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Original Research Article

QUALITY AND MICROBIAL ASSESSMENT OF WATER USED BY THE RESIDENTS OF KABIANGA FROM THE DIFFERENT SOURCES.

Abstract.

Lack of clean piped water in the rural areas is a phenomenon experienced in the third world countries. Microbial assessment of the water quality used by these people becomes of crucial importance as only such tests can indicate the status of water in terms of bacteriological analysis. Techniques used in this research project included test for indicator organism preferable *E. coli*, total viable count, and enumeration of filamentous fungi and yeasts. For bacteriological analysis of the water, indicator organisms were used to indicate the presence of pathogenic microorganisms. The indicator and model organism used was *E. coli.* The report showed that Water obtained from springs and wells were safe for human usage and consumption as it was free from indicators of contamination. The presence of colorless colonies in well water was not considered hazardous because the colonies are well below the lethal colony number which is set to be a hundred colonies per plate. The microbial populations identified showed that the water is contaminated with E. coli an indicator of microbial contamination of water sources. This water is likely to be unsafe to young children and highly immunocompromised people who may suffer from diarrheal related diseases. Spontaneous outbreaks related to water-borne diseases could also be attributed to increase in the number of indicator organisms.

Key words: Microbial, **B**acteriological**, W**ater Quality.

INTRODUCTION

The provision of a safe and sustainable drinking water supply is one of the hallmarks of a successful society. Without safe drinking water, public health is always at risk and the economic wellbeing of the community cannot be realized. High-quality water sources may be required only for drinking purposes, while the quality of water for other domestic uses can be quite variable. Therefore, water polluted to only a certain extent can be considered pure [1]. Lack of clean piped water for the residents of Kabianga has prompted them to seek other means to acquire water. The region, being in the rural areas is thus characterized by rivers, wells, rain harvesters, and springs, which the residents use as their water sources. The springs and rivers in turn serve as direct water sources for the water vendors who bridge the gap and take water to residential homes. The quality of this water from the different sources is yet to be determined even though they believe that their water sources provide good quality. When it comes to wells especially, due to lack of education, they believe that the ground water is of a better quality than any other source including municipal water. With an increasing population density of the study area, the scarcity and pollution of surface water poses a serious problem for urban drinking water supplies of metropolitan areas [2, 5]. Consequently, water resources are a key factor, particularly for planning a sustainable socioeconomic development [6, 7]. Bottled water, however, is being widely consumed because it contains fewer impurities. Therefore, it can also be beneficial to

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detect deterioration in the quality of water resources and to facilitate appropriate and timely corrective actions with a minimal negative impact on public health [8, 11].

For the last three decades, many countries in arid and semi-arid regions have depended heavily on the desalination of seawater to meet their growing needs. Kenya is considered as one of the biggest efficient producers of freshwater from its fresh rivers and lakes [12, 13]. In many parts of Kenya surface water sources (i.e. dams, lakes, and open water reservoirs) are considered to be very common resources and are exploited for almost every use. They are also exposed to urban wastewater disposal from both wastewater stations (that has not reached secured stages 3–4 in most of the wastewater stations 70%, causing an expected environmental pollution especially around metropolitan areas), which has made the surface water resources highly polluted, especially in parched valleys. The frequent outbreaks of waterborne diseases are the result of a direct discharge of untreated or partially treated domestic sewage water sources located beside local gutters [1]. Groundwater is still and will continue to be the main source of safe and reliable drinking water, especially in rural areas in Kenya. Water taken from such sources (different types of shallow and deep wells) is often of better quality than surface water or other open water sources if the soil is fine-grained and its bedrocks do not have cracks, crevices, and bedding plants, which permit the free passage of polluted water especially within metropolitan zones [5, 25]. It is often assumed that natural, uncontaminated water from deep wells is clean and healthy, and this is usually true with regard to bacteriological composition [16]. However, bacterial pollution of water sources may occur and is mostly derived from watershed corrosion as well as drainage from sewage, swamps, or soil with a high humus content. This type of hazard exists particularly in limestone areas where underground chambers or fissures may permit water to flow in the freely moving streams without substantial filtrations. Such suspected water sources cannot be used without caution for human drinking purposes because of the inherent health risks [22, 16, 27, 22, and 29]. The major interest of public health authorities in developing quality standards for urban water uses is focused on the recognition, enumeration, identification, and assessment of microorganisms related to waterborne diseases that are considered indicators of microbiological parameters [20]. These indicators are of great importance to assess the microbial condition of the examined water sources [22]. Moreover, the bio-indicator of faecal pollution is a non-pathogenic microorganism, whose detection suggests the presence of enteric pathogens. Usually, coliforms, faecal coliforms, and faecal streptococci are recognised as the main indicators of microorganisms in water sources [21]. These indicators are of significance to assess the microbial condition of the water supply [23]. Microorganisms as an indicator of faecal pollution should satisfy several criteria [24]. For instance, they should be present in faeces in greater numbers and have more resistance than any pathogen to the stresses of an aquatic environment [26]. The evaluation of total coliforms may sometimes give erroneous information regarding faecal contamination [27, 28]. The main objective of this study was to assess the bacteriological water quality and its geospatial relations of the major urban water sources in the study area (which have been the focus of the community). The findings may be considered as a basis for water health policy decisions at different administrative levels in the study area.

Abbreviations: EMB: Eiosin Methylene Blue; **TSI:** Triple Sugar Iron; **MIO:** Motility Indole Orthidine; **NA:** Nutrient Agar; **SDA:** Sobouard Dextrose Agar

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MATERIALS AND METHODS.

Sample Collection

The samples were collected from households in Kabianga; 4 samples, Kapmaso/Tea farm 8 samples, and Chepnyogaa 8 samples. Random households were selected to provide samples.

Determination of the microbial load.

A serial dilution of 10^{-3} was done for all samples. 1 mL of the sample was transferred into a test tube containing 9 mL of distilled water; from this tube another 1 mL was drawn and transferred to another tube containing 9 mL of distilled water and another 1mL was drawn and transferred to a tube with 9 mL distilled water. At this point the desired dilution was achieved. 1mL of each of the final dilutions was drawn using sterile syringe (each sample) and transferred to a Petri plate which was then added the Nutrient agar media, swirled and left to solidify. The plates were then incubated at 37℃ for 24 hrs and observations recorded

Plates containing SDA amended with 2 mL of penicillin were incubated at 37℃ (for yeasts) and another set at 25℃ for 24hrs and observations recorded

Determination of the bacteriological properties.

In the presumptive test, a series of lactose tubes were inoculated with measured amounts of the water sample to be tested. A series of 3 test tubes in a group of 3 were used to establish the presence of fecal coliforms. 3 tests tubes had double strength MacConkey, and the rest had single strength MacConkey (5 mL). All the tubes had 1mL of the media but with different inoculums. For double strength tubes 10 mL of sample was inoculated, then 1mL of the sample to one set of single strength and lastly 0.1mL to the last set of tubes. They were all incubated at 37℃ for 24 hours.

Confirmative tests

MacConkey agar was streaked using a minute of an inoculum drawn from a test tube that would turn positive. A plate of MacConkey agar was used for one each of the different inoculums used in the presumptive test and incubated at 37℃.

From MacConkey agar the pink colonies that grew were then transferred to a plate EMB agar for the characteristic identification of E. coli the target fecal coliform. These tests were done on the metallic green sheen.

Biochemical tests

Using a sterile straight inoculating wire a minute of the sample was picked and used to inoculate TSI agar by first stabbing through the center of the media to the bottom and then streaking on the surface of the agar slant. The slant was then left with a loose incubated at 37℃ for 24 hours.

For MIO test; using a sterile straight inoculating wire the picked colony was inoculated into the media through a single stab through the media to the bottom and exited through that line. The tube was then sealed slightly and incubated at 37℃.

Simmon citrate agar green in color was also inoculated by streaking the slant using a picked minute colony and the tube incubated at 37℃.

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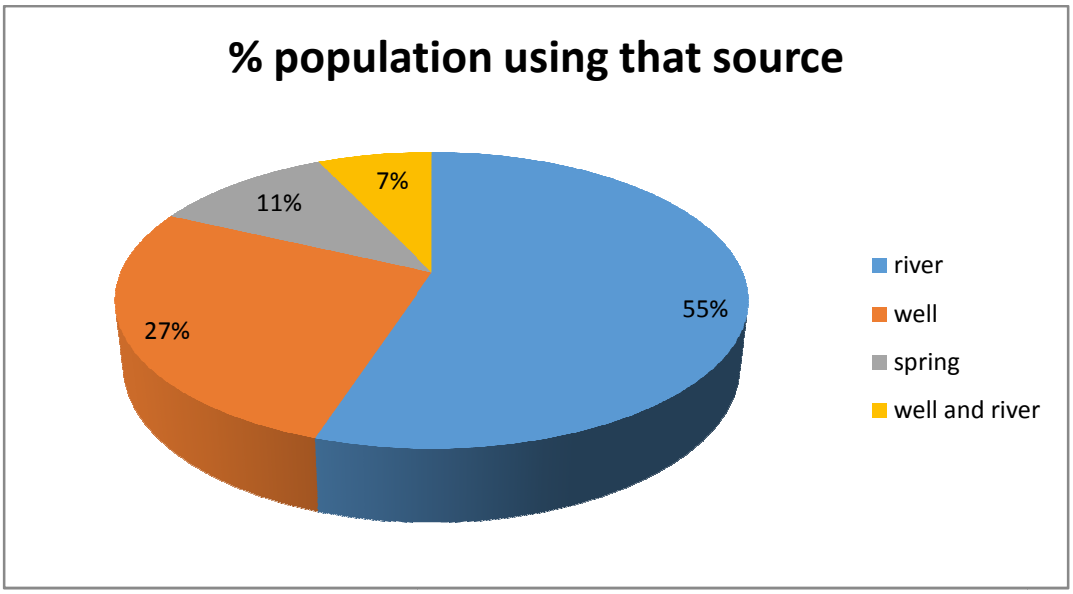
RESULTS AND DISCUSSION

Sources of household water used by the residents of Kabianga

From the data collected 55 % of the residents obtain their drinking water from the river. This source is majorly used by residents living in the Kapmaso-tea farm region and the market area of Chepnyogaa. 27 % of residents get their water from open deep wells that are mechanically pumped. 11 % of the residents obtain their water from the spring found in Kabianga location. 7 % use both well water and river water; these are the residents living in the upper regions of Chepnyogaa and a small population in the lower sides of tea farm.



A total of 20 samples were collected from all the sites. 4 from Kabianga, 8 from Kapmaso/tea farm, and 8 from Chepnyogaa.

Microbial load of water.

Total viable count

This gave out a quantitative analysis of the total bacterial load in water samples (Table 1, 2 and 3). The bacteria use the nutrient agar media components to grow and agar provides a support matrix. Since it is a pour plate technique all sorts of bacteria grow including anaerobic bacteria that appear as small colonies in the media. Anaerobic colonies that grow will grow on the surface. This test however, assumed that each colony originated from a single cell that was in the sample. Since dilutions were made prior to inoculation the diluent factor was put as a factor of the total count using a colony forming unit and since the estimate was made in 100 mL this was also considered.

TABLE 1: River water samples

SAMPLE ID	TOTAL COUNT	CFU/ML
1	37	3.7×10^4
2	21	2.1×10^4
3	55	5.5×10^4

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4	52	5.2×10^4
5	53	5.3×10^4
6	51	5.1×10^4
7	61	6.1×10^4
8	48	4.8×10^4
9	69	6.9×10^4
10	51	5.1×10^4
11	40	4.0×10^4

TABLE 2: Well water samples

1	31	3.1×10^4
2	27	2.7×10^4
3	22	2.2×10^4
4	35	3.5×10^4

TABLE 3: Spring water samples

Sample ID	Colony count	CFU/ML
1	6	6.0×10^3
2	4	4.0×10^3
3	5	5.0×10^3
4	3	3.0×10^3
5	3	3.0×10^3

Bacteriological analysis of water for portability

In this test lactose broth with inverted Durham tubes was the media used. The presence of coliforms in the media was indicated by gas production and change in color of the media from yellow to turbid pale. This is because coliforms have the ability to ferment the lactose in the media raising the pH and producing carbon IV oxide which are indicated by the change in color and presence of gas bubbles in the Durham tubes respectively. Out of the twenty samples that were tested for the presence of coliforms, 15 samples showed positive results. 5 samples showed neither gas production nor change in media color. Other four showed change in media color but no gas production (false negative/positive (Table 4).

TABLE 4: Most probable number

SAMPLE ID	**Number of tubes showing positive reactions.**			
	3tubes of 10ml each	**3tubes O1ml each**	**3 tubes of 0.1ml each**	**MPN INDEX**
1	**3**	**3**	**3**	**1100**
2	**3**	**3**	**3**	**1100**

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3	**3**	**3**	**3**	**1100**
4	**3**	**3**	**3**	**1100**
5	**3**	**3**	**3**	**1100**
6	**3**	**3**	**3**	**1100**
7	**3**	**3**	**3**	**1100**
8	**3**	**3**	**3**	**1100**
9	**3**	**3**	**3**	**1100**
10	**3**	**3**	**3**	**1100**
11	**3**	**3**	**3**	**1100**
12	**3**	**3**	**3**	**1100**
13	**3**	**3**	**3**	**1100**
14	**3**	**3**	**3**	**1100**
15	**3**	**3**	**3**	**1100**
16	**0**	**0**	**0**	**3**
17	**0**	**0**	**0**	**3**
18	**0**	**0**	**0**	**3**
19	**0**	**0**	**0**	**3**
20	**0**	**0**	**0**	**3**

Confirmative tests for presence of coliforms.

15 samples were tested for the confirmation of true fecal coliforms. The tubes in the presumptive test were streaked in MacConkey Agar with NaCl which prevents the swarming of proteus species. Out of the 15 test tubes that were confirmed in this test, 11 showed flat pink colonies and pink Mucoid colonies growing on MacConkey media. The other 5 had pink Mucoid colonies only.

MacConkey agar acts as a selective and a differential media. It actively selects gram negative and inhibits the growth of gram positive bacteria as a result of bile salts and as a differential media it differentiates between lactose fermenters and non-lactose fermenters. Lactose fermenters are characterized by localized pink colonies due to production of mixed acids byproducts which reduce the pH of the media while none lactose fermenters by colorless colonies. In the same media E. Coli the target coliforms grows with a distinctive flat pink

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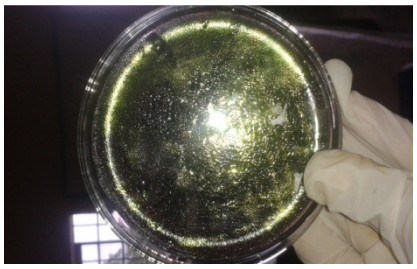
colonies while other coliforms like Enterobacter species have Mucoid colonies. The pink colonies are then cultured in EMB agar to confirm the presence of *E. coli* (Figure 2).

Results in EMB Agar to confirm the presence of E.coli.

The 11 samples with pink colonies in MacConkey agar were inoculated in EMB agar to confirm the presence of *E. coli.* EMB agar is a sensitive media and is both a selective and differential media used to isolate fecal coliforms. The presence of the fecal coliform E. coli is indicated by metallic green sheen that tend to spreads i.e. not localized (Figure 1). The metallic sheen is due to the metachromatic effects of the dyes in the media, flagella movement of the bacteria and an indicator of vigorous lactose/sucrose fermentation.

All the 11 samples showed the characteristic metallic green sheen and hence E. coli was confirmed to be present. The sheen was then biotyped in an IMViC series of tests.



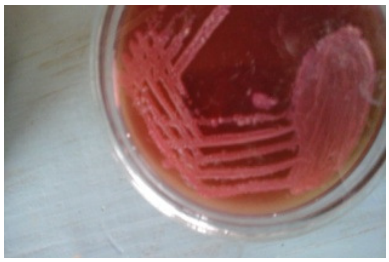


Figure 1 **Figure 2.**

The media used in these tests were: Triple Sugar Iron (TSI, Motility media, Indole/motility, and Simmon citrate agar. TSI media had a butt and slant while Simmon citrate had a slant. Different strains of E. coli react differently in biochemical media and thus it is possible to identify the number of strains available. Reactions on biochemical media

i. TSI

This is a differential media that contains lactose, sucrose, a small amount of glucose, ferrous sulfate and pH indicator Red. It differentiates microorganism on their ability to ferment the three sugars, produce gas or hydrogen sulfide. Prior to inoculation the media is Red in both slant and butt. After inoculation the following changes occur; when the media changes from red to yellow in both slant and butt that is deducted as positive slant and butt meaning that the microorganism can ferment all the three sugars. A negative slant (Red indicates that the organism only ferments one sugar I.e. dextrose. Gas production is indicated by the cracks seen in the media. Positive butt and slant, Gas production with no Hydrogen sulfide

ii. MIO

This media has three parameters which it tests, motility, Indole production, and ornithine-decarboxylase. Itcan distinguish enteric Bacilli based on their reactions to affirm to the parameters indicated. Motility is exhibited by growth of the inoculants from the line the line of inoculation outwards. Tryptophan, present in the basal media, is degraded by organisms that possess the enzyme tryptophinase. Degradation of tryptophan produces indole. The kovac'sreagent added to the media a compound called p-dimethyllaminobenzaldehyde that reacts with indole to form a red quinodial compound to indicate a positive Indole test. Ornithine is

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tested using the ability of an organism to possess the enzyme decarboxylase which degrades ornithine to produce a byproduct called diamineputrescine which causes an alkaline shift turning the media from purple to dark purple. This happens with E. coli. For other coliforms like Klebsiella which can ferment dextrose but do not have decarboxylase the acid produced causes the media to change to yellow throughout or a yellow with a purple top indication that that environment is acidic.

iii. Simmon citrate

This media tests the ability of an organism's ability to utilize citrate as the sole carbon source. Organisms that can utilize citrate do so by using the enzymes citrase or citrate-permease and the give out a blue colored slant due to a raise in pH from acidic to alkaline. E. coli is unable to utilize the sodium citrate in the media and hence shows neither color change nor growth in the slant. The results are tabulated in Table 5.

Table 5: Biochemical reactions

SAMPLE ID	TRIPLE SUGAR IRON				MIO			CITRTATE
	BUTT	SLANT	GAS	H2S	MOT	IND	ORN	
CONTROL	-	-	-	-	-	-	-	-
1	+	+	+	-	+	+	+	-
2	+	+	+	-	+	+	-+	-
3	+	+	+	-	+	+	+	-
4	+	+	+	-	+	+	+	-
5	+	+	+	-	+	+	+	-
6	+	+	+	-	+	+	+	-
7	+	+	+	-	+	+	+	-
8	+	+	+	-	+	+	+	-
9	+	+	+	-	+	+	+	-
10	+	+	+	-	+	+	+	-
11	+	+	+	-	+	+	+	-

CONCLUSSION

Water obtained from springs and wells was concluded to be safe for human usage and consumption as it was free from indicators of contamination. The presence of colorless colonies in well water is not considered hazardous because the colonies are well below the lethal colony number which is set to be a hundred colonies per plate. However boiling of the water or treating the water could make it safer River water is not safe for human consumption without further treatment. The microbial populations identified showed that the water is contaminated with E. coli an indicator of microbial contamination of water sources. However the residents do not normally get sick when they consume the water probably because of adaptation and high immunity. This water is likely to be unsafe to young children and highly immunocompromised

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people who may suffer from diarrheal related diseases. Spontaneous outbreaks related to water-borne diseases could also be a result of further increase in the number of indicator organisms.

Residents who drew water from the river in the early morning hours had safer water than those who did so in late afternoons and evenings. This is probably from the disturbance of water by people and animals alike and hence reduces risk of infection.

Tenwek mission hospital has done appraisable work in providing cleaner healthy water to the residents through the provision of biofilters. Residents using biofilters spend less income in purchasing water and probably in hospital bills.

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