AN ASSESSMENT OF BACTERIOLOGICAL QUALITY, WITH SPECIAL EMPHASIS ON THE PRESENCE OF *Escherichia coli*, IN THE ROOF-COLLECTED RAINWATER FROM SOME PERI-URBAN AREAS OF NAIROBI, KENYA.

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A project report submitted in partial fulfilment of the requirements for the degree of Master of Veterinary Public Health, University of Nairobi

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Department of Public Health, Pharmacology and Toxicology: Faculty of Veterinary Medicine, university of Nairobi.

MAY, 2003

Declaration:

This project report is my original work and has not been presented for a degree in any other University.

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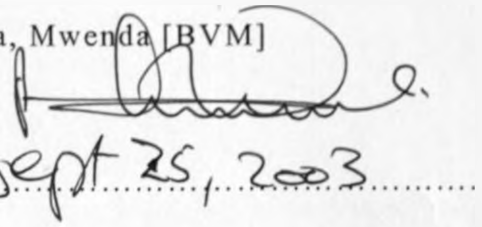

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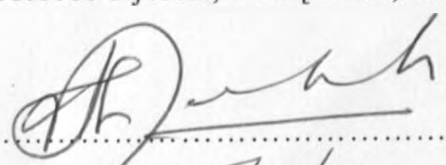

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Date .....25/9/03.....

Dedication:

- To my father, Mwathani Mbaka, for his dedicated support and unwavering faith in his children; my mother, Tabitha Mbaka, for her gentle faith in us, and to my wife and children, for their silent but unrelentingly powerful support!

- To the poor in the world, for whom the privileges of technology are mostly mirages!

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Abstract

Rainwater is gaining importance as a supplementary source of drinking water, especially in the peri-urban zones of Nairobi, where piped water is not available, or the supply is irregular. The ground water sources, boreholes and wells, may be unsuitable due to unpleasant odours and taste, or microbial contamination.

Studies addressing the public health suitability of rainwater for drinking purposes are lacking in this country and the region. The present study was conducted to determine the presence of *Escherichia coli [E. coli]* in rainwater harvested for human consumption. This study was conducted in Ngong Division of Kajiado District, Kikuyu Division of Kiambu District and Dagoretti Division of Nairobi Province. These divisions are found in the neighbourhood of the city of Nairobi, Kenya, and were purposely selected. Collection of samples was done from regions where people consume rainwater stored in reservoir tanks.

An area with a rich tree cover, moderate tree cover, and a predominantly residential region were selected for sampling. Livestock keeping and crop farming was done in these areas to varying degrees. The homes selected for sampling from were those conveniently situated along the commonly used roads in the study area.

Rainwater samples were collected from water tanks. The roofs were made of either concrete roofing-tiles or galvanized corrugated iron sheets. The water tanks were made of galvanized iron sheets, concrete (or ferro-concrete), or plastic.

Bacteriological analyses were done to determine the microbiological quality of water from the sources. The total viable count of the microorganisms was assessed using the pour plate method. The most probable number of coliforms was determined using the multiple tube fermentation technique. The presence of *E. coli* was determined by biochemical reactions of Indole, Methyl Red, Voges Proskauer, and fermentation of Citrate.

Eighty-nine (89) water samples were collected; 29 from Dagoretti Division, 31 from Ngong Division, and 29 from Kikuyu Division. The TVC per ml of the water samples ranged between 0 and $1.02x10^7$ in Dagoretti and Ngong while it ranged between 0 and $8.0x10^5$ in Kikuyu. In Dagoretti, the mean TVC was $1.665x10^6$ with a standard deviation (SD) of $2.995X10^6$ while in Ngong it was $8.236x10^5$ with an SD of $2.545X10^6$ and in Kikuyu it was $7.756x10^4$ with an SD of $1.837X10^5$. The most probable number (MPN) of coliforms per 100mls of water sample ranged between 0 and 1609 in the 3 Divisions. The means were 684 with an SD of 780 for the area, 740 and an SD of 734 for Dagoretti, 652 and an SD of 641 for Ngong, and 891 with an SD of 729 for Kikuyu. In the study area, 89% of the samples screened tested positive for general coliforms, with 40% testing positive for *E. coli*. Area-wise, 83% of the samples from Dagoretti tested positive for coliforms and 31% for *E. coli;* 87% from Ngong were positive for coliforms and 55% for *E. coli,* while in Kikuyu 97% tested positive to coliforms and 34% for *E. coli.*

An analysis of the variance in the TVC of water samples from the three divisions showed that there was no significant difference between Dagoretti and Ngong, but that both

divisions had a significantly higher TVC than Kikuyu [p = 0.033].

Of the 29 samples from Dagoretti, 24% had less than 3 coliforms per 100mls, 28% less than 10 coliforms, and 72% more than 10 coliforms per 100mls; in Ngong it was 19%, 23% and 77% respectively, while in Kikuyu it was 7%, 17% and 83% respectively.

Seventy-eight percent (69/89) of the samples collected contained more than 10 coliforms per 100mls of water, and therefore failed the Kenya Bureau of Standard's requirement for un-piped drinking water. Eighty-three percent (74/89) of the samples of the samples in the study area had more than 3 coliforms per 100mls of water and therefore failed the World Health Organization's standard for untreated drinking water. It was therefore concluded that rainwater from the study region should be treated before consumption.

1. INTRDUCTION

Sources of water for domestic use include: boreholes, wells, rivers, springs, dams and rainwater. Rainwater is usually collected from roofs directly into receptacles from where it is collected for consumption. The roofs are made of either concrete tiles or metal sheets. This being a peri-urban area, no grass-thatched roofs were encountered.

Faecal contamination of rainwater harvested from roofs emanates from: wind-blown dust containing particulate matter from animal faeces, and defecation onto roofs by birds. Since the primary habitat for *E. coli* is the gastro-intestinal tract of warm-blooded animals, it is a good indicator for faecal contamination. Livestock may play an important role as a source of the microbial load found in the dust. Work done by Khalid, (1993) showed that storage tanks in homes with animals are more prone to microbial contamination than those without.

Contamination of rainwater will occur on the roof, or after harvesting, in the reservoir. Studies on the public health suitability of rainwater for drinking purposes are lacking in

Kenya. It is conceivable that contamination occurs. The Centre for Disease Control (CDC), Atlanta, Georgia, (1988) reported that rats contaminate rainwater sources. As the rats use the gutters and roofs as portals of invasion into houses, their faeces and contaminated fur are washed into the water reservoirs. Dust contaminated by faecal matter from livestock may be transferred to the roofs by wind, from where it is washed into the water storage tanks.

The population within the city of Nairobi has increased tremendously over the last few years, forcing people to move to its suburbs. These areas do not have enough piped water supply and the residents have resorted to using ground and river water as alternatives (Mohammed, 1971, Githui, 1990). Ground water has to be pumped out for it to be available. In some cases, it has an objectionable taste due to high mineral content, and so alternative sources of drinking water are needed. Among these is rainwater which is harvested and stored specifically for drinking purposes.

The primary habitat of *E. coli* is the gastro-intestinal tract of mammals and birds. It is one of the predominant bacterial species that form the normal flora of intestines in humans and

other animals (Atlas, 1984). The transfer of bacteria has been shown to occur among different animal species, between humans, from animals to man and vice versa (Levy *et al.*, 1985; Marshall *et al.*, 1990). The organism may be passed through contact with faecal material or faecal contaminated water. The pathological consequences in the victim will depend on the pathogenic characteristics of the particular serotype involved. Drug-resistant serotypes of this organism remain a major problem in the world.

Although most of the strains of *E. coli* are non-pathogenic, some show acquired or opportunistic pathogenicity. *E. coli* is an important cause of acute gastroenteritis with high morbidity and mortality in children (Senerwa *et al.*, 1989). In addition, *E. coli* causes urinary and wound infections (Holts *et al.*, 1994).

In view of the conceivable contamination of rainwater from the roofs, this project aimed to assess the microbiological quality and the prevalence of *E. coli* in the roof-harvested water from some peri-urban zones of Nairobi, Kenya. The selected study areas in the vicinity of Nairobi were chosen for this survey purposely, based on the following criteria: their wide variation

in types of shelter; different intensities of livestock and crop farming; actual utilization of roof-harvested rainwater; storage of the rainwater in water tanks, and proximity to a city characterized by deteriorated garbage collection and sewage handling services.

The specific objectives of this study were:

1) To assess the microbiological quality of roof-harvested stored rainwater.

2) To assess the presence of *E. coli* in the roof-harvested rainwater.

3) To evaluate the design, erection and location of the storage rainwater tanks.

2. REIEW OF LITERATURE

2.1 Water and water sources

Water in its various forms is so abundant in the environment that it is taken for granted until it becomes scarce. Environmental analysts frequently indicate that violent human conflicts are likely to occur in the future as water supplies reduce drastically. This reduction can be attributed to the fact that rainfall and run-off patterns are likely to be altered by atmospheric warming due to present day environmental pollution (Goldfarb, 1993).

Rainwater harvesting is becoming more widespread in Africa and Kenya in particular, where numerous projects have been initiated mainly by the non-governmental organizations. Little attention has been given to rainwater harvesting in urban centres, which also suffer periodic water shortages though ironically, have excellent catchments (Ngigi, 1996). However, it appears that people at the individual level are increasingly paying attention to roof catchments, especially after the severe water shortages experienced in the last few years.

2.2 Quality of drinking water

The quality of drinking water is affected by bacteriological, physical and chemical factors. The World Health Organization (WHO, 1996) has published guidelines for drinking water quality. Individual countries are supposed to use these guidelines to formulate their own standards based on local specific conditions.

The most important parameter is the bacteriological content of drinking water. A wide variety of microorganisms are found in natural waters, forming a balanced ecosystem. However, the presence of pathogens in drinking water poses a public health hazard. The World Health Organization guidelines (WHO, 1971) state that untreated water for human consumption must not contain more than 3 coliforms per 100mls, and must not have a single faecal coliform at any one sampling. The Kenya Bureau of Standards (KBS) require that un-piped water should contain no faecal coliforms per 100mls, and a maximum of 10 coliforms at any single sampling (KBS, 1985).

2.3 Physical and chemical characteristics of water

Untreated water contains numerous materials. These materials determine its physical characteristics (colour, turbidity, taste,

and odour), chemical characteristics (pH, hardness, dissolved oxygen, oxygen demand, nutrients, chlorides and other elements), and biological characteristics (microbial content) (APHA, 1981). The impurities in water affect its public health aspects to various degrees.

2.4 Water pollution

All natural and wastewater contains a variety of living organisms. The contamination of water by pathogenic bacteria, viruses and metazoal parasites can occur either at the water sources during its conveyance from the sources to the consumers or in the storage tanks. The pathogenic agents may be from faecal and urinary excretions of human and animal origin, sewage and sewage effluents, and washings from the soil (WHO, 1972). Rainwater is likely to be contaminated in the storage tanks, on the roof, or in the atmosphere before it comes in contact with the roof.

Atmospheric pollution is a common problem in the world today (Dix, 1981; Miller, 1990; Gourlay, 1995). The natural aerosolisation of bacteria through dust, vapour, wind, and other means, also introduces potentially harmful microorganisms to the atmosphere, and eventually into rainwater. Simmons (2001)

showed that roof-tapped rainwater had bacteria known to pose public health hazards. In his work in Auckland, New Zealand, he demonstrated that roof-collected rainwater systems provide potable supplies of relatively poor physicochemical and bacteriological quality. Samples of cold faucet water were analyzed for physico-chemical and bacteriological determinants, including metals (zinc, copper and lead), bacterial indicator organisms, heterotrophic plate count, total coliforms, faecal coliforms, enterococci, bacterial pathogens including *Salmonella spp*, *Legionella spp*, *Campylobacter spp*, *Aeromonas spp;* and the protozoa, *Cryptosporidium spp* and *Giardia spp*. The presences of the indicator organisms were all significantly correlated with one another. *Aeromonas spp* were identified in 16.0% of the supplies. There was a positive association between the presence of *Aeromonas* and the bacterial indicator organisms. Households reporting at least one member with gastrointestinal symptoms in the month prior to sampling were more likely to have *Aeromonas* species identified in their water supply than those households without symptoms. It would appear that further work is required on *Aeromonas spp*, as potential indicators of both bacteriological quality and public health risk of roof-collected drinking water supplies.

Although the work by Simmons (2001) does not indicate whether the contaminants were as a result of being washed off the roof by the rainwater, or as a result of direct atmospheric pollution, work by Angstrom *et al.* (1952) supports the possibility of direct atmospheric contamination being a contributing factor. It has been reported that small showers tend to have a much higher mineral-nitrogen concentration than do larger falls, and that concentration decreases with shower size in an approximately exponential manner (Angstrom *et al.*, 1952). Experiments carried out at Samaru, Nigeria, in 1969, produced similar results (Jones, 1978). Jones (1978) therefore concluded that the greater part of the rain mineral-nitrogen derives from "washout" of mineral-nitrogen compounds from the air through which the rain falls. Empirically, microorganisms and other atmospheric contaminants may be found in rainwater as a result of the "washout" phenomenon.

Work in the Middle East suggests that the bacterial content of rainwater falling over cities and their environs is different from that falling in rural areas (Herut *et al.*, 2000). In this work, the relationship between the acidity and chemical composition of rainwater and climatological conditions along a

transition zone between large deserts and Mediterranean climate were studied. During five winters in 1981/1982, 1982/1983, 1983/1984, 1988/1989 and 1989/1990, 569 rainwater samples were collected for chemical analyses. The geographical and meteorological variations of rain chemistry were studied in relation to natural and anthropogenic sources and transport of the constituents. The regional effect on rain chemical composition is a latitudinal function in a transition zone between large deserts in the south and a Mediterranean climate in the centre and northern Israel, and a longitudinal function in the land-sea configuration. The average rainwater salinity, mainly contributed by non-sea-salt fraction (NSSF), varies by more than one order of magnitude from south to north. These variations were attributed to higher input of continental components at the southern regions and to differences of annual precipitation between the regions. In a West-East cross-section through Israel, sea-salt fraction (SSF) is influenced mainly by the distance from the Mediterranean Sea (Herut *et al.*, 2000).

Bacteria discharged into the atmosphere may be deposited in rainwater within the neighbourhood. The mechanism of this bacterial deposit would partly be the "washout" phenomenon of

mineral-nitrogen measured in Samaru by Angstrong *et al.* (1952), and the incorporation of microorganisms in raindrop-forming particulate milieu.

Pinfold (1990), reported that water used for toilet, washing dishes and cooking-related activities was more contaminated than water used for drinking, due to the carelessness of the handlers. He reported that *E. coli* was strongly associated with childcare, food and water-related activities. Water contaminated by the handlers, or pre-contaminated water may be the cause of this observation. From these sources, *E. coli* may show opportunistic pathogenicity by causing enteritis, urethritis, and neonatal meningitis, especially in children (MacDonald *et al,* 1970; Kariuki, 1996).

2.5 Bacterial indicators of faecal water pollution

Coliform organisms are easy to detect and enumerate in water and so they have for a long time been regarded as suitable indicators of drinking water quality. These organisms are defined as microorganisms which display H-galactosidase activity (WHO, 1996). Not all coliforms are useful as indicators of water pollution from faecal sources. The so-called faecal [thermotolerant] coliforms are used for this

purpose. Unlike the rest, these ferment lactose at 44-45°C. They include *Escherichia coli, Citrobacter, Enterobacter* and *Klebsiela spp* (WHO, 1985).

Although other coliforms may be of faecal origin, the primary habitat of *E. coli* is the gastro-intestinal tract of mammals and birds. This has made it an important indicator organism of faecal contamination of food and water. Measurement of faecal coliforms is a good indicator of water contamination by materials of faecal origin. Some coliforms such as *Klebsiella pneumoniae* and *Enterobacter spp* may also, besides a faecal origin, be associated with other decomposing matter and water-soaked wood. Some species of the faecal streptococcus group may also multiply in soil and surface water, especially in combination with plant debris decay material. However, *E. coli* is exclusively of faecal origin (WHO,1985). The average human excretes around 2 billion *E. coli* bacteria each day (Adrian, 1999)

2.5.1 Pathogenic bacteria

Most microorganisms found in water are harmless. However, pathogenic ones may be accidentally introduced.

Enteropathogenic strains of *E. coli* are some of the organisms

reported among the pathogenic bacteria that may contaminate water (Mohammed and Morrison, 1975; WHO, 1984; Ihona *et al.*, 1988; Pinfold, 1990). Antibiotic resistant *E. coli* may be transmitted from animals to humans through contact with faecal material or faecal contaminated water. Normal *E. coli* flora acquire resistance plasmids from the ingested resistant *E. coli* strains. The antimicrobial resistant normal *E. coli* flora disseminate resistance plasmids to pathogenic bacteria (Espinasse, 1993).

2.5.2 Viruses

Enteroviruses, e.g. polioviruses and coxsackie viruses, adenoviruses and rhinoviruses are some of the viruses commonly found in polluted waters and sewage (WHO, 1979; 1984). The viruses transmitted through water are those, which multiply in the intestines and are excreted in large numbers in faeces of infected individuals (WHO, 1979). Outbreaks of viral hepatitis and gastroenteritis resulting from sewage-contaminated water are frequently reported in many countries all over the world (WHO, 1984). Rats that hide in sewage ducts and toilets later move into houses via the roof gutters and may therefore transmit these viruses, either through their excretions

or fur, into the storage tanks, thus contaminating rain water (Centre for Disease Control, 1988).

2.5.3 Parasites

Common human protozoal waterborne pathogens such as *Entamoeba hystolytica, Giardia lamblia, Cryptosporidium spp, and Balantidium coli,* and helminths such *Echinococcus granulosus, Taenia solium* and *Taenia saginata* may be transmitted through drinking water and have been associated with disease outbreaks after consumption of contaminated water. These organisms may contaminate rainwater in the storage tank or after such rainwater is handled by infected patients.

2.5.4 Free-living organisms

Phytoplankton free living bacteria, fungi and algae, zooplankton free-living protozoa, Rotifers, Cladocera and worms, and macro-invertebrates can grow in rainwater, especially that which stays in storage tanks for prolonged periods.

2.6 Public health aspects of water

Many communicable infections are related to both water and excreta. Although a water-washed faecal-oral route transmits many excreta-related diseases, especially diarrhoea, a waterborne route can also spread them.

Safe water supplies and better sanitation have been accepted as some of the major basic necessities for a healthy living. However, measuring the health benefits that accrue from them remains controversial. Some studies indicate that improved water quality and sanitation facilities are not efficacious in improving health status and are not particularly cost effective (Walsh and Warren, 1979). In contrast, a review of 67 studies from 28 countries found that investments in water and sanitation can reduce diarrhoeal morbidity and mortality rates by a median of 22% and 21% respectively (Esrey *et al.*, 1985). The health benefits resulting from investment in clean water and sanitation have been measured by case-control studies since the end of the 1980's. These studies have been conducted in Malawi (Young and Briscoe, 1988), the Philippines (Baltazar, 1988), Lesotho (Daniels, 1990) and in Columbia (Bersh and Osorio, 1985). It was found that water and sanitation investments can produce a 20% - 24% reduction in

the incidence of diarrhoea. Chunge *et al.* (1992), in a study to compare the aetiology of childhood diarrhoea in Kiambu and Kakamega Districts of Kenya, found that water sources accounted for some occurrences of diarrhoeal cases.

2.7 Standard Techniques For Testing Microbial Water Quality

2.7.1 Multiple Tube Fermentation Technique

The Multiple Tube Fermentation Technique (MTT) has been used for the enumeration of total coliforms for over 70 years as an indicator of water quality. The technique is still in use today in many countries for monitoring water supplies and food quality control (Evans *et al.*, 1981). The method utilizes the ability of coliforms to ferment lactose with production of acid and gas within 24 hours at 37°C.

In the initial presumptive coliform test, water samples are inoculated into fermentation tubes containing a suitable liquid medium, and then incubated for an appropriate period of time for the reaction to take place. The test is called presumptive because the reaction observed may occasionally be due to the presence of other organisms or combination of organisms

(WHO, 1971). The presumption that the reaction is due to coliforms has to be confirmed.

Several workers (Leitch, 1925; Hossong, 1981) have reported false reactions which are thought to be dependent on the bacterial flora of water and the method used. Hossong (1981) recovered members of the *Enterobacteriaceae* as the predominant group from the false positive presumptive test and also a large number of atypical and anaerobic lactose fermenting strains. He concluded that no single specific bacterial group can be identified as being responsible for the false positive reaction of the presumptive coliform test.

Problems of false negative reactions using Standard Most Probable Number (S-MPN) technique have been reported (Evans *et al.*, 1981). Using a Modified Most Probable Number (M-MPN) method, Evans *et al.*, (1981) managed to recover different species of coliforms from the false negative tests including *Citrobacter, Enterobacter, Klebsiella, and Escherichia*. The recovery of these organisms depended on the methods used since each coliform detection technique tended to select for a different profile of coliform species from water samples (Evans *et al*, 1981).

2.7.2 Membrane Filtration Technique

Since the Membrane Filtration (MF) Technique was introduced as a tentative method for coliform enumeration, it has gained wide usage for not only enumeration of total coliforms, but also for faecal coliforms, total bacterial count, and a variety of other bacterial tests. The unique advantage of the MF over other test methods is its ability to concentrate and localize the bacteria from large sample volumes, hence the sensitivity of quantitative bacteriology. The ideal characteristics of a membrane filter for qualitative bacteriology would appear to be pores small enough to retain the bacteria, but open enough to permit optimal diffusion of media and a hospitable surface for growth. Membrane filters currently recommended for enumeration of faecal coliforms have a pore retention size of 0.45µm. Filters from different manufacturers have a retention size of 0.45µm, but they may have different surface morphologies and thus exhibit considerable differences in coliform recovery (Sladek *et al.*, 1975).

It has been reported that membrane composition of either cellulose ester, cellulose acetate, or polyaryl ester, is not an important factor in bacterial recovery; neither are there

significant differences between various sterilization methods and the number recovered (Sladek *et al.*, 1975).

Barbra *et al.* (1975) compared membranes from different manufacturers for their recovery sensitivity and concluded that membranes could be rated in order of decreasing sensitivity as follows:
Millipore HC>Gelman>Johns-Manville>Sartorious>Millipore HA>Schleicher & Schnell.

Dutka *et al.* (1974) reported various problems encountered when using various brands of filters. On several occasions, Sartorious membranes were found to have hydrophobic areas, which limited the effective filtering areas. Both Millipore and Sartorious membrane filters have areas, which upon autoclaving become distorted and somewhat fragile mainly due to shrinking. When testing for faecal coliform density, Dutka *et al.* (1974) found that Millipore membrane filters often produced a beige-yellow background that in some instances made counting more difficult.

Other complications of using the filter membranes include the reduction in the apparent recovery of organisms by the

membrane filters in cases of high colony concentration due to overlapping, and the difficulty in counting individual colonies for spreading organisms. The filter membranes have been modified to include a square grid pattern printed in hydrophobic material on the conventional filters. This appears to separate the colonies from one another and prevents lateral growth, spreading and confluence, since the colonies grow upwards instead of sideways.

It appears that conflicting reports will continue until a standardized procedure for filter evaluation, including the source of the test organism and statistical analysis, is established and accepted (Brodsky; 1975).

2.7.3 Other techniques

An enzymatic procedure for the identification of coliforms has been proposed (Lechevallier *et al*, 1983). This procedure is based on O-nitrophenyl-b-galactopyranoside (ONPG) hydrolyses and cytochrome oxidase activities. Enzymes are used as surrogate measures of coliform concentration (Berg and Fiksdal, 1988). A direct MF method incorporating MU-b-D-galactoside into the agar medium allowed the detection of as few as 1 faecal coliform per 100mls of water within 6 hours.

The use of Presence-Absence Test, as an alternative to the Membrane Filtration and Multiple Tube Technique, for monitoring the quality of drinking water was first suggested by Clark (1968). Initial studies used double strength MacConkey broth modified by addition of 10g Tryptone per litre. The test was performed by adding 50mls of water sample to 50mls of the modified broth in glass-screwed bottles containing inverted Durham tubes. The P-A bottles were incubated at 37°C for 5 days and checked daily for growth and acid or acid and gas production. Inoculum from positive presumptive bottles were transferred to confirmatory media and on MacConkey agar plate. The first test showed that the P-A test produced 478 confirmed positive results for coliforms, whereas Membrane Filtration test produced only 317 confirmed positive results. The P-A test produced a higher number of positive tests, demonstrating a greater sensitivity for detection of coliforms than the Membrane Filtration Technique (Clark *et al.*, 1982).

A defined substrate method (Edburg *et al.*, 1989) has been applied to drinking water to simultaneously enumerate total coliform and total *E. coli* directly from water samples. After incubation at 35°C for 24 hours, the development of yellow

colour from the initially colourless solution is specific for coliforms. Fluorescence at 266 nm in the same tube(s) demonstrates the presence of *E. coli*. The method is known as Autoanalysis Colilert (AC) and has been reported to give results comparable to standard methods in presence/absence format.

A Hydrogen sulphide Screening Test for water quality was compared to the Standard Multiple Tube Technique and found to be highly sensitive and specific and showed a high predictive value (Bukenya, 1990). This test has been recommended for the developing countries where resources are very limited.

The Endo agar medium has been found to be selective for coliforms and also the colonies have a metallic sheen unlike other bacterial colonies, which may grow on the medium. Problems have been reported with this method since the medium is not adequately inhibitive to other bacteria from water and as a result, the metallic sheen of crowded colonies is not clear-cut (Sharp and Michard, 1974). Mohammed and Morrison (1975) reported a high degree of false negative colonies on Endo agar medium, which they attributed to the

presence of chemical impurities in the water supplies, interfering with the proper development of the normal reaction.

2.8 *Escherichia coli*

Escherichia coli (E. coli) is a facultatively anaerobic, gram-negative, rod-shaped, non-spore-forming, glucose-fermenting bacterium belonging to the family *Enterobacteriaceae* (Atlas, 1984). Most strains ferment lactose although this fermentation may be delayed or even absent in some cases (Holts *et al.*, 1994). Like some other members of its group, it is motile by peritrichus flagella (Holts *et al.*, 1994). It belongs to the genus *Escherichia*, and is found as a commensal organism in the gastro-intestinal tracts of warm-blooded animals, including humans. However, it is an important cause of acute gastro-enteritis in children, with high morbidity and mortality rates (Senerwa *et al.*, 1989). In developed countries, it rarely causes gastro-enteritis except 'travellers' diarrhoea (Dupont *et al*, 1982). Kariuki, (1996) reported it as the most important pathogen in the young and old individuals of poor economic and social status. It has been isolated from wounds (Holts *et al.*, 1994) and also causes urinary tract infections (Boyd, 1984).

2.8.1 History

Theodor Escherich, a German pediatrician, was the first person to isolate the organism, in 1885 (Escherich, 1885; 1886). It was then named *Bacteria coli commune*, but has since been renamed *Escherichia coli* (Sussman, 1995).

2.8.2 Biochemical reactions

The major biochemical characteristics of *E. coli* according to Holt *et al.* (1994) are shown in appendix 1.21.

2.8.3 Serology

The most useful way of classifying *E. coli* is serology, based on the antigenic properties of its various surface structures. The serological classification, as reviewed by Kaufman (1966), forms the basis of its typing.

The antigens used in this classification include the O or somatic antigens, which denote the polysaccharide moiety of the cell wall; the K, or capsular antigens, are acidic polysaccharides; and the H or flagella antigen, are protein in nature. One hundred and seventy one (171) different O antigens (01 to 171), 103 K antigens, and 55 H antigens have been identified.

2.8.4 Enteropathogenicity

E. coli is a commensal organism in the intestinal tract of humans but is potentially pathogenic (Mundell *et al.*, 1976, Field, 1979). It shows opportunistic pathogenicity by causing enteritis, urinary tract infections, and neonatal meningitis in humans, and mastitis in cows. The most important diseases due to this organism are enteritis in children and young animals.

De *et al.* (1956) demonstrated the first specific virulence factors associated with the bacteria. They reported that some strains of *E. coli* were associated with diarrhoea caused by secretion of excessive fluids and electrolytes into the ileal loops of rabbits.

The different pathogenic mechanisms involved in the enteric infections in humans caused by the organism have been classified (Honda, 1992) as follows:

1. Enteropathogenic *Escherichia coli* (EPEC);
2. Enterotoxigenic *Escherichia coli* (ETEC);
3. Enterohaemorrhagic *Escherichia coli* (EHEC);
4. Enteroinvasive *Escherichia coli* (EIEC);
5. Enteroadherent *Escherichia coli* (EAEC);
6. Enteroaggregative *Escherichia coli* (EAggEC);

2.8.4.1 Enteropathogenic *E. coli*

Enteropathogenic *E. coli* belong to the O serogroups and are capable of causing diarrhea without producing enterotoxins, and are not invasive (Levin *et al,* 1983). About 15 to 20 of the known *E. coli* serogroups O, K and H have been designated EPEC with the most prevalent serogroups worldwide being O11, O26, O119, O127 and O128 (Rowe, 1979). EPEC is responsible for many cases of infantile gastrointestinal diseases with high mortality worldwide (Rowe, 1983).

2.8.4.2 Enterotoxigenic *E. coli*

Enterotoxigenic *E. coli* cause diarrhoea by elaborating heat-labile (LT) and heat stable (ST) toxins (Clements and Finkelstein, 1979). The production of both LT and ST is encoded by transferable plasmids with specific plasmids governing production of LT alone, LT and ST and ST alone (Wachsmuth *et al.,* 1976).

ETEC possesses accessory virulence factors. The best characterized are the adherence or colonization factors, which permit attachment of ETEC to the mucosa of small intestines (Nagy *et al.,* 1977; Levin *et al,* 1983), permitting release of enterotoxin close to the reactive sites. Clinically, the disease

caused by ETEC is indistinguishable from cholera (Mundell *et al.*, 1976; Field, 1979).

2.8.4.3 Enterohaemorrhagic *E. coli*

E. coli O157:H7 belongs to this group. This organism produces cytotoxins called verocytotoxin (VT) or shiga-like toxin that appear to play a major role in the pathogenesis of haemorrhagic colitis (Pai *et al.*, 1986). There is significant incrimination of VT-producing *E. coli* (VTEC) as cause of Hemolytic Uraemic Syndrome (HUS) (Spika *et al.*, 1986).

Since the 1983 outbreaks of hemorrhagic colitis associated with *E. coli* O157:H7 from the United States, it has been reported with increasing frequency (Riley *et al.*, 1983), particularly in industrialised countries.

2.8.4.4 Enteroinvasive *E. coli*

Enteroinvasive *E. coli* does not produce enterotoxins, but it invades the colonic mucosal cells. This invasive property was demonstrated by the guinea pig keratoconjunctivitis test (Sereny, 1955). Strains O114:K, O43:K, O136:K, O124:K and O28A, C:K74 were isolated (Ogawa *et al.*, 1968) and shown to possess invasive properties characteristic of virulence factors found in *Shigella* strains.

2.8.4.5 Enteroadherent *E. coli*

This term was designated to *E. coli* isolated from a diarrheic patient in 1985 and found not to belong to the typical EPEC serotypes, but adhered to Hep-2 cells. Two types of adherence are known: localized and diffuse (Mathewson *et al.*, 1985).

2.9.4.6 Enteroaggregative *E. coli*

These organisms are often isolated from patients with diarrhoea persisting longer than 2 weeks. The bacteria show characteristic 'stacked brick' adherence on Hep-2 cells and even on a glass surface (Vial *et al.*,1988).

3. MATERIALS AND METHODS

3.1 Area of Study:

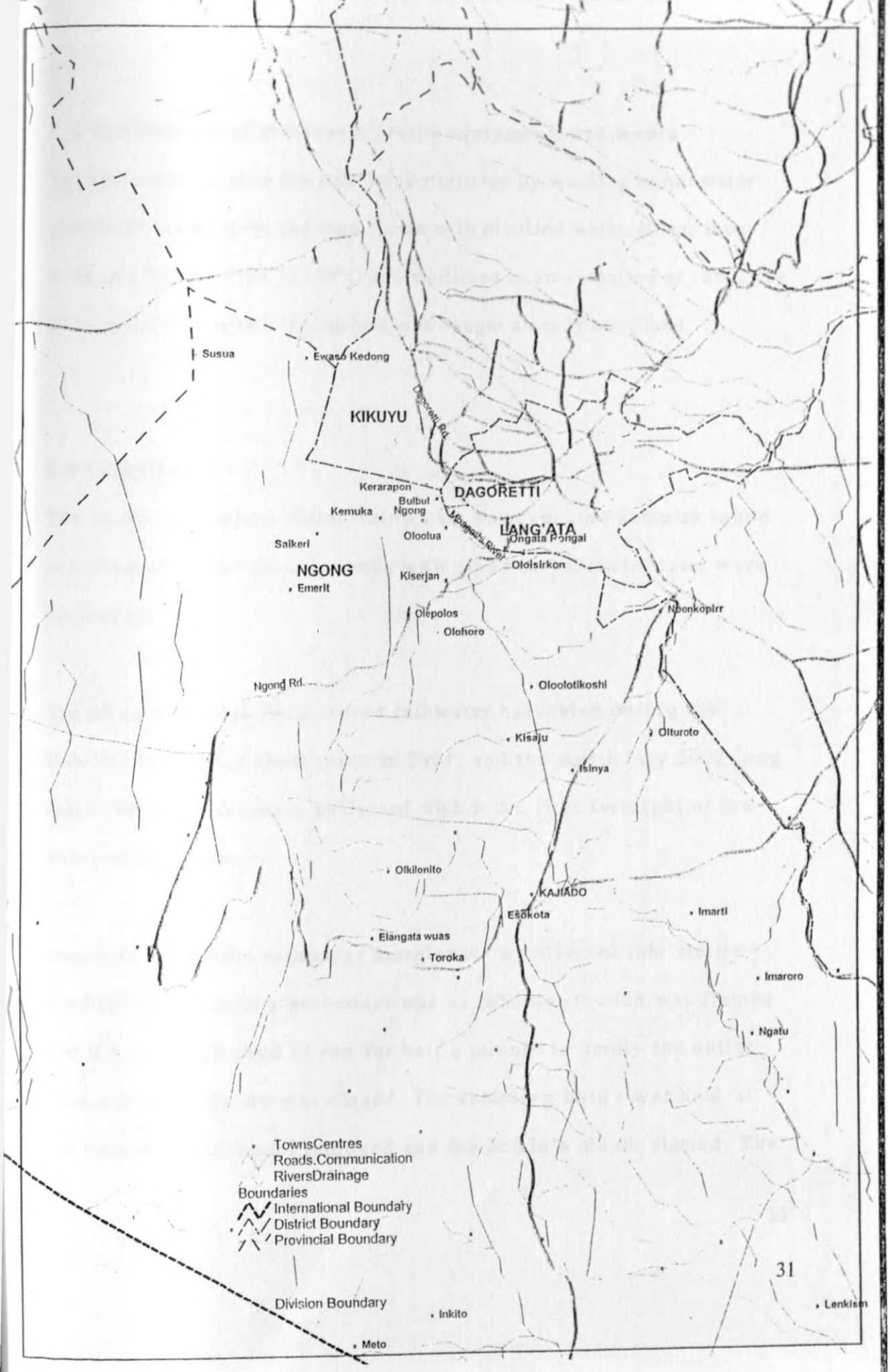
The study was conducted in the peri-urban areas of Nairobi (figure 1). Purposive selection, aiming to collect samples for analyses from regions where people consume rainwater which has been stored in reservoir tanks, was done. Factors considered for the selection of the study areas were: their wide variation in types of shelter; different intensities of livestock and crop farming; actual utilization of roof-harvested rainwater; and proximity to a city with deteriorated garbage collection and sewage handling services.

Convenient sampling was carried out from homes situated along the access roads, which so richly crisscross the area that the selected tanks were regarded representative of the area.

The samples were collected from Dagoretti Division of Nairobi District in Nairobi Province; Ngong Division of Kajiado District in Rift Valley Province; and Kikuyu Division of Kiambu District in Central Province, Kenya. Samples in Dagoretti were collected from: Kawangware, Riruta, and Ruthimitu Locations; in Ngong Division: from Ngong, Oloolua, Kiserian, Nkai Murunya and

Ongata Rongai Locations; and in Kikuyu Division: from Kinoo, Kabete, and Kikuyu Locations.

Figure 1 (overleaf) is a map of the study area:


Susua
Ewaso Kedong
KIKUYU
Dagoretti Rd.
Kerarapon
DAGORETTI
Bulbul
Kemuka
Ngong
Mbagathi River
LANG'ATA
Ongata Pongai
Oloolua
Saikeri
NGONG
Kiserjan
Ololsirkon
Emerit
Olepolos
Nkonkopirr
Olohoro
Ngong Rd.
Oloolotikoshi
Kisaju
Olturoto
Isinya
Olkilonito
KAJIADO
Esokota
Imarti
Elangata wuas
Toroka
Imaroro
Ngatu
TownsCentres
Roads.Communication
RiversDrainage
Boundaries
International Boundary
District Boundary
Provincial Boundary
Division Boundary
Inkito
Lenkisen
Meto

3. 2 Sterilization of glassware, plastic equipment, and media

The equipment used in the study was sterilized by washing in hot water containing a detergent and then rinsed with distilled water. It was then dried in a hot air oven at 100°C and sterilized in an autoclave at 120°C for 30 minutes. The plastic equipment was bought already sterilized.

3.3 Sampling

The study focused on stored rainwater; however, the samples found to be mixed in the storage tanks with piped or borehole water were discarded.

Sampling was done from stored rainwater harvested during the October-December short rains in 2001, and the April-July 2002 long rains. The samples were collected within the first fortnight of the onset of the rains.

About 250ml of the rainwater samples were collected into sterile bottles. The sampling procedure was as follows: the tap was flamed and the water allowed to run for half a minute to empty the outlet pipe and then the tap was closed. The sampling bottle was held at the bottom, the stopper removed and the bottle's mouth flamed. The

tap was flamed again, opened, and the water allowed to run for another 30 seconds. The mouth of the bottle was then placed under the tap and the water sample collected. The bottles in each case were then labelled with the following information: location; date; time; type of sample (tank-collected); roof type (whether metal or concrete tiles); type of tank (whether plastic, metal, or concrete); environment (whether slum or other residence, livestock or mixed farm); name and address of respondent. The sample was placed in a cool-box and taken to the laboratory.

Photographs of some of the tanks sampled from were taken to illustrate the conditions under which the rainwater was harvested. A deliberate effort was made to ensure that every situation of rainwater harvesting was represented. A total of 9 photographs were taken of the various situations in which rainwater was found to be collected.

3.4 Bacteriological Analyses

The samples were analysed for total viable count (TVC) and the coliform count. *E. coli* was analysed for physical and biochemical characteristics.

The media were prepared and sterilized according to the manufacturers' recommendations, which involved sterilization in a pressure cooker at 121°C for 15 minutes.

3.4.1 Total Viable Count

The Oxoid dehydrated plate count agar (PCA) was reconstituted and prepared according to the manufacturer's recommendations (Appendix 6.1). The agar was maintained at between 45°C and 50°C in a water bath until ready for use. The bottled water sample was shaken several times, the stopper flamed and opened, and the mouth of the bottle flamed. One millilitre (1ml) of the water sample was collected using a sterile pipette and transferred into a test tube containing 9ml of sterile physiological saline. This was serially diluted ten fold up to 10^5. One millilitre (1ml) was drawn from each diluted sample using a sterile pipette and aseptically dispensed into sterile petri dishes in duplicate.

Approximately 20ml of the PCA at 45°C was added into each petri dish containing the water sample and gently mixed. The plates were allowed to cool and then incubated at 30°C for 48 hours. The plates with countable colonies were selected and the colonies in each plate counted with the aid of a colony counter.

The mean count for each sample was recorded as the total viable count (TVC) of bacteria per ml of the water sample.

3.4.2 Coliform Count

3.4.2.1 Presumptive Coliform Test

The MacConkey Broth (Oxoid, 1980) was prepared according to the manufacturer's instructions (Appendix 6.2). For each water sample, 10ml of the double strength broth was distributed into 5 universal bottles and 5mls of the single strength distributed into 2 sets of 5 fermentation tubes. Inverted Durham tubes were introduced into the universal bottles and the fermentation tubes. The broth was then sterilized in a pressure cooker at 121°C for 15 minutes and allowed to cool.

The water samples were thoroughly mixed. 10ml of the water samples was then transferred into each universal bottle, 1ml into each of the bottles in one set of the 5 fermentation tubes, and 0.1ml into the other set of 5 bottles, using a sterile pipette. The bottles and the tubes were incubated at 37°C for 48 hours, and thereafter observed for acid (Bromocresol dye turning orange from purple) and gas (in the Durham tubes) production. The number of bottles and tubes showing acid and gas production was noted. The Most Probable Number of

Coliforms per 100ml of the water sample was read from the McCrady's Statistical Table (appendix 1.14).

3.4.2.2 Confirmation of The Presence of *E. coli*

Eosin Methylene Blue Agar (EMBA) (Oxoid, 1980) was prepared according to the manufacturer's instructions (Appendix 6.4), poured into petri dishes, and allowed to cool. The plates in duplicate were streaked with culture from the positive presumptive tubes, and incubated for 24 hours at 37°C. Medium-sized round colonies, 2-3mm diameter, with a dark centre, some showing a metallic greenish sheen, were regarded suspect *E. coli*. The suspect colonies were then subjected to biochemical tests to confirm the presence of *E. coli*, as described below.

Methyl-Red Voges-Proskauer medium (MRVP) (Appendix 6.5), Simmons Citrate agar (Appendix 6.6), Indole reagent (Appendix 6.7), Methyl Red solution (Appendix 6.8), Creatine solution (Appendix6.9), and 40% Potassium Hydroxide (Appendix 6.10) were prepared according to the manufacturer's instructions. A portion of the suspect colony was inoculated into a fermentation tube of Tryptone water. Two portions from the same colony were each inoculated into a tube of MRVP medium and another portion into Simmons citrate agar. All

tubes were incubated at 37°C for 48 hours. Thereafter Indole production was tested by addition of Kovac's reagent into the Tryptone water. Methyl-Red pH indicator was added into one of the MRVP medium tubes, and Creatine solution and Potassium Hydroxide into the other.

Colonies producing the reactions: Indole positive (I+), Methyl-Red positive (M+), Voges-Proskauer negative (Vi-), and Creatine negative (C-) were designated IMViC ++-- and considered to be *E. coli*.

Nutrient Agar (Appendix 6.11) and Cooked Meat Medium (Appendix 6.12) were prepared according to the manufacturer's instructions. The confirmed *E. coli* organisms were preserved under refrigeration in these media for future reference.

3.5 Statistical Analyses

The data were entered and handled in a database (dBase IV, Ashton-Tate, Torrance, CA, USA). PROC FREC in SAS (PC-SAS version 6.04, SAS institute, Inc., Cary, NC, USA) was used to generate tables of descriptive statistics and for computing the X^2 statistics and odds ratio (OR) in tests of association.

RESULTS, DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4. RESULTS

4.1 The Samples Analysed

Out of 115 respondents who were contacted to participate in the study, 104 (90%) complied, but only 89 water samples (86%) were usable. The rejected 15 water samples were from tanks known to have been mixed with treated piped-water, but which were collected because the owners' cooperation in the exercise was based on the understanding that all the tanks in their compound would be sampled, in order to establish the bacteriological quality of their water.

4.2 Photographic illustration of the types of tanks, their design, location and erection.

During the study, no water tank was found to be ideally designed, constructed and located; the tanks are illustrated in figures 2 to 10.

Only one tank was found to be properly designed and constructed (Figure 10), but there was no provision made for the first rainfall to rinse off the dust on the roof and gutters before the water meant for storage was allowed into the tank.

Figure 2: A photograph showing a concrete rainwater storage tank in Ngong.

Note: The tank is improperly covered, exposing the collected rainwater to easy contamination. The gutter is also open to contamination. Note the livestock grazing nearby.

Figure 3: A photograph showing a tap fitted on the side of the concrete tank (right hand side bottom of tank between the 2 shadows)

Note: The pipe is so high above the floor of the tank that complete cleaning with drainage of debris is not possible. Contamination from one rain can therefore be carried on to subsequent ones.

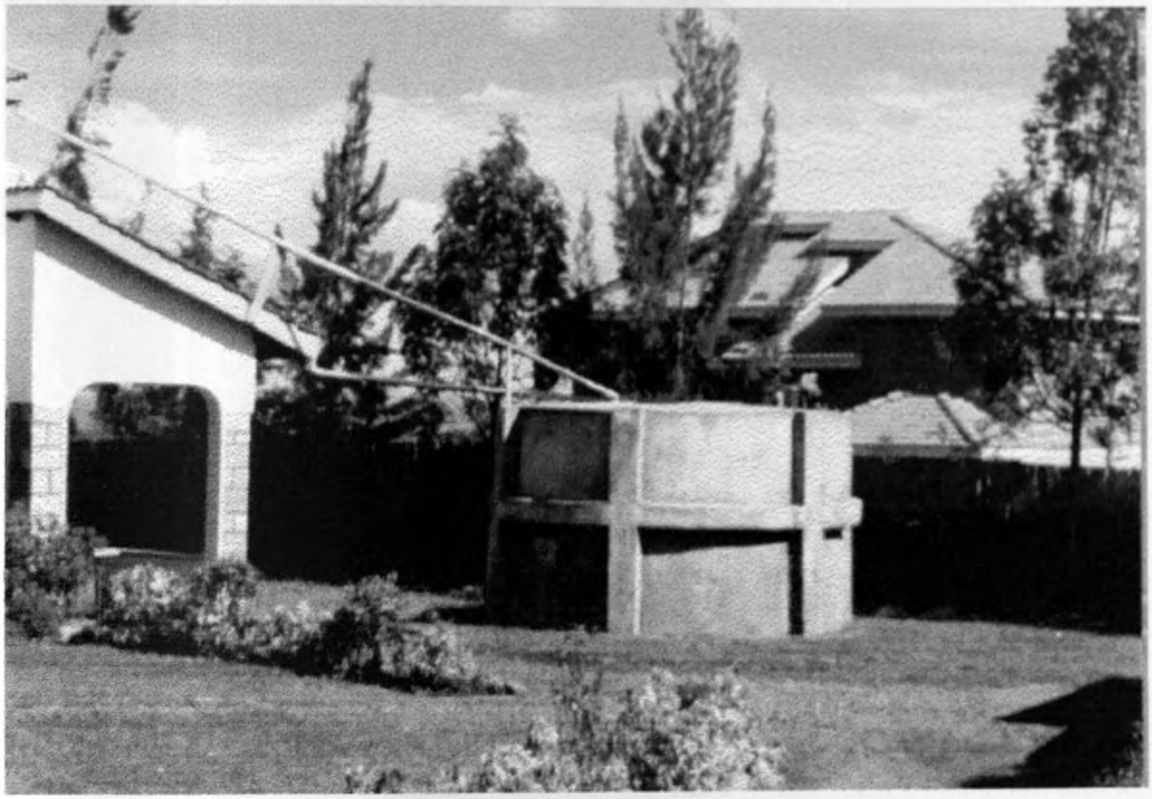


Figure 4: A photograph showing a sturdy concrete rainwater tank in a modern homestead in Ngong Division.

Note: he ornamental-cum-windbreaker exotic trees, and the concrete flat roof to prevent contamination of the stored water. However, the poorly finished roof top does not effectively prevent the introduction of dirt into the tank.

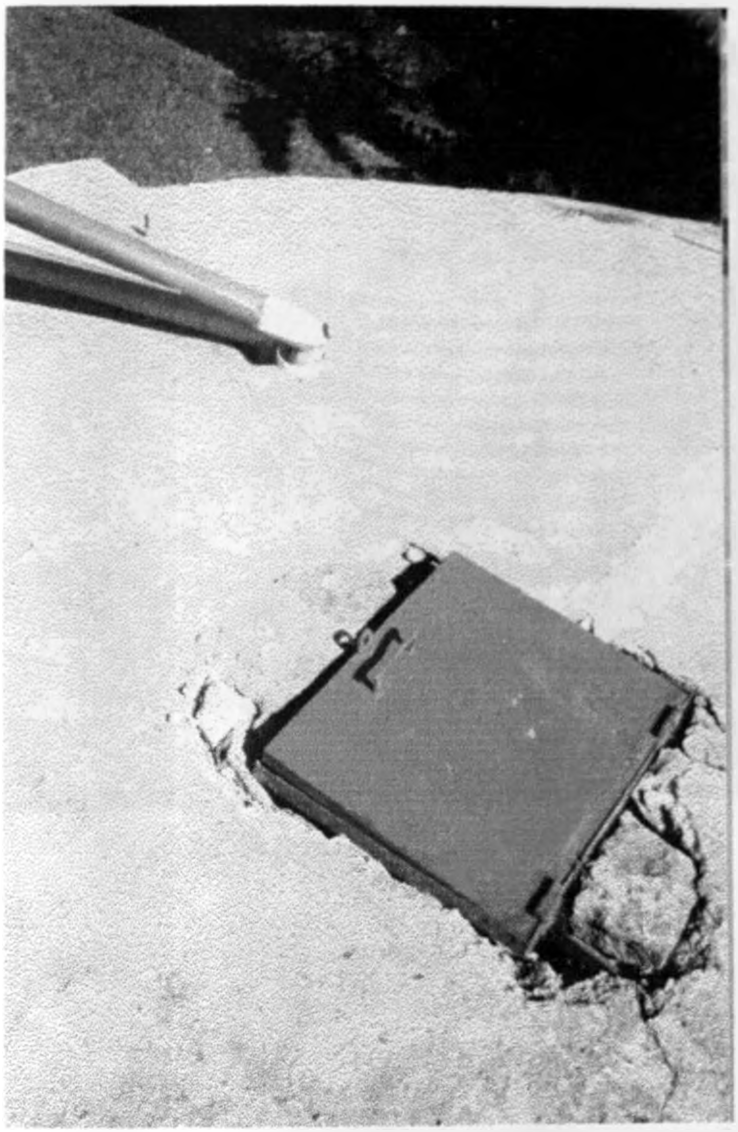


Figure 5: A photograph taken on top of the water tank in figure 4.

Note: the poorly fitted inspection cover and the poorly fitted connection to the connection pipes, which allow contaminants to be washed into the water

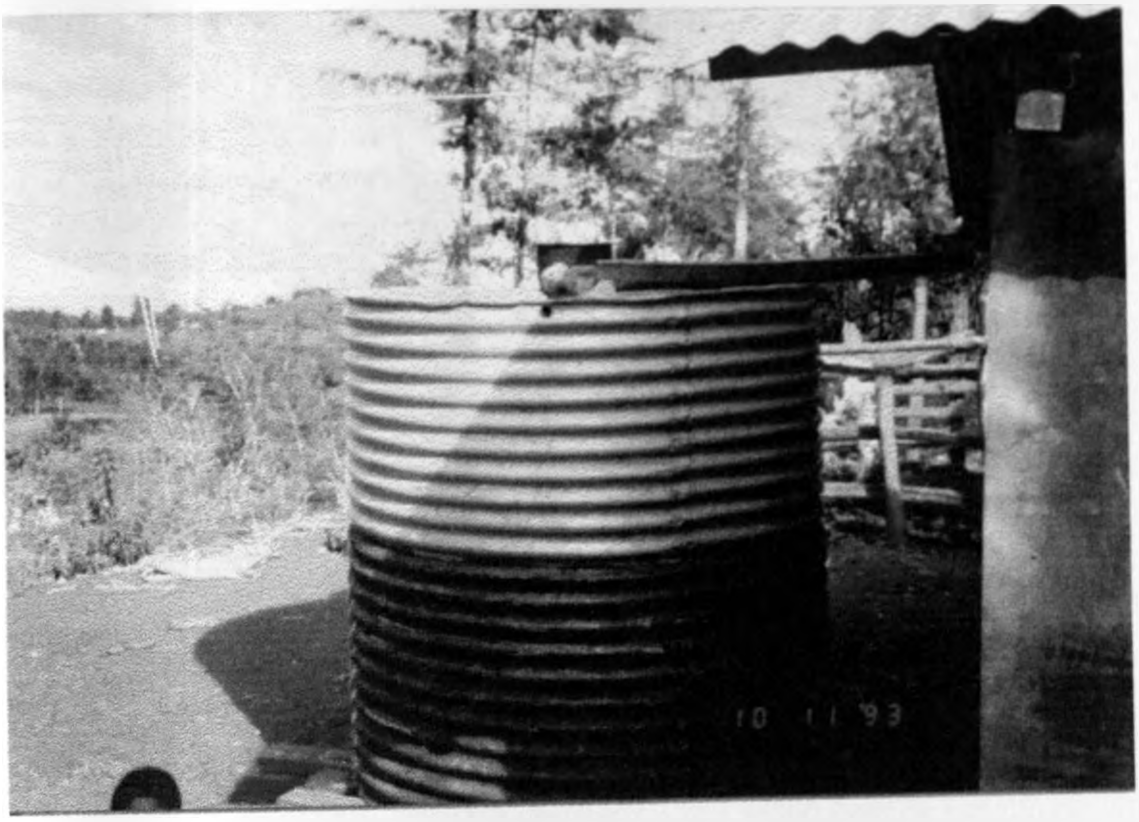


Figure 6: A photograph showing a metal water tank next to a zero-grazing cow shed in Ngong.

Note: The manure accumulated in the cow shed, the open gutters, and the trees overhead. Also note the flat tank roof and the objects on top of it (a cooking pot); all of which allow contamination of the stored water.

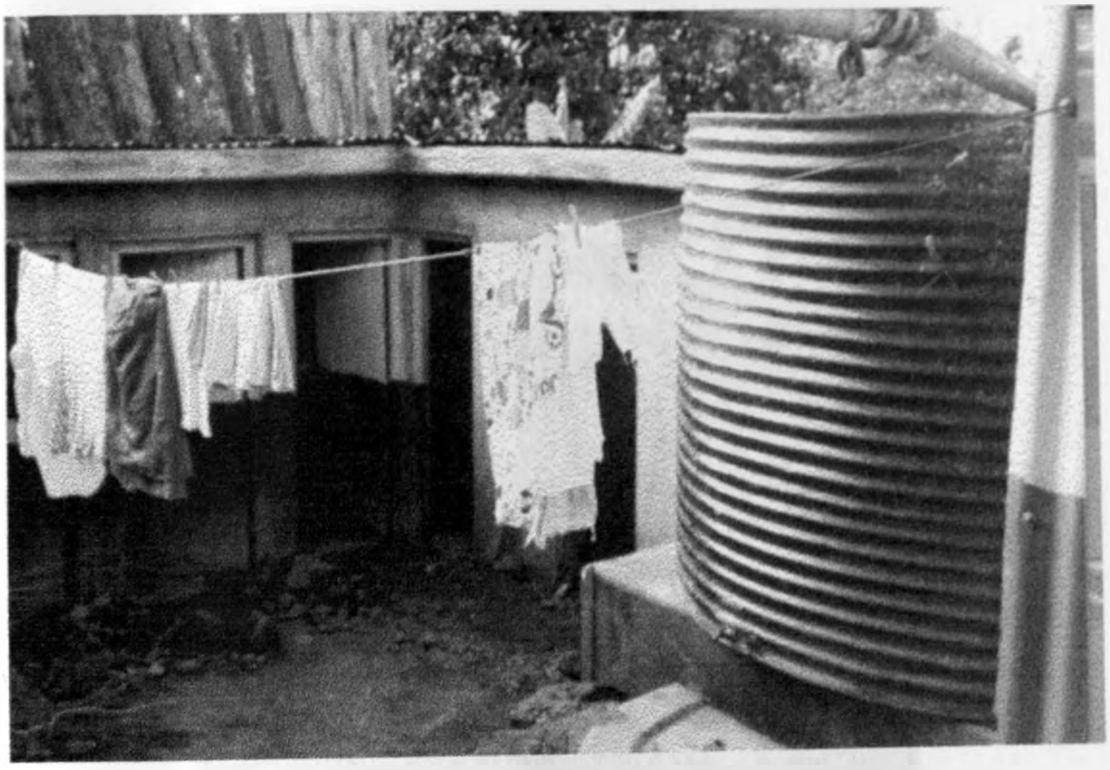


Figure 7: A photograph showing a metal rainwater storage tank in Dagoretti.

Note: The tank has been put up next to a pit latrine. This was in a crowded residential area.

Figure 8: A photograph showing an improperly erected plastic water tank in Kikuyu.

Note: Contamination of the water is introduced through the improperly closing inlet. Immediately next to the tank and the fence (between this property and the next one) is a dust road. Note the open gutters.

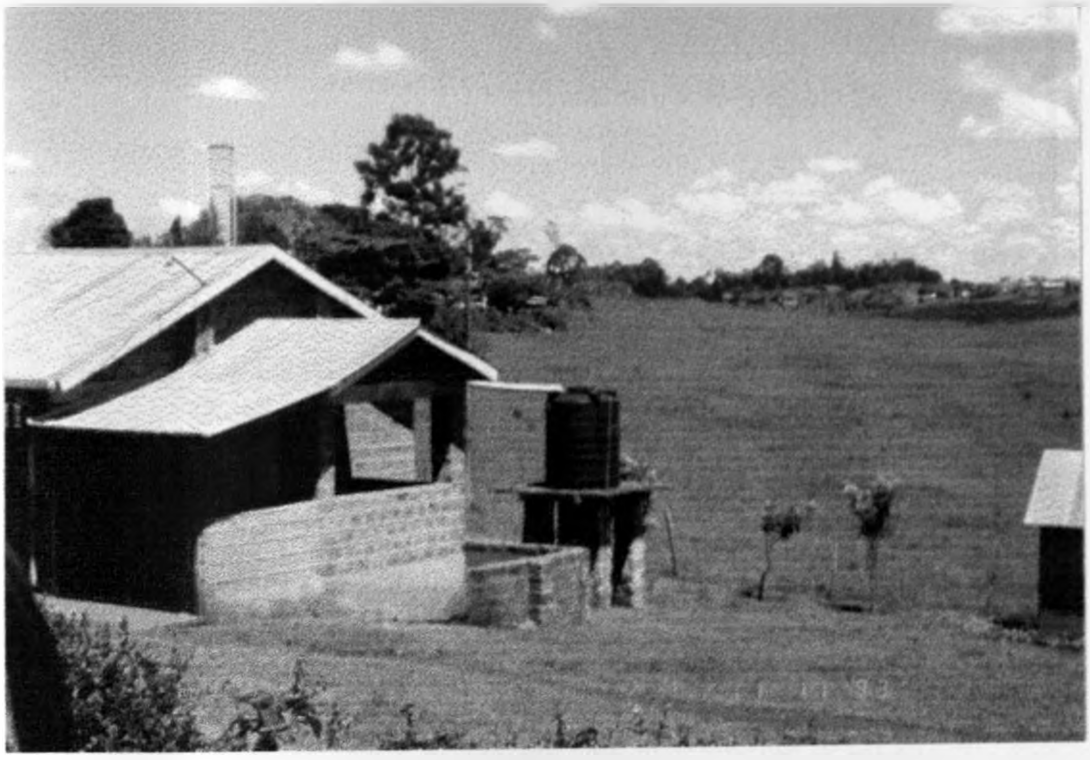


Figure 9: A photograph showing a plastic water tank modified to correct the manufacturer's design error of lack of a proper inlet point.

Note: there was a modification to collect water into the tank without contamination on the tank's roof but there was no allowance to discard the first rainwater washing off the roofs and gutters.

Figure 10: A photograph showing a properly constructed rainwater collection water tank in Ngong Division.

Note: The inlet, inspection and 'breather' points are built into the sides of the sloping top of the tank to facilitate an easy flow-off of contaminants from the surface of the tank during the rains. However, there is the lack of a facility to allow discarding of the first rainwater from the roof and gutters.

4.3. Livestock Density

Cattle, poultry, pigs, sheep, goats, rabbits and donkeys are kept in the Divisions (MOA annual reports, 2001). Except for cattle, the rest of the animals were not uniformly distributed in the study area; only a few farmers reared them as a main income generating enterprise.

Table 1: Types of animals and numbers kept per square kilometre of land in the study area

Division	Area	Cattle	Poultry	Pigs	Sheep and goats	Rabbits	Donkeys
Kikuyu	128	364	2677	220	180	61	27
Ngong	40	1446	2873	176	-	39	-
Dagoretti	38.2	166	691	77	93	40	-

The cattle were kept in zero grazing units within small parcels of land in the backyard or right adjacent to the family house (Figure 6). Cattle contribute large amounts of faeces vis-à-vis the unit area of land. Therefore they were chosen as the livestock density indicator.

Kikuyu Division is 232 Km^2 with 183 Km^2 in agro-eco-zone II but only 70% (128 Km^2) is under mixed agriculture. The cattle density for this Division is 364 per Km^2 (table2). Ngong

Division is 3,142 Km^2 with 40 Km^2 agro-eco-zone II. This zone of Ngong is under mixed agriculture, with a cattle density of 1446 per Km^2 (table 2). Large numbers of cattle, sheep, goats, and donkeys are kept in the predominantly pastoralist zone III and IV of the Division, which are outside the study area. Dagoretti Division is 38.2 Km^2, all of which is in agro-eco-zone II, with a cattle density of 166 per Km^2 (table 2).

Table 2. Cattle densities (animals per Km^2) per Division in the study area

Division	Area (Km^2)	Cattle
Kikuyu	128	364
Ngong	40	1446
Dagoretti	38.2	166

4.4 Total Viable Count

A total of 89 water samples were collected; 29 from Kikuyu Division, 31 from Ngong Division, and 29 from Dagoretti Division. The TVC for Dagoretti ranged from 0 to $1.02X10^7$ (appendix 1.13a) with a mean of $1.665X10^6$ (table 3) and a standard deviation of $2.995X10^6$ (appendix 1.13a). The TVC for Ngong ranged from 0 to $1.02X10^7$ (appendix 1.13b) with a mean of $8.236X10^5$ (table 3) and a standard deviation of $2.545X10^6$

(appendix 1.13b). The TVC for Kikuyu ranged from 0 to $1.02X10^7$ (appendix 1.13c) with a mean of $7.756X10^4$ (table 3), and a standard deviation of $1.837X10^5$ (appendix 1.13c). The TVC for the study area ranged from 0 to $1.02X10^7$ (appendix 1.13d) with a mean of $8.547X10^4$ (table 3) and a standard deviation of $2.278X10^6$ (appendix 1.13d). The mean TVC for Dagoretti Division was significantly higher ($p=0.033$) than the mean TVC for both Ngong and Kikuyu Divisions.

Table 3 Bacteriological quality of rainwater samples from tanks in Dagoretti, Ngong, and Kikuyu Divisions, Kenya

Division	No. water samples	Mean Total Viable Count (MTVC)	Mean Most Probable no. (MPN)	No. with *E. coli*
Dagoretti	29	1,665,000	684	9(31%)
Ngong	31	823,600	652	17(55%)
Kikuyu	29	775,600	729	10(34%)
Total	89	854,700	733	36(40%)

4.5.0 Coliforms

The MPN for water samples from Dagoretti ranged from 0 to 1609 (appendix 1.13a) with a mean of 684(table 3) and a standard deviation of 729 (appendix 1.13a). Those for samples from Ngong ranged from 0 to 1609 (appendix 1.13b) with a mean of 652 (table 3) and a standard deviation of 741(appendix 1.13b) while those for samples from Kikuyu ranged from 0 to 1609 (appendix 1.13c) with a mean of 891 (table 3) and a standard deviation of 729 (appendix 1.13c). The MPN for

samples from the overall study area ranged from 0 to 1609 (appendix 1.13d) with a mean of 740 (table 3) and a standard deviation of 733 (appendix 1.13d). There was no significant difference between the mean MPN for the three Divisions (p=0.4042).

4.4.1 *E. coli*

Of the 89 water samples tested for the presence of *E. coli*, 36 (40%) were positive (Table 3). The proportion of water samples testing positive for *E. coli* was 31% (9/29), 55% (17/31) and 34% (10/29) in Dagoretti, Ngong, and Kikuyu Divisions, respectively (Table 4). There was no difference between the occurrence of *E. coli* in water samples from the three Divisions (p=0.1249).

Seventy-eight percent (78%) of the water samples contained more than 10 coliforms per 100ml of water.

Table 4: The proportions of water samples with either less or more than 10 coliforms per 100mls of water sample among those testing positive for coliforms in each Division

Division	Number of samples	Proportion of samples with less than 10 coliforms	Proportion of samples with over 10 coliforms
Dagoretti	29	8(28%)	21(72%)
Ngong	31	7(23%)	24(77%)
Kikuyu	29	5(17%)	24(83%)
Total	89	20(22%)	69(78%)

Most of the plastic water tanks sampled from were found in Dagoretti. Most of the homesteads sampled from in Ngong and Kikuyu Divisions had concrete and metal-sheet water tanks.

We observed that most of the metal and concrete water tanks were old, some of which were in a poor repair condition.

Table 5: The types of tanks found in the study area

Division	Plastic	Metal	Concrete	Total
Dagoretti	17	9	5	31
Ngong	5	13	11	29
Kikuyu	3	12	14	29
Total	25(28%)	34(38%)	30(34%)	89

5. DISCUSSION

About 97.3% of the water on earth is seawater, 0.65% is fresh water and the rest (2.05%) occurs in the form of frozen water (Anon, 1977). Water is found in the troposphere as vapour and as clouds, with a little water vapour found in a narrow band of the lowest portion of the stratosphere (Dix, 1981). The water in the troposphere is continually being exchanged with the earth through the hydrological cycle. In the troposphere, water vapour condenses to form rain, which is precipitated over land and oceans (Dix, 1981). It is conceivable that pollution can be spread from a source to distant places through the hydrological cycle.

The demographic proportion of the poor in the world is on the increase. We find that among other things, poverty creates urbanization as people move to urban areas in search of employment. Urbanization and poverty creates slums and a general overcrowding in the low-income residential areas, both in the cities and peri-urban areas. The inability of the affected cities to adjust to the increased demand for basic services such as waste disposal and water supply drives people to search for alternative sources of these needs, or to the misuse of what is available. Garbage-strewn streets in

residential areas, slums with overflowing pit latrines and septic tanks, consumption of poor quality water, etc, become common. Poultry and other livestock are kept by these urbanites as a supplement for nutrition as well as an income generation. Waste generation is high, although its disposal is poor. Domestic and sewage wastes are mainly organic material, which under moist conditions are found to be conducive for microbial multiplication.

Rainwater is an important substitute for piped water. The rainwater is collected from roofs into water tanks of houses and dwellings which are constructed from plastic, metal, or concrete (or ferro-concrete) materials. During this study it was found that most water tanks are improperly designed, improperly located, or improperly erected. This exposes the water collected to contamination either at the time of collection, or during storage. Khalid, (1993) reported that livestock and human faeces are probable sources of contamination to water in storage tanks. The human population distribution in the 3 study areas is similar and so it is reasonable to draw such conclusions from this study since the major difference in the possible sources of faecal; contamination of water is domestic animals.

The high incidence of microbial contamination in the water samples collected from the areas poor in tree cover; Dagoretti and Ngong; compared to the tree-rich Kikuyu Division, suggests that wind scatters microbe-bearing dust, especially in the drier periods preceding the rains, onto roofs or flat-roof water tanks. Among other methods, the organisms could also be introduced into the particulate milieu around which raindrops are formed. Dix, (1981) reported that chemical pollutants may be dispersed into the atmosphere through smoke and other exhausts carried in it. Likewise, microorganisms can find their way into the atmosphere through dust particles and other windborne means.

During sampling, it was observed that many exotic trees are planted in agro-residential areas of Kikuyu and Ngong Divisions, for shade, wood and ornamental purposes. Kikuyu Division was seen to have the richest tree cover, followed by Ngong Division. The area of Dagoretti Division from which water samples were taken is a predominantly residential area, with very few trees. The results from this study showed that the mean TVC of water samples from Dagoretti was significantly higher ($p=0.033$) than for both Kikuyu and

Ngong Divisions. Probably, this difference is related to the wind-breaker role of the tree cover, which reduces the distribution of the microbe-containing dust onto the roofs.

In each Division, the Ministry of Agriculture has classified the various zones into 'agro-eco-zones', and also identified the percentage of each zone that is used for agricultural activities (MOA annual reports, 2001). This has made it possible to define the agricultural performance per unit of land per zone, which is more accurate than a reference to the production per unit of the total administrative area of the Division since some of them have large unproductive tracts of land.

Livestock reared in the study area included dairy cattle, poultry, pigs, sheep and goats, rabbits and donkeys (MOA annual reports, 2001). A heavy human population density is found in zones I and II, and this is where a conflict of livestock keeping and public health would be encountered. Purposive sampling of the rainwater was therefore carried out within these zones of the Divisions.

The results obtained indicate that the mean MPN for the three Divisions was almost similar. However, Ngong Division,

which had the heaviest cattle density, showed the highest proportion of water samples containing *E. coli* as compared to the other 2 Divisions. This difference was significant ($p=0.0059$). Khalid, (1993), similarly reported that storage tanks in homes with animals were more prone to microbial contamination than those without.

Of the faecal coliforms, *E. coli* is always present in the faeces of humans and animals. It is rarely found in water which has not been contaminated with faecal matter, and it is therefore an essential indicator of water contamination by faecal matter of human and animal origin. In this study, 40% (36/89) of the samples collected were positive for *E. coli*. Chemuliti (1999), reported that 40% (32/80) of the samples collected from her study in Kibera were positive for *E. coli*, while Khalid (1993), reported that 73% (44/60) of the samples he collected from water tanks in Kiambu were positive for *E. coli*. In this study, samples collected were from roof-trapped rainwater. The samples collected by Chemuliti (1999), were from Nairobi City Council piped water sources while Khalid (1993a) collected water samples of borehole origin. Chemuliti observed that human contamination of the water after it had been fetched from the out-house tanks and stand pipes was an

important contributor to the presence of *E. coli*. Khalid (1993b) observed that contamination from human or animal sources through seepage of contaminants into storage tanks through cracks and loose pipe joints, or contamination by humans during or after fetching from the storage tanks were important factors. Both observations are consistent with the findings of this study.

Kibera is a residential area quite similar to the area of Dagoretti in which the samples for this study were collected. This study showed 30% (9/29) of the samples from Dagoretti to be positive for *E. coli* while Chemuliti in Kibera found 40% (32/80) of the samples to be positive. However, of the 32 samples from Kibera that were positive for *E. coli*, 93.75% were from in-house containers while only 6.25% were from the out-house tanks. Most of the water fetched for storage indoors therefore did not have faecal contamination. Likewise, the rainwater from the roof sources in the livestock-free slum areas such as Kibera and Dagoretti is most probably contaminated by human activity, hence the similarity in the proportion positive for *E. coli*. In Ngong Division where livestock are the most probable cause of

faecal contamination, the proportion of samples with *E. coli* (55%; 17/31) is much higher.

Watt (1986) reported that storing water for several weeks at 10°C-15°C would destroy most of the main disease causing pathogens. During this study, it was noted that the collected rainwater was consumed within only a few weeks after the rains ended. Were it stored for longer durations, the design of most of the tanks would support the survival of micro-organisms in the mud accumulated within the tanks.

Twenty eight percent (25/89) of the storage tanks were plastic, 38% (34/89) metal, and 34% (30/89) concrete. Most of the plastic tanks were relatively new, and had a conically shaped top. However, the usage of the plastic tanks did not necessarily utilize the conical shape to facilitate the washing off of the dust collected on them. Most of the concrete and metal tanks were old. One concrete tank was found to be designed in the modern plastic-tank-design. The old fashioned concrete and metal tanks had flat tops, where dust easily gathered, and easily got washed into the tanks when it rained. A pipe in the wall of the tank, 5 to 10 cm above the base and fitted with a tap, was used to collect the stored water, and

therefore complete cleaning of a tank after the stored water is exhausted is not possible. Water was scooped from shallow tanks and drums using a jug.

It was noted that adequate hygienic measures to protect the water in the storage tanks from contamination were lacking; for example, some tanks were erected near pit latrines, livestock barns, and roadsides; sometimes due to lack of space. The inlets of most storage tanks were not sealed to ensure that contamination of the water in the tank did not happen. Gutters were open and therefore liable to contamination by dust, infesting rodents or lizards, and faeces from overhead birds. It was observed that rainwater was usually not treated before consumption.

To most people, clear odourless water is safe for drinking. However, people sometimes boil or chemically treat discoloured water. It was found that if rotten leaves from overhead trees imparted bad odour to the rainwater stored in tanks, it was boiled before consumption; the foul smell having been associated with some sort of spoilage. The common situation is that the rainwater which is discoloured by dust from the roofs at the time of collection will have

settled in the storage tanks long enough to come out clear at the time of consumption. People therefore take it raw, especially in the belief that what comes from the sky has not been polluted.

Seventy-eight percent (78%) of the water samples contained more than 10 coliforms per 100ml of water, and therefore failed the Kenya Bureau of Standard's requirement for un-piped drinking water (KBS, 1985). Lower numbers of micro-organisms are needed to cause disease when transmitted in water than in other foods, since unlike the latter whose presence in the stomach causes secretion of hydrochloric acid in the gastric juice, which kills some of the bacteria, water does not.

5.1 Conclusion

1. Wind appears to distribute contaminants, including those of faecal origin, onto the roofs from where it is washed into storage water tanks by rain, especially in situations of poor sewage and garbage disposal.
2. Untreated rainwater collected in the study area is unfit for human consumption according to the World Health Organisation's standard for untreated drinking water.

3. Untreated rainwater collected in the study area is unfit for human consumption according to the Kenya Bureau of Standard's requirement for un-piped drinking water.
4. The number of animals kept in an area appears to influence the number of samples testing positive for *E. coli.*
5. Most water tanks are improperly designed, improperly located, and / or improperly erected. Where properly located and designed, there lacks a design to allow the first down pour to wash off the roof and gutters before the cleaner water is collected.
6. Many users of rainwater do not treat it in the assumption that it is safe, and are therefore vulnerable to infections

5.2 Recommendations

1. Water from the study area should be treated before human consumption.
2. An ideal collection of rainwater for human consumption should be based on the following points:
    - Trees should not grow over the catchment roof and gutters
    - The gutters should have an inclination so that water easily flows unhindered to prevent pockets of

dust and debris accumulation. They should also have wire-mesh at the outlet to prevent rodents, lizards and geckos entering the tanks.

- A diversion of the first heavily contaminated rainwater at the beginning of the rains before it gets into the tank should be provided.
- Water should flow from the gutters to the tank through a collection pipe rather than an open gutter.
- The roof of the tank should be sloping to facilitate washing off of any dust, and to discourage placing of objects on the tank
- A policy specifically addressing the design, location and erection of rainwater tanks should be made and enforced to ensure that the growing important rainwater resource does not lead to serious public health problems.

3. Users should be enlightened on the safe harvesting and use of rainwater.
4. Further research should be carried out to assess the presence and importance of faecal coliforms and other pathogens in the water harvested from roofs in Nairobi.

5. **Architects should be compelled to include the design for safe rainwater harvesting receptacles in the designs for their clients.**

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7. APPENDICES

1.1 Preparation of Plate Count Agar (Oxoid, Basingstoke, England).....

	grams per liter
Lab M yeast extract	2.5
Lab M Tryptone	5.0
Glucose	1.0
Lab M Agar No.1	15.0

This agar was prepared by suspending 17.5 grams of the medium in 1.0 liter of distilled water and dissolved by bringing to boil with constant stirring and mixing. It was then sterilized in a pressure cooker at 121°C for 15 minutes and cooled to 55°C. The PH of the agar was 7.0. Approximately 20 ml of the medium were dissolved into sterile petri dishes and allowed to solidify.

1.2 Preparation of Mac Conkey Broth (Biotec, England

	grams per liter
Peptone	20.0

Lactose	10.0
Bile salts	5.0
Sodium Chloride	5.0
Bromocresol purple	0.01

Thirty-five grams of powder was weighed and dissolved in 1 liter of deionised water. It was mixed and dispensed into tubes or bottles with inverted Durham tubes. All were sterilized by autoclaving for 15 minutes at 121°C. The double strength MacConkey broth was prepared by dissolving 70 grams of the powder. The PH was brought to 7.4.

1.3 Preparation of Brilliant Green Lactose Bile (2%) Broth (Oxoid, Basingstoke, England).

	grams per liter
Peptone (Oxoid L37)	10.0
Lactose	10.0
Ox-Bile (purified)	20.0
Brilliant Green	0.0133

Forty grams of the broth were suspended in 1.0 liter of distilled water. The broth was distributed into fermentation

tubes fitted with Durham tubes. The mixture was sterilized in a pressure cooker at 121°C for 15 minutes. The PH of the broth was adjusted to 7.4.

1.4 Preparation of the Eosin Methylene Blue agar (Oxoid, Basingstoke, England)

	grams per liter
Peptone (OXOID L37)	10.0
Lactose	10.0
Dipotassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene Blue	0.06
Agar no.3 (OXOID L13)	15.0

A weight of 37.5 grams of a mixture of the above chemicals was suspended in 1.0 liter of distilled water and boiled to dissolve completely. The mixture was sterilized in a pressure cooker at 121°C for 15 minutes, and cooled to 60°C. The medium was shaken in order to oxidize the Methylene blue (i.e. restore its blue color) and to suspend the precipitate that is an essential part of the medium. The PH of the agar was

brought to 6.8. The agar was dispensed into sterilized petri dishes and left to solidify.

1.5 Preparation of Methyl-Red Voges-Proskauer Medium (MRVP) (Oxoid Basingstoke, England).

	grams per liter
Peptone (OXOID L49)	5.0
Phosphate buffer	5.0
Dextrose	5.0

Fifteen grams of the above medium were put into 1.0 liter of distilled water and then dissolved by boiling. The mixture was sterilized in a pressure cooker at 121°C for 15 minutes, and cooled to 60°C. The PH of the medium was 7.5. The medium was then dispensed on sterile fermentation tubes.

1.6 Preparation of Simmons' Citrate Agar (Oxoid, Basingstoke, England)

	grams per liter
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1.0

Dipotassium phosphate	1.0
Sodium chloride	5.0
Lab M Agar No.2	15.0
Bromothymol	0.08

A weight of 24.2 grams of the above medium were suspended in 1.0 L of distilled water and dissolved by boiling with constant stirring and mixing. The agar was sterilized in a pressure cooker at 121°C for 15 minutes. The PH of agar was 6.8.The medium was distributed into sterile universal bottles and allowed to solidify.

1.7 Preparation of Indole reagent (EHRLICH'S Reagent) (Cowan and steel's 1974)

p-dimethylaminobenzaldehyde	1.0 gram
Absolute Ethanol	95 ml
Conc. HCL	20 ml

One gram of p-dimethylaminobenzaldehyde was dissolved in 95ml of absolute ethanol. Twenty ml of concentrated

hydrochloric acid were added to the solution. The final solution was put in a brown bottle to protect it from light.

1.8 Preparation of Methyl Red solution (Cowan and steel's 1974)

Methyl red	0.04 gram
Ethanol	40 ml
Distilled water to	100 ml

A weight of 0.04 gram of the methyl-red was dissolved in 40ml of ethanol and diluted using 100 ml of distilled water.

1.9 Preparation of 1% Creatine solution (Cowan and steel's 1974)

Creatine	1gram
0.1 N-HCL	100ml

A weight of 1 gram of Creatine was dissolved in 100 ml of 0.1 N-HCl acid.

1.10 Preparation of 40% Potassium hydroxide (Cowan and steel's 1974

Potassium hydroxide (KOH) 40gram

Distilled water to 100ml

Forty grams of potassium hydroxide were added to 100ml of distilled water.

1.11 Preparation of Nutrient Agar

	grams per liter
LAB M Peptone	5.0
LAB M Beef Extract	5.0
Sodium chloride	8.0
LAB M Agar No.2	12.0

Twenty-eight grams of the medium were suspended into 1.0 liter of distilled water. This was distilled by boiling with constant stirring and mixing .It was then sterilized in a pressure cooker at 121°C for 15 minutes. The solution was dispensed into universal bottles and fermentation tubes, and allowed to solidify. The PH of the agar was 7.3.

1.12 Preparation of cooked meat medium

One part LAB. M meat granules is equivalent to 5 parts of fresh ox heart trimmed free of fat.

1.5g of cooked meat medium were added to 20mls of Nutrient broth. The mixer was sterilized in a pressure cooker at 121°C for 15 minutes, and allowed to cool. It was then dispensed into sterile universal bottles.

1.13a: Findings for water samples from the Dagoretti Division

Sample number	TVC	MPN	*E. coli*	Faecal *E. coli*
1	0	2	+	
2	9,200	17		
3	7,800	542		
4	1,120,000	1609		
5	320,000	1609		
6	0	0		
7	9,500,000	0		
8	10,200,000	22		
9	187,000	4		
10	2,210,000	0		
11	1,600,000	2		
12	0	5		
13	0	0		
14	5,800	1609	+	
15	2,400,000	1609		
16	1,340,000	34	+	+
17	1,920,000	1609	+	+
18	41,000	918		
19	169,000	1609		
20	1,320,000	0		
21	1,080,000	130	+	+
22	10,200,000	1609		
23	138,000	1609		
24	2,000,000	542	+	
25	2,500,000	1609		
26	0	918	+	+
27	21,200	542	+	
28	0	1609		
29	0	70	+	+
Range	**0 to $1.02X10^7$**	**0 to 1609**	-	-
Mean	**$1.665X10^6$**	**684**	-	-
Standard deviation	**$2.995X10^6$**	**729.70**		

1.13b: Findings for water samples from the Ngong Division

Sample number	TVC	MPN	*E. coli*	Faecal *E. coli*
1	173,000	1609	+	+
2	10,200,000	240	+	+
3	2,000,000	0		
4	79,000	34		
5	10,200,000	1609		
6	960,000	1609		
7	5,700	1609	+	+
8	1,500,000	1609		
9	16,200	1609	+	+
10	7,200	1609		
11	0	1609	+	+
12	21,400	346	+	+
13	0	1609		
14	0	23	+	+
15	0	0		
16	0	1609	+	+
17	0	33	+	+
18	120,000	109	+	+
19	0	221	+	
20	0	22		
21	0	130		
22	141,000	1609		
23	0	918	+	+
24	0	278	+	+
25	0	130	+	+
26	10,900	2	+	+
27	0	2		
28	4,800	0		
29	8,600	0		
30	85,000	9	+	+
31	0	22	+	+
Range	**0 to $1.02X10^7$**	**0 to 1609**	-	-
Mean	**$8.236X10^5$**	**652.19**	-	-
Standard deviation	**$2.545X10^6$**	**741.30**		

1.13c: Findings for water samples from the Kikuyu Division

Sample number	TVC	MPN	*E. coli*	Faecal *E. coli*
1	0	22	+	+
2	0	2		
3	0	12		
4	0	172		
5	137,000	9		
6	45,000	1609		
7	137,000	1609		
8	128,000	1609	+	+
9	15,000	918		
10	17,300	23		
11	0	918		
12	0	0		
13	800,000	918	+	
14	87,000	918		
15	0	1609		
16	3,100	1609	+	
17	0	1609		
18	7,100	1609	+	
19	5,600	33		
20	43,000	26		
21	91,000	1609	+	+
22	630,000	1609		
23	6,800	1609		
24	92,000	1609	+	+
25	4,400	1609	+	+
26	0	13	+	
27	0	918		
28	0	1609		
29	0	22	+	+
Range	**0 to $8.000X10^5$**	**0 to 1609**	-	-
Mean	**$7.756X10^4$**	**891.07**	-	-
Standard deviation	**$1.837X10^5$**	**728.90**		

1.13d: Findings for water samples from the study area

Sample number	TVC	MPN	*E. coli*	Faecal *E. coli*
Range	**0 to $1.02X10^7$**	**0 to 1609**	-	-
Mean	**$8.547X10^4$**	**740.42**	-	-
Standard deviation	**$2.278X10^6$**	**733.52**		

1.14 McCrady's Statistical table for reading MPN of coliforms per 100ml of water

Number of tubes giving positive reaction out of:			MPN	95% confidence limit	
5 of 10ml	5 of 1ml	5 of 0.1ml		Lower limit	Upper limit
0	0	1	2	0.5	7
0	1	0	2	0.5	7
0	2	0	4	0.5	11
1	0	0	2	0.5	7
1	0	1	4	0.5	11
1	1	0	4	0.5	11
1	1	1	6	0.5	15
1	2	0	6	0.5	15
2	0	0	3	0.5	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	1	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	78
4	3	0	27	9	80
4	3	1	33	11	93
4	4	1	34	12	96
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	114
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	154

5	2	0	49	17	126
5	2	1	70	23	168
5	2	2	94	28	219
5	3	0	79	25	187
5	3	1	109	31	253
5	3	2	141	37	343
5	3	3	175	44	503
5	4	0	130	35	302
5	4	1	172	43	486
5	4	2	221	57	698
5	4	3	278	90	849
5	4	4	345	117	999
5	5	0	240	68	754
5	5	1	346	118	1005
5	5	2	542	180	1405
5	5	3	918	303	3222
5	5	4	1609	635	5805

1.15: **Descriptive statistics for the TVC in the study area**

Feature	Dagoretti	Kikuyu	Ngong
Sample size	29	29	31
Sum	48289000	2249300	25532800
Lower 95% confidence interval	$5.259X10^{5}$	$7.673X10^{3}$	$-1.099X10^{5}$
Mean	$1.665X10^{6}$	$7.756X10^{4}$	$8.236X10^{5}$
Upper 95% confidence interval	$2.804X10^{6}$	$1.475X10^{5}$	$1.757X10^{6}$
Standard Deviation	$2.995X10^{6}$	$1.837X10^{5}$	$2.545X10^{6}$
Standard Error of Mean	$5.562X10^{5}$	$3.412X10^{4}$	$4.571X10^{5}$
Minimum	0.0000	0.0000	0.0000
Maximum	$1.020X10^{7}$	$8.000X10^{5}$	$1.020X10^{7}$

1.16 **ANOVA for the means of the TVC in the study area**

Source	DF	SS	MS	F	P
Between	2	$3.659X10^{13}$	$1.830X10^{13}$	3.52	0.0330
Within	86	$4.465X10^{14}$	$5.191X10^{12}$		
Total	88	$4.830X10^{14}$			

Variable	Mean	Sample size	Group Standard Deviation
Dagoretti	$1.665X10^{6}$	29	$2.995X10^{6}$
Kikuyu	$7.756X10^{4}$	29	$1.837X10^{5}$
Ngong	$8.236X10^{5}$	31	$2.545X10^{6}$
Total	$8.547X10^{5}$	89	$2.278X10^{6}$

1.17: **LSD (T) Pair-wise comparisons of the means of the TVC in the study area**

Variable	Mean	Homogenous groups
Dagoretti	$1.665X10^{6}$	I
Ngong	$8.236X10^{5}$	I I
Kikuyu	$7.756X10^{4}$	I

There are 2 groups in which the means are not significantly different from one another

Critical T value 1.988. Rejection level 0.050. Standard errors and critical values of differences vary between comparisons because of unequal sample sizes.

1.18: **Descriptive statistics for the MPN in the study area**

Feature	Dagoretti	Kikuyu	Ngong
Sample size	29	29	31
Sum	19838	25841	20218
Lower 95% confidence interval	406.41	613.81	380.27
Mean	684.07	891.07	652.19
Upper 95% confidence interval	961.63	1168.3	924.12
Standard Deviation	729.70	728.90	741.33
Standard Error of Mean	135.50	135.35	133.15
Minimum	0.0000	0.0000	0.0000
Maximum	1609	1609	1609

1.19 **ANOVA for the means of the MPN in the study area**

Source	DF	SS	MS	F	P
Between	2	$9.915X10^5$	$4.958X10^5$	0.92	0.4042
Within	86	$4.527X10^7$	$5.380X10^5$		
Total	88	$4.726X10^7$			

Variable	Mean	Sample size	Group Standard Deviation
Dagoretti	684.07	29	729.70
Kikuyu	891.07	29	728.90
Ngong	652.19	31	741.33
Total	740.42	89	733.52

1.20: **LSD (T) Pair-wise comparisons of the means of the MPN in the study area**

Variable	Mean	Homogenous groups
Dagoretti	891.07	I
Ngong	684.07	I
Kikuyu	652.19	I

There are no significant pair-wise differences among the means

Critical T value 1.988. Rejection level 0.050. Standard errors and critical values of differences vary between comparisons because of unequal sample sizes.

1.21: **The major biochemical characteristics of *E. coli* (Holt *et al*, 1994):**

Optimum growth temperature	37^0c
Catalase	+
Oxidase	-
D- Galactosidase	+
Gas from glucose at 37^0c	+
Growth on potassium cyanide	-
Mucate(acid)	+
Nitrate	+

Carbohydrates

Acid production from:	
Adonitol	-
Arabinose	+
Dulcitol	d*(1)
Esculin	D*
Inositol	-
Lactose +or * (2) X	
Maltose	+
Mannitol	+
Salicin	d*
Sorbitol	+
Sucrose	D*
Trehalose	+
Xylose	D*
Other carbon sources:	
Citrate	-
Malonate	-
d-Tartrate	d*
Methyl Red Reaction	+
Voges-proskauer	-
Protein utilization:	

Arginine	d*
Gelatin hydrolysis	-
H_2S from Triple Sugar Metal Medium	-
Indole production	+
Lysine decarboxylation	+
Ornithine	d*
Urea	-
Glutamic acid	-
Phenylalanine	-

d*(1) refers to different reactions by different serotypes.
*(2)X refers to late and irregularly positive (mutative)