# BACTERIOLOGICAL AND PHYSICO-CHEMICAL QUALITY OF WATER FROM VARIOUS SOURCES IN SAMBURU DISTRICT AND EFFICACY OF SELECTED PLANT PRODUCTS IN WATER PURIFICATION

**By**

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## DECLARATION

I Cheluget Kipkemboi, declare that this thesis is my original work and has not been presented for the award of a degree in any other University or for any other award.

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## DEDICATION

To my entire family and mostly my dear wife Doreen, and children; Tracy, Sharon, Emmanuel, Ian and Christian, who despite my long stay away from home during the course of this work, stood by me, persevered and provided me with much inspiration and energy to go on.

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## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| APHA | American Public Health Association |
| BOD | Biological Oxygen Demand |
| $CaCO_3$ | Calcium Carbonate |
| CFU | Colony Forming Units |
| DO | Dissolved Oxygen |
| EMB | Eosin Methylene Blue |
| FC | Faecal Coliforns |
| $H_2SO_4$ | Sulphuric Acid |
| HPC | Heterotrophic Plate Counts |
| IMViC | Indole, Methyl-red, Voges Proskauer and Citrate Utilization |
| IZD | Inhibition Zone Diameter |
| KEFRI | Kenya Forest Research Institute |
| KEMRI | Kenya Medical Research Institute |
| MBC | Minimum Bactericidal Concentration |
| MIC | Minimum Inhibitory Concentration |
| MPC | Maximum Permissible Concentration |
| MPN | Most Probable Number |
| MRVP | Methyl red Voges Proskauer |
| NOM | Natural Organic Matter |
| NTU | Nephelometric Turbidity Units |
| $PO_4$-P | Phosphorus |
| SPSS | Statistical Package for Social Sciences |
| TCBS | Thiosulphate Citrate Bile Salt |
| THMs | Trihalomethanes |
| TOC | Total Organic Carbon |
| TSI | Triple Sugar Iron |
| VBNC | Viable But Non Culturable |
| WHO | World Health Organisation |

## ABSTRACT

Limited access to safe drinking water and information on water quality in sparsely populated arid and semi-arid regions has contributed to frequent outbreaks of diarheal disease. There is therefore urgent need to determine the bacteriological and physico-chemical quality of water in various sources in these regions. This study was undertaken in order to determine water quality in Wamba Division of Samburu District and to asses the efficacy of plant extracts in purifying water. Bacteriological analyses was carried out using multiple tube fermentation technique and heterotrophic plate counts technique, while physicochemical analyses were carried out using standard methods. Qualitative bacterial determination confirmed the presence of thermotolerant coliforms, *Shigella* and *Salmonella* spp. in most water samples examined. The same samples frequently recorded high levels of turbidity (range, 5 to 6100 NTU), alkalinity (range, 20 to 1577 mg $L^{-1}$ $CaCO_3$) and low salinity (range, 0 to 0.2 ppt). Faecal coliform load in dry river bed wells (mean 471.63) was higher than in the other categories of water sources (dams, rivers, springs and tap water). This study also found that the boreholes had the highest mean conductivity (830.8 µS $cm^{-1}$) while wells had the widest range (4.6 to 5940.0). High levels of conductivity in water from groundwater sources can be attributed to the long period of contact between the water and mineral sources. Water treatment with alum, sodium hypochlorite and extracts from *Boscia coriacea* Pax. *Maerua decumbens* (Brogn.) Dewolf roots and *Moringa oleifera* Lam. seeds resulted in a varied reduction of bacterial and sediment loads of the water samples. Overall, all the treatments were found to be effective in reducing bacteria and sediment load in water samples collected from various sources, except for some unidentified residual bacteria that resisted the disinfection properties of plant extracts. Changes in the percentage load of heterotrophic plate counts (HPC) among the treatments used differed significantly ($P < 0.05$, DF = 5). Overall mean percentage change in HPC were 26.51, 46.00, 30.20 and 14.50 for *M. Oleifera* Lam., *M. decumbens*, *B. coriacea* and the control respectively compared to 74.76 and 90.95 in the case of alum and sodium hypochlorite in the same order. These values indicate that changes in bacterial density during water treatment may be due to loss of viability or alteration in culturability. Results obtained in this study further indicated that there was no significant difference in water turbidity reduction ($P < 0.05$) by *M. oleifera*, *M. decumbens* and *B. coriacea.* Both *B. coriacea* and *M. decumbens* chelants resulted in a high removal of the initial turbidity by 50.36 % and 43.87 % respectively during 30 minute treatment period while *M. oleifera* were 40.53 %. As such the three species can be considered potentially useful chelants, and should be subjected to further study. During this study, it was noted that, plant extracts changed the water pH. This observation suggests that pH change possibly plays a vital role in inactivating bacteria in water. Bacteriological water quality analyses revealed that water from most sources had bacterial loads that exceeded the WHO value/guidelines for drinking water. Isolated species of *E. coli, Salmonella* and *Shigella* spp from water samples also showed varied antibacterial sensitivity to crude plant water extracts. This study therefore concludes that water from most sources is contaminated and must therefore be treated before consumption. It is recommended that further studies be conducted to identify the mechanism and active ingredients present in the plant extracts responsible for reducing sediment and bacterial load in water and how their efficacy is affected by the physico-chemical properties of water.

# CHAPTER ONE: INTRODUCTION

## 1.1 Background to the study

The Millennium Development Goals include halving the proportion of people without access to safe drinking water by 2015. The assessment of the health risk from naturally occurring microbes in drinking water continues to be of a high interest to microbiologists, public health practitioners and water supply regulators (Richards *et al.*, 1992; Hunter 1993). Although a number of studies have investigated water supply and quality in sub-Saharan Africa (Shier *et al.*, 1966), very limited information is available from the more sparsely populated arid and semi-arid regions.

Samburu district is semi arid with annual rainfall of between 250 - 500 mm. As such, water is scarce and is the most critical resource in the region. The area supports pastoral communities that coexist with wildlife. Hence humans, wildlife and livestock compete for available water resource in ephemeral laggas, natural ponds, man made dams and the only permanent water source, the Ewaso Nyiro river. Competition intensity varies with season. During the dry season, the available surface water sources considerably reduce. This coupled with increased intensity of use by humans, livestock and wildlife leads to a deterioration of water quality. Contamination results when animal wastes are released into the water.

Livestock movement and migratory wildlife in search of water can contaminate water by carrying pathogens from one source to another. High water evaporation rates increase the concentration of dissolved ions in the water, which favour high microbial growth. Ground water is invariably cleaner than surface water sources in rural areas. However the latter may need treatment to reduce the load of suspended solids and to kill microorganisms.

Removal of suspended solids presents the greatest treatment challenge, and there is a need to develop and choose technologies that will be sustainable in the medium to long term. In general, complex solutions should be avoided. Most drinking waters, even with residual disinfectants, have a natural bacterial population. This is because they use naturally available carbon and nitrogen sources in water to multiply (Manaia *et al.*, 1990; Reasoner, 1990). Addition of phosphates to water bodies influences growth of bacteria even at concentrations of less than 20 µg $L^{-1}$ (Sathasivan *et al.*, 1997; Lehtola *et al*., 2002). Animal and human wastes are major sources of nitrogen, phosphates and pathogenic bacteria contamination of aquatic environments. These nutrients can promote growth of pathogenic bacteria implicated in causing disease in humans, wildlife and domestic animals. The type of treatment technology or combination of technologies to be used depends on the quality of water to be treated. Hence, there is need for monitoring the quality of water bodies and to explore the efficacy of various treatment options such as coagulants from plant extracts to reduce bacterial load and dissolved solutes.

## 1.2 Problem statement and justification

Information on the bacteriological quality of water from various sources used for domestic purposes and for livestock watering in Samburu is limited. Livestock faecal wastes may contain pathogenic microorganisms such as *Salmonella* and *Escherichia coli.* When livestock drink water contaminated with enteric bacteria, they may be exposed to potential pathogens like *Salmonella,* which cause salmonellosis in cattle and enteric fever (Typhoid) in humans. Such waterborne diseases have been shown to be capable of infecting large numbers of animals over a short time.

The water treatment potential of natural coagulants to purify water in Samburu district has not been explored. Use of chemical treatments like chlorine as a disinfectant of polluted drinking

water is only practiced to a small extent in Samburu. Apart from the high cost of commercial disinfectants, their use has been reported to lead to the formation of trihalomethane products (Milot *et al.,* 2000), which are potentially carcinogenic (King *et al.,* 1996). There is, therefore, a need to develop water purification methods that are cost effective, locally available and environmentaly friendly. Determination of the physico-chemical properties of water such as turbidity, pH, salinity, temperature, dissolved oxygen, nitrogen and phosphorus will provide useful information on overall water quality and its potential to support bacterial growth (APHA, 2005).

This study describes the microbial quality of water from various sources commonly used by humans, livestock and wildlife in Wamba Division, Samburu District. The study also examines the relations between the physico-chemical properties and the microbial properties of water sources, and assesses the efficacy of plant extracts in purifying water to acceptable levels for human consumption.

### 1.3 Research questions

i. What is the level of bacterial contamination of water from common sources in Wamba Division of Samburu District?
ii. How does the bacterial load and type compare with the type of water source?
iii. How does the bacterial load and type compare with the physico-chemical properties of the water body?
iv. How effective are the roots extracts of *Boscia coriacea* Pax., *Maerua decumbens* (Brogn.) Dewolf and of seeds of *Moringa oleifera* Lam. in reducing the bacterial and sediment load in water?

## 1.4 Hypotheses

i. There is no significant bacterial contamination of water from common water sources in Wamba Division of Samburu District.

ii. There is no relationship between bacterial load, type and water source.

iii. There is no relationship between bacterial load, type and the physico-chemical properties of the water.

iv. The roots extracts of *Boscia coriacea* Pax., *Maerua decumbens* (Brogn.) Dewolf and seeds of *Moringa oleifera* Lam. are not effective in reducing the bacterial and sediment load in water.

## 1.5 Objectives

### 1.5.1 General objective

To determine water quality and to and to assess the efficacy of plant extracts in purifying water in Wamba Division, Samburu District.

### 1.5.2 Specific objectives

i. To determine the level of bacterial contamination of water from common water sources in Wamba Division, Samburu District.

ii. To compare bacterial load, type with type of water source.

iii. To compare bacterial load, type and physico-chemical properties of water.

iv. To determine the efficacy of roots extracts of *Boscia coriacea* Pax., *Maerua decumbens* (Brogn.) Dewolf and seeds of *Moringa oleifera* Lam. in reducing the bacterial and sediment load in water.

## 1.6 Significance of the study

This study evaluated the level of health risk that the residents of Wamba division are exposed to through direct use of water from the various water sources. The documented information will be important in the formulation of guidelines on water resource use in the division. Information on the bacterial load in water from different sources will be used by local public health officers to determine the sources of contamination and to educate the local community on how to protect the water sources from contamination. Information on the efficacy of the plant extracts in water purification validates their use as an alternative to chemical treatment, or forms the basis of their use combination with other water treatment methods to achieve safe drinking water. This will in the long run provide a cost effective and user-friendly option to be adopted for domestic household purification of water.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1 Bacterial water quality

Dirty and polluted water can contain many harmful organisms including pathogenic bacteria, which cause diseases like cholera, bacillary dysentery, typhoid, and diarrhea. Disinfection of water aims to kill these pathogens without leaving any harmful chemical substances in the water. Coliform bacteria, thermotolerant (faecal) coliforms and *Escherichia coli* have for almost a century been used as indicators of the bacterial safety of drinking water (Leclerc *et al.,* 2001). Water quality guidelines state that drinking water must not contain waterborne pathogens. More specifically, *Escherichia coli* or thermotolerant coliforms should not be present in any 100 ml sample of drinking water (WHO 2004). The guidelines further state that should this value be exceeded, immediate investigative action must be taken, including repeated testing, thorough inspection of the water source, and the general hygiene of the water distribution system.

Unlike other indicators, such as *Escherichia coli* or total coliforms, low concentrations of heterotrophic plate count (HPC) bacteria will still be present after treatment of drinking water. In general, water purification can achieve heterotrophic bacteria concentrations of 10 colony-forming units (CFU) per millilitre or less in finished water (Fox and Reasoner, 1999).

## 2.2 Water quality changes

Natural waters are subject to important changes in their microbial quality. These changes have direct impacts on the decisions made by water authorities striving to maintain safe conditions in catchments or distribution systems. Correct decision making by water authorities relies heavily on having access to rapid and accurate bacteriological data (Daniel *et al.,* 2003). This can be obtained by using HPC, which is a suitable tool for monitoring changes in bacterial water quality over time for a particular catchment or distribution system (Daniel *et al.*, 2003).

Changes in the microbial quality of water may arise from agricultural use, discharges of sewage, wastewater resulting from human activity, and storm or surface water runoff. Previous studies have suggested that sewage effluents contain a wide variety of pathogenic microorganisms whose density and variety are related to the size of the human population, the seasonal incidence of the illness, and dissemination of pathogens within the community (Pipes, 1982). Discharge of domestic sewage into water bodies also depletes dissolved oxygen leading to low dissolved oxygen concentrations and high numbers of enteric bacteria. An improvement of water quality is associated with an increase in the concentration of dissolved oxygen and a decrease in the load of faecal coliforms.

Changes in water conductivity results from changes in the mineral composition of water, which may be caused by seasonal variations in the chemical composition of the various sources of water. It may also indicate sewage, industrial or agricultural pollution or intrusion of saline waters. Determination of various water quality properties on a regular basis may, therefore reveal a need to adjust water treatment according to changes in raw water quality.

## 2.3 Water quality challenges

Livestock practices that can impact on water quality include both intensive and non-intensive operations. Intensive agricultural livestock operations (waste management and disposal) have been identified as point sources of pollution to streams. In water scarce areas, livestock and wildlife density tends to be high in water catchment areas and near water sources due to the presence of pasture and water. These animals generate large quantities of wastes. Water quality changes associated with livestock production include changes in nutrients loads, (nitrogen and phosphorus), microorganisms (e.g. bacteria, faecal coliforms, *Cryptosporidium*, *Giardia*) and organic material such as livestock wastes. Localized concentration of animal waste is considered a point source of pollution for surface or ground water. Lack of manure

management can adversely affect the water quality of receiving streams, its aquatic life, and reuse of the water downstream for agricultural, recreational and drinking water purposes. High animal densities within the catchment area of a water source may lead to the loss of protective cover of grasses, herbs, and shrubs due to trampling, grazing and browsing action. This exposes the soil to agents of erosion. Increased erosion results in a loss of organic matter, fine soil particles, nutrients, and microbes in the soil (Harper and Marble 1988; Schimel *et al.*, 1985; Belnap, 1995). They may be transported by surface runoff to eventually contaminate drinking water.

## 2.4 Biological indicators of water quality

The presence of faecal coliforms (over 99 % of which are *Escherichia coli*) in a water body is an indication of possible human/animal waste contamination and the possible presence of pathogenic bacteria. The detection of *Escherichia coli* provides definite evidence of faecal contamination. However, in practice, the detection of thermotolerant (faecal) coliform bacteria is an acceptable alternative. According to World Health Organisation (WHO, 1997, 2004) standards, faecal coliforms should be absent (0 colony forming units per 100 ml water) in portable water while total coliforms should be less than 10 colony forming units in any 100 ml water sample. The measurement of faecal coliforms can give an indication of the likely chlorine demand and also indicates where more intensive treatment is needed.

### 2.4.1 Heterotrophic plate counts

Heterotrophs are those microorganisms that use organic compounds for most or all of their carbon requirements (Singleton and Sainsbury, 2001). Most bacteria, including those associated with drinking water systems, are heterotrophs. A common and universal water testing method for the general bacteriological quality of water is the heterotrophic plate count (HPC). This technique assesses the number of bacteria in water that are able to replicate and

form visible colonies on a solid nutrient medium under specified test conditions (Australian Drinking Water Guidelines, 1996). Although not a direct method for the detection of pathogenic organisms, HPC can be used as a tool to assess the overall quality of water. In particular, HPC can be used to asses the effectiveness of water treatment processes and to detect bacterial regrowth within a distribution system (Geldreich, 1996).

The HPC technique has some inherent limitations. One important limitation is that the media used do not allow for the detection of all bacteria of interest, such as the chemolithotrophic ammonia-oxidizing bacteria that often colonize chloraminated distribution systems (Cunliffe, 1991). Furthermore, the incubation conditions of HPC, leaves slower growing heterotrophic organisms undetected (Australian Standard, 1995). Additionally, some bacteria, when exposed to environmental stresses, are capable of maintaining metabolic activity whilst developing recalcitrance to culture, commonly referred to as viable but nonculturable (VBNC) bacteria (McDougald *et al.*, 1998). Chlorine disinfection used in the water treatment process may also cause sublethal injury of some bacteria (McFeters *et al.*, 1986; Du Preez *et al.*, 1995), thereby rendering them nonculturable by routine HPC. This has been shown for both coliform and enteropathogenic bacteria in drinking water (McFeters *et al.*, 1986; du Preez *et al.*, 1995). The standard HPC procedure may also dilute vital signalling molecules which may be required for bacterial growth (Kaprelyants and Kell, 1996). Finally, HPC culture may be impeded by substrate-accelerated bacterial cell death, promoted by the presence of certain substrates in the HPC medium that were limiting in the environment when bacterial starvation was initiated (Barer and Harwood, 1999).

### 2.4.2 Thermotolerant coliforms

Thermotolerant (faecal) coliforms are a subset of total coliforms that possess a more direct and closer relationship with homeothermic faecal pollution (Geldreich, 1967). These bacteria

conform to all the criteria used to define total coliforms (all are aerobic and facultatively anaerobic, gram-negative, non-spore forming rod-shaped bacteria that ferment lactose with gas and acid production in 24 - 48 hours at 36 ± 1°C), but in addition they grow and ferment lactose with production of gas and acid at 44.5 ± 0.2°C within the first 48 hours of incubation. For this reason, the term "thermotolerant coliforms" rather than "faecal coliforms" is a more accurate name for this group (WHO, 1993).

Thermotolerant coliforms are physiologically adapted to temperatures found in the enteric tracts of animals (Clark, 1990). Thermotolerant coliforms include strains of the genera *Klebsiella* and *Escherichia* (Dufour, 1977). *E. coli* is, however, the only biotype of the family Enterobacteriaceae that is almost always faecal in origin (Hardina and Fujioka, 1991). Therefore, the thermotolerant coliform group when used should ideally be replaced by *E. coli* as an indicator of faecal contamination. For the purpose of water testing, most *E. coli* can be confirmed by a positive indole test and by their inability to use citrate (as the only carbon source) in the culture medium.

Several studies have, however, pointed out the limitation of both the thermotolerant coliform group and *E. coli* as ideal faecal indicators or pathogen indicator organisms. Several thermotolerant *Klebsiella* strains have been isolated from environmental samples with high levels of carbohydrates in the apparent absence of faecal pollution (Niemi *et al.,* 1997). Similarly, other members of the thermotolerant coliform group, including *E. coli,* have been detected in some pristine areas (Rivera *et al.,* 1988; Ashbolt *et al.,* 1997) and have been associated with regrowth in drinking water distribution systems (Lechevallier, 1990). The principal disadvantages of *E. coli* as an indicator of faecal contamination in water are its detection in other environments without faecal contamination (Hazen and Toranzos, 1990;

Hardina and Fujioka, 1991) and its low survival capability in aquatic environments when compared with faecal pathogens (Borrego *et al.*, 1983; Cornax *et al.*, 1990).

### 2.4.3 Faecal *Streptococci* and *Enterococci*

Faecal *Streptococci* have received widespread acceptance as useful indicators of faecal pollution in natural aquatic ecosystems. These organisms show a close relationship with health hazards (mainly for gastrointestinal symptoms) associated with bathing in marine and freshwater environment, (Cabelli *et al.*, 1982, 1983; Dufour, 1984; Kay *et al.*, 1994) and persistence patterns are similar to those of potential water-borne pathogenic bacteria (Richardson *et al.*, 1991). The group called faecal *Streptococci* includes species of different sanitary significance and survival characteristics (Gauci, 1991; Sinton and Donnison, 1994). In addition, the proportion of the species of this group is not the same in animal and human faeces (Rutkowski and Sjogren, 1987; Poucher *et al.*, 1991). The taxonomy of this group comprise species of two genera *Enterococcus* and *Streptococcus* (Holt *et al.*, 1993), and the most predominant species in polluted aquatic environments are *Enterococcus faecalis, E. faecium* and *E. durans* (Volterra *et al.*, 1986; Sinton and Donnison, 1994; Audicana *et al.*, 1995).

### 2.4.4 Pathogenic bacteria

Waterborne diseases are typically caused by enteric pathogens which belong to the group of organisms transmitted by the faecal-oral route. Many of these pathogens are of animal origin and water may also play a role in their transmission. Some of these pathogens are natural inhabitants of certain water environments. Most waterborne pathogens are distributed worldwide, but outbreaks of some, for instance cholera and hepatitis E, tend to be regional. The main goal of drinking water treatment is to remove or kill pathogenic organisms in order to reduce the risk of illness. Although it is impossible to completely eliminate the risk of

waterborne disease, adopting multi-barrier, source to tap approach to safe drinking water will reduce the numbers of microorganisms in drinking water. This approach includes protection of the water source (where possible), the use of appropriate and effective treatment methods, well-maintained distribution systems, and routine verification of drinking water safety. All drinking water supplies should be disinfected, unless specifically exempted by the responsible authority. In addition, all surface and groundwater sources under the direct influence of surface run-off water should be filtered before treatment.

#### 2.4.4.1 *Salmonella* spp.

*Salmonella* is a gram-negative facultative rod-shaped bacterium in the same proteobacterial family as *Escherichia coli*, the family *Enterobacteriaceae.* The principal habitat of *Salmonella* is the intestinal tract of humans and animals. In humans, *Salmonella typhi* and *Salmonella paratyphi* A cause two diseases called salmonellosis and enteric fever (typhoid), respectively (Todar, 2005). The diseases result from bacterial invasion of the bloodstream, and acute gastroenteritis, resulting from a foodborne infection/intoxication. *Salmonella* are disseminated in the natural environment (water, soil, sometimes plants used as food) through human or animal excretion. Humans and animals (either wild or domesticated) can excrete *Salmonella* when clinically diseased or after having had salmonellosis, if they remain carriers. *Salmonella* organisms do not seem to multiply significantly in the natural environment (out of digestive tracts), but they can survive for several weeks in water and for many years in soil if conditions of temperature, humidity, and pH are favourable (Todar, 2005).

During the last decade, antibiotic resistance of *Salmonella* spp. have increased a great deal due to increased and indiscriminate use of antibiotics in the treatment of humans and animals and the addition of growth-promoting antibiotics to the food of breeding animals. Antibiotics are usually ineffective on *Salmonella* carriage (even if *Salmonella* are susceptible to them)

because the site of carriage may not allow penetration by the antibiotic. Resistance to ampicillin, streptomycin, kanamycin, tetracycline, and sulfonamides is commonly observed. Colistin resistance has not yet been observed. Recently *Salmonella typhi* strains resistant to chloramphenicol (the antibiotic most commonly used against typhoid) strains have been isolated in India, Thailand, and Vietnam.

#### 2.4.4.2 *Shigella* spp.

*Shigella*, a well known pathogen that causes gastrointestinal infection in human, is prevalent in less developed countries where poor sanitation increases incidences of this disease. The low infectious dose (DuPont *et al*., 1989) allows the disease to be spread effectively by infected food or water. Like two other species of *Shigella* (*S. flexineri and S. dysentriae*) both *S. sonnei* and *S. boydii* are almost equally important as diarrheal pathogens, since a number of reports have been published on the outbreak of shigellosis caused by these two species (Alamanos *et al.*, 2000; McCall *et al.*, 2000). Medium pH has been reported to affect the survivability of *S. flexineri* and *S. dysentriae* (Sultana *et al.*, 2002). Their survival in acidic conditions may have clinical significance, because enteric pathogens must pass through the stomach (pH less than 3) for upto 2 hours before colonizing the intestinal tract (Giannella *et al*., 1972). It has been reported that, *Shigella* spp. are more acid tolerant (pH 2 to 2.5) than are *Salmonella* and *E. coli* (Gorden and Small, 1993).

### 2.4.5 Bacterial water quality risk assessment

The World Health Organization recommends sanitary inspections of water points as part of the comprehensive risk-based assessment of drinking water quality (World Health Organization, 2004). The objectives of the sanitary inspection include supporting operation and maintenance of the water point by providing clear guidance for remedial action to protect and improve the water supply. In Samburu, sanitary risk-based assessment can only apply to

37 % of the water sources (mainly springs and boreholes) since 63 % (majority) are temporary sources. According to Lloyd and Bartram (1991) sanitary inspection is not designed to replace microbiological water quality testing, but rather is a complementary assessment designed to identify risks to water quality. Owing to the nomadic lifestyle of the Samburu population, and the increasing competition for water, a risk assesment approach which aims at determining the most suitable water source for use and enhancing immediate water treatment before use will be of more immediate health significance than visual sanitary risk inspection.

Waterborne pathogens have been recognized as a significant risk to public health for more than a century. The risk is based on microbiological standards of drinking water (zero faecal coliforms and pathogens). Quantitative risk assesment has demonstrated that the risks from pathogens are greater than the risks from disinfection by products (Craun *et al.,* 2001). The greater risks of waterborne bacterial disease are known to be associated with contaminated drinking water. The pathogens that are most widely recognized to cause dangerous waterborne diseases by the public are *Salmonella typhi*, *Vibrio cholerae*, *Shigella* and pathogenic *E. coli*

### 2.4.6 Physico-chemical indicators of water quality

#### 2.4.6.1 Hydrogen ion potential - pH

The pH of water is a measure of whether it is acidic or alkaline. However, its significance depends on the buffering capacity of the water. pH affects the acceptability of water for various uses and influences the solubility of metal ions in water.

#### 2.4.6.2 Turbidity - NTU (Nephelometric Turbidity Units)

Turbidity is a measure of the reduction in the transparency of a water body as a result of light scattering by suspended particulate matter (Ziegler, 2002). It is a measure of the relative

clarity of water (Sadar, 1996). Levels of turbidity in raw water can range from less than 1.0 NTU to more than 1000.0 NTU. Turbidity is not an absolute value, but a relative value representing a qualitative measurement that can yield different readings based on the method used (Ankcorn, 2003). Turbidity can also be used to estimate loads for contaminants typically bound to sediment particles, such as nutrients and bacteria (Ankcorn, 2003). Turbidity readings may vary between water sources due to water colour, suspended particle size and particle composition (Packman *et al.,* 1999). Organic particles in water have been shown to absorb light, and therefore provide different turbidity values compared to water carrying primarily mineral soils (Lewis, 1996).

The microbiological quality of drinking water can be significantly affected by turbidity. This is because microbial growth in water is most extensive on the surfaces of particles and inside loose flocs, which may occur naturally or be formed during treatment. Microbial growth occurs because nutrients adsorb to surfaces, allowing bacteria to grow more efficiently than when in free suspension (Brock, 1966 and Stotzky, 1966). A study by Reilly and Kippin (1983) suggested that turbidity of around 1.0 NTU has no impact on coliforms or HPC. According to WHO, the indicator value only applies at the treatment works since turbidity above 1.0 NTU can compromise disinfection by increasing chlorine demand, and hence the cost of water treatment. However, it is important to optimise the removal of turbidity during water treatment in order to remove all micro-organisms.

#### 2.4.6.3 Total organic carbon (TOC)

TOC is a non specific measure of the dissolved organic matter present in water. High concentration can lead to the formation of high levels of chlorination by-products such as Trihalomethanes (THMs). An abnormal increase in TOC can be an indicator of treatment failure in the system and it is associated with an increase in assimilable organic carbon

(biologically available carbon). This may give rise to an increase in bacterial growth and formation of biofilms in water distribution systems.

### 2.4.7 Water pollution by organic matter

Input of organic matter is a normal feature of aquatic systems. The total load usually depends on the catchment area of the water body and the density of humans and wildlife in areas where organic wastes are discharged into water bodies through point and non point sources. Organic wastes discharge into waterbodies increases water turbidity, organic compounds, nutrients, and the bacterial load in the waterbody (Bitton and Gerba, 1984). Organic wastes contain a high concentration of both phosphorus and nitrogen. A single cow can produce as much phosphorus (about 18 kg per year) as 212 ha of forest or more than 57 ha of cropland. It can also excrete as much nitrogen (58 kg per year) as 68 ha of forest or more than 6 ha of arable land (Brian, 1980).

Phosphorus enters water bodies either as inorganic phosphate ions or organic polymers and biodegradable organic compounds in living organisms and detritus. Presence of phosphorus in a water body promotes bacterial growth (Geildreich, 1996; LeChevallier, 1990; LeChevallier *et al.,* 1991). A number of studies carried out through batch experiments using drinking water have shown that the addition of phosphates may lead to increased bacterial culturability (Miettinen *et al.,* 1997; Sathasivan and Ohgaki, 1999) and sometimes to bacterial growth (Sathasivan *et al.,* 1997; Lehtola *et al.,* 2002). However, in drinking water in which carbon is the limiting nutrient for bacterial growth, the addition of phosphates does not induce bacterial growth (Lyons *et al.,* 1995; Cohen *et al.,* 1999; Appenzeller *et al.,* 2001; Batté *et al.,* 2003).
Treatment of water contaminated by organic matter has always been one of the major public health concerns. The primary inorganic compound of concern to public health in groundwater contaminated with livestock wastewater is nitrate-nitrogen, which may cause

methemoglobinemia (also called blue-baby syndrome) in infants (Canter, 1997). In most cases, the recommended treatment method requires the removal of a large part of the organic load. This is often accompanied by the elimination of nitrogen and phosphorus (Rotereau, 1969; Moletta and Torrijos, 1999; Hamdani, 2002).

## 2.5 Common water treatment methods

### 2.5.1 Solar disinfection

Solar disinfection involves storing contaminated drinking water in a transparent container that is placed in direct sunlight for periods of up to eight hours before consumption (Conroy *et al.*, 1996; Sommer *et al.*, 1997). This technique is highly effective against a broad range of pathogens (Conroy *et al.*, 2001; Kehoe *et al.*, 2004; Smith *et al.*, 2000; Wegelin *et al.*, 1994). Coliforms in water and sewage have been completely inactivated by exposure to sunlight for about one hour in the presence of methylene blue or rose Bengal (Archer and Juken, 1977). This method is cheap and can easily be applied by rural communities to disinfect water but cannot be used to sediment dissolved particulate matter in water.

### 2.5.2 Chemical water treatment methods

#### 2.5.2.1 Alum coagulation

Use of alum has been traditionally practiced for centuries in many parts of the world (Jahn and Dirar, 1979; Gupta and Chaudhuri, 1992). In one study, potash alum was evaluated for household water treatment in a suburban community in Myanmar by adding it to water in traditional storage vessels (160 L capacity) at 500 mg $L^{-1}$. Faecal coliform contamination was reduced by 90 - 98 % and consumer acceptance of the treated water was high (Oo *et al.*, 1993). However, because the coagulation-flocculation treatment with alum, iron and other coagulants requires knowledge and skills to optimize treatment conditions, this type of treatment is less likely to be performed reliably at point of use for water treatment, especially

by semi literate rural communities. Similarly, the relatively high costs of alum and ferric salts, makes this treatment option not affordable. Some studies have reported that aluminium, a major component of alum, may induce Alzheimer's disease (Martyn *et al.,* 1989). Alum also reacts with the natural alkalinity of water leading to a reduction of pH and low efficiency in coagulation in cold water (Haarhoff and Cleasby, 1988). This limits the acceptance and use of alum.

#### 2.5.2.2 Chlorine treatment

Among the drinking water disinfectants, free chlorine is the most widely used and the most affordable. It is also highly effective against nearly all waterborne pathogens, with notable exceptions being *Cryptosporidium parvum* cysts and *Mycobacteria* species (Sobsey, 1989). At doses of a few mg $L^{-1}$ and contact times of about 30 minutes, free chlorine generally inactivates more than 99.99 % of enteric bacteria and viruses. Despite its disinfection property, it does not sediment dissolved solids in water hence must be combined with another sedimentation method.

In water with turbidities ranging from 3.8 to 84.0 NTU, coliforms were detected after the water was treated with chlorine (free chlorine residuals between 0.1 and 0.5 mg $L^{-1}$) after a minimum contact time of 30 minutes (Sanderson and Kelly, 1964). While studying the efficiency of chlorination in killing coliforms in unfiltered surface water supplies, LeChevallier *et al.*, (1981) observed a negative correlation between the effectiveness of chlorination and turbidity. Chlorine (as hypochlorous acid) also reacts readily with organic matter containing unsaturated bonds, phenolic groups and nitrogen groups, giving rise to taste and odour producing compounds (Sawyer and McCarty, 1967) and trihalomethanes (Rook, 1977). Chlorine is also expensive and requires skills to apply, hence not feasible for use by rural households.

### 2.5.3 Treatment with plant extracts

The mucilage extracted from the seeds of *Tamarindus indica* Linn. has been used as a flocculant for removal of sulphate and phosphate ions in aqueous medium. A mucilage dose of 50 mg $L^{-1}$ has been reported to remove 74 % and 76 % of sulphates and phosphates respectively after 30 minutes (Anuradha and Malvika, 2005). Seeds of *Moringa* species have been reported to be an effective, simple and low-cost flocculant for turbid surface water that can be used for household water treatment (Jahn and Dirar, 1979; Jahn, 1981; Olsen, 1987). *Moringa oleifera* Lam. seed is a non toxic (Grabow *et al*., 1985) and natural organic flocculant.

The treatment potential of other vegetable materials have also been investigated. These include extracts of Okra and *Strychnos potatorum* Linn. seeds (Al Samawi and Shokralla, 1996) and Tamarind (Bhole, 1995). The effectiveness of *Strychnos potatorum* Linn. to flocculate or precipitate microbes and turbidity in water has also been investigated (Tripathi *et al*., 1976). Microbial reductions of about 50 % and 95 % have been reported for plate count bacteria and turbidity respectively. Past studies have documented an 80 to 99 % turbidity removal by *Moringa oleifera* Lam. as the primary coagulant both for raw waters and synthetics turbid waters (distilled water with turbidity added as kaolin) (Muyibi and Okufu, 1995; Ndbigengesere *et al.,* 1995; Muyibi and Evison, 1996). However, according to Muyibi and Okufu (1995), *Moringa oleifera* Lam. might not be an efficient coagulant for water with low turbidity. Although the water treatment potential of *Moringa oleifera* Lam. has been extensively investigated and the plant recommended as a coagulant (Muyibi and Okufu, 1995; Muyibi and Evison, 1995, 1996; Ndabigengesere and Narasiah 1996, Ndabigengesere *et al.,* 1995) it is important to establish its effectiveness in waters from different sources and with different physico-chemical properties.

## 2.6 Microbial reduction by coagulation-flocculation

Optimum coagulation to achieve maximum reduction of turbidity and microbes requires careful control of coagulant dose, pH, consideration of the quality of the water being treated as well as appropriate mixing conditions for optimum flocculation. Lack of attention to these details can result in poor coagulation-flocculation and inefficient removal of particles and microbes. Under optimum conditions, coagulation-flocculation and sedimentation with alum and iron can achieve microbial reductions of between 90 to 99 % for all classes of waterborne pathogens (Payment and Armon, 1989). Greater microbial reductions (more than 99.99 %) can be achieved with lime coagulation-flocculation at high pH levels (pH more than11).

## 2.7 Bacterial antibiotic resistance

Antibiotic resistant bacteria have been isolated from a variety of sources such as domestic sewage, hospitals drinking water, rivers and lakes (Magee and Quinn, 1991; Ogan and Nwiika 1993; Boon and Catanach, 1999). These strains include bacteria that are pathogenic to humans and many of these show multiple resistance (MAR) (Lancini *et al.*, 1995). Increasing resistance to commonly used antimicrobial agents in *Shigella* and *Salmonella* species has become a major public health concern worldwide (Mandell *et al.,* 2000). These antibiotic resistant bacteria are significant environmental contaminants (Huber, 1971) and calls have been made for antibiotic resistance to be considered when establishing bacteriological water quality criteria (Bell *et al.,* 1983; El-Zanfaly, 1991).

# CHAPTER THREE: MATERIALS AND METHODS

## 3.1 Study area

The study was carried out in Wamba Division, Samburu District in the Rift Valley Province, Kenya (Figure 1). The area has four conservancies namely Barsalinga, Namunyak, Ngaroni and Ngutuk Ongiron. The landscape is home to many ethnic groups including the Samburu, Masai and Borana communities. Samburu nomadic herdsmen in the area follow traditional migration patterns determined by rainfall and available pasture.

Rainfall in the Samburu District follows a fairly erratic pattern, varying significantly both in time and space. The district does, however, receive short and long rains. The driest months are January and February. The long rains start at the end of March, and continue into April and end in May. Short rains occur in January and August in most of the district but in October and November in two divisions. The Lorroki Plateau receives between 500-700 mm of the rain annually, but rainfall varies with altitude (Kasusya, 1998).

Some variations are unfavourable to pastoralist communities, especially during dry years when rainfall does not exceed 250 mm. In such arid areas, limited access to water of adequate quantity and quality is a major risk factor in environmental health (Bannaga and Pickford, 1978) and therefore there is need to asses the quality of available water in various sources in order to advise consumers on its health implications when used.

Ewaso Nyiro River is the only permanent source of water. Numerous ephemeral laggas and natural ponds contain water during the rainy season and some continue providing water during the dry season. There are also man-made dams for water harvesting to supplement the scarce water resources. Temperatures vary with altitude and generally range between 24 °C mean minimum and 33 °C mean maximum. Lower lying areas experience higher evaporation

losses (3106 pan-data), whereas higher areas experience relatively lower evaporation (1786 penman estimate) (Kasusya, 1998).

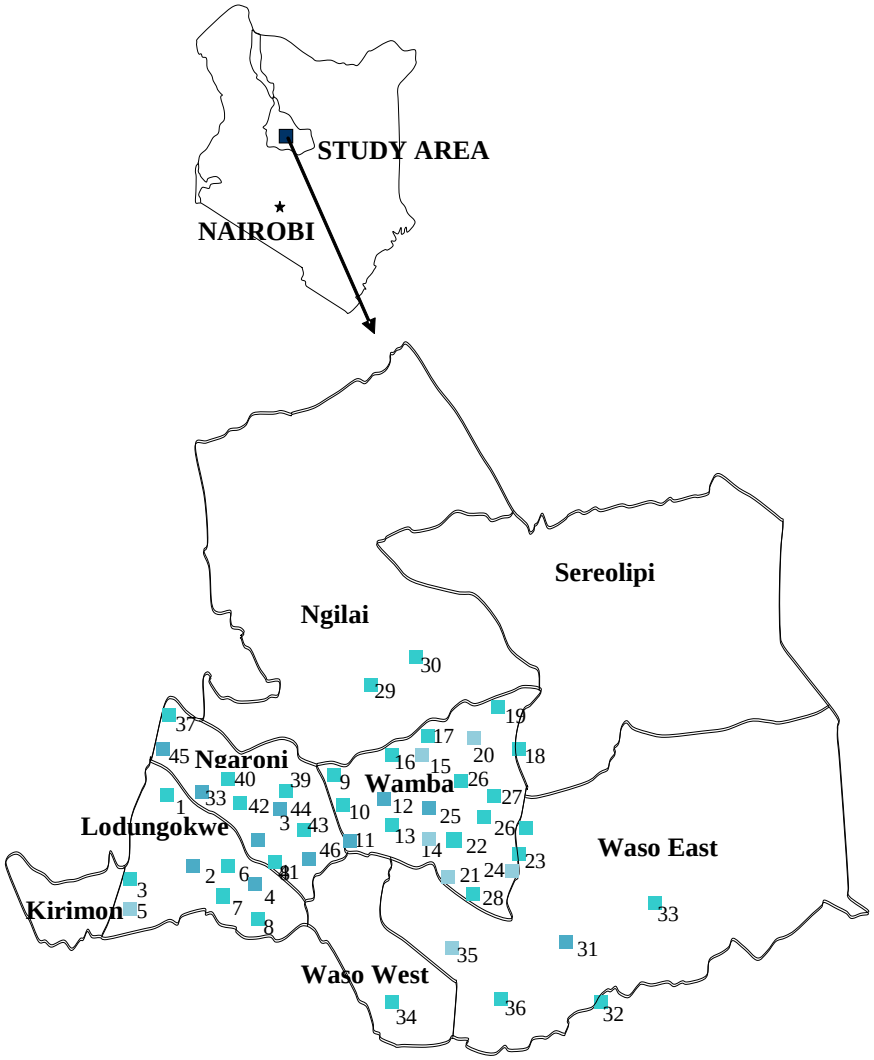


**Figure 1 Map of the study area showing the sampling sites**

## 3.2 Water sampling

During this study, water from forty six sources in Wamba Division, Samburu District was sampled and analyzed (Appendix 1). Water from all these sources was water used for drinking and other household purposes. Some of these sources also served as watering points for both livestock and wildlife.

Preliminary results of water quality sampled through purposive and random sampling were used to choose the sampling sites with high microbial loads and diverse pathogenic bacteria. The water sampling, preservation and tests were performed according to standard methods (APHA, 2005). Water samples for microbial and chemical analyses were collected from each source mostly in the morning hours (between 9.00 am to 12 noon) in sterile water sampling bottles. Where possible, water samples for microbiological analyses were drawn directly from the water body using sterile 125 ml plastic bottle. When this was not possible, the samples were drawn using a sterile scooper. At each site, two sterile 125 ml plastic bottles fitted with screw caps were used for water collection. Water samples for microbial and physico-chemical analyses were transported to the laboratory in an iced cool box. Microbial analyses were carried out at Wamba Mission Hospital while physico-chemical analyses were carried out at Earthwatch camp laboratory.

## 3.3 Field measurements

### 3.3.1 pH (pH units)

Water pH was determined using a portable WTW Multiline P4 meter (Weilheim, Germany), which uses a probe fitted with automatic temperature compensation to 25 °C. This meter measures hydrogen ion concentration by direct potentiometry. pH readings were taken to the nearest one decimal place. Where possible, the probe was lowered directly into water and the meter readings allowed to stabilize for about three minutes before the pH value was taken.

### 3.3.2 Temperature (°C)

Temperature was taken in the field using the dissolved oxygen probe (CellOx325) of a portable WTW Multiline P4 meter (Weilheim, Germany). The dissolved Oxygen probe has an in-built temperature sensor, which gives water temperature readings in degrees celcius to one decimal point. Where possible, the probe was lowered directly into water and the meter readings allowed to stabilize for about three minutes before the temperature value was taken.

### 3.3.3 Electrical conductivity (µS $cm^{-1}$)

Electrical conductivity was measured in the field using a portable universal multiline P4 WTW (Wilheim Germany) meter. The multiline meter uses a Tetra Con 325 electrical conductivity probe to measure conductivity. Where possible, the probe was lowered directly into the water and the meter readings allowed to stabilize for about three minutes before the electrical conductivity value was taken.

### 3.3.4 Dissolved oxygen (DO µg $L^{-1}$)

Dissolved oxygen was determined in the field using the dissolved oxygen probe (Ox325) of the universal multiline P4 WTW (Wilheim Germany) meter. Where possible, the probe was lowered directly into water and the meter readings allowed to stabilize for about three minutes before the dissolved oxygen value was taken (APHA, 2005). When this was not possible, samples were carefully collected with a water scooper and readings taken immediately.

## 3.4 Laboratory physico-chemical analyses

### 3.4.1 Total alkalinity (TA mg $CaCO_3$ $L^{-1}$)

Total alkalinity was determined by the titration of 100 ml water samples with 0.02N standard HCl using mixed methyl red bromocresol green indicator to determine titration end point. Sample total alkalinity was computed using the procedure outlined in APHA (2005).

### 3.4.2 Phosphorus (mg $L^{-1}$)

Orthophosphate phosphorus was determined by ascorbic acid reduction procedure (APHA, 2005). Water samples were first filtered with pre-washed glass fiber filters (GF/C). To determine total phosphorus, all forms of phosphorus in water samples were first oxidized to orthophosphate ($PO_4$ – P). This was achieved by autoclaving a 25 ml water sample at 140 °C for 40 minutes in the presence of 0.2 g potassium persulfate oxidizing agent. A reagent blank and standards in a suitable range were prepared from a standard phosphate solution (APHA, 2005). Colour intensity was measured using a digital grading spectrophotometer (Nanocolor 300 D) at a wavelength of 690 nm and the phosphates concentrations determined based on the standards curve of known phosphate phosphorus concentrations.

### 3.4.3 Turbidity (NTU)

Turbidity of the water samples were determined by using a colorimeter (Smart - 26617). The water sample was gently swirled and 10 ml was drawn out using a clean sterile syringe and transferred into a suitable curvette and readings taken immediately. The turbidity of the sample was measured against a distilled water blank. Initial turbidity was determined before treatment of the water sample while change in turbidity was determined after 30 minutes and 24 hours of treatment in the laboratory using a colorimeter.

### 3.4.4 Most probable number (MPN) of total and faecal coliforms per 100 ml

Analysis of water for the presence of total coliforms was carried out using the multiple tube fermentation technique (APHA, 2005) which involves three steps: the presumptive, confirmed and the completed tests.

#### 3.4.4.1 Presumptive test

The Presumptive test was carried out to determine total and faecal coliforms present in the water samples (Figure 2). Double and single strength lactose broth was prepared and

dispensed into tubes in 10 ml volumes. Durham tubes were inverted in the broth and then sterilised at 121 °C for 15 minutes using an autoclave. Five tubes containing double strength broth were inoculated with 10 ml of sample water. Two sets of five tubes containing single strength broth were inoculated with 1 ml and 0.1 ml of the water sample respectively using a sterile pippete.

The tubes were incubated at 37 °C for 24 $\pm$ 2 hours after which each tube was swirled gently. Presence of gas in the Durham's tubes as well as growth and acid production evidenced by colour change to yellow were the presumptive evidence for the presence of coliform bacteria in the sample. The negative tubes (no gas, no colour change and turbidity) were re-incubated for a further 24 hours and then re examined for gas production (Figure 2). The total coliforms per 100 ml of water were estimated using the most probable number (MPN) index as described in APHA (2005).

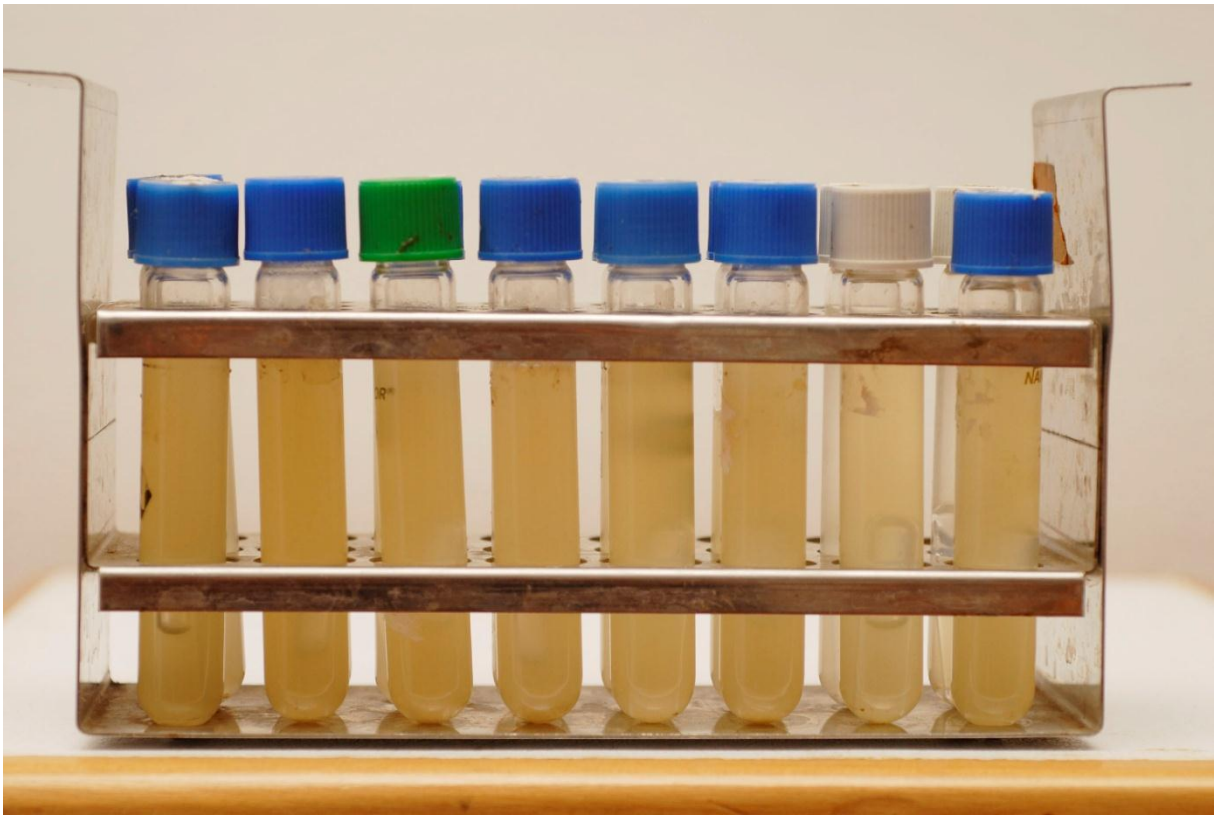

**Figure 2 Multiple tube fermentation technique showing positive presumptive test for faecal and total coliforms in a 24 hour lactose broth culture**

### 3.4.4.2 Confirmatory test

This test was used to confirm the presence of coliform bacteria in all presumptive tubes which showed growth, gas production or acidic reaction within 24 ± 2 hours of incubation (Figure 3). Additional presumptive tubes which showed active fermentation or acidic reaction at the end of 48 ± 3 hours of incubation were also submitted to the confirmatory test. Using a sterile loop (3 mm in diameter), loopfuls of culture were aseptically transferred from positive presumptive tubes to fermentation tubes containing brilliant green lactose bile broth and then incubated at 37 °C for 48 ± 3 hours. The MPN value was then calculated from the number of positive brilliant green lactose bile broth tubes which showed gas formation in the inverted vial (Figure 3).

**Figure 3 Multiple Tube Fermentation technique showing positive confirmatory test for faecal and total coliforms in a 24 hour brilliant green bile lactose broth culture**

#### 3.4.4.3 Completed test

This test was carried out by submitting 10 % of positive confirmed tubes of brilliant green lactose bile broth into Eosin Methylene Blue agar (EMB). Using a sterile 3 mm diameter loop, a culture from each tube of brilliant green lactose bile broth showing gas was streaked on plates containing Eosin Methylene Blue agar and incubated at 37 °C for 24 ± 2 hours. Typical lactose fermenting colonies were then isolated and transferred to a single strength lauryl tryptose broth fermentation tubes (with inverted fermentation vials) and incubated at 37 °C for 24 ± 2 hours. Presence of turbidity in lauryl tryptose broth and gas in Durham tube within 24 ± 2 hours indicates positive completed test for the presence of total coliforms.

### 3.4.5 Faecal coliforms (thermotolerant *Escherichia coli*)

The test for faecal coliforms was conducted simultaneously with the test for total coliforms at the presumptive stage. Three sets of five tubes containing lauryl tryptose broth were each inoculated with10 ml, 1 ml and 0.1 ml portions of water samples respectively and incubated at 37 °C for 48 hours. Presence of turbidity in lauryl tryptose broth and gas in Durham tube within 24 ± 2 hours indicates positive completed test for the presence of faecal coliforms.

#### 3.4.5.1 Confirmatory test

This test was carried out by transferring a loopful culture from positive presumptive tubes of the total MPN test to EC media and incubated at 44.5 °C for 18 to 24 hours (APHA, 2005). Gas production in fermentation tube within 24 hours confirmed the presence of faecal coliform bacteria. The MPN value was then calculated from the EC media based on the number of positive tubes which showed gas formation in the inverted vial.

#### 3.4.5.2 Completed test

This test was carried out by carefully streaking Eosin Methylene Blue (EMB) agar plates from each of the tubes of EC medium showing gas. The plates were then incubated at 37 °C for 18 to 24 hours. Dark blue colonies with green metallic sheen on the EMB agar indicates the possible presence of *Escherichia coli.* Colonies with metallic sheen appearance were then subjected to Indole, Methyl Red, Voges - Proskauer and Citrate utilization tests, ("IMViC" tests) to confirm *Escherichia coli* identity.

### 3.4.6 Heterotrophic plate counts (CFU $mL^{-1}$)

Serial dilutions of water sample of $10^{-3}$ and $10^{-4}$ were prepared by adding 1 ml water sample to 9 ml distilled sterile water. Using a sterile pipette, an amount of 0.5 ml of serially diluted water sample was inoculated aseptically on nutrient agar contained in presterilized plastic petri dishes and spread with a bend rod. The plates were incubated at 35 °C for 48 hours after which all bacterial colonies were counted in all dilutions (APHA, 2005). The dilution factor was used to calculate the number of total heterotrophic plate counts in the original water sample.

### 3.4.7 Isolation of *Salmonella* and *Shigella* spp (presence/absence)

One ml water sample was enriched with 10 ml sterile selenite F broth and incubated at 35 °C for 24 hours. The culture was then carefully streaked on *Salmonella - Shigella* agar and then incubated at 35 °C for 24 hours. Typical *Salmonella* colonies are colourless with a black center while those of *Shigella* are colourless, shinny with no black center. Presence of *Salmonella* and *Shigella* spp were confirmed and differentiated using Motility and IMViC test.

### 3.4.8 Isolation of *Vibrio cholera* (presence/absence)

An amount of 1 ml of water sample was enriched with 10 ml peptone water and incubated for 6 - 8 hours at 35 °C. The culture was then streaked on to thiosulphate citrate bile salts (TCBS) agar plates, and then incubated at 37 °C for 24 $\pm$ 2 hours. Typical *Vibrio cholerae* colonies are 2 - 3 mm in diameter, yellow in colour, and oxidase positive (Cheesbrough, 1985).

## 3.5 Identification of bacteria

### 3.5.1 Gram staining

A pure colony of bacteria was removed using a sterile wire loop and smeared on a clean slide then heat fixed. The smear was then flooded with gram crystal violet primary stain to stain for 1 minute, after which it was washed off with cold water. The slide was then flooded with grams iodine mordant and left to seat for 1 minute, and then washed off with safranin counterstain solution. More counterstain solution was added to the slide to stain for 50 seconds after which it was washed off with cold water and the slide blot dried. Using a light microscope, the slide was mounted and observed under oil immersion (APHA, 2005).

### 3.5.2 Oxidase test

A drop of overnight pure bacterial peptone culture was put on the surface of oxidase discs. An immediate change of the disk from white to purple colour indicated a positive result for *Vibrio cholera* (APHA, 2005).

### 3.5.3 Motility test

SIM medium (CM435) agar was used to detect the presence or absence of motility in bacteria. This was done by preparing the SIM agar deep tubes and inoculating each tube with appropriate bacteria using aseptic technique by inserting a straight wire to about one third of the depth of the medium. The cultures were then incubated for 24 hours at 35 °C after which it

was examined for motility and hydrogen sulphide production. *Salmonella* spp are motile and produce hydrogen sulphide while *Shigella* spp are non motile (APHA, 2005).

### 3.5.4 Indole test

Indole, a nitrogenous compound and a degradation product of amino acid tryptophan of various bacteria, was detected using Kovac's method. This was done by preparing an overnight peptone bacterial culture and adding 0.2 ml of Kovac's reagent to cover it. The mixture was then shaken and the reagent allowed to stand for 10 minutes. The appearance of dark red colour at the top indicated a positive result for presence *of E. coli* and *Vibrio* while no change from the original colour indicated negative result for *Salmonella* (APHA, 2005).

### 3.5.5 Methyl red test

A 10 ml tube of Methyl red-Voges-proskauer **(**MRVP) medium (CM43) was prepared and aseptically inoculated with the bacterial culture and incubated for 48 hours at 37 °C. A test for acid production was done by adding 5 drops of 0.4 % w/v methyl red to the test culture and the colour on the surface was observed immediately. A distinct red colour is a positive test for *E. coli*, while yellow is negative.

### 3.5.6 Voges-proskauer test

A tube of MRVP broth was prepared and aseptically inoculated with 6 hour old bacterial culture and incubated for 3 days at 35 °C in a water bath. To test for acetyl methyl carbinol, 2 drops of a 0.3 % w/v solution of creatine and 5 ml of 40 % KOH solution was added and then gently shaken for 30 seconds. The appearance of pink to red colour was a positive result for the presence of acetyl methyl carbinol, while no colour change (negative) indicate a positive result for *E. coli* and *Shigella* spp (APHA, 2005).

### 3.5.7 Citrate test

Simmons citrate agar (CM155) slopes in test tubes were prepared and aseptically inoculated with bacteria by streaking the slopes and stabbing the butt of the medium. The slopes were then incubated for 48 hours at 35 °C. Change of media colour from green to bright blue constituted a positive result for *Vibrio* whilst in a negative test the colour of the medium remained unchanged (APHA, 2005).

## 3.6 Bacterial risk level determination

Bacterial risk level determination was carried out by first determining the faecal coliform numbers in various types of water sources as described in section 3.4.5. Based on the number of faecal coliforms obtained, the water sources studied were grouped into the following risk categories as described in WHO (1997).

- 0 colony forming units / 100 ml (conformity to WHO standards, hence no risk);
- 1–10 colony forming units /100 ml (low risk);
- 10–100 colony forming units /100 ml (intermediate risk);
- 100–1000 colony forming units/100ml (high risk);
- More than 1000 colony forming units/100 ml (very high risk).

For better visualization, different colours were used to indicate the risk level/type and action required as shown (Table 1).

**Table 1 Bacterial risk level classification based the number (MPN/ 100 mL) of faecal coliforms present**

| Risk Level | Colour | Risk type | Faecal coliforms (MPN/ 100 mL) | Action required |
|---|---|---|---|---|
| A | Blue | Conformity | 0 | No action required |
| B | Green | Low risk | 1 – 10 | Low action priority |
| C | Yellow | Intermediate | 10 – 100 | Higher action priority |
| D | Orange | High risk | 100-1000 | Urgent action required |
| E | Red | Very high risk | > 1000 | Urgent action required |

### 3.7 Preparation of plant material

Roots of *Boscia coriacea* Pax., and *Maerua decumbens* (Brogn.) Dewolf were collected from Wamba, Samburu District with the help of traditional healers. These two plants, indigenous and common in Samburu are used to treat water and infections of microbial origin. Seeds of *Moringa oleifera* Lam. were obtained from KEFRI. All the plant materials collected in the field were identified and processed in the Department of Plant and Microbial Sciences, Kenyatta University.

The roots of *Boscia coriacea* Pax. and *Maerua decumbens* (Brogn.) Dewolf were washed then chopped into small pieces and dried in the shade. Dried seeds of *Moringa oleifera* Lam. were shelled to remove the seed coat and to obtain the kernels. The root choppings and seed kernels were each milled using a sterile mill (Thomas–Wiley, Model 4) and stored in a sterile airtight container at a temperature of 3 °C in a refrigerator until time for use. From each plant, an amount of 2 g of the ground plant materials was weighed and packed in filter sachets in readiness for use.

### 3.8 Water treatment

The study involved 15 different surface water samples with different physico-chemical and bacteriological qualities. Treatment tests were carried out at laboratory temperatures (24 $\pm$ 2 °C) inside a laminar flow (Figure 4). The effectiveness of the treatment was assessed analytically by determining changes in bacterial heterotrophic plate counts (HPC) and turbidity over a 24 hour period. Heterotrophic plate counts were determined using the spread plate method while turbidity was determined with the aid of a colorimeter.

An amount of 200 ml of turbid water samples with predetermined bacterial density and turbidity was dispensed into six 250 ml sterile glass bottles. Using sterile forceps, a sachet of each plant material was immersed in different water samples. Additionally, 2 grams of alum

(aluminium sulphate) and 2 ml of 1.2 % sodium hypochlorite was also added to a different 200 ml of the test water samples for comparison purposes, and one 200 ml of test water sample served as a negative control. Each treatment was then allowed to stand for 24 hours, during which change in turbidity and bacterial counts were determined using a colorimeter and predried nutrient agar plates respectively. Change in water sample pH was also determined using a portable pH meter. The aluminium sulphate used in this study was of industrial grade 3 ($Al_2(SO_4)_3.18H_2O$), which conforms to the Kenya Bureau of Standard KS-03-85:1979 (Figure 4).

**Figure 4 Setup showing incubation of water samples during treatment. Different samples show different turbidity levels**

### 3.9 Antibacterial activity

#### 3.9.1 Preparation of extracts

Dried and milled plant materials were extracted sequentially with water and were allowed to remain in contact with the plant material for 48 hours. After filtration of total extracts, the extracts were evaporated to dryness in vacuo. 1 gram of each plant extract was weighed and

dissolved in 1ml of sterile distilled water. Sterile disks of 6 mm diameter were soaked in the extract water mixture for one hour. Disks were also soaked in water containing 1 g of alum and 1ml of 1.2 % sodium hypochlorite.

### 3.9.2 Sources of test bacteria

Environmental isolates from most polluted water sources in Wamba were used. The isolates used were *E. coli, Salmonella* spp, and *Shigella* spp. as identified based on the IMViC reactions, cultural characteristics and gram stain reactions. The reference strains used for the screening were *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). These bacteria were obtained from stock cultures from Department of Public Health and Pharmacology, Faculty of Veterinary Medicine, University of Nairobi.

### 3.9.3 Antibacterial sensitivity testing

Three to five identical colonies from each agar plate were transferred with a sterile wire loop into a tube containing 5 ml of nutrient broth. The turbidity of each bacterial suspension was adjusted to give a turbidity value similar to that of a 0.5 McFarland standard, resulting in a suspension containing approximately 1 to 2 x $10^8$ CFU/ml. Mueller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface. After allowing the inoculum to dry at room temperature, 6 mm diameter disks each with fixed concentration of the extract was tested for antibacterial activity by introducing duplicate disks per plate. Streptomycin (10 μg $ml^{-1}$) was used as a positive control.

The plates were allowed to stand at room temperature for 1 hour and then incubated at 37 °C for 18 hours. Subsequently, the plates were examined for bacterial growth inhibition and the inhibition zone diameter (IZD) measured to the nearest millimetre using a transparent ruler. A standard table of antibiotic susceptibilities (Performance Standards for Antimicrobial

Susceptibility Testing (2007).17th informational Supplement.M100-517 volume 27, no 1 available at www.microbiolab-bg.com/cls) was used to determine whether the strain was resistant (R), intermediate (I) or susceptible (S) to the specific treatment tested (Table 2).

**Table 2. Showing inhibition zone diameter interpretive standards**

| | | | Zone diameter (mm) | | |
|---|---|---|---|---|---|
| Reference bacteria | Antimicrobial agent | Disk content | Resistant (R) | Intermediate (I) | Sensitive (S) |
| *E.coli* ATTC 25922 | Streptomycin | 10µg | ≤ 11 | 12-14 | ≥ 15 |

### 3.9.4 Data analysis

Both descriptive and inferential statistics were used to analyse and report the data. A one way ANOVA was used to compare the physico-chemical parameters and HPC bacterial loads from different water sampling sites and the efficacy of treatments in reducing sediment and HPC bacterial load in water. Mean separation was done using Tukey technique.

# CHAPTER FOUR: RESULTS

## 4.1 Introduction

Water quality determination involved the measurements of among others pH, conductivity, dissolved oxygen, temperature, alkalinity, salinity, turbidity, nutrient levels and the load of various waterborne bacteria. These properties are fundamental for evaluating water quality.

## 4.2 Physico-chemical properties of water

### 4.2.1 Temperature

Water temperatures varied widely among the study sites with a range of 18.2 to 35.6 °C. The water sources investigated recorded the following temperature (°C) ranges: springs, 18.2 to29.4; wells, 18.2 to 32.3; dams, 20.8 to 35.6; rivers, 23.2 to 30.8; boreholes, 25.5 to 29.8; tap, 29 to 31.2 (Table 3). The lowest mean temperature (22.8 °C) was recorded in spring water while highest mean temperature (30.5 °C) was recorded in tap water (Table 3). Using the one way ANOVA test, it was established that there was a significant difference in water temperature of the different sources (P =0.05, DF = 5).

### 4.2.2 pH

The median pH of water at the various sources ranged from 6.1 to 7.9. The lowest (5.68) and the highest (10.46) pH values were measured in a shallow well and a dam respectively. Dams recorded the highest median value of 7.9 with the springs and tap water having a median pH value of 7.1 and 6.1 respectively (Table 3).

### 4.2.3 Electrical conductivity (µS $cm^{-1}$)

Electrical conductivity (EC) of water at various sources ranged from 3.0 (tap) to 5940 (well) µS $cm^{-1}$. The lowest mean EC (11.17 µS $cm^{-1}$) and the highest (830.83 µS $cm^{-1}$) were measured in tap and borehole sources respectively. Using a one way ANOVA test, it was

noted that there was no significant difference in mean EC of water from the different sources (P =0.05, DF= 5) (Table 3).

**Table 3 Physico-chemical properties of water from the various types of sources in Wamba Division over the period 26/2/06 to 11/3/07**

| | SPRINGS | | WELLS | | DAMS | | RIVERS | | BOREHOLES | | TAPS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Property | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean |
| Turbidity (NTU) | 12-188 | 58.4 | 75-720 | 119.6 | 17-6100 | 1192 | 20-3300 | 620.8 | 5-156 | 35.5 | 12-20 | 15.8 |
| Temp. (°C) | 18.2-29.4 | 22.8 | 18.2-32.3 | 27.3 | 1 | 20.8-35.6 | 28.3 | 23.2-30.8 | 28.1 | 25.5-29.8 | 27.5 | 29-31.2 | 30.5 |
| EC (µS $cm^{-1}$) | 22-2610 | 327.2 | 4.6-5940 | 397.8 | 4.6-930 | 366.7 | 4.4-404 | 234.4 | 20-2540 | 830.8 | 3-245 | 11.2 |
| DO (mg $L^{-1}$) | 1.5-11.9 | 5.8 | 0.8-8.4 | 3.2 | 1.8-15 | 5.7 | 1.9-10.5 | 7.3 | 0.8-9.6 | 3.8 | 4.5-5.6 | 5.6 |
| TA (mg $L^{-1}$ $CaCO_3$) | 33-890 | 159 | 600-115 | 262 | 20-522 | 257 | 63-190 | 137 | 73-1577 | 568 | 70-90 | 81 |
| TP (µg $L^{-1}$) | 2.0-418.7 | 44.5 | 5 | 6.0-263.3 | 104.3 | 2.9-454.0 | 126.0 | 23.1-125.0 | 68.6 | 20.0-774.3 | 318.0 | 27.6-135.0 | 131.0 |
| $PO_4$-P (µg $L^{-1}$) | 3.6-200.7 | 22.7 | 16-126.0 | 55.8 | 6 | 0.8-120.4 | 57.1 | 10.5-59.5 | 32.4 | 59.1-371.7 | 223.7 | 0-12.68 | |
| Salinity (ppt) | 0-1.2 | 0.12 | 0-1.2 | 0.29 | 0-0.2 | 0.48 | 0-0 | 0 | 0-1.2 | 0.78 | 0 | 0 |
| pH (median) | 6.5-8.9 | 7.2 | 5.7-9.3 | 7.59 | 6.2-10.5 | 7.9 | 6.2-7.8 | 7.8 | 6.7-9.0 | 7.6 | 6.0-7.6 | 7.3 |

### 4.2.4 Dissolved oxygen (DO mg $L^{-1}$)

Dissolved oxygen values in water from the different sources ranged from 0.8 to 15 mg $L^{-1}$. The lowest mean DO values were recorded in wells (3.2 mg $L^{-1}$) while springs and rivers had the highest mean DO values of 5.8 mg $L^{-1}$ and 7.3 mg $L^{-1}$respectively (Table 3). Using a one way ANOVA test, it was observed that the difference in DO among the waters sources investigated was not significant (P = 0.413, DF = 5).

### 4.2.5 Salinity

Sample water salinity ranged from below the limit of detection to 1.2 ppt in springs, wells and boreholes, while in river and tap water salinity was consistently below the detection limit by the instrument used (Table 3). Borehole water had the highest mean salinity (0.78 ppt). Using a one way ANOVA test, it was observed that there was a significant difference (P = 0.001, DF = 4) in salinity of water from the different sources. Mean separation using Tukeys technique revealed that borehole water had the highest salinity compared to all other water sources.

### 4.2.6 Turbidity (NTU)

Sample water turbidity ranged from 5 NTU (boreholes) to 6100 (dams). Dams and rivers recorded the highest mean turbidity of 1192.02 NTU and 393.92 NTU respectively (Table 3). Using a one way ANOVA test, it was observed that, there was a significant difference in mean water turbidity (P = 0.018, DF = 5). Mean separation using Tukeys technique revealed that dam water had the highest turbidity compared to water from the other sources.

### 4.2.7 Total alkalinity (TA, mg $L^{-1}$ $CaCO_3$)

Total alkalinity values of water from various sources ranged from 20 (rivers) to 1577.5 (boreholes) mg $L^{-1}$ $CaCO_3$. The lowest mean TA (81.25 mg $L^{-1}$ $CaCO_3$) and the highest mean TA (567.66 mg $L^{-1}$ $CaCO_3$) values were measured in tap water and borehole water respectively (Fig. 10). Using a one way ANOVA test, it was observed that there was a

significant difference in mean total alkalinity of the water sources investigated (P = 0.01, DF = 5). Mean separation using Tukeys technique revealed that borehole water had a significantly higher TA compared to that of other sources.

### 4.2.8 Total phosphorus (TP, µg $L^{-1}$) and orthophosphate phosphorus ($PO_4$-P, µg $L^{-1}$)

Sample water total phosphorus and orthophosphate phosphorus ranged from 2.0 (springs) to 774.3 (boreholes) µg $L^{-1}$ and from below the detection limit (tap) to 371.7 (borehole) µg $L^{-1}$ respectively (Table 3). Using a one way ANOVA test, it was observed that there was a significant difference in total phosphorus (P = 0.004, DF = 5) and orthophosphate phosphorus (P = 0.001, DF = 4) of water from the various categories of water bodies investigated. Mean separation using Tukeys technique revealed that boreholes had significantly higher total phosphorus levels compared to other water sources.

## 4.3 Bacterial properties of water sources

### 4.3.1 Heterotrophic plate counts (HPC, CFU $mL^{-1}$)

Heterotrophic bacterial plate counts recorded in water from different sources varied widely with a range from 22 (in a river water sample) to 2 x $10^7$ colony forming units (CFU) $mL^{-1}$ (in a dam water sample). Using a one way ANOVA test, it was found that the different categories of water differed significantly in their mean HPC, CFUs (P = 0.01, DF= 5). Mean separation using Tukey's technique revealed that dams, boreholes and rivers had a significantly higher mean HPC, CFUs of 2.75 x $10^6$ compared to tap water (Table 4).

**Table 4 Heterotrophic plate counts (HPC) in water from various sources in Samburu**

| | HPC CFU $mL^{-1}$ | |
|---|---|---|
| Type of water sources | Mean | Range |
| 1. Dams | $2.75 \times 10^6$ a | $2.2 \times 10^3$ - $2.0 \times 10^7$ |
| 2. Boreholes | $2.04 \times 10^6$ a | $1.0 \times 10^2$ - $1.38 \times 10^7$ |
| 3. Rivers | $1.44 \times 10^6$ a | $2.15 \times 10^1$ - $1.56 \times 10^7$ |
| 4. Wells | $6.42 \times 10^5$ ab | $1.0 \times 10^2$ - $3.6 \times 10^6$ |
| 5. Springs | $6.31 \times 10^5$ ab | $6.0 \times 10^2$ - $1.2 \times 10^6$ |
| 6. Tap | $2.82 \times 10^4$ b | $3.0 \times 10^3$ - $1.35 \times 10^5$ |

**NB**: Means indicated by the same letters are not significantly different at P = 0.01

### 4.3.2 Total coliforms MPN/100 mL

Total coliform counts (MPN/ 100 mL) in water from various sources varied widely, ranging from 2 (wells and boreholes) to 1600 (wells, rivers, dams, springs and boreholes) (Table 5). The water in each source category exhibited a wide variation in total coliforms (Table 5). Mean MPN of water in each source category ranged from 217 (in boreholes) to 1310 MPN/ 100 mL (in wells). Using a one way ANOVA test, mean total coliform counts were noted to differ significantly among the categories of water sources investigated ($P < 0.01$, DF= 5). Mean separation using Tukey's technique revealed that wells and rivers had significantly higher mean total coliform counts as compared to tap and borehole water (Table 5).

**Table 5 Total coliforms counts (MPN/100 mL) in water from various sources in Samburu**

| | Total coliforms MPN/100 Ml | |
|---|---|---|
| Type of water source | Mean | Range |
| Wells | $1.31 \times 10^3$ a | $2 – 1.6 \times 10^3$ |
| Rivers | $1.29 \times 10^3$ a | $40 – 1.6 \times 10^3$ |
| Dams | $9.63 \times 10^2$ ab | $20 – 1.6 \times 10^3$ |
| Springs | $7.14 \times 10^2$ abc | $9 – 1.6 \times 10^3$ |
| Tap | $3.73 \times 10^2$ bc | $14 – 9.0 \times 10^2$ |
| Boreholes | $2.17 \times 10^2$ c | $2 – 1.6 \times 10^3$ |

**NB**: Means indicated by the same letters are not significantly different at $P < 0.01$

### 4.3.3 Faecal coliforms

The study confirmed the presence of faecal coliforms in most water samples tested. Faecal coliform counts in water from all sources ranged from 1 to 1600 MPN/ 100 mL. Mean faecal coliform counts in water from various sources varied widely. Using a one way ANOVA test, it was found that faecal coliform bacterial load were significantly different in water from various sources ($DF = 5$, $P < 0.01$). Mean separation using Tukey's technique revealed that water from wells had significantly higher mean faecal coliforms counts than water from other sources while boreholes had significantly lower mean faecal coliform counts than the other sources (Table 6).

**Table 6 Faecal coliforms counts (MPN/ 100 mL) in water from various sources in Samburu**

| | Faecal coliforms MPN/100 mL | |
|---|---|---|
| Type of water source | Mean | Range |
| 1. Wells | $4.71 \times 10^2$ a | 1 – 1600 |
| 2. Dams | $3.92 \times 10^2$ b | 2 – 1600 |
| 3. Rivers | $2.72 \times 10^2$ b | 2 – 1600 |
| 4. Springs | $1.55 \times 10^2$ b | 2 – 1600 |
| 5. Boreholes | $2.3 \times 10^1$ c | 2 – 188 |
| 6. Tap | 6d | 2 – 70 |

**NB**: Means indicated by the same letters are not significantly different at $P < 0.01$

### 4.3.4 Occurrence of *Vibrio cholerae*, *Salmonella* and *Shigella* species

*Salmonella* spp. was more frequent in water samples from springs (86 %) and least frequent in boreholes (17 %). Two tap water samples collected during the study were both positive for the presence of *Salmonella* spp. Tests for the presence of *Shigella* spp. revealed that this bacterium was present in all spring samples (100 %) and most river samples (75 %). Presence of *Shigella* spp was lowest in borehole water samples (33 %) (Table 7). All the samples tested were negative for the presence of *Vibrio cholera* during study period.

**Table 7 Frequency of occurrence (%) of *Salmonella* and *Shigella* spp in water from different sources.**

| Category | Tap | Springs | River | Dams | Wells | Boreholes | Overall |
|---|---|---|---|---|---|---|---|
| *Salmonella* spp. | 100 % | 86 % | 75 % | 70 % | 47 % | 17 % | 59 % |
| *Shigella* spp. | 50 % | 100 % | 75 % | 90 % | 65 % | 33 % | 72 % |
| Total no of sources | 2 | 7 | 4 | 10 | 17 | 6 | 46 |

## 4.4 Risk levels

Based on the number of faecal coliforms obtained the water sources studied were categorized into different categories ranging from conformity to drinking water standards to a very high risk (Table 1). The categories chosen represented a progressive increase in the risk of infection by pathogenic bacteria following the consumption of water from these sources. The majority of the water sources (43 %) posed an intermediate risk to the consumers of its water. None of the water sources were in conformity with WHO drinking water standards, while 35 % posed a high risk. Different water sources are presented using their sample codes (Tables 8 and 9).

**Table 8 Bacterial risk level classification for specific water sources**

| Risk type | Water source codes | | | | | | % of sources |
|---|---|---|---|---|---|---|---|
| Conformity | | | | | | | 0% |
| Low risk | Nka.5A | Ngi.7A | Ngu.7A | Nam.8B | Ngu.6C | Nam.7B | 13.04% |
| Intermediate | Nka.1A<br>Nam.1B<br>Bar.5A<br>Nka.1B | Bar.1A<br>Nka.1C<br>Bar. 6C | Nam.5A<br>Nam.5B<br>Nam.5E | Nka.6A<br>Nam.6F<br>Nam.8A | Nam.7A<br>Nka.7B<br>Nka.7A | Nam.1D<br>Nam.6E<br>Nam.3A | 43.48% |
| High risk | Nam.1A<br>Nka.1D<br>Bar.6D | Nam.6B<br>Nam.5C<br>Nam.6D | Ngu.3A<br>Nka.6B<br>Nam.5D | Ngu.1A<br>Nam.6C<br>Bar.6B | Bar.6A<br>Nka.6C | Ngu.6A<br>Nam.6A | 34.78% |
| Very high risk | Bar.3A | Bar.3B | Nam.1C | | | | 8.70% |

**KEY**

| Waterbody name | Code | Waterbody name | Code |
|---|---|---|---|
| Lodungokwe dam | BAR.1A | Lodungokwe Nantasim/Sesia well | BAR.6C |
| Mukur Omuny river | BAR.3A | Lodungokwe Seiya well | BAR.6D |
| Ewaso Nyiro river (bridge) | BAR.3B | Dalambo dam | NAM.1A |
| Mukur Omuny spring | BAR.5A | Lcharo nyiro Sarara dam | NAM.1B |
| Ladasaoo well | BAR.6A | Loidikidiko dam | NAM.1C |
| Mukur Omuny sessia well | BAR.6B | Sere Wamba dam | NAM.1D |
| Ngong'o river | NAM.3A | Ngilai borehole | NGI.7A |
| Margwe upstream. Spring | NAM.5A | Naisunyai dam | NGU.1A |
| Margwe spring (lower) | NAM.5B | Ewaso nyiro river | NGU.3A |
| Sere Wamba - upstream | NAM.5C | Lengusaka laga well | NGU.6A |
| Sere Wamba - downstream | NAM.5D | Lengusaka well | NGU.6B |
| Lkanto spring | NAM.5E | Noolosilale well | NGU.6C |
| Margwe well | NAM.6A | Ngutuk borehole | NGU.7A |
| Ngong`o well | NAM.6B | Loturu(Ndikir) dam | NKA.1A |
| Njorong'iro well | NAM.6C | Nagorworu dam | NKA.1B |
| Nompitiro well - Sarara | NAM.6D | Nguass dam | NKA.1C |
| Sere Wamba well | NAM.6E | Lesiteti dam | NKA.1D |
| Sionta well | NAM.6F | Nagoruworu laga billabong/spring | NKA.5A |
| Mjiriman borehole | NAM.7A | Lkisin well | NKA.6A |
| Tingatinga borehole | NAM.7B | Nagoruworu well | NKA.6B |
| Sarara camp tap 1 | NAM.8A | Nagoruworu well | NKA.6C |
| Sarara camp tap2 | NAM.8B | Nkaroni borehole | NKA.7A |
| Ngilai well | NGI.6A | Nagoroworu borehole | NKA.7B |

**Table 9 Levels of risk in water from various sources**

| Risk Level | Risk type | Dams (N= 10) | Wells (N= 17) | Springs (N= 7) | Rivers (N=4) | Boreholes (N= 6) | Tap (N= 2) |
|---|---|---|---|---|---|---|---|
| A | Conformity | 0 | 0 | 0 | 0 | 0 | 0 |
| B | Low risk | 0 | 5.88 % | 14.29 % | 0 | 50 % | 50 % |
| C | Intermediate risk | 60 % | 29.41 % | 57.14 % | 25 % | 50 % | 50 % |
| D | High risk | 30 % | 58.82 % | 28.57 % | 25 % | 0 | 0 |
| E | Very high risk | 10 % | 5.88 % | 0 | 50 % | 0 | 0 |

The results of the risk analysis showed that dams, rivers and shallow wells pose a very high risk compared to boreholes and springs. Based on the presence or absence of pathogens, it

was observed that, 23.91 % of the water sources contained no pathogens, while 21.74 % and 54.35 % contained 1or 2 types of pathogens respectively, that is *Salmonella* or *Shigella* spp. None of the water samples analyzed contained *Vibrio cholera*.

## 4.5 Water purification experiments

### 4.5.1 Change in heterotrophic bacterial counts

The efficacy of *Boscia coriacea* Pax., *Maerua decumbens* (Brogn.) Dewolf roots and *Moringa oleifera* Lam. seeds extracts in reducing heterotrophic bacterial load in water were determined by considering the percentage change in HPC after treatment of water with the extracts at various time intervals. Overall mean percentage reduction in HPC in extracts of *M. oleifera* Lam., *M. decumbens* (Brogn.) Dewolf, and *B. coriacea* Pax. were 26.51, 46.00, and 30.20 respectively compared to percentage reduction of 14.5, 74.8 and 91 percent for the control, alum and sodium hypochlorite (Table 10). A one way ANOVA test revealed a significant difference in HPC reduction among treatments ($DF = 5$, $P < 0.05$). Mean separation using Tukey's test revealed that HPC reduction by sodium hypochlorite and alum was significantly higher than that of the other treatments. Although the HPC reduction by plant extracts was not significantly different, only *M. decumbens* (Brogn.) Dewolf posted a HPC reduction that was significantly different from that of the control (Table 10).

**Change in HPC after 30 minutes.**

The percentage change in number of HPC obtained after 30 min of water treatment varied between 35.88 % - 83.22 %. A one way ANOVA test revealed a significant difference in percentage reduction of HPC among treatments ($DF = 5$, $P = 0.05$). Mean separation using Tukey's test revealed that HPC reduction by sodium hypochlorite, alum, *M. decumbens* (Brogn.) Dewolf and *B. coriacea* Pax. were significantly higher than that of the control (Table 10). Among the plant extracts, HPC reduction by *M. decumbens* (Brogn.) Dewolf was significantly higher than that of *M. oleifera* Lam. (Table 10).

**Table 10 Mean percentage reduction in HPC of water samples from various sources after treatment with plant extracts, alum and sodium hypochlorite**

| Treatment used | Mean % change in HPC | | |
|---|---|---|---|
| | Overall | After 30 min | After 24 hrs |
| 1. *M .oleifera* Lam. | 26.51 bc | 35.88 b | 17.15 b |
| 2. *M. decumbens* (Brogn.) Dewolf | 46.00 b | 68.88 a | 23.12 b |
| 3. *B. coriacea* Pax. | 30.20 bc | 53.92 ab | 18.49 b |
| 4. Alum | 74.76 a | 67.20 ab | 82.32 a |
| 5. 1.2 % sodium hypochlorite | 90.95 a | 83.22 a | 98.91 a |
| 6. Control | 14.50 c | 5.91 c | 24.62 b |

Nb: Mean denoted by the same letters are not significantly different at $P < 0.05$

**Change in HPC after 24 hours**

The percentage change in number of HPC obtained after 24 hours of water treatment varied between 17.15 % - 98.91 % (Figure 5). A one way ANOVA test, revealed a significant difference (DF = 5, P = 0.05) between effects of plant extracts and those of alum and sodium hypochlorite (Table 10). Mean separation using Tukey's technique revealed that sodium hypochlorite (98.91 %) was the most effective treatment in reducing the number of HPC followed by alum while *B. coriacea* Pax. (18.49 %) and *M. oleifera* Lam. (17.15 %) were the least respectively (Table 10).

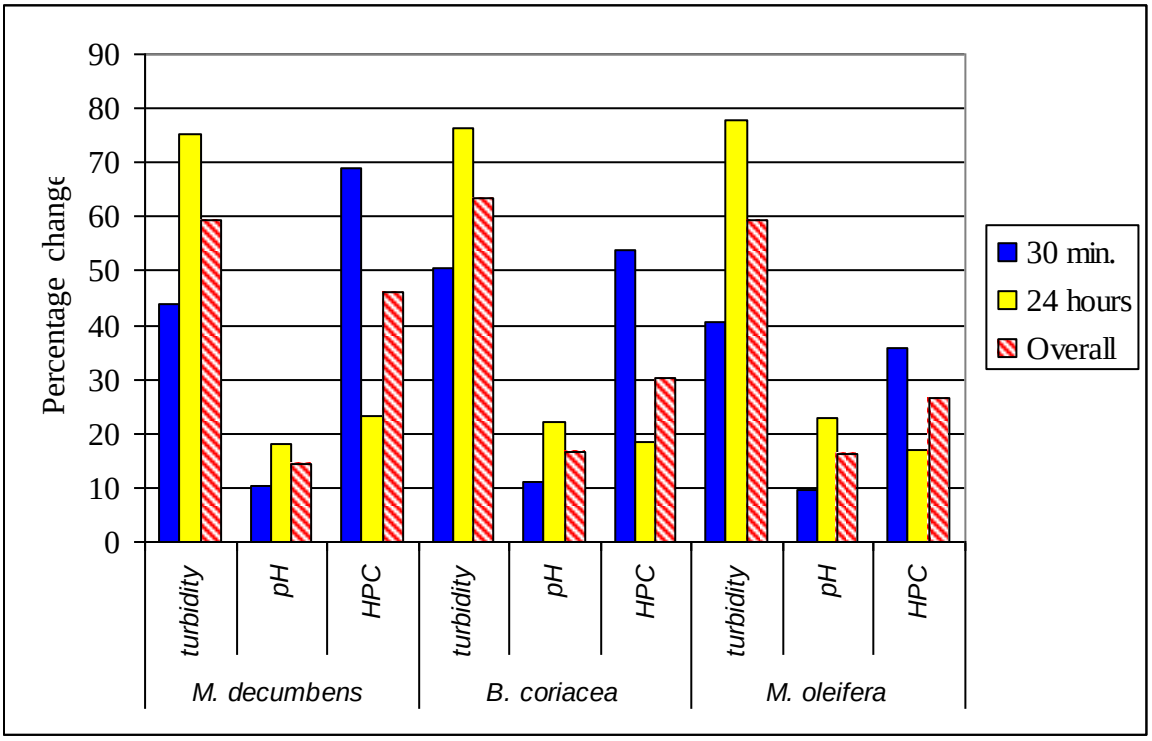


**Figure 5 Percentage changes in water pH, turbidity and HPC of water samples treated with extracts of the three plant species**

### 4.5.2 Change in water turbidity

The efficacy of *Boscia coriacea* Pax., *Maerua decumbens* (Brogn.) Dewolf roots and *Moringa oleifera* Lam. seeds extracts in reducing sediment load in water were determined by considering the percentage change in turbidity after treatment of water samples with the extracts at various time intervals. Turbidity reduction after treatment with extracts from the three plant species ranged from 53.94 to 94.45 NTU (Table 11). Using a one way ANOVA test, it was found that there was a significant difference in mean percentage reduction in water turbidity among the treatments used (DF = 5, P = 0.05), but there was no significant difference in the effect of *M. oleifera* Lam., *M. decumbens* (Brogn.) Dewolf and *B. coriacea* Pax. on change in water turbidity (Table 11, Figure 5). Mean separation using Tukey's technique revealed that alum and the three plant extracts had the highest percentage reduction of water turbidity that was significantly greater from that of the control. Overall, alum was the

most effective treatment for reducing sediment load followed by plant extracts, while sodium hypochlorite was the least effective.

**Table 11 Percentage reduction in water turbidity after treatment of water samples with extracts from the three test species, alum and sodium hypochlorite**

| Treatment used | % Change in turbidity | | |
|---|---|---|---|
| | Overall | 30 Min | 24 Hrs |
| 1. *M. oleifera* | 59.22 ab | 40.53 a | 77.91 ab |
| 2. *M. decumbens* | 59.52 ab | 43.87 a | 75.17 ab |
| 3. *B. coriacea* | 63.44 ab | 50.36 a | 76.53 ab |
| 4. Alum | 67.96 a | 41.47 a | 94.45 a |
| 5. 1.2 % sodium hypochlorite | 32.88 bc | 17.81 b | 53.94 b |
| 6. Control | 28.56 c | 6.16 c | 50.97 b |

NB: Means denoted by the same letters are not significantly different $P < 0.05$.

**Effects of the plant extracts after 30 minutes on water turbidity**

The percentage change in turbidity recorded after 30 minutes of water treatment, ranged from 17.81% (control) - 50.36 % (*B. coriacea* Pax.) (Table 11). Using a one way ANOVA test, it was found that there was a significant difference in the percentage reduction of turbidity among treatments ($DF = 5$, $P < 0.05$). Mean separation using Tukey's technique revealed that alum and the plant extracts gave the highest percent reduction of turbidity that was significantly greater than that of sodium hypochlorite and the control (Table 11).

**Effects of the plant extracts after 24 hours on water turbidity**

The percentage change in turbidity recorded after 24 hours of water treatment, ranged from 53.94 % - 94.45 %. Using a one way ANOVA test, it was found that there was a significant difference in the percentage reduction of turbidity among treatments ($DF = 5$, $P = 0.05$). Mean separation using Tukey's technique revealed that alum and the plant extracts gave the highest

percent reduction of turbidity. Turbidity reduction by alum was significantly greater than that of sodium hypochlorite and the control (Table 11).

### 4.5.3 Changes in water pH

The various treatments brought about changes in water pH. Treatment with plant extracts (from *M. oleifera* Lam., *M. decumbens* (Brogn.) Dewolf and *B. coriacea* Pax.) and alum reduced water sample pH (became more acidic) while 1.2 % sodium hypochlorite increased water sample pH (became more alkaline). Among the treatments, extracts from *Moringa oleifera* Lam. recorded the lowest median pH (6.0), followed by extracts from *Boscia coriacea* Pax. (6.3), *Maerua decumbens* (Brogn.) Dewolf, (6.37) and alum (6.4) after 30 minute period of water treatment.

However, after a 24 hour period, exctracts of *Moringa oleifera* Lam. and *Boscia coriacea* Pax. recorded similar pH (5.49) while alum recorded the lowest median pH (5.46). The median pH following treatment with 1.2 % sodium hypochlorite after 30 minutes and 24 hours was 7.44 and 7.39 respectively (Table 12).

**Table 12 Changes in water sample pH obtained following treatment of water samples with the three plant species investigated as well as alum and sodium hypochlorite over 24 hour period of treatment.**

| Treatment used | *Moringa oleifera* | *Maerua decumbens* | *Boscia coriacea* | Alum | NaOCl | Control (untreated water) |
|---|---|---|---|---|---|---|
| Median pH before treatment | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 | 7.9 |
| Median pH after 30 minutes of treatment | 6 | 6.37 | 6.33 | 6.4 | 7.44 | 7.16 |
| Median pH after 24 hours of treatment | 5.49 | 5.77 | 5.49 | 5.46 | 7.39 | 7.13 |

### 4.6 Bacterial inhibition effects of plant extracts

The antibacterial activity of plant water extracts was determined using the disk diffusion (Kirby-Bauer) method (Kirby *et al.,* 1966) and the percentage inhibition recorded in milimeters. *E. coli* (ATCC 25922), *Staphylococcus aureus* (ATTC 25923) and Streptomycin drug were used as positive controls in the experiment. Preliminary results showed that all test bacteria strains were sensitive to the treatments used. The mean zone of inhibition recorded for all treatments ranged between 7.57 mm to 17.92 mm. This study found that *S. aureus* (ATCC 25923) was more sensitive to extracts from *M. oleifera* Lam. (24.6 mm) compared to other treatments (Table 13). Using a one way ANOVA test, it was found that bacterial zones of inhibitions were significantly different among all treatments (DF = 5, P = 0.05). Mean separation using Tukey's technique revealed that treatment with streptomycin achieved a significantly greater inhibition than all other treatments followed by treatment with extracts from *M. oleifera*, which gave a significantly greater inhibition than the remaining treatments.

**Table 13 Mean zones of inhibition (mm) of selected test bacterial strains by treatment with plant extracts, alum, sodium hypochlorite and Streptomycin**

| Test bacterial strains | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | *M. oleifera* | *M. decumbens* | *B. coriacea* | 1.2% NaOCl | Alum | Streptomycin |
| *S. aureus* (ATCC 25923) | 24.6 (S) | 17.7 (S) | 9.25 (R) | 10.17 (R) | 7.67 (R) | 19.33 (S) |
| *E. coli* (ATCC 25922) | 9.60 (I) | 7.67 (I) | 8.75 (I) | 14.17 (I) | 8.00 (I) | 19.67 (S) |
| *E. coli* 6 | 10.20 (I) | 10.83 (I) | 10.71 (I) | 11.20 (I) | 6.20 (I) | 17.00 (S) |
| *E. coli* 3 | 10.40 (I) | 8.83 (I) | 15.60 (S) | 10.25 (I) | 7.50 (I) | 17.67 (S) |
| *E. coli* 5 | 10.80 (I) | 9.83 (I) | 6.00 (I) | 10.67 (I) | 8.17 (I) | 12.00 (R) |
| *Shigella* spp 6 | 16.80 (S) | 9.14 (I) | 14.57 (S) | 11.00 (I) | 7.40 (I) | 15.33 (S) |
| *Shigella* spp 9 | 14.00 (R) | 10.17 (I) | 7.20 (I) | 17.40 (S) | 7.25 (I) | 21.33 (S) |
| *Salmonella* spp 7 | 11.75 (R) | 10.57 (I) | 7.25 (I) | 10.00 (I) | 8.00 (I) | 21.00 (S) |
| *Salmonella* spp 8 | 11.80 (R) | 9.14 (I) | 6.00 (I) | 9.40 (I) | 7.75 (I) | |

**Key: (R) Resistant, (I) Intermediate, (S) Susceptible**

# CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Physico-chemical quality of water

Among the physico-chemical properties of water from various sources investigated, pH, dissolved oxygen, total alkalinity and conductivity values met the requirements for drinking water as per the guidelines of the World Health Organization (WHO, 2004). However, turbidity in most dams, rivers, and some wells exceeded the maximum permissible concentration (MPC) according to WHO (2004) on most occasions.

In general, mean water temperatures in all water sources ranged from 22.8 to 30.5 °C. Water temperature variation at the various water sources can be attributed to differences in weather conditions at the time of sampling, the amount of water present in the source and presence of vegetation shielding water source from direct insolation. During this study it was observed that compared to other types of water sources, most springs were mainly located in high altitude areas where they are on most occasions sheltered by trees. Such an environment is likely to experience cool air temperature which influences water temperature. The dams, rivers and the shallow wells however were located in lowlands exposed to direct insolation. Of all the water sources, tap water recorded the highest mean (30.5 °C). This can be attributed to the fact that the metallic pipes carrying the water at some points in bare rocky areas, were exposed to direct sunlight. This enabled the water to absorb the heat conducted by the metallic pipes.

Mean turbidity was highest in dams (1192 NTU) and lowest in boreholes (35.5 NTU). High turbidity in dams may be due to deposition of soil and organic matter by surface runoff water coupled with wind erosion during wet and dry seasons respectively. However, animals too may excrete substantial amount of faecal matter into water directly especially when watering in dams. Hence non point pollutants mainly affect surface waters, which are exposed

compared to water in boreholes, which are protected and therefore usually have a comparatively lower turbidity (35.5 NTU). According to WHO (2004) it is recommended that water with a turbidity level of more than 5 NTU, should undergo some form of treatment to remove turbidity before the water can effectively be disinfected with chlorine. This therefore means that most water sources in Samburu require some initial treatment to remove sediments if chlorine disinfection is to be employed.

The electrical conductivity is acknowledged as a quick and reliable measure to indicate the general chemical quality of water (Hem, 1982; Auer, 1997). The conductivity values obtained in this study therefore indicate that some water sources have a high concentration of dissolved solids (minerals). High conductivity values recorded in boreholes can be attributed to the longer period of contact between the water and the parent rock material whose dissolution releases mineral ions into the water. The dissolution and infiltration of faecal matter into sandy aquifer and lateral movement of groundwater along the sandy river bed account for the high levels of contamination in wells. However the relatively high conductivity and salinity levels of water observed at some study sites are not likely to affect water consumption by cattle and sheep and wildlife and their health. Previous studies (Weeth and Haverland, 1961; Wilson, 1966; Potter and McIntosh, 1974) have demonstrated that conductivity above 15,150 $\mu S\ cm^{-1}$, lead to reduced water intake and growth rate of cattle while salinities above 26,620 $\mu S\ cm^{-1}$cause health disorders and subsequent death.

The low values of dissolved oxygen recorded in springs and boreholes are likely to be due to the fact that they were sheltered from direct wind action by vegetation and concrete cover respectively. A high mineral content in borehole water (evidenced by the higher salinity) could have further reduced the solubility of oxygen in water.

The median pH recorded in different water sources, were within the WHO recomended range for drinking water. Although water sources that recorded a pH of around 9 and above are likely to have reduced levels of faecal bacteria, such high pH values are above the recommended range for drinking water. Acidic conditions are known to be favourable for the survival of *E. coli* both in fresh and saline waters (Rozen and Belkin, 2001).

Mean total alkalinity was highest in boreholes (757.2) and lowest in rivers (136.8) and tap water (131). The high level of alkalinity and electrical conductivity in boreholes suggests a possible dominance of bicarbonate, and carbonate ions in water from these sources. Such highly mineralized, alkaline hard water have an objectionable "soda" taste and hence not very suitable for drinking. Additionaly, use of mineralized hard water to wash clothes increases the cost as it does not easily lather with soap. It also causes excessive drying of the skin (remove natural skin oils) when used for bathing.

Boreholes had the most saline water (mean 0.78 ppt) while rivers had the least (less than 0.1 ppt). These results suggest the existence of a higher load of dissolved salts in deeper water sources (boreholes) as compared to other shallow ones. The salinity difference in between surface and bottom water can be attributed to variation in the chemistry of rocks and soils at different depths, which in turn influences the concentration of introduced cations and anions such as $Na^{+}$, $K^{+}$, $Mg^{2+}$, $Fe^{2+}$, $NO_3^{-}$, $CO_3^{2-}$, $SO_4^{2-}$, $Cl^{-}$ etc. (Fasunwon *et al*., 2010).

During this study it was noted that most phosphorus entering water sources originates from non-point sources. Possible non point sources of phosphorus include the natural decomposition of rocks and minerals, storm water runoff, erosion and sedimentation, and direct input by animals/wildlife. The presence of higher loads of phosphorus and low levels of dissolved oxygen in boreholes compared to wells confirms the generalization that phosphorus is often scarce in the well oxygenated waters (Ricklefs and Schluter 1993). This is due to the

fact that as aerobic bacteria decompose organic wastes, they consume oxygen, and in return release more phosphorus into the water.

Total coliform in all types of water sources exceeded WHO (2004) maximum permissible load (0/100 mL) for drinking water. Although total coliform organisms may not always be directly related to the presence of faecal contamination or pathogens in the drinking water, this study found that all water samples contained both total coliform and faecal coliform. Hence the total coliform test would still be useful in monitoring the microbial quality of the raw and treated piped water supplies in the area. During the study it was noted that total and faecal coliforms counts in wells differed significantly from those in springs with the wells recording higher total and faecal coliforms counts compared with the springs. These findings are in agreement with the previous observations (Feachem 1980; Lindskog and Lindskog 1988; Sandiford *et al*., 1989; Tensay, 1991; Utkilen and Sutton, 1989; White *et al.,* 1972; Wright, 1985), which suggested that a protected hand-dug well is usually one of the least contaminated with only spring water being usually cleaner. The higher total and faecal coliforms counts recorded in wells in Samburu can be attributed to the contamination caused by lateral movement of water along the sandy laggas where the majority of the wells are closely situated. Although some shallow wells were well protected from direct access by animals, the protection appears not to have been effective in reducing water contamination. This is perhaps due to the fact that most of the animals move along the laggas in search of water, salt licks and pasture. In the process, they deposit a lot of organic wastes on the lagga floor. When it rains, the seasonal floods wash off bacteria and organic water into the wells hence contaminating them.

A higher faecal coliform load in wells (mean 471.63) compared to that in other types of water sources suggests a recent contamination of the ground water with bacteria of faecal origin.

Wells in the study area are mostly shallow and located in dry river beds. Since most river beds are made of loose sand, they are easily filled up with sand. Although some community members enclose their wells to protect them from direct fecal contamination by livestock, the high population of livestock and wildlife that visit the river beds at different times exposes the wells to some contamination. Hence each morning, these wells have to be cleaned before drawing drinking or livestock water. These activities contaminate the groundwater sources and are likely to contribute to high levels of faecal coliforms. Although it has been reported that indicator and pathogenic bacteria are efficiently retained in soils and are detected at only low levels in groundwater under field conditions (Liu, 1982; Alhajjar *et al.,* 1988), other studies have found that heavy rainfall promotes the movement of bacteria and other inorganic contaminants through soil (Zyman and Sorber, 1988; Nikolaidis *et al.,* 1998). It is therefore clear that should the groundwater be qualified as drinking water, it must be fully treated and the wells must be protected from pollutants accordingly.

The risk analysis results of bacteriological water quality shows that consumption of untreated water from various sources poses a risk to the users (Table 8). Presence of thermotolerant *E. coli* in all water sources indicates possible presence of gastro-intestinal pathogens in water. This is likely since 21.74 % and 54.35 % of water sources contained 1or 2 types of pathogens respectively that is *Salmonella* spp or *Shigella* spp. These pathogens can infect both human beings and animals. Transmission of these pathogens occurs through faecal contamination of water in heavily used and unprotected water sources. Hence the consumption of water from dams, rivers and shallow wells pose very high risk compared to boreholes and springs (Table 7 and 8).

Water treatment using extracts from natural and renewable vegetation has been widely practiced and appears to be an effective and accepted physico-chemical treatment for

household water in some parts of the world (Jahn, 1988). Water treatment results obtained in this study revealed the capacity of extracts of *Boscia coriacea* Pax., *Maerua decumbens* (Brogn.) Dewolf roots and *Moringa oleifera* Lam. seeds to reduce heterotrophic bacterial load in water to some degree. Changes in bacterial density recorded during water treatment may have been due to loss of viability or alteration in culturability, persistence or aftergrowth of bacteria. Under treatment conditions it is probable that bacteria may experience metabolic stress, and as such bacterial cells may enter into a vulnerable but non culturable state (VBNC).

The ability of pathogenic micro-organisms to exist in VBNC state is well known (Islam *et al*., 1993). Rollins and Colwell (1986) have reported that the non culturable cells may remain viable for a prolonged period of time. Since non culturable cells may still remain metabolically active and if pathogenic, might maintain their infectiveness (Oliver, 1993), it is important to determine the viable state of non culturable cells. Rollins and Colwell (1986) have also reported that non-culturable cells may remain viable for a prolonged period of time. Some investigators have claimed that non culturable bacteria of selected species can be resuscitated to the culturable state (Roszak *et al.,* 1984). It is therefore important to use a highly selective and sensitive method to detect VBNC bacteria prior and after treatment of water using plant extracts. Such a method should be considered for use during bacteriological water quality testing.

The results of this study demonstrate that the plant extracts have some water soluble compounds which have disinfection properties, which lead to the reduction of HPC, especially within a 30 minute period. However, a decrease in the percentage reduction of bacterial density after a 24 hour period of water treatment suggests that this disinfection property is lost after sometime. The results also indicate that the treatments differ in their stability and ability

to persist in water to maintain a disinfectant residual. These results are in agreement with previous work (Eilert *et al*., 1981; Madsen *et al*., 1987), which showed that *Moringa oleifera* Lam. seed extracts flocculate bacteria and possess antimicrobial activity. According to Gassenschmidt *et al*., (1991), the agents responsible of the coagulation and flocculation in *Moringa oleifera* Lam. are water soluble proteins.

Among the plant extracts investigated, the greatest antimicrobial activity was due to *Moringa oleifera* Lam. (inhibition zone 8 to 13 mm). This suggests that the plant has some metabolic toxins or broad-spectrum antibiotic compounds. It is known that *Moringa oleifera* Lam. is rich in derivatives of benzyl isothiocyanates, a class of compounds with remarkable antimicrobial activity. It is apparent that *Boscia coriacea* Pax., and *Maerua decumbens* (Brogn.) Dewolf also have some active compounds. However, the nature of the active compounds present in the two plants remains unknown. Among the test bacteria used *Staphylococcus aureus* showed the highest sensitivity to all plant extracts compared to *E. coli, Salmonella* and *Shigella* species. This observation is in agreement with the findings of earlier studies on medicinal plants (McCutcheon *et al*., 1992) that showed that, medicinal plants were less active against gram-negative bacteria than to gram-positive bacteria. Failure of the plant extracts to completely eliminate the bacteria present suggests that some bacteria present in the water being treated may have spontaneously developed resistance to antimicrobial activity of plant extracts or alternatively that the bacteria may have escaped the bacteriocidic effect of the plant extracts, for instance, after degradation of the active ingredients. It is also possible that the concentration of active ingredients in the extracts were too low to fully inhibit bacterial growth. The results of the antimicrobial properties of the plant extracts investigated and changes over time suggests a need to determine the minimum bactericidal concentration (MIC) and the optimum time of water storage during treatment to avoid degradation of the microbial quality of water and biofilm accumulation due to bacterial regrowth.

Comparison of efficacy of plant extracts in reducing sediment and bacterial load in water demonstrates that the three species are almost similar in their performance and that the potential of *Boscia coriacea* Pax. and *Maerua decumbens* (Brogn.) Dewolf extracts needs to be further evaluated. Although alum, sodium hypochlorite and the three plant extracts significantly reduced the bacterial load of sample water within a 24 hour period, all cases had a residual bacterial population that remained in the treated water. The findings of this study are in close agreement with other studies that reported resistance of bacteria to chlorine (LeChevallier *et al*., 1988; Mathieu *et al*., 1992; Camper *et al*., 1997). These results suggest that there were variations in the kinetics of inactivation by the disinfectants, depending on the bacterial populations involved.

The interpretation of the results of heterotrophic bacteria counts obtained after water treatment may not be straightforward. This is because indicator bacteria are discrete in water, generally have a non-random distribution (Lightfoot *et al*., 1994) and are more likely to be found in clumps following treatment, rather than being uniformly spread out in the water (Gale *et al*., 1997). During this study, the spread plate method was used to culture the heterotrophic bacteria present in treated water. It is therefore possible that the bacterial cells from the clumps were dispersed leading to increased false colony forming units counts being obtained. These observations indicate that other cultural methods of determining residual bacterial colonies in treated water should be explored. Nevertheless, the results clearly demonstrates that no single water treatment method is highly effective in reducing water turbidity and bacterial loads to levels recomended by WHO for drinking water. The varied effectiveness of plant extracts in purifying water from the various sources may be due to variation in the physico-chemical properties of water treated. There is, therefore, a need to establish the effect of these physico-chemical properties of water on efficacy of plant extracts in order to optimize their use. These findings also indicate the need to determine changes in each type of bacterial

population present during water treatment in order to formulate a more effective water treatment intervention which will guarantee total elimination of all bacterial types.

The efficacy of *Boscia coriacea* Pax., *Maerua decumbens* (Brogn.) Dewolf roots and *Moringa oleifera* Lam. seeds extracts in reducing sediment load in water were determined by considering the percentage change in turbidity after treatment of water samples within 24 hour period. A high reduction of initial turbidity by both *B. coriacea* Pax. (50.36 %) and *Maerua decumbens* (Brogn.) Dewolf (43.87) coagulants after an initial 30 minutes treatment period suggests that the two plants are potentially useful candidates for further examination. Previous studies (Muyibi and Okuofu, 1995) on use of the aqueous extract of *Moringa oleifera* Lam. seeds to treat three surface water sources in Nigeria found that on average, a 50 % removal of turbidity could be obtained when the *Moringa oleifera* Lam. extract was used as the primary coagulant. In the present study, the percentage removal of turbidity after 30 minutes by the three plant extracts (*Moringa oleifera* - 41%, *B. coriacea* - 50% and *Maerua decumbens* - 44%) was roughly similar to the above observation while the turbidity reduction after 24 hours was much higher (*Moringa oleifera* - 77.91 %, *B. coriacea* - 76.53 % and *Maerua decumbens* - 75.17 %).

An observed decrease in water turbidity appeared to have resulted in a decrease in pH of water samples. This contradicts the findings of earlier work that reported that the use of *Moringa oleifera* Lam. does not cause alteration in pH (Ndabigengesere *et al.*, 1995, Ndabigengesere and Narasiah, 1996). From the study findings, it is clear that pH reduction was accompanied by reduction in final turbidity. During the study, it was noted that the final pH obtained during a 24 hour contact period ranged between 5.50 – 5.80 for all coagulants used. This range is close to a pH of 5 obtained in studies by Ghosh *et al.*, (1994), who

suggested that the only mechanism for turbidity removal is charge neutralization, which favours low concentrations of coagulant.

It is clear from the results that *Boscia coriacea* Pax. extracts have higher turbidity removal efficiency than *Maerua decumbens* (Brogn.) Dewolf and *Moringa oleifera* Lam. extracts. Results obtained after 24 hours with alum in the range studied were significantly different ($P < 0.01$, $DF=5$) from those of plant extracts It is therefore apparent that a change in the coagulation mechanism occurs at low pH since all the treatments which where effective in reducing turbidity also lowered the pH of the treated water sample.

Despite the results showing that *B. coriacea* Pax. had the least antibacterial activity compared to *M. oleifera* Lam. *and M. decumbens* (Brogn.) Dewolf it was able to record equally the same percentage reduction of heterotrophic bacterial counts with extracts of these plants. This observation suggests that pH reduction plays a vital role in inactivating bacteria in water. A reduction of heterotrophic bacterial counts by alum appears to have resulted from a reduction in water pH. Although both the plant extracts and alum lowered water pH, their contributions to reduction in HPC were significantly different. A dismal decrease in HPC counts in the water samples treated with plant extracts after 24 hours compared to 30 minutes of treatment, suggests that the plant extracts may have factors which favour resuscitation of bacteria or certain bacteria quickly adapted to the low (acidic) pH. The gram-negative anaerobic bacteria (coliforms) detected in high densities in treated water might have fermented the organic matter present in the plant extracts leading to the production of organic acids. Responses to pH stress are of particular interest because organisms can be exposed to extremes of pH in aquatic environments, in animals and human bodies (Rowbury *et al.,* 1989). It has been reported that *Shigella* spp are more acid tolerant (pH 2 to 2.5) than *E. coli* (Gorden and Small, 1993). A similar type of acid response was reported in *E. coli* (Rowbury *et al.,* 1994),

*Salmonella dysentriae* and *Salmonella flexineri* Ishrat *et al*., (2002). This adaptive acid tolerance has been attributed to several genes isolated from *E. coli* 0157:H7 and *Salmonella* spp (Foster, 1991; Benjamin and Datta, 1995). Survival in acid may have clinical significance, because enteric pathogens must pass through the stomach pH < 3 for upto 2 hours before colonizing the intestinal tract (Giannella *et al.,* 1972). As most water sources were found to be contaminated with *E. coli*, *Salmonella* spp and *Shigella* spp, these bacteria could have developed tolerance to low pH after 30 minutes of water treatment and hence multiplied and increased in number after 24 hours.

The close correspondence between coagulation activity and a decrease in pH (from median of 7.3 to 5.3) during water treatment using plant extracts and alum (aluminium sulphate) point to the fact that all treatments exert similar effects on the sediments and bacteria in water. The dramatic reduction of HPC after 30 minutes of water treatment by both plant extracts and alum, confirm the existence of bactericidal compounds and sensitivity of the HPC to these compounds. This observation agrees with that made by Ishrat *et al*., (2002). In a study using a number of salts ($CaCl_2$, KCl, $NH_4Cl$, and $Na_2SO_4$), bacterial counts (e.g. *Salmonella sonnei)* incubated at 37 ° C drastically reduced when the pH tended towards acidic (pH 3.0) and this was rapidly noted in 30 minutes. It is therefore apparent that the general similarities in reduction of heterotrophic bacteria and turbidity observed between *Moringa oleifera* Lam., *B. coriacea* Pax. and *Maerua decumbens* (Brogn.) Dewolf in purifying water in the current study are sufficient to suggest that their modes of action might be similar.

## 5.2 Conclusions

From the results of the water quality analysis, the following conclusions can be drawn:

1. Only 25 % of the water samples analyzed in Wamba meet the standards permitted by the WHO regarding water for human use and consumption. The high numbers of

faecal coliforms, recorded in most water sources indicate that faecal matter is a major water pollutant. Hence consumption of water contaminated by feacal bacteria poses a health risk to consumers.

2. Among the water sources present in Wamba division, wells have significantly higher mean faecal coliforms counts than water from other sources while boreholes are the least contaminated of the water sources. Among the pathogens investigated, *Salmonella s*pp. and *Shigella s*pp. were present in all water sources. This means that water from most sources should be treated to avoid outbreaks of gastro-intestinal diseases among the inhabitants of the area.
3. The high density of faecal coliforms in many water sources indicates that treatment of such water will require high chlorine demand. This will increase the cost of treating water and as such the use of chlorine may not be feasible for the population of Wamba due to the economic constraints they encounter.
4. Use of plant extracts in water treatment proved to be effective in the elimination of heterotrophic bacteria and suspended solids. Plants extracts may therefore be considered as alternative methods of purifying water for the inhabitants of Samburu.

## 5.3 Recommendations for further research

1. There is need to characterize the active ingredients in extracts of *Boscia coriacea* Pax., and *Maerua decumbens* (Brogn.) Dewolf responsible for reducing water turbidity and its bacterial load.
2. There is also an urgent need to determine the optimum concentration of the plant extracts and contact time that would not induce resistance in bacteria and which inhibits its growth.
3. The observed decrease and subsequent increase in HPC bacterial population during water treatment indicate that there is need to determine changes in

specific type of bacterial population during water treatment and to understand the reasons for the observed changes in bacterial populations as a result of treatment.

4. The sedimentation and antibacterial effects of various combinations of extracts of *Boscia coriacea* Pax., *Maerua decumbens* (Brogn.) Dewolf and *Moringa oleifera* Lam., should also be investigated in order to evaluate whether they have a synergistic effect that would guarantee total elimination of bacteria.
5. Further research on the effects of physico-chemical properties of water on the purification efficacy of the plant extracts should be carried out.
6. Before adoption of plants extract for water treatment, further research is necessary to define, optimize and standardize conditions for the use of these extracts in the treatment for household water or determine its acceptability, sustainability, costs and effectiveness in reducing waterborne infectious diseases.

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# APPENDICES

## Appendix 1 Location and identity of specific water bodies sampled in Wamba Division, Samburu District.

| LOCATION | | Conservancy | Waterbody name | Waterbody type | Waterbody code |
|---|---|---|---|---|---|
| Northing | Easting | | | | |
| 279402 | 95156 | Barsalinga | Lodungokwe dam | Dam | BAR.1A |
| 298062 | 89710 | Barsalinga | Mukur omuny | River | BAR.3A |
| 288785 | 86843 | Barsalinga | Ewaso nyiro river (bridge) | River | BAR.3B |
| 298065 | 89589 | Barsalinga | Mukur omuny | Spring | BAR.5A |
| 288587 | 87264 | Barsalinga | Ladasaoo | Well | BAR.6A |
| 298062 | 89710 | Barsalinga | Mukur omuny sessia | Well | BAR.6B |
| 299042 | 87442 | Barsalinga | Lodungokwe Nantasim/Sesia | Well | BAR.6C |
| 27683 | 98984 | Barsalinga | Lodungokwe seiya | Well | BAR.6D |
| 311235 | 106775 | Namunyak | Dalambo dam | Dam | NAM.1A |
| 329100 | 98622 | Namunyak | Lcharo nyiro Sarara | Dam | NAM.1B |
| 329093 | 98622 | Namunyak | Loidikidiko dam | Dam | NAM.1C |
| 315322 | 107719 | Namunyak | Sere wamba dam | Dam | NAM.1D |
| 317182 | 10963 | Namunyak | Margwe upstream. | Spring | NAM.5A |
| 316871 | 109688 | Namunyak | Margwe spring(lower) | Spring | NAM.5B |
| 306420 | 104573 | Namunyak | Sere wamba upstream.spring | Spring | NAM.5C |
| 31496 | 107413 | Namunyak | Serewamba downstream | Spring | NAM.5D |
| 322662 | 112526 | Namunyak | Ngongo | River | NAM.3A |
| 315747 | 112169 | Namunyak | Nkanto spring | Spring | NAM.5E |
| 312006 | 111919 | Namunyak | Margwe well | Well | NAM.6A |
| 322557 | 112510 | Namunyak | Ngong`o well | Well | NAM.6B |
| 322976 | 115339 | Namunyak | Njorong'iro well | Well | NAM.6C |
| 322763 | 111634 | Namunyak | Nompitiro well sarara | Well | NAM.6D |
| 314423 | 107988 | Namunyak | Sere wamba well | Well | NAM.6E |
| 313191 | 97771 | Namunyak | Sionta well | Well | NAM.6F |
| 312787 | 106742 | Namunyak | Mjiriman | Borehole | NAM.7A |
| 334990 | 86297 | Namunyak | Tingatinga | Borehole | NAM.7B |
| 315330 | 107708 | Namunyak | Sarara camp | Tap | NAM.8A |
| 315319 | 107708 | Namunyak | Sarara camp | Tap | NAM.8B |
| 303482 | 126930 | Ngilai | Ngilai well | Well | NGI.6A |
| 306340 | 111934 | Ngilai | Borehole | Borehole | NGI.7A |
| 319905 | 92598 | Ngutuk ongiron | Naisunyai dam | Dam | NGU.1A |
| 324829 | 63993 | Ngutuk ongiron | Ewaso nyiro river | River | NGU.3A |
| 312433 | 96287 | Ngutuk ongiron | Lengusaka laga well | Well | NGU.6A |
| 312448 | 96228 | Ngutuk ongiron | Lengusaka laga | Well | NGU.6B |
| 312923 | 97036 | Ngutuk ongiron | Borehole | Borehole | NGU.7A |
| 310501 | 95113 | Ngutuk ongiron | Noolosilale | Well | NGU.6C |
| 295494 | 103425 | Nkaroni | Loturu(Ndikir) dam | Dam | NKA.1A |
| 298347 | 100316 | Nkaroni | Nagorworu dam | Dam | NKA.1B |
| 307450 | 113530 | Nkaroni | Nguass dam | Dam | NKA.1C |
| 29834 | 104316 | Nkaroni | Lesiteti dam | Dam | NKA.1D |
| 300929 | 103884 | Nkaroni | Nagoruworu laga bilabong | Spring | NKA.5A |
| 305523 | 111953 | Nkaroni | Lkisin laga | Well | NKA.6A |
| 3000790 | 103424 | Nkaroni | Nagoruworu laga | Well | NKA.6B |
| 300761 | 103512 | Nkaroni | Nagoruworu | Well | NKA.6C |
| 300883 | 103868 | Nkaroni | Nkaroni borehole | Borehole | NKA.7A |
| 300883 | 103868 | Nkaroni | Nagoroworu borehole | Borehole | NKA.7B |

**Appendix 2 Selenite F broth used to enrich *Salmonella* and *Shigella* spp bacteria. On the left is sterile broth while on the right is a 24 hour positive enriched bacterial culture.**

**Appendix 3 Peptone water used to enrich *Vibrio cholerae* spp bacteria. On the left is sterile Peptone water while on the right is a 24 hour enriched bacterial culture.**

**Appendix 4 Measurement of water turbidity using a colorimeter during treatment.**

**Appendix 5 Laboratory measurement of water pH using a portable pH meter during treatment.**

**Appendix 6 Assortment of some bacteriological media used to culture bacteria.**