990). Even after filtration and coagulation, the possibility still exists that water may contain some bacteria hence the need for disinfection (Okafor, 1985). This is

accomplished 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 (Volk and Wheeler, 1988).

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 numbers (Clark, 1969; Okafor, 1985; Cheesbrough, 1985). Holderness et al. (1982) stated that pure water does not exist in natural state but supplies of water are obtainable all over the world in degrees of purity from rain water (which contains 0.0005% of solid impurities) to sea water (in which impurities reach 3.6%).

It is for this reason that the government of various countries sets standards to be met for drinking water. For instance, the United States E.P.A. in 1977 produced sets of standards for that country while WHO (1970, 1971) produced regional standards for drinking water. WHO (1970) 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 standard however, may not be attainable in all countries, particularly in the third world countries like Nigeria where about 75% of her populace live in rural areas.

2.4 Water-borne diseases

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

a. Cholera: Cholera is a water-borne disease, caused by Vibrio cholerae, a Gram-negative, comma-shaped bacterium. The disease is characterised by sudden diarrhoea with profused watery stool, vomiting, rapid dehydration, fall of blood pressure, subnormal temperature, increased blood acidity of the body tissues and collapse. Except urgent medical care is taken death may occur within 48 hours (Nester et al; 1983; Okafor, 1985).

Cases of cholera outbreak have been recorded in Nigeria. For instance, Njoku-Obi (19h0) reported an outbreak of cholera in Ohaozara in Imo State. The investigator found that personal hygiene of the people played a

major role in the outbreak. The people had no good water supply and their environmental sanitation left much to be desired. Besides, the people had no toilets and they defaecated indiscriminately in the nearby bushes. The Pathogenic V. cholerae from carriers therefore infected the vegetation around, fruits, water supply and fresh cases resulted which gave rise to the outbreak (Njoku -Obi, 1980).

b. Amebic dysentery: This is caused by a protozoan, Entamoeba histolytica. According to Prescott et al. (1990), this parasite is endemic in warm climates where adequate sanitation and proper personal hygiene is lacking. The disease is characterised by mucus, and pus with blood in the faeces. Other clinical features are intestinal inflammation, diarrhoea and watery stool (Pelczer et al. 1977). Prevention of the disease is achieved by avoiding water or food that might be contaminated with human faeces in endemic areas. Unfortunately, Chlorination of water does not destroy E. histolytica (Prescott et al; 1990).

c. Shigellosis. Shigellosis is caused by Shigella belonging to the Enterobacteriaceae family. Shigellosis can be contacted through person-to-person contact, poor quality

drinking water, or contaminated food. Okafor (1985) stated that shigellosis is also known as bacillary (rod) dysentery. The disease is characterized by frequent passage of blood stained, mucous-containing stool. It is reported (Mitchell, 1971) that summer and early autumn are peak periods for the spread of this disease, but, in areas of year-round cold climate the incidence peak occurs in February and March. Shigellosis surveillance indicates that there are at least 32 Shigella serotypes of S. sonei and the sub groups of S. flexneri account for over 90% of all isolates from the human population.

d. Typhoid fever. Typhoid fever is also a water-borne disease that occurs as an acute gastroenteritis with diarrhoea and abdominal cramps. Fever, inflammation of intestine, formulation of intestinal ulcers, enlargement of spleen, a characteristic rose-spot eruption on abdomen, and a toxema also characterize this disease. The incubation period is 10-14 days. It is reported (Okafor, 1985) to be a world wide disease, but can be reduced with good sanitation.

It is caused by a Gram-negative, lactose-fermenting rod-like bacterium, Salmonella species.

e. Infectious Hepatitis. Hepatitis is a common, acute, systematic infectious disease primarily affecting the Liver, and is caused by two viruses, designated hepatitis type A (causing infectious hepatitis) and hepatitis type B (causing serum hepatitis). Infectious hepatitis is usually transmitted by faecal-oral contamination of food, drink, or infected shellfish that live in contaminated water (Prescott et al; 1990). Symptoms last from 2 to 20 days and include anorexia, general malaise, nausea, diarrhea, fever and chills, If the liver becomes infected, jaundice ensues. It is reported (Prescott et al; 1990) that the mortality rate is low (less than 1%), and infections in children are usually asymptomatic. Most cases resolve in four to six weeks and yield a strong immunity.

f. Giardiasis. The disease is caused by Giardia lamblia, a flagellated protozoan. The clinical features or manifestation may vary from the passage of cysts without major malfunctions to severe malabsorption. It is a common disease of tourists particularly in the United States of America where it has been reportedly spread from tourists returning to that country after holidays abroad (Okafor, 1985). Transmission is most frequent with

cyst contaminated water supplies (Prescott et al; 1990). It is also reported (Prescott et al; 1990) that endemic outbreak have been recorded in wilderness areas, suggesting that humans may be infected from "clean water" with various animal Giardia species harboured by rodents, dear, cattle, or household pets. This implies that human infections can also be a zoonosis. As many as 200 million humans may be infected world wide (Prescott et al; 1990). As is the case with the cysts of Entamoeba histolytica, the cysts of this protozoan are not destroyed by Chlorination at the level of contact normally employed in water treatment (Okafor, 1985).

2.5 Some physicochemical parameters of drinking water

2.5.1 Hydrogen ion concentration (pH). The pH of a solution is a measure of hydrogen ion concentration (Vesiland, 1976). The determination of pH in drinking and potable water is vital since the acidity and alkalinity of the medium do affect the growth of microorganisms and taste of water (WHO, 1963, 1971; Vesiland, 1976) 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 values of

7.2 and below than above 9.2.

Fortunately, the pH of most water ranges from pH 6.0 to 7.5.

(WHO, 1963; Best et al 1976; Boro, 1982, SMEWW, 1989), hence 50 - 95% of the free residual chlorine will be present as hypochlorous acid (HOCl). Hypochlorous acid is germicidal (Okafor, 1985; Prescott et al; 1990). At neutral pH, bacteria predominate over fungi but at pH below 6.5 fungi take over (Okafor, 1985).

2.5.2 Nitrate

A significant level of nitrate in water (i.e. over 45mg/L or even over 10ppm) indicates pollution and may be harmful to infants though not to growing children and adults (Moran et al; 1983). The disease caused is known as blue-baby disease or cyanosis; this is due to the reduction in oxygen transport in the blood. Therefore, for potable water nitrate concentration should not exceed 45mg/L or 10ppm. Stewart et al. (1967) investigated nitrate pollution of groundwater in the South Platte Valley of Colorado, USA; an area intensively farmed with many concentrated livestock feeding operations. Ground water samples obtained contained high concentrations of nitrate,

ammonium, nitrogen and organic carbon. Similarly, Karnchanaway and Koottalep (1983), working on well waters quality near a waste disposed site found that the well water was not suitable for drinking due to the moderate contamination by nitrate and manganese besides heavy loads of total and faecal coliforms in the water samples.

2.5.3 **Temperature**

Rivers are used frequently as sources of cooling for factories, and electricity generating units driven by team turbines. The cooling waters from a generating station can raise water temperature by some degrees. This thermal pollution affects the organisms in two ways: First, directly, when the temperature of the water is raised, and, indirectly because the oxygen content of waters is inversely related to temperature (Boro, 1982; Okafor, 1985). This rise in water temperature which inversely affects the oxygen content of waters has adverse effect on aquatic life.

Temperature of 30 - 37°C favours growth of enteric organisms, while thermophiles may survive at higher temperature (Vesiland, 1976). At low temperatures of 4°C to 10°C, growth of psychrophiles is encouraged (Vesiland, 1976).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 DESCRIPTION OF SAMPLING SITES

i) Maikunkele is 15 kilometers (km) from Minna, and it is along Minna-Zungeru road. It is the headquarters of Bosso Local Government Area of Niger State. Many of those working in the Nigerian Airport Authorities live there and not less than three hundred Federal low-cost houses are situated in Maikunkele. Even with this population, there is no major source of tap water in the area.

ii) Bosso Village is another settlement along the same route; it is 6km from Minna township. Federal University of Technology, Federal lowcost houses, Bosso Housing Estate and Secondary Schools, in addition to a market and business centres are situated in this satelite village. The inhabitants occasionally have supply of pipe-borne water.

iii) Tunga is along Minna-Chanchaga-Suleja road. It is 7km from Minna. Some parts of this settlement have fairly good supply of pipe-borne water while some parts are not adequately supplied, particularly the inhabitants of Tunga Lowcost Houses and Rainbow Clinic areas. As a result, many households constructed wells for their domestic water supply.

3.2 Sampling of Water

A total of thirty wells were sampled. These include ten each in Maikunkele, Bosso and Tunga respectively. For the purposes of identification, these wells were coded as follows: Maikunkele (M), Bosso (B) and Tunga (T). Each of the Wells was sampled three times during the rainy season (Mid-June to July 1993) and three times in the dry season (Mid-January to February 1994.

The samples were collected from the wells fortnightly using sterile sampling bottles of 200ml capacity. The wells were shallow, ranging from 2.0m to 3.7m deep (Table 1a)). Each sample taken was analysed not later than six hours, according to Cheesbrough (1985).

Features of each of the wells in the three locations are presented in tables 1b - 1d.

Table 1a. Depth (metres) of Wells sampled in Maikunkele, Bosso and Tunga areas of Minna

Range of depth of well (m)	Number of well: Rainy Season Maikunkele	Bosso	Tunga	Dry Season Maikunkele	Bosso	Tunga
2.0 - 2.5	5	0	0	0	0	0
2.6 - 3.0	5	10	10	0	0	0
3.1 - 3.5	0	0	0	10	10	8
3.6 - 4.0	0	0	0	0	0	2

Table 1b Characteristics of the wells sampled in Maikunkele

Sampling Station	Characteristics of Wells
1.	The well was not elevated or covered nor was it plastered within and outside. It was not fenced.
2.	This was elevated but had no good cover. The inside of the well was plastered. The surroundings were untidy. There was no fence.
3.	It was neither elevated or covered nor plastered within and outside. It was without fence.
4.	It was elevated, covered and plastered within and outside. It was constantly under lock and key. Though there was no fence, the surroundings were plastered. A pumping machine was installed to pump water to the overhead tank.
5.	It was not properly elevated. There was neither good cover nor plastering within and outside.
6.	The well was elevated but had no adequate cover. The well was plastered within and outside. The well had no fence but was located within the court yard.
7.	The well was elevated and plastered within and outside. It was located within a fence and adequately covered. The surrounding was not plastered.
8.	The well was neither elevated nor covered. It was also neither covered nor fenced. The surrounding was not clean.
9.	The well was elevated and plastered but adequately covered. The surrounding was not plastered.
10.	It was not elevated and there was no good cover. The well was not plastered within or outside. There was also no fence.

ıble 1c Characteristics of the wells sampled in Bosso Village

ımpling Station	Characteristics of Wells
1.	The well was elevated but had no proper cover. It was not plastered and there was no fence.
2.	It was elevated without proper cover. The well was neither plastered nor neat. In addition, it was not fenced.
3.	The well was elevated and covered but it was not plastered. There was no fence.
4.	The well, though elevated, was neither plastered nor fenced.
5.	It was elevated but without plastering and fencing.
6.	The well was elevated and covered. It was neither plastered nor fenced.
7.	The well, though elevated, had no proper cover. It was neither plastered nor fenced.
8.	The well was elevated but had no proper cover. It was neither plastered nor fenced. The surrounding was not very neat.
9.	The well was elevated but not adequately covered. It was neither plastered nor fenced.
10.	This well was elevated but not covered. In addition, it was neither plastered nor fenced.

Table 1d Characteristics of the wells sampled in Tunga

Sampling Station	Characteristics of Wells
1.	The well was elevated though adequately covered. It was constructed within the court yard, and was plastered.
2.	It was elevated, plastered and covered. The well was located within a fence. It was under lock and key.
3.	This was not properly elevated nor covered. It was neither plastered nor fenced.
4.	There was neither elevation nor proper cover for the well. In addition, it was neither plastered nor fenced. The surrounding was untidy.
5.	The well was elevated and covered. It was not plastered within and outside. Also, there was no fence.
6.	The well was elevated but without proper cover. It was not plastered within and outside. It was also without fence.
7.	It was elevated, covered properly and plastered outside. It was under lock and key. The well was located within the court yard.
8.	The well was elevated but not covered. It was not adequately plastered within but the outside was not plastered.
9.	It was neither elevated nor covered. It was neither plastered nor fenced.
10.	The well was neither elevated nor covered. It was neither plastered nor fenced.

3.3 Glassware and Media Sterilization

The glasswares were thoroughly washed and dried after rinsing with distilled water. They were sterilized in hot air oven at 160°C for two hours. All the media used were sterilized by autoclaving at 121°C for 15 minutes with the exception of few that were only dissolved by boiling as directed by the manufacturers.

3.4 Enumeration of bacteria (viable counts) in water samples collected from wells in Maikunkele, Bosso and Tunga areas of Minna

The enumeration of viable bacteria was done using the plate count technique. The initial dilution was prepared by pipetting 1ml of water sample into 9ml of sterile distilled water (SMEWW, 1989). The test tube containing the initial dilution was shaken vigorously and rotated between the palms to obtain a uniform distribution of organisms. Serial dilutions were continued until the fourth (10^{-4}) dilution tube.

Each dilution was thoroughly shaken and mixed by rotating the test tubes between the palms before removing aliquots for subsequent dilutions.

Then 1ml each of the dilutions was pipetted aseptically into sterile petridishes into which 20ml of the Blood Agar cooled to 45°C has been poured. The petridishes were then rotated gently to distribute the inoculum throughout the medium.

The petridishes were allowed to solidify and were later incubated in the inverted position to prevent water vapour from condensing on the surface of the Agar which may affect the distribution of bacterial colonies (APHA, 1985). Incubation was at 37°C for 24 to 48 hours. The plates were then observed for bacterial growth and numbers of colonies were counted in each plate according to the method used by Brenniman et al. (1981). The mean of the three plates was recorded.

3.5 Enumeration of coliform bacteria

The coliform group of organisms as determined by the Most Probable Number technique includes all of the aerobic and facultatively anerobic Gram-negative, non-spore forming rods which ferment lactose with gas formation between 24 to 48 hours at 37°C (Pathak et al., 1991).

1. Presumptive test for coliform bacteria

Lactose broth was used in the fermentation tubes as the presumptive test medium. Durham's tubes were inserted into the fermentation tubes. The procedure was carried out as described for the Most Probable Number by Breed et al. (1957; 1974) and Vesiland (1976).

a) Each of the three tubes of double strength presumptive test broth was inoculated with 10ml of each water sample.

b) Each of the three tubes of single strength presumptive test broth was inoculated with 1ml of each water sample.

c) Each of the three tubes of single strength presumptive test broth was inoculated with 0.1ml of each water sample.

All the fermentation tubes were incubated at 37°C. Each tube was examined at the end of 24 hours and where there was no gas formation, incubation period was extended for another 24 hours.

2. Confirmed test for coliform bacteria

Where active fermentation appeared in the fermentation tube before the expiration of the 24 hours period of incubation, the cultures were used for the "confirmed test".

a) The positive presumptive test tubes were mixed by gentle shaking. Then

b) Levine's Eosin Methylene Blue Agar (E M.B. Agar) plate was streaked by taking some loops from positive presumptive test in (a). Eosin methylene blue agar

contains methylene blue which inhibits Gram-positive bacteria such as Clostridium perfringens.

c) The plates were then incubated at 37°C for 24 hours. Typical colonies were examined for the presence of any coliform, isolated colonies which appeared bluish black with metallic sheen or greenish metallic by reflected light and dark purple centres by transmitted light indicate the characteristic presence of E. coli. Colonies of Enterobacter aerogenes appear brownish and tend to coalease, the colonies are mucoid without metallic sheen (Breed et al., 1957).

3. The Completed test for coliform bacteria

a) From the E.M.B. plate, two colonies considered to be organisms of coliform group were picked and each transferred to nutrient agar slant and lactose-broth fermentation tube.

b) The gas production in the lactose broth culture completes the test for coliforms.

c) Microscopy - Gram-stain preparation from the agar slant was done. The appearance of Gram-negative, non-sporing bacilli

indicated that coliforms were present

d) Methylred-Voges Proskauer (MR-VP). These tests are used in distinguishing between Escherichia coli and Enterobacter aerogenes (Schlegel, 1985; Okafor, 1985). Escherichia coli ferments carbohydrates accompanied with acid production and hence the colour of methyl red retains its red (acid) colour, while E. aerogenes ferments carbohydrates without acid production and hence methyl red changes to yellow (Breed et al., 1957).

3.6 Test for Faecal Streptococci

Winter and Sandholzer (1946) described the composition and the use of enterococcus presumptive broth and in the same paper, enterococcus confirmatory broth was described. These media were used in this work to identify and confirm the presence of faecal Streptococci in all water samples. The media were used in exactly the same way as described for that of coliform bacteria, except that Durham's tubes were not inserted into the fermentation tubes. The turbidity after 24 or 48 hours of incubation shown in the fermentation tubes indicated that faecal streptococci were present.

A portion of the positive tubes were streaked on the Pbizer selective Enterococcus agar (P.S.F.) and the plates were incubated at 37°C for 24 hours. Brownish - black colonies confirmed the presence of faecal streptococci. Faecal streptococci densities were estimated from the number of tubes in each dilution series that were positive on P.S.E. (BEWS, 1969; Cheesbrough, 1985).

3.7 Test for Clostridium perfringens

i) The stormy fermentation of milk with the formation of acid and strong (quick) gas production by Clostridium perfringens, in litmus milk as described by Cheesbrough, (1985) was the method used to detect the presence of this organism in this project.

ii) Isolation of pure cultures

Isolated colonies were transferred to sterile nutrient broth under aseptic conditions and incubated at 37°C for 24 to 48 hours. After 24 hours, the isolated cultures were streaked unto solid nutrient agar plates and incubated at 37°C for 24 to 48 hours. Pure colonies were then picked and transferred to nutrient agar slants under aseptic conditions. After incubation at 37°C for 24 hours the slants were removed and kept in a refrigerator (8 ± 2°C) until required.

iii) Microscopic Examination

A small portion of each colony was picked with a wire loop from the streaked plates. A smear of this was made and Gram-stained. After Gram-staining, the examination of the morphology was carried out under light microscope.

3.8 Selective Isolation of Salmonella and Shigella from water samples.

Both the fresh water samples from wells and positive presumptive test tubes obtained with gas production during Most Probable Number (M.P.N.) test were used.

The Salmonella - Shigella (SS) agar plates were aseptically streaked with samples from these two sources using wire loop. The plates were then incubated at 37°C for 24 hours.

Distinct colonies of Salmonella and Shigella species were subcultured on Deoxycholate Citrate Agar (D.C.A.). The result obtained were compared to the colour plates as illustrated by Cheesbrough (1985).

To confirm that the isolates obtained were species of Salmonella and Shigella, isolated colonies on SS agar were sub-cultured on Brilliant Green Agar (BGA). This medium has greater advantage than others in that it exhibits greater inhibition on E. coli, Proteus species and

restriction on Pseudomonas species (BEWS, 1976)

The distinct colonies of Salmonella and Shigella were streaked on Triple Sugar Iron (TSI) slant and the but was made to distinguish between the two species. The Shigella species did not produce hydrogen sulphide (H_2S) gas while Salmonella and proteus species produced H_2S gas. The organisms were also gram-stained and examined under the microscope.

Indole test was carried out using tryptone water. The organisms were inoculated and incubated at 37°C for 48 hours. After this, Kovac's reagent (2 drops) was added to the broth culture. A dark-red layer at the surface indicated that it was indole positive. This test was also carried out to differentiate between Salmonella and Proteus species. The former is motile and the latter non-motile.

3.9 Determination of temperature of water samples

Temperature of the water samples was determined using a conventional laboratory thermometer graduated in degree centigrade (SMEWW, 1989). The water temperature was measured as soon as the water samples were collected (that is, the temperature was taken at the sampling sites).

3.10 Determination of pH

The hydrogen iron concentration (pH) of water

was measured by the use of Electronic pH meter (Model 7010, KENT, England). This was carried out as soon as the samples were brought to the Laboratory, before any other analysis was done.

First, the pH meter was examined to ascertain that it functions properly. The instrument was calibrated with at least one standard buffer solution. The electrode was rinsed in the sample whose pH was then determined. The pH was read to the nearest tenth of a pH unit (Best and Ross, 1977; Boro, 1982). The reading was taken 3 times and the mean recorded.

3.11 Determination of nitrate content of well water samples

Exactly 100ml of water samples from each well was placed in a porcelain dish and then evaporated to dryness on a boiling water-bath. The residue was then cooled and 1ml of phenol-disulphonic acid solution added, care was taken that the reagent made contact with all the residue particles. It was allowed to stand for 10 minutes. Then 10ml of distilled water was added and after cooling the mixture, 10ml of 10% ammonia solution was added and cooled. It was then diluted with water to 25ml (SMEWW, 1985).

At the same time a "blank" solution containing the same quantities of the reagents but omitting the sample under test was similarly treated. One

of the comparator test tubes with test solution was filled and placed in the right-hand compartment of the comparator while the other test tube with blank solution was filled and placed in the left-hand compartment of the Lovibond comparator (Model COH 550, Gallenkamp, England). The colour produced in the test solution with the colour in the standard disc was compared by rotating the standard disc until a match or nearness of colour was obtained. The nitrate content of the sample was calculated as outlined in Water Analysis Handbook (1985).

CHAPTER FOUR

4.0 RESULTS

4.1 VIABLE COUNT OF BACTERIA IN WATER SAMPLES IN MAIKUNKELE, BOSSO AND TUNGA AREAS OF MINNA

The results (Table 2) show that, in Maikunkele, well number M3 had the highest number of viable bacteria, 2.4 x 10^6 colony forming units per millilitre (cfu/ml). However, by the end of the rainy season the well collapsed and it was observed that at this period, well number M10 had the highest bacteria count of 1.2 x 10^5cfu/ml. Well number M4 had the lowest number of bacteria of 7.3 x 10^3cfu/ ml and 4.5 x 10^3cfu/ml in the rainy and dry seasons respectively.

In Bosso, well number B10 had the highest viable bacteria of 9.5 x 10^5cfu per ml and 8.4 x 10^5cfu/ml in the rainy and dry seasons respectively. Well number B7 in both rainy and dry seasons had the lowest bacterial load of 5.4 x 10^3cfu/ml and 5.1 x 10^3cfu/ml respectively.

In Tunga, well number T8 had the highest count of 7.3 x 10^5 cfu/ml and 4.1 x 10^5cfu/ml in the rainy and dry seasons respectively. Well number T2 had the lowest bacterial count in both rainy season (5.8 x 10^3cfu/ml) and dry season (3.2 x 10^3 cfu/ml,. The results are shown in Table 2.

Figure I shows that at the three locations (Maikunkele, Bosso and Tunga) there were higher bacterial loads (cfu/ml) in the rainy season. However, water samples collected from wells in Maikunkele had the highest bacterial load whereas Tunga had the lowest bacterial load.

Table 2

Mean viable counts of bacteria in wells sampled in Maikunkele, Bosso and Tunga areas of Minna.

Sampling Station		No. of Micro-organisms per ml June to July	No. of Micro-organisms per ml January to February 1994
Maikunkele	1	1.6×10^{6}	9.6×10^{5}
	2	2.3×10^{4}	1.8×10^{4}
	3	2.4×10^{6}	-
	4	4.3×10^{2}	2.5×10^{2}
	5	1.5×10^{5}	1.7×10^{4}
	6	1.7×10^{4}	1.3×10^{4}
	7	1.3×10^{6}	1.4×10^{5}
	8	1.9×10^{5}	1.9×10^{5}
	9	4.3×10^{5}	3.8×10^{6}
	10	1.7×10^{6}	1.2×10^{6}
Bosso	1	6.2×10^{4}	5.3×10^{4}
	2	5.8×10^{4}	4.7×10^{4}
	3	8.4×10^{5}	7.9×10^{4}
	4	7.1×10^{4}	6.4×10^{4}
	5	4.2×10^{4}	3.5×10^{4}
	6	3.3×10^{5}	3.0×10^{5}
	7	5.4×10^{3}	5.1×10^{3}
	8	6.8×10^{4}	4.6×10^{4}
	9	5.4×10^{4}	4.5×10^{4}
	10	9.5×10^{5}	8.4×10^{5}
Tunga	1	6.2×10^{3}	5.7×10^{3}
	2	5.8×10^{3}	3.2×10^{3}
	3	6.3×10^{4}	4.5×10^{4}
	4	7.5×10^{4}	6.1×10^{4}
	5	8.3×10^{3}	5.9×10^{3}
	6	5.2×10^{4}	4.7×10^{3}
	7	6.4×10^{5}	8.5×10^{3}
	8	7.3×10^{5}	4.1×10^{5}
	9	4.1×10^{5}	3.6×10^{5}
	10	9.2×10^{5}	7.1×10^{3}

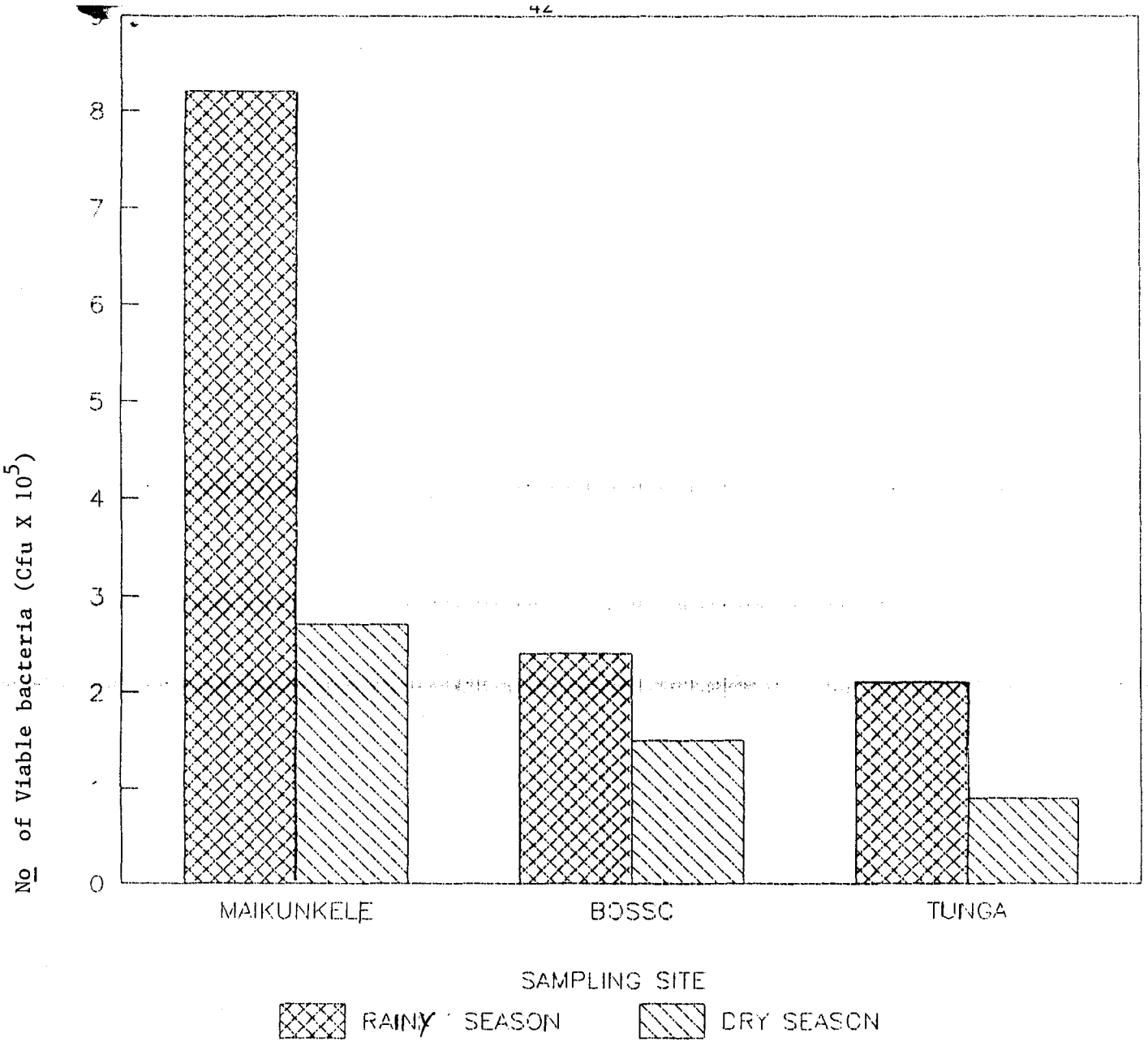

Figure: 1 Mean Viable counts of bacteria in water Samples in Maikunkele, Bosso and Tunga areas of Minna.

4.2 COLIFORM COUNTS IN WELLS IN MAIKUNKELE, BOSSO AND TUNGA AREAS OF MINNA

The Coliform counts (MPN) show that, in Maikunkele, well numbers, M1, M3, M8 and M10 had high number of coliforms, that is, >1100 coliform bacteria per 100 ml of water each. Well number M4 showed the presence of 7 coliforms/100 ml of water, which is the lowest, in the rainy season. In the dry season, well number M8 and M10 had the same number of coliforms (>1100 coliform) bacteria per 100 ml (Table 3) as in the rainy season.

In Bosso, well number B10 had the highest count of >1100 coliform bacteria per 100 ml in the rainy season. Well numbers B1 and B4 had 28 coliforms each (Table 3). In the dry season, well number B10 had the highest coliform count of 250 per 100 ml while well number B4 had the lowest (14) coliform count per 100 ml of water (Table 3).

In Tunga, well number T8 had the highest coliform bacteria (>1100 ml of water) in the rainy season. In the dry season, this well had as low as 210 coliform bacteria per 100 ml of water although well number T2 had the lowest count (11 coliform bacteria per 100 ml of water) in both rainy and dry seasons (Table 3).

In general, the results (Figure 2) show that higher coliform counts were recorded in the rainy season than in the dry season at the three locations studied.

Table 3

Mean Coliform counts in Wells sampled in Maikunkele, Bosso and Tunga area of Minna.

Sampling Station		No. of Positive Tubes Out of 3			Most Probable Number of Coliform per 100ml	No. of Positive Tubes out of 3			Most Probable Number of Coliform per 100ml of sample
		10ml	1ml	0.1ml		10ml	1ml	0.1ml	
Maikunlele (M)	1	3	3	2	>1100	3	2	1	250
	2	2	2	1	28	2	0	1	14
	3	3	3	2	>1100	Collapsed			
	4	1	1	0	7	1	1	0	7
	5	2	2	1	28	2	1	1	20
	6	2	1	1	20	1	1	0	7
	7	2	1	1	20	1	1	1	11
	8	3	3	2	>1100	3	3	2	>1100
	9	3	1	1	75	3	1	0	43
	10	3	3	2	>1100	3	3	2	>1100
Bosso (B)	1	2	2	1	28	2	1	0	15
	2	3	2	1	150	2	2	1	28
	3	3	2	2	210	3	2	1	150
	4	2	1	2	28	2	0	1	14
	5	3	2	1	150	3	2	0	93
	6	3	2	2	210	3	2	1	150
	7	3	1	0	43	2	2	1	28
	8	3	1	1	75	3	0	1	43
	9	3	1	1	75	3	2	0	64
	10	3	3	3	>1100	3	2	2	210
Tunga	1	2	1	1	20	2	1	0	14
	2	1	1	1	11	1	1	0	11
	3	3	1	1	75	3	1	0	43
	4	3	2	2	210	3	2	1	150
	5	3	1	0	43	2	2	1	28
	6	2	1	1	20	2	1	0	14
	7	3	2	2	210	2	2	1	28
	8	3	3	2	>1100	3	2	2	210
	9	3	2	1	150	3	0	2	93
	10	3	0	1	43	2	2	0	21

4.3 INDICATOR ORGANISMS OF FAECAL POLLUTION IN MAIKUNKELE, BOSSO AND TUNGA AREAS OF MINNA

(a) **Test for Escherichia coli**

Table 4 shows that in the rainy season, only well numbers M4, M7 and M9 did not show the presence of E. coli. The same happened in the dry season though with a decrease in the number of organisms in the other wells. In Bosso, well numbers B1, B4, B5, B7 and B9 also did not show the presence of the organism. All others were positive. In Tunga, well numbers T3, T4, T7, T8 and T9 showed the presence of E. coli, in the rainy season while in the dry season only well number T8 was positive.

(b) **Test for Streptococcus faecalis**

Table 4 also shows that S. faecalis was present in Six wells in Maikunkele (M1, M3, M5, M6, M8 and M10) in the rainy season. In the dry season, it was present in three wells (M1, M8 and M10) only. In Bosso, well numbers B2, B3, B6 and B10 showed the presence of the organism in the rainy season. In the dry season, S. faecalis was present in well numbers B3, B6 and B10. In Tunga, well numbers T3, T4, T5, T7, T8 and T9 showed the presence of S. faecalis, only in the dry season.

(c) **Test for Clostridium perfringens**

Table 4 indicates that Cl. perfringens was present in well numbers M1 and M8 in the rainy season. In the dry season, well numbers M1, M8 and M10 showed the

presence of Cl. perfringens. In Bosso, during the rainy season, well numbers B6 and B10 were positive for the organism, and in the dry season, only well number B10 showed the presence of the organism. In Tunga, Cl. perfringens was present in well numbers T4, T7 and T8 in the rainy season. However, in the dry season, the presence of the organism was not observed in any of the wells. Generally, it was observed that E. coli, S. faecalis and Cl. perfringens were more prevalent in the rainy season than in the dry season (Figures 3a,b,c, respectively).

Table 4

Indicator Organisms of faecal pollution in wells samples in Maikunkele, Bosso Tunga areas of Minna.

Sampling Station	Eschericia coli		Streptococcus faecalis		Clostridium perfringens	
	R	D	R	D	R	D
M1	+++	++	+++	+	+	+
2	+	+	-	-	-	-
3	+++	Collapsed	++	Collapsed	-	Collapsed
4	-	-	-	-	-	-
5	+	-	+	-	-	-
6	+	+	+	-	-	-
7	-	-	-	-	-	-
8	++	+	+++	+	+	+
9	-	-	-	-	-	-
10	+++	+	++	+	-	+
B1	-	-	-	-	-	-
2	+	-	+	-	-	-
3	+++	+	++	+	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	++	+	+	+	+	-
7	-	-	-	-	-	-
8	+	+	-	-	-	-
9	-	-	-	-	-	-
10	+++	+	+++	++	+	+

Sampling Station	Escherichia coli		Streptococcus faecalis		Clostridium perfringens	
	R	D	R	D	R	D
T 1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	+	+	-	-	-	-
4	++	-	+	-	+	-
5	-	-	+	-	-	-
6	-	-	-	-	-	-
7	+	-	+	-	+	-
8	++	+	+	-	+	-
9	+	-	+	-	-	-
10	-	-	-	-	-	-

KEY

R = Rainy Season

D = Dry Season

- = Signifies the three sampled plates are negative

+ = Signifies only one sampled plate is positive

++ = Only two sampled plates are positive

+++ = All the three sampled plates are positive.

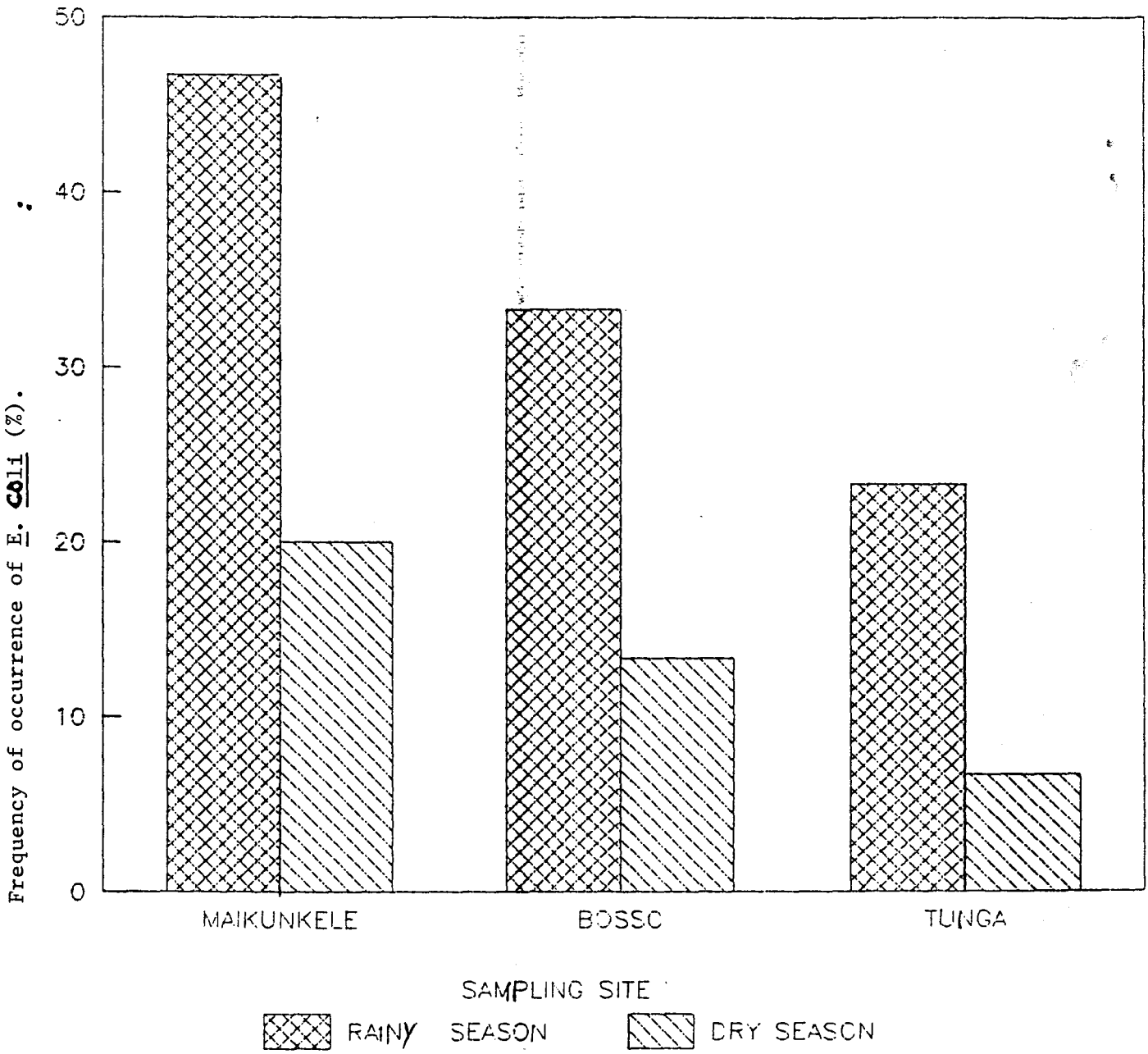

FIgure: 3a Occurrence of E. Coli in wells in Maikunkele, Bosso and Tunga areas of Minna.

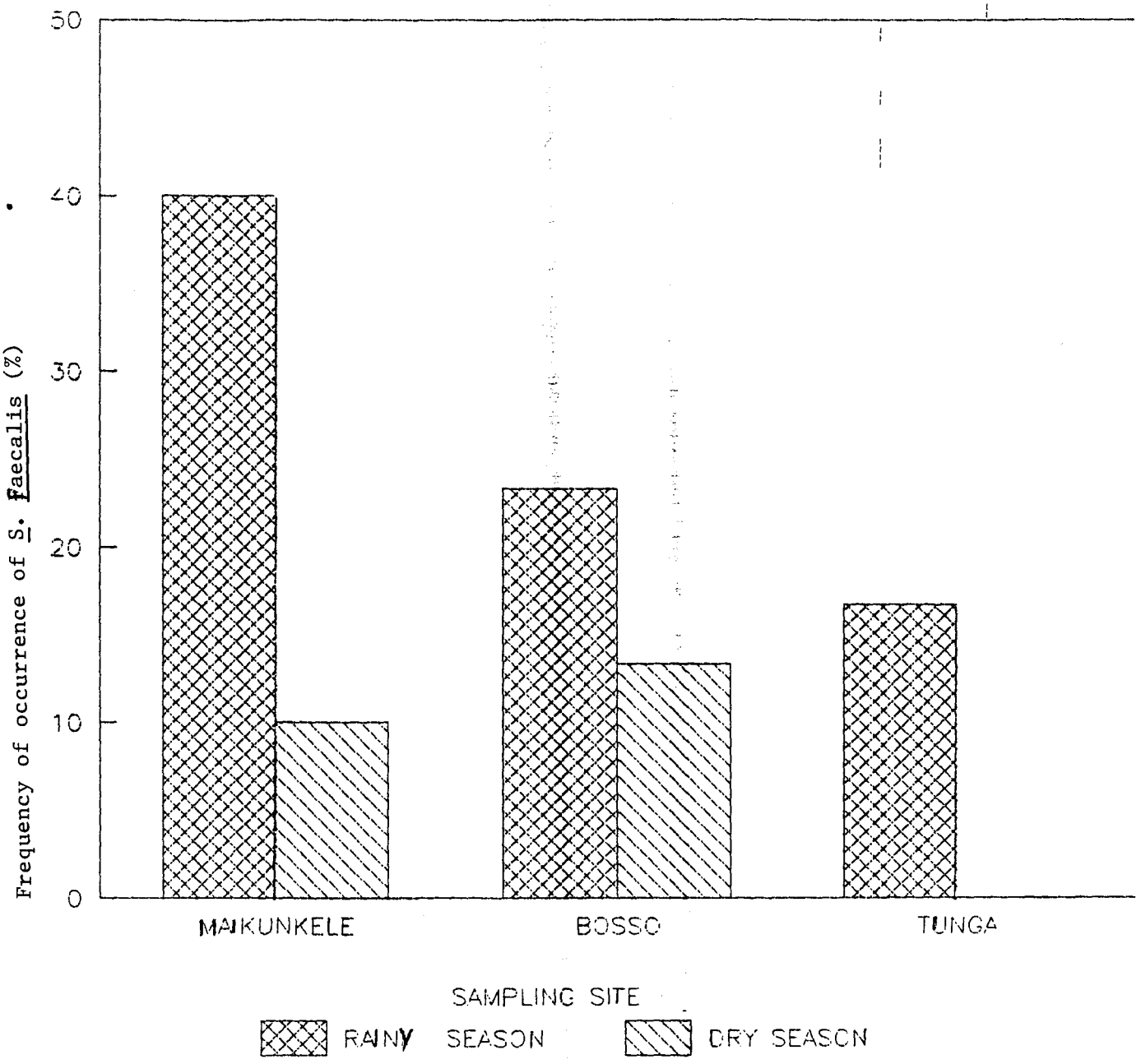

Figure: 3b Occurrence of S. faecalis in wells in Maikunkele, Bosso and Tunga areas of Minna.

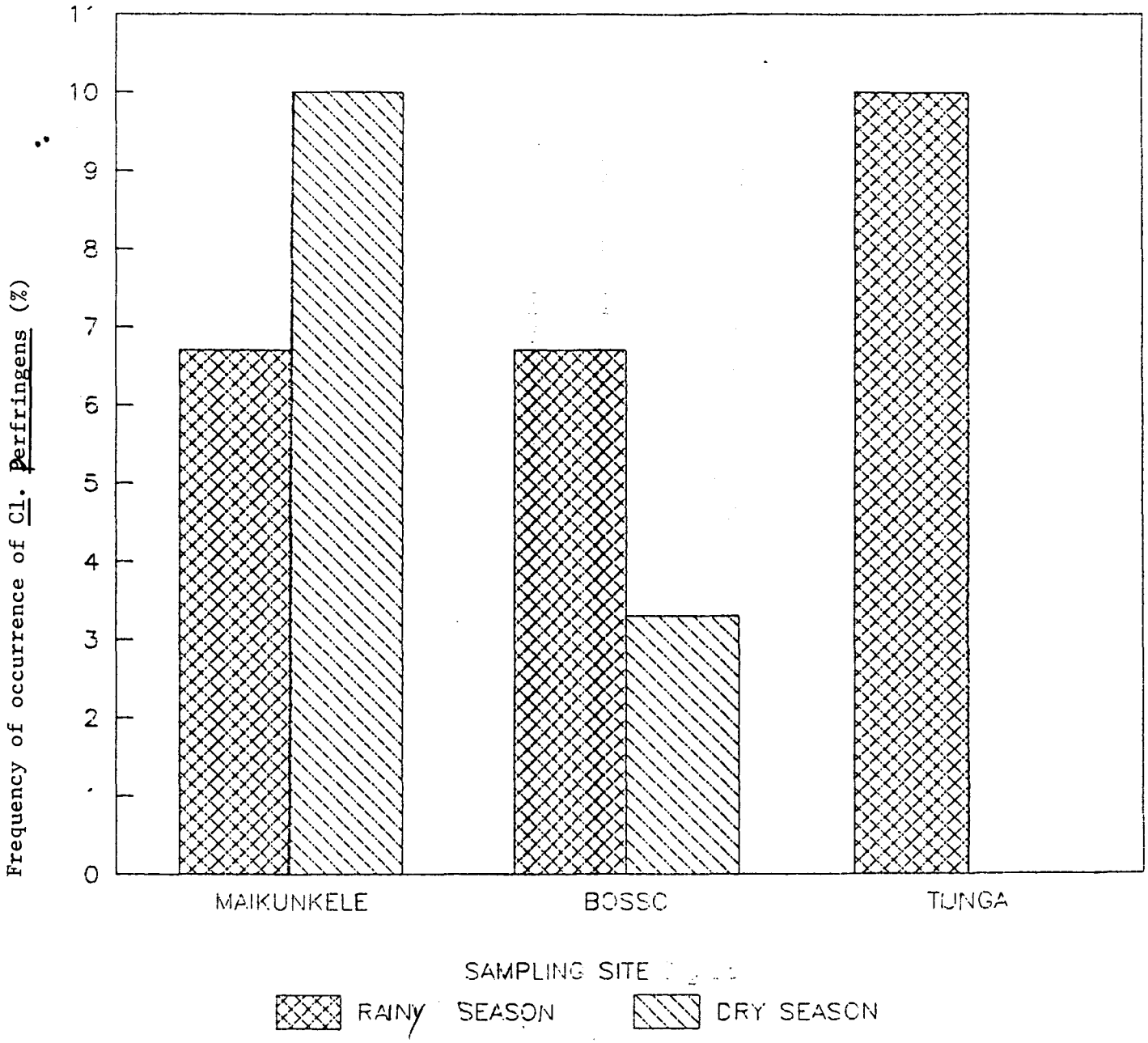

Figure: 3c Occurrence of Cl. Perfringens in wells in Maikunkele, Bosso and Tunga areas of Minna.

4.4 ISOLATION OF OTHER MEMBERS OF THE ENTEROBACTERIACEAE IN MAIKUNKELE, BOSSO AND TUNGA AREAS OF MINNA

(a) **Isolation of Proteus species**

Table 5 shows that in Maikunkele, well numbers M1, M3, M8 and M10 showed the presence of Proteus species in the rainy season. In the same season, it was present in well numbers B3, B6 and B10 in Bosso and T4, T7, T8 and T9 in Tunga respectively. However, in the dry season, in Maikunkele, well numbers M1, M8 and M10, in Bosso well numbers B6 and B10 and in Tunga well numbers T4, T7 and T8 showed the presence of the organism (Table 5).

(b) **Isolation of Shigella species**

In Maikunkele, Shigella species were present in well numbers M1, M3 and M10, while in Bosso well numbers B3, B6 and B10 and in Tunga well numbers T4, T7 and T8 also showed the presence of the organism in the rainy season. During the dry season, only well numbers M1, M8 and M10 in Maikunkele, B10 in Bosso and T4 and T8 in Tunga showed the presence of Shigella species (Table 5).

(c) **Isolation of Salmonella species**

The Salmonella species were isolated from well numbers M1, M3 and M10 in Maikunkele, in the rainy season, and only well numbers M1 and M10 showed its

presence in the dry season. In Bosso, in both the rainy season and dry season, only well number B10 showed its presence of the organism. In Tunga, well numbers T4 and T8 showed the presence of Salmonella species in the rainy season, while in the dry season, none of the wells showed its presence.

(d) **Isolation of Pseudomonas Species**

In Maikunkele, Pseudomonas species were present in well numbers M1, M3, M8 and M10 and well numbers M1 and M8 in the rainy season and dry season respectively (Table 5). In Bosso, it was found to be present in well numbers B3, B4, B6 and B10 and B4 and B10 in the rainy and dry seasons respectively. In Tunga, in the rainy season well numbers T3, T4, T7, T8 and T9 showed the presence of Pseudomonas species, while in the dry season, well numbers T3, T4, T8 and T9 had the organism. Figures (4a) and (4b) show that the degree of occurrence of both Proteus and Shigella species respectively was higher in the rainy season than in the dry season. However, in Bosso Salmonella species did not show any difference in either seasons (Figure 4c). Pseudomonas species (Figure 4d) did not show a marked difference in its occurrence in the two seasons in the three locations studied.

Table 5

Isolation of members of the enterobacteriaceae in wells sampled in Maikunkele, Bosso and Tunga areas of Minna.

Sampling Station	Isolates							
	Proteus Spp		*Shigella* Spp		*Salmonella* Spp		*Pseudomonas* Spp	
	R	D	R	D	R	D	R	D
M 1	++	+	+	+	+	+	+	+
2	-	-	-	-	-	-	-	-
3	++	Collapsed	+	Collapsed	+	Collapsed	+	Collapsed
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-
8	++	+	+	+	-	-	+	+
9	-	-	-	-	-	-	-	-
10	+	+	++	+	+	+	+	-
B 1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	+	-	+	-	-	-	+	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	+	+	+	-	-	-	+	-
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-
10	++	+	+	+	+	+	+	+

Table 5 Cont'd

Sampling Station	Isolates							
	Proteus Spp		Shigella Spp		Salmonella Spp		Pseudomonas Spp	
	R	D	R	D	R	D	R	D
T 1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	+	+
4	+	+	+	+	+	-	+	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	+	+	+	-	-	-	+	-
8	++	+	+	+	+	-	+	+
9	+	-	-	-	-	-	+	+
10	-	-	-	-	-	-	-	-

KEY

R = Rainy Season

D = Dry Season

- = All the three sampled plates are negative

+ = Only one of the three sampled plates is positive

++ = Two of the sampled plates are positive

+++ = All the three sampled plates are positive.

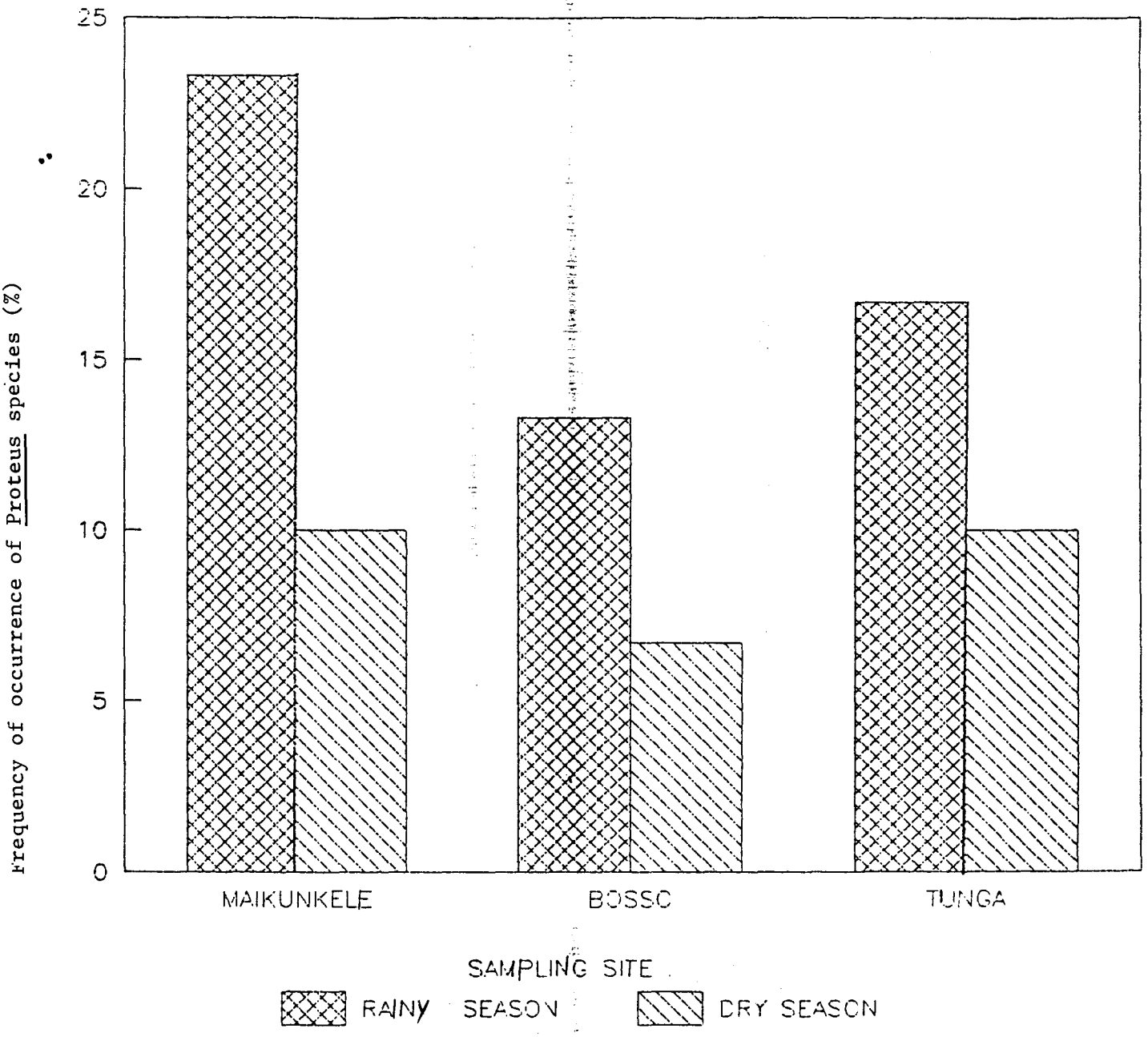

Figure: 4a Occurrence of Proteus species in wells in Maikunkele, Bosso and Tunga ares of Minna.

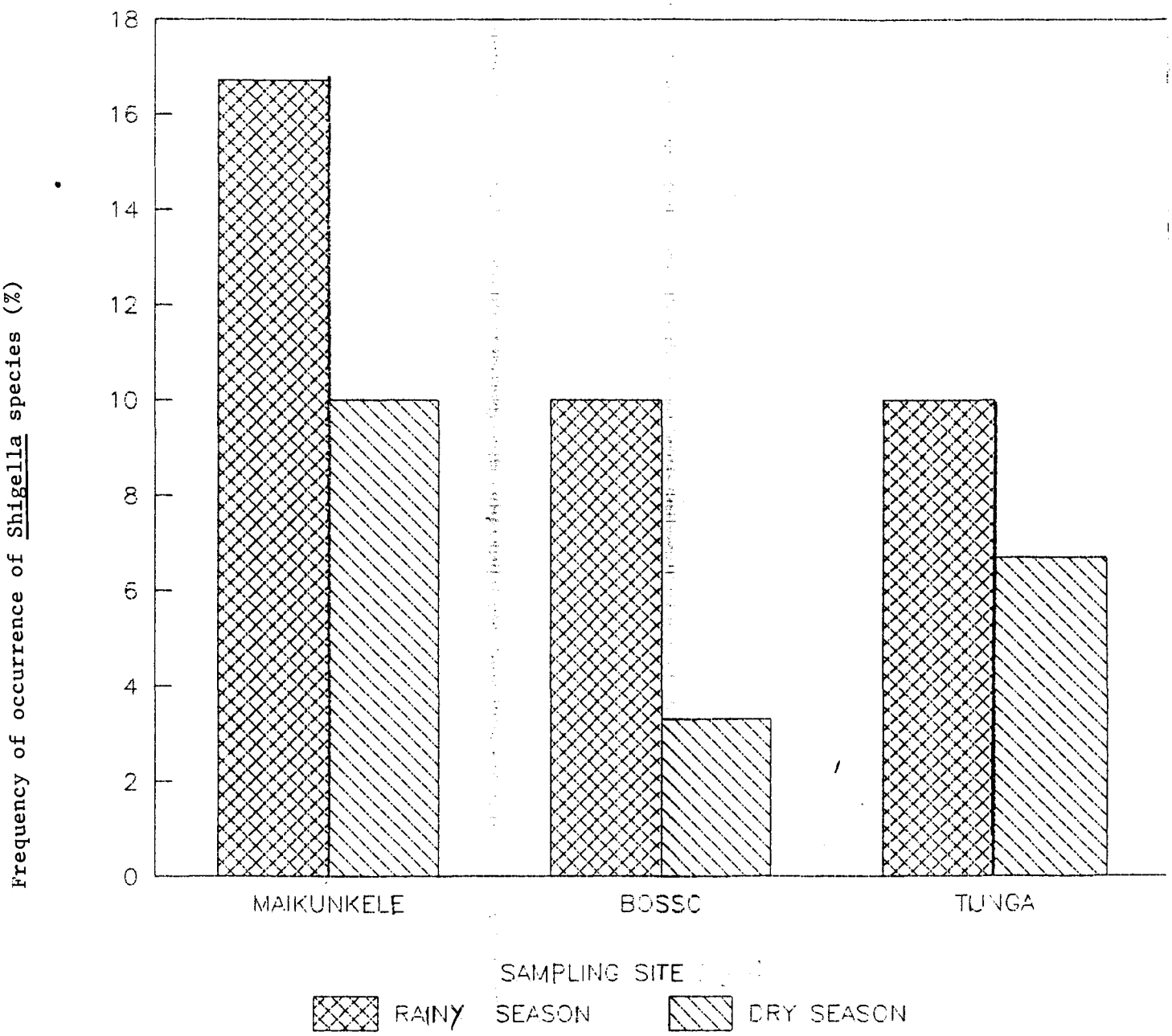

Figure: 4b Occurrence of Shigella species in wells in Maikunkele, Bosso and Tunga areas of Minna.

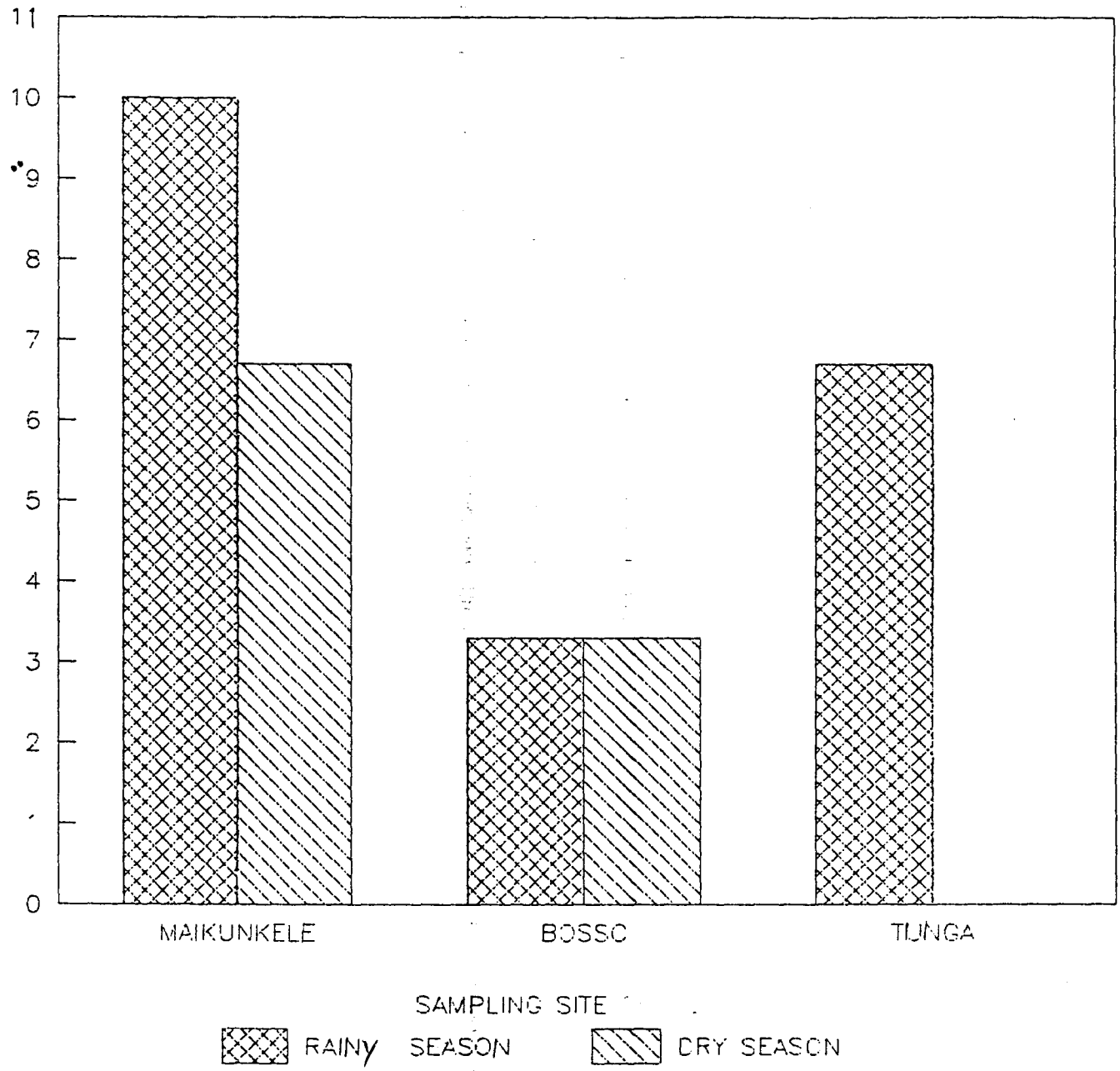

Figure: 4c Occurrence of Salmonella species in wells in Maikunkele, Bosso and Tunga areas of Minna.

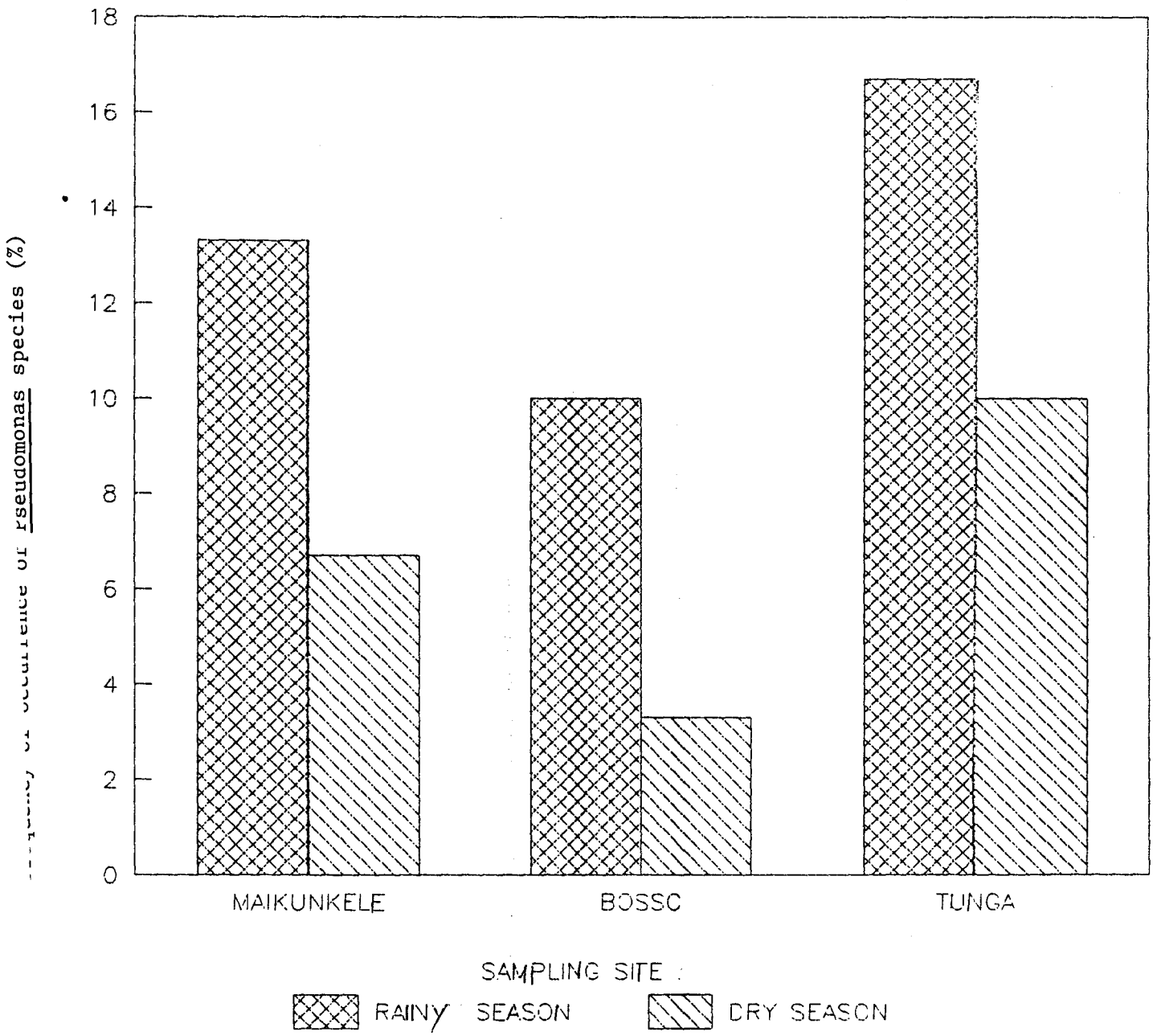

Figure: 4d Occurrence of Pseudomonas species in wells in Maikunkele, Bosso and Tunga areas of Minna.

4.5 PHYSICOCHEMICAL PROPERTIES OF WELL WATER SAMPLES

(a) **Hydrogen ion concentration (pH)**

The results (Table 6) obtained in the three areas show the highest pH of the water sample to be 7.2 and the lowest 6.9 in both the rainy and dry seasons.

(b) **Nitrate Content in Water Samples**

Table 6 shows that the nitrate concentration in well numbers M1, M3, M8 and M10 was over 10ppm in Maikunkele in the rainy season. In the dry season, only well numbers M1, M8 and M10 had over 10ppm. In Bosso, well numbers B6 and B10 had over 10ppm in the rainy season. In the dry season, the same amount of nitrate was recorded for well number B10 whereas well number B6 had less than 10ppm (Table 6). In Tunga, only well number T8 had over 10ppm in the two seasons. Generally, the results (Figure 5) obtained show that nitrate concentrations of the water samples in the three sites were higher in the rainy season than in the dry season.

(c) **Temperature of water samples**

The lowest temperature recorded was 28.5°C while the highest was 30°C in the three sampling sites during both rainy and dry seasons.

Table 6

Physical and Chemical Tests of Water samples of Wells in Maikunkele, Bosso and Tunga areas of Minna.

Nitrate		pH	
R	D	R	D
26.6ppm	26.6	7.1	7.1
8.9	6.6	6.9	6.9
22.1	Collapsed	7.1	Collapsed
<2.2	2.2	6.9	6.9
8.9	6.6	6.9	6.9
6.6	6.6	6.9	6.9
<2.2	2.2	7.0	6.9
17.7	8.9	7.2	7.1
8.9	6.6	7.0	6.9
22.1	17.7	7.1	7.1
4.4	4.4	6.9	7.0
8.9	8.9	7.0	7.0
8.9	8.9	7.1	7.1
6.6	4.4	6.9	6.9
8.9	6.6	7.0	7.0
17.7	8.9	7.0	7.0
8.9	8.9	7.0	7.0
8.9	6.6	7.1	7.1
8.9	6.6	6.9	6.9
22.1	17.7	7.0	7.0
4.4	2.2	6.9	6.9
2.2	<2.2	6.9	6.8
8.9	8.9	7.0	6.9
8.9	6.6	7.0	7.0
8.9	6.6	7.1	7.0
4.4	4.4	7.0	7.0
8.9	8.9	7.0	7.0
22.1	17.7	7.0	6.9
8.9	6.6	6.9	6.9
8.9	8.9	6.9	6.9

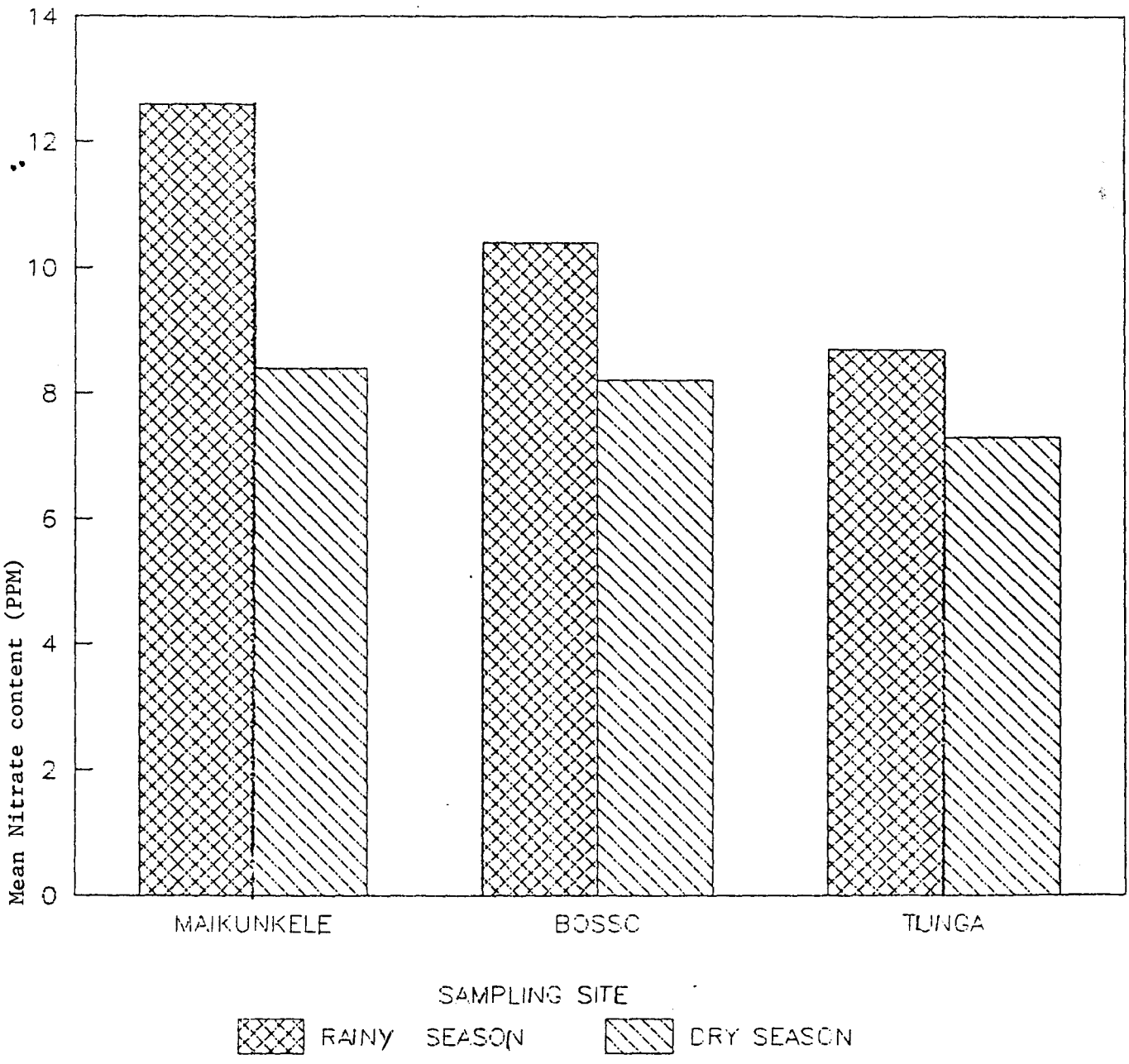

Figure: 5 Mean nitrate content of wells in Maikunkele, Bosso and Tunga areas of Minna.

Table 7

Morphology, Gram-reaction, growth and biochemical characteristics with probable identity of isolated members of the family eterobacteriaceae and *Pseudomonas* species.

Characteristics	ISOLATES					
	A	B	C	D	E	F
Gram-reaction	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
Morphology	short Rods	Short Rods	Rods	Short Rods	Rods	Rods
Growth in Gelation stab.	Undulate growth NO liquefaction	No liquefaction	Stratiform	Rapid Liquefaction with bluish-gree fluid	NO liquefaction	No liquefaction
Indole test	±	-	-	-	-	+
Methyl Red test	+	-	-	+	+	±
Voges-proskauer test	-	±	±	-	-	-
Citrate utilization	-	+	±	+	±	-

Table 7 Cont'd

	ISOLATES					
	A	B	C	D	E	F
Hydrogen Sulphide-Production	-	-	+	-	-	-
Lactose test	AG	AG	-	-	-	-
Mannitol	+	+	-	-	-	-
Urease production	-	-	+	-	-	-
Sucrose test	+	+	-	+	-	-
Probable - identiy	Esherichia Coli	Enterobacter Species	Proteus Species	Pseudomonas aeruginosa	Salmonella SP	Shigella SP

- Means Negative
± " Positive
± " slightly Positive
AG " Acid and gas produced
A " Acid produced.

CHAPTER FIVE

5.0 DISCUSSION

The results obtained for the viable counts of bacteria in Maikunkele, Bosso and Tunga areas of Minna show that the bacterial load (cfu/ml) was higher in the rainy season than in the dry season in the three locations. This finding agrees with the reports of McCoy (1970) and Males (1975). Most of the wells (except M4, M7, M9, B1, B2, T1 and T2) did not meet WHO standard. The high microbial counts in wells sampled can be attributed to the nature of the wells. That is, many of the wells were shallow, not fenced and plastered and were neither erected nor covered. In addition, many were not far from the soak-away, pit latrines or bathroom passages or sewage. When it rains wastes and debris including animal and human excrements could be washed into the wells. These wastes have been reported (Okafor, 1985; Prescott et al., 1990) to harbour a lot of microorganisms including pathogens. Besides, Kriss et al. (1967) reported that numbers of bacteria indicate the level of readily available organic matter.

However, well numbers M4, M7, B1, B2, T1 and T2 had viable bacterial counts close to WHO Standard probably because of the facts that these wells were deep, erected and covered, fenced and plastered, hence the low

microbial counts in these wells. A well may contain high microbial count, if however, it does not contain the pathogenic forms then its fitness for consumption is not questionable (WHO, 1971).

The much higher counts recorded in Maikunkele compared to Bosso and Tunga could be due to the topography of the area. Since there are no proper toilet facilities (Water system) many inhabitants defecate in the bush. In addition to this, poultry and animal are allowed to roam in the area and thus, there are poultry and animal droppings and farmlands with lots of decayed organic matter. These might have led to an increase in the bacterial flora in this area. Poultry droppings harbour a lot of bacteria including pathogenic forms (Ijah, 1992).

On the enumeration of coliforms, Moran et al., (1983) put a tolerable limit of 10-25 coliforms/100ml of water as safe. For many years standards have existed in most developed countries. The research by Kehr and Butterfield (1943) and the work of the Public Health Service of the United States of America since 1925 indicated a density of one coliform bacterium per 100ml of drinking water as a safe limit. Also according to WHO publications "International Standards for Drinking Water " (1958 and 1971), drinking water should be free from pathogenic microorganisms.

The results obtained in the present study show that the coliforms were more prevalent in the rainy season compared to the dry season in the three locations. The closeness of soak-away and pit latrines to the wells could be responsible. Run-off carrying bacteria may also infiltrate into the wells when it rains. This finding is in line with the report of Males (1975). It was also noted that of the three locations investigated, Maikunkele had the highest density of coliforms. This was followed by Bosso and Tunga in descending order for the two seasons. This could be attributed to the topography and lack of adequate pipe-borne water in the areas. The presence of coliforms indicates recent faecal pollution (Geldreich, 1972; Wolf, 1972; Cheesbrough, 1985).

The high occurrence of E. coli at Maikunkele in both seasons is likely to be due to topography, closeness of wells to soak-away, pit latrines, animal droppings and human excrement. Animal and human excrements contain E. coli (Schlegel, 1985). In Bosso and Tunga, the above mentioned factors are very much reduced because most homes have pipe-borne water and proper toilet facilities. However, during the rainy season the frequency of occurrence of E. coli was higher than in the dry season in the three locations. This is probably due to socio-cultural practices affected by seasons (Males, 1975) observed higher counts of E. coli in well water samples in rainy season than during the dry season. The investigator attributed this to climatic conditions.

Thus the present finding agrees with the report of Males (1975).

The magnitude of occurrence of S. faecalis (Figure 3b) was higher in the rainy season than in the dry season in the three locations studied. This may be due to shallowness of the wells, in addition to the indiscriminate discharge of excrement, as well as animal and poultry droppings (SMEWW, 1989). This finding agrees with the report of McCoy (1971) that uses of water are dependent upon climatic conditions. This organism, like coliforms is an indicator organism of fairly recent faecal pollution (Galvani, 1974; Cheesbrough, 1985).

For Cl. perfringens there was not much difference in results in both season in the three locations. This could be due to the fact that Clostridial spores can survive in water for a longer time than organisms of faecal origin (Moran et al., 1983). The results on E. coli, S. faecalis and Cl. perfringens are in agreement with the findings of Bonde (1962) and Cheesbrough (1985). These workers reported that though Cl. perfringens, can regularly be found in faeces, it is always in smaller number compared to E.coli.

The presence of Proteus species (Figure 4a) and Shigella species (figure 4b) in Maikunkele, Bosso and Tunga was higher in the rainy season than in the dry season in Maikunkele, Bosso and Tunga areas. The

occurrence of Salmonella species did vary in both the rainy season and dry season except for Bosso where there was no difference in the frequency of occurrence of the orgnaism in the two seasons (Figure 4c). The results (Figure 4a,b,c) obtained could be due to the fact that faecal matter harbouring these organisms may have been introduced by surface run-off into the wells. Contaminated water and sewage infiltration during the rainy period could also be responsible. Proteus, Shigella and Salmonella species inhabit human and animal faeces as well as sewage (Okafor, 1985; Prescott et al., 1990).

Also for Pseudomonas species, the frequency of occurrence was more in the rainy season than in the dry season in the three locations. However, its occurrence was low in Bosso compared to Maikunkele and Tunga areas. Pseudomonas species, according to Nester et al. (1985) can grow on many media and solutions at 37°C. Such media are any aqueous solutions, distilled water and some disinfectant solutions. Thus, the environmental conditions during rainy season may have encouraged the proliferation of the organism than during the dry period. Moran et al. (1983) showed that the mere presence of the indicator and pathogenic organisms in water no matter how few constitute a serious health hazard. For instance, Shigella species cause bacillary

dysentery, while Salmonella species cause typhoid fever and gastroenteritis in humans (Prescott et al., 1990). Since they are pathogenic, their presence in water renders such water unfit for human and animal consumption (WHO, 1971).

The highest pH was 7.2 and the lowest 6.9 in the two seasons at the three locations. This means that the water samples were neither highly acidic nor highly alkaline. Thus, the pH range observed conforms with the international standard that gives the pH range of potable water to be from 6.5 to 9.2 (WHO, 1963, 1971).

Nitrate contents of the water samples were higher in the rainy season than in the dry season. Males (1975) reported that climatic conditions such as rains could influence the physicochemical properties of water. Closeness of soak-away, pit latrines sewage and refuse dumps to the wells may be responsible for high nitrate content. This finding agrees with the report of Lee (1969) and Bennett and Linstedt (1975) that wells sited on or near these areas could cause an increase in nitrate level of water from such wells. From the results (Table 6) obtained 7 out of the 30 wells sampled are not fit for consumption since they contain higher amount of nitrate than the permissible level in drinking water as specified by WHO (1971). Nitrate content over 10ppm causes cyanosis or blue-baby disease in infants (WHO, 1963; Ridder el al., 1974; Ivanor et al., 1975).

The lowest temperature recorded was 28.5°C and the highest was 30 C in the two seasons. This range favours the growth of most enteric organisms, as too high or too low temperatures affect the growth of microorganisms (Okafor, 1985). Experiments carried out by the Pacific North West Water Laboratory Oregon in the United States of America showed that the density and viscosity of water are decreased at higher temperatures, permitting suspended solids to settle at a faster rate (Okafor, 1985). Also evaporation increased rapidly and the rate at which chemical reactions occur increases at increased temperature. The high temperature leads to greater assimilation of waste, hence greater depletion of Oxygen (Hodges, 1977) while very low temperature could inhibit the growth or multiplication of bacteria (Pathak et al., 1991).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY AND CONCLUSION.

This study was carried out on well water samples collected from Maikunkele, Bosso and Tunga areas of Minna, to determine the bacteriolgical quality of the 30 wells sampled. In addition, the wells were sampled to determine some physicochemical parameters of the water. Sampling was done from Mid-January to July, 1993 (Rainy Season) and from Mid-January to February, 1994 (dry season). Three samples were taken from each well in both seasons.

Most of the wells sampled were shallow which probably contributed to the high occurrence and isolation of pathogenic bacteria from the water samples analysed. Other factors that may have been responsible for this are that many of the wells were not properly constructed, not erected or covered. They were also neither plastered nor fenced. Unhygienic conditions characterised by indiscriminate defecation and disposal of animal and domestic wastes contributed to high counts of bacteria.

The results (Table 2) showed higher counts of viable bacteria in the three locations. However, they were more prevalent in the rainy season than in the dry season. Figure I showed the mean viable bacteria count in the three sampling stations to be 8.2 X 10^5cfu/ml, 2.4 X 10^5

cfu/ml and 2.1 X 10^3cfu/ml, in the rainy season, compared to 2,7 X 10^3cfu/ml, 1.5 X 10^3cfu/ml and 0.9 X 10^3cfu/ml in the dry season in Maikunkele, Bosso and Tunga areas respectively. Maikunkele also had more bacterial load than Bosso and Tunga which could be attributed to the topography and lack of Adequate toilet facilities (water system) in Maikunkele. The mean coliform counts were 458, 207 and 188 coliforms/100ml of water in the rainy season and 255, 80 and 61 coliforms/100ml of water in the dry season in Maikunkele, Bosso and Tunga respectively. However, higher coliform counts were recorded in the rainy season than in the dry season.

For Escherichia coli, the frequency of occurrence was 46.7%, 33.3% and 23.3% in the rainy season and 20%, 13.3% and 6.7% in the dry season in Maikunkele, Bosso and Tunga respectively. Figure 3 shows the frequency of occurrence of S. faecalis to be 40%, 23.3% and 16.7% in the rainy season while in the dry season, it was 10%, 13.3% and 0.0% in Maikunkele, Bosso and Tunga respectively.

The frequency of occurrence of Cl perfringens was 6.7%, 6.7% and 10% in the rainy season compared to 10%, 3.3% and 0.0% in the dry season in Maikunkele, Bosso and Tunga respectively. Of these three indicator organisms (E. coli, S. faecalis and Cl. perfringens), E. coli was more prevalent while Cl. perfringens was the least prevalent organism.

Proteus species as indicated in Figure 4 occurred at the frequency of 23.3%, 13.3% and 16.7% in the rainy season compared to 10.0%, 6.7% and 10.0% in the dry season in Maikunkele, Bosso and Tunga respectively. The frequency of occurrence of Shigella species in the rainy season was 16.7%, 10.0% and 10.0% whereas in the dry season, 10.0%, 3.3% and 6.7% frequencies of occurrence were recorded in Maikunkele, Bosso and Tunga respectively. For Salmonella species, the frequency of occurrence was 10%, 3.3% and 6.7% in the rainy season compared to 6.7%, 3.3% and 0.0% in the dry season in Maikunkele, Bosso and Tunga respectively. Salmonella species did not occur in Tunga in the dry season. The frequency of occurrence of Pseudomonas species was 13.3%, 10% and 16.7% in the rainy season and 6.7%, 3.3% and 10% in the dry season in Maikunkele, Bosso and Tunga respectively. The occurrence of Pseudomonas species was higher in Tunga than in Maikunkele and Bosso.

The highest pH was 7.2 and the lowest was 6.9 in both the rainy and dry seasons in the three locations. For temperature, the lowest temperature recorded was 28.5°C while the highest was 30°C in both seasons in Maikunkele, Bosso and Tunga. The mean nitrate content of the water samples was 12.6, 10.4 and 8.7ppm in Maikunkele, Bosso and Tunga respectively in the rainy season compared to 8.4, 8.2 and 7.3ppm in the three locations respectively in the dry season. Figure 5 shows

that nitrate content was higher in the rainy season than in the dry season in the three locations. In general, the nitrate content of the water samples was higher than the permissible level (less than 10ppm) in drinking water by World Health Organization (WHO).

In conclusion, many of the wells are shallow and have not met the international standard of untreated water and although only few did not contain pathogenic microorganisms, many of the wells sampled are not considered potable and should be sealed off. The few that are close to WHO standard are M4, M7, M9, B2 and T2 and should be used by the people.

6.2 RECOMMENDATIONS

Based on the results of this study and in the light of the fact that the World Health Organization states that 80% of sicknesses and diseases in developing countries like Nigeria is caused by lack of adequate or potable drinking water and poor sanitation, the following recommendations become necessary:

1. A National standard that could take cognisance of the topography and nature of the communities (Rural or Urban) should be established for Nigerian Communities similar to International Standards. In addition to the above, public enlightenment campaign should be mounted by the three tiers of government (Federal, State and Local governments), and other agencies to create awareness in the people to provide the necessary facilities when constructing wells. As a matter of seriousness, it should be incorporated into Primary Health Care System and the campaign termed "Operation Provide Potable Water" as this would agree with the programme on the eradication of guinea-worm and other water borne diseases.
2. Compliance should be enforced so that wells should be deep enough and constructed on good soil (topography) in the area. Government should embark on the development of a good sewage disposal system, a canal method would be of advantage.

Constant surveillance or monitoring of the microbiological and chemical qualities of the well should be maintained by primary Health Workers.

3. The wells should be properly built with concrete ring from inside and elevated above the ground level. It should be adequately covered and under lock and key. In addition, the immediate surroundings should be plastered and fenced to prevent children and domesticated animals going near to discharge excreta.

4. The construction should be at least 30 meters (De Araoz and Sabrahmanyan, 1970) from the pit latrines, sewage, soak-away or refuse dump sites. Well construction should be far from farm land where a lot of fertilizer is applied.

The importance of clean potable water and hygienic conditions around the wells cannot be over-emphasized. In this decade, it is not feasible for every settlement in Niger State in particular and Nigeria in general to be provided with pipe borne water. The immediate remedy is the provision of wells and bore-holes which should not only be safe for utilization but must meet an established standard. It is hoped that, if these suggestions are strictly followed, wells and drinking water sources will be free of pathogenic

microorganisms and toxic materials. Thus, health of the citizens would be guaranteed and more positive result on productivity would be realised.

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APPENDIX 1

The following media and materials were used during the investigations.

A. **Blood Agar Base (BAB):**

A non-selective media was used for the enumeration of viable bacteria. It consisted of the following:

Lab-Lemco Powder	-	10.0g
Peptone	-	10.0g
Sodium Chloride	-	5.0g
Agar	-	15.0g
Distilled Water	-	1 Litre.

The Components were dissolved by boiling and sterilized by autoclaving at 121°C for 15 minutes. The final pH was adjusted to 7.3.

B. **Enterococcus Confirmatory Broth:**

This was used as described by Winter and Sandholzer (1946), Cheesbrough (1985) and Ogundana (1989) for confirming the presence of Streptococcus faecalis. The broth consisted of:

Tryptone	-	5.0g
Yeast extract	-	5.0g
Dextrose	-	5.0g
Sodium azide	-	0.40g
Sodium chloride	-	65.0g
Methylene blue	-	0.01g
Distilled water	-	1 Litre.

The final pH of this confirmatory broth was adjusted to 8.0.

C. **Litmus Milk:**

This was used for the detection of Clostridium perfringens. It contained:

Skimmed milk powder	-	2g
Litmus indicator	-	2 drops
Distilled water	-	20ml

D. **Lactose broth:**

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

Gelysate peptone	-	5.0g
Beef extract	-	3.0g
Lactose	-	5.0g
Distilled Water	-	1 Litre.

The final pH was adjusted to 6.9.

E. **Levine's Eosin Methylene Blue Agar (EMB):**

The medium was used to confirm the presence of Coliform bacteria. This medium inhibits the growth of Gram-positive bacteria such as Cl.perfingens. It contains:

Peptone	-	10.0g
Dipotassium hydrogen phosphate	-	2.0g
Eosin Y	-	0.4g
Methylene blue	-	0.065g

Agar	-	15.0g
Distilled water	-	1 Litre.

F. **Salmonella - Shigella (SS) agar:**

The medium was used in the growing of Salmonella and Shigella species. It consists of the following:

Lab-lemco powder	-	5.0g
Peptone	-	5.0g
Lactose	-	10.0g
Bile Salts	-	8.5g
Sodium Citrate	-	1.0g
Sodium Thiosultphate	-	8.5g
Ferric Citrate	-	10.0g
Brilliant Green	-	0.00033g
Neutral Red	-	0.025g
Agar	-	15.0gg
Distilled Water	-	1 Litre.

G. **Deoxycholate Citrate Agar (D.C.A):**

This medium was used in the isolation of distinct colonies of Salmonella and Shigella species. DCA consists of the following:

Lab-lemco powder	-	5.0g
Peptone	-	5.0g
Lactose	-	10.0g
Sodium Citrate	-	8.5g
Sodium Thiosulphate	-	5.4g
Ferric Citrate	-	1.0g

Sodium deoxycholate	-	5.0g
Neutral Red	-	0.02g
Agar	-	12.0g
Distilled Water	-	1 Litre.

H. **Triple Sugar Iron (T.S.I) Agar:**

Both colonies of Salmonella and Shigella Spp were streaked on this medium to differentiate between the two organisms. T.S.I. agar had the following compositions:

Lab-lemco powder	-	3.0g
Yeast Extract	-	3.0g
Peptone	-	20.0g
Lactose	-	10.0g
Sucrose	-	10.0g
Dextrose	-	1.0g
Ferric Citrate	-	0.3g
Sodium Thiosulphate	-	0.3g
Phenol red	-	q.SI
Agar	-	15.0
Distilled water	-	1 Litre
Sodium chloride	-	5.0g

I. **Glassware and media sterilization:**

The glasswares were thoroughly washed and dried after rinsing with distilled water. After this, all glasswares were sterilized in hot air oven at 160°C for two hours. All media used were sterilized by autoclaving at 121°C for 15 minutes

with the exception of few that were not autoclaved but only dissolved by boiling as directed by the manufacturers.

Indole test medium

Tryptone	10.0g
Sodium chloride	5.0g
Distilled water	1 Litre

It was dissolved by boiling and later sterilized by autoclaving at 121°C for 15 minutes. Kovac's reagent consists of:

paradimethlamino benzaldenyde	5.0g
Amyl alcohol	75ml
Concentrated Hcl	25ml.

APPENDIX 2A

Anova for number of bacteria in well water sample in Maikunkele, Bosso and Tunga areas of Minna

Source	DF	SS	MS	F
Seasons (S)	1	9.806E+11	9.806E+11	4.20*
Location (L)	2	1.891E+12	9.457E+11	4.05*
S/L	2	6.271E+11	3.136E+11	1.434N.S
Error	54	1.262E+13	2.336E+11	
Total	59	1.611E+13	2.731E+11	

* - Significantly different
N.S - not significantly different.

APPENDIX 2B

Anova for number of Coliforms found in Wells in Maikunkele, Bosso and Tunga areas of Minna

Source	DF	SS	MS	F
Season (S)	1	348082	348082	2.86 N.S
Location (L)	2	663803	331901	2.72 N.S
S/L	2	18951	9475	0.08 N.S
Error	54	6577316	121802	
Total	59	7608151	128952	

APPENDIX 2C

nova for number of Indicator organisms in Maikunkele, Bosso and unga areas of Minna

E. coli [APPENDIX 2C (1)]

Source	DF	SS	MS	F
Season (S)	1	7.3500	7.3500	8.96**
Location (L)	2	3.0333	1.5167	1.85 N.S
S/L	2	0.0333	0.1500	0.18 N.S
Error	54	44.3000	0.8204	
Total	59	54.9833	0.9319	

S. faecalis [APPENDIX 2C (2)]

Source	DF	SS	MS	F
Season (S)	1	6.017	6.0167	9.53**
Location (L)	2	1.633	0.8167	1.29 N.S
S/L	2	1.233	0.6167	0.98 N.S
Error	54	34.100	0.6315	
Total	59	42.983	0.7285	

Cl. Perfringens [APPENDIX 2C (3)]

Source	DF	SS	MS	F
Season (S)	1	0.2667	0.26667	1.47 N.S
Location (L)	2	0.0333	0.01667	0.09 N.S
S/L	2	0.2333	0.11667	0.64 N.S
Error	54	9.8000	0.18148	
Total	59	10.3333	0.17514	

APPENDIX 2D

Anova table for some members of the Enterobacteriaceae in Maikunkele, Bosso and Tunga areas of Minna

Proteus SP [APPENDIX 2D (1)]

Source	DF	SS	MS	F
Season (S)	1	1.3500	1.3500	3.00 N.S
Location (L)	2	0.2333	0.1167	0.26 N.S
S/L	2	0.3000	0.1500	0.33 N.S
Error	54	24.3000	0.4500	
Total	59	26.1833	0.4438	

Shigella SP [APPENDIX 2D (2)]

Source	DF	SS	MS	F
Season (S)	1	0.6000	0.60000	2.19 N.S
Location (L)	2	0.2333	0.11667	0.43 N.S
S/L	2	0.1000	0.05000	0.18 N.S
Error	54	14.8000	0.27407	
Total	59	15.7333	0.26667	

Salmonella SP [APPENDIX 2D (3)]

Source	DF	SS	MS	F
Season (S)	1	1.3500	1.35000	3.78 N.S
Location (L)	2	0.4333	0.71667	2.01 N.S
S/L	2	0.1000	0.05000	0.43 N.S
Error	54	8.4000	0.15556	
Total	59	8.9333	0.15141	

Pseudomonas SP [APPENDIX 2D (4)]

Source	DF	SS	MS	F
Season (S)	1	1.3500	1.35000	3.78 N.S
Location (L)	2	0.4333	0.71667	2.01 N.S
S/L	2	0.1000	0.05000	0.43 N.S
Error	54	19.3000	0.35741	
Total	59	22.1833	0.37599	

APPENDIX 2E

ınova table for some physicochemical test of some Well water in laikunkele, Bosso and Tunga areas of Minna

PH [APPENDIX 2E (1)]

Source	DF	SS	MS	F
Season (S)	1	0.9882	0.9882	1.21 N.S
Location (L)	2	1.4943	0.7472	0.92 N.S
S/L	2	1.7583	0.8792	1.08 N.S
Error	54	44.0410	0.8156	
Total	59	48.2818	0.8183	

Nitrate content [APPENDIX 2E (2)]

Source	DF	SS	MS	F
Season (S)	1	101.40	101.40	2.62 N.S
Location (L)	2	64.31	32.16	0.83 N.S
S/L	2	21.62	10.81	0.28 N.S
Error	54	2093.91	38.78	
Total	59	2281.24	38.67	

APPENDIX 3A

Mean viable counts of bacteria in water samples from Maikunkele, Bosso and Tunga areas of Minna during rainy and dry seasons.

Location	Season		Difference
	Rainy season	Dry season	
Maikunkele	8.2×10^5	2.7×10^5	5.5×10^5
Bosso	2.4×10^5	1.5×10^5	0.9×10^5
Tunga	2.1×10^5	9.0×10^4	2.4×10^4
Mean Total	4.3×10^5	1.7×10^5	

APPENDIX 3B

Mean Coliform counts in wells in Maikunkele, Bosso and Tunga areas of Minna during rainy and dry seasons.

Location	Season		Difference
	Rainy season	Dry season	
Maikunkele	458.	255.	203.
Bosso	207.	80.	127.
Tunga	188.	61.	127.
Total	284.	132	

APPENDIX 3C

Indicator organisms of faecal pollution in Maikunkele, Bosso and Tunga areas of Minna

i) **E. coli**

Location	% occurrence of isolate Season		Difference (a-b)
	(a) Rainy Season	(b) Dry Season	
Maikunkele	46.7	20.0	26.7
Bosso	33.3	13.3	20.0
Tunga	23.3	6.7	16.6

ii) **S. faecalis**

Location	% occurrence of isolate Season		Difference (a-b)
	(a) Rainy Season	(b) Dry Season	
Maikunkele	40.0	10.0	30.0
Bosso	23.3	13.3	10.0
Tunga	16.7	0.0	16.7

iii) **Cl perfringens**

Location	% occurrence of isolate Season		Difference
	(a) Rainy Season	(b) Dry Season	(a-b)
Maikunkele	6.7	10.0	-3.3
Bosso	6.7	3.3	3.4
Tunga	10.0	0.0	10.0

APPENDIX 3D

Members of the Enterobacteriaceae in Maikunkele, Bosso and Tunga areas.

i) ***Proteus*** species

Location	% occurrence of isolate Season		Difference
	(a) Rainy Season	(b) Dry Season	(a-b)
Maikunkele	23.3	10.0	13.3
Bosso	13.3	6.7	6.6
Tunga	16.7	10.0	6.7

ii) ***Shigella*** species

Location	% occurrence of isolate Season		Difference
	(a) Rainy Season	(b) Dry Season	(a-b)
Maikunkele	16.7	10.0	6.7
Bosso	10.0	3.3	6.7
Tunga	10.0	6.7	3.3

iii) **Salmonella species**

Location	% occurrence of isolate Season		Difference
	(a) Rainy Season	(b) Dry Season	(a-b)
Maikunkele	10.0	6.7	3.3
Bosso	3.0	3.3	-0.3
Tunga	6.7	10.0	-3.3

iv) **Pseudomonas species**

Location	% occurrence of isolate Season		Difference
	(a) Rainy Season	(b) Dry Season	(a-b)
Maikunkele	13.3	6.7	6.7
Bosso	10.0	3.3	6.7
Tunga	16.7	10.0	6.7

APPENDIX 3E

Nitrate content of Well water samples in Maikunkele, Bosso and Tunga areas, Minna.

Location	% occurrence of isolate Season		Difference
	(a) Rainy Season	(b) Dry Season	(a-b)
Maikunkele	12.6	8.4	4.2
Bosso	10.4	8.2	2.2
Tunga	8.7	7.3	1.4