

**WATER QUALITY AND DISTRIBUTION OF PATHOGENIC MICROORGANISMS
ALONG KABIANGA RIVER WATER BETWEEN JANUARY AND MARCH 2016 IN
KERICHO COUNTY, KENYA.**

ABSTRACT

Safe and clean water is of major concern to mankind because it is directly linked with the health and their wellbeing. The disposal of different kinds of pollutants and nutrients through sewage, agricultural runoff and industrial effluents into rivers and water bodies effect a sequence of changes in its physicochemical characteristics. Kabianga River is known for provision of clean drinking water, a habitat for many aquatic plants and living organisms and irrigation water to farmlands. Excessive industrialization and consequent urbanization has led to several problems of water quality management of the river. This research was aimed at assessing the quality of Kabianga river to ascertain the levels of microorganisms and if it meets the local and international microbiological standards. Standard bacteriological techniques was used to describe bacteria content from water samples. The water samples were collected during rainy and dry seasons. The results indicates that water is highly contaminated with human pathogenic bacteria. The most dominant bacteria in both seasons was *Escherichia coli*, *salmonella*, *shigella*, *proteus*, *pseudomonas aeruginosa*. From the findings it can be concluded that the water from this river is not fit for human consumption or for domestic uses. Therefore regular monitoring of the water microbiological quality and public health education should be carried out to sensitize the residence on the dangers of using the polluted water.

Key words: Pathogens, Microbiological, Coliforms, Contamination,

INTRODUCTION

The causes of climate change and many diseases is pollution, this results to emergence of many contagious diseases. Water pollution is responsible for many of such death which is estimated to be about 14,000 people every day. Water pollution can be defined according to online dictionary as any damage in its natural features by adding anthropogenic pollutants, which interrupt the normal usage of water. This can be achieved through drinking directly by humans or by supporting biotic communities. This problem of water contamination is alarming in third world countries compared to industrialized ones. The main variations in the ecological position and quality of water due to natural disasters like earthquakes and tremors, volcanic activities, algal blooms and storms are also contributing factors. Fresh water is limited resource, vital for agriculture, industry and even human existence, without fresh water of adequate quantity and quality, sustainable development will not be possible [1]. The buildup of many contaminants and nutrients through dirt, industrial seepages and sewages and unindustrialized overflows into the water bodies results into a series of variations in the physicochemical and features of water, which are the subject matter of several inquiries. This pollution are caused widely due to Mechanization and abstraction of natural resources. The main area of concerns in the whole world today is pollution of air, soils, underground water, sedimentation, and surface water runoff. The recommendations of the possible solutions for contamination of these natural resources has resulted to the development of novel know-hows that put importance on the demolition of the contaminants and impurities relatively instead of the conservative method of dumping. This is due to their probability to get into the food chain. High concentrations of insecticides and pesticides have been identified and reported in air, water, soil and in foods among

others. Many of such contaminations have also been reported to be toxic, mutagenic, and carcinogenic and tumorigenic which means a more new approach needs to be followed [2].

Pollution of river waters with deleterious microbes, including bacteria, viruses, parasites as well as fungi has been in a steady increase in the recent past. The major source of microbes in water is feces from human and other animals. Entry of pathogens into rivers can occur either from a point source, non-point source or both. Non-point source microbial pollution of rivers occurs from rainwater surface run-offs, storm sewer spillages or over flow, while point source pollution comes from discharge of untreated or partially treated effluents from waste water treatment plants. The impact of river pollution on human depends mainly on the water use, as well as the concentration of pathogen in water. In order to reduce water borne disease outbreaks, World Health Organization (WHO) developed microbiological quality guide lines based on intended water uses. The guidelines stipulates, that fecal coliforms (FC) should not exceed 103 per 100 mL of water to be used in irrigation of crops that are eaten uncooked, sports fields and public parks in unrestricted regions (WHO,2004). Environmental Protection Agency (EPA) is stricter and requires zero (0) FC/100 mL of water to be used in irrigation of crops not commercially processed including crops eaten raw. Kenya standard of drinking water quality states that no *Escherichia coli*, *Shigella*, *pseudomonas aeruginosa* or coliforms should be detectable in 250 mL of drinking water. Bacteriological threat valuation and measurement of water worth is an urgent concern which focuses on different legislations of the governmental and scientific inquiry and validation. [3]. Bacteria, protozoa and viruses are universal in aquatic settings. Since most of this pathogens are important biologically and can serve the vital environmental functions, many are harmful to the ecology and human health. Polluted drinking water with pathogens has been a concern in people's infection and for a long time attention has concentrated on end product. However, such tactics are progressively observed as inadequate and not sufficient to prevent occurrences of sickness among many people. Research has shown that more attention has been paid to preventing illness from source-to-tap rather than the causal agents themselves [4, 5]. Besides, it has been shown that poor bacteriological water quality is not safe for aquatic life and other ecosystem function [5, 6, and 1]. It can therefore be deduced that people's health and environmental health are obliquely related, mostly if pathogenic risk are assessed along the source-to-tap spectrum [7, 8].

An inclusive thoughtful and well calculated understanding of the dangers and risks of both existing and possible dangers of polluted water versus its quality should be assessed. This can be achieved through assessment of the entire water supply system. The concept 'source-to-tap', is where a high value source, in combination with most effective treatments and distribution can yield water which is safer for consumption. Therefore as a milestone of water quality risk assessment [9, 10], the source-to-tap basis has been mobilized for use with a focus on human health. These interventions which include the entire source-to-tap gradient are most probable to successfully report not only point-source contamination of water but also the more composite influence of non-point sources of water pollutants within a given watershed [11]. Complete risk valuation and administration is critical for both drinking water provision [4, 12, and 13] as well as larger environment protection [7]. Thus risk managing in a source to tap framework is believed to be serious given the appreciation that hazards are countless and resources to deal with them are inadequate [14, 15, 12 and 9]. This research was therefore aimed at characterization and determination of microbial concentration in Kabianga River during the rainy and dry seasons to ascertain its quality for domestic uses. This will help both the policy makers and implementers to ascertain the safety of the water and hence come up with a better solution or recommendations

which can alleviate or reduce human death due to water contamination and microorganisms pollutions.

Abbreviations: MPN: Most Probable, EMB: Eosin Methylene Blue, XLD: Xylose Lysine Deoxy cholate.

MATERIALS AND METHOD

Sample Collection

Samples were collected from Kabianga River Kericho West sub- county Kenya, and were analyzed at university of Kabianga microbiology laboratory. Water samples to be analyzed were collected in sterilized 250 mL plastic bottles pre-cleaned and rinsed in distilled water. After collection the samples were filtered and kept at 4°C, in a refrigerator.

Isolation of Microorganisms

The number of coliforms in water were determined by statistical estimation. Most probable number (MPN) test was used. This test involved a multiple series of Durham fermentation tubes and was divided into three parts: The presumptive, Confirmed and Completed test. In presumptive test, dilution from the water samples were added to lactose broth fermentation tubes. Water was poured in each tube to a volume of 10 mL in double strength lactose broth. The tubes were incubated at 35°C and observed after 24 hours and 48 hours for evidence of gas production. In the confirmed test one transfers material from the highest dilution of those lactose broth tubes that showed growth and gas production were streaked onto Eosin methylene blue (EMB) agar plates. EMB prevents growth of Gram-positive organisms. Coliforms which are Gram- negative produced acid. Under acidic conditions the Eosin and methylene blue dyes were absorbed by the organisms of a colony. In the completed test, the colonies formed were inoculated into lactose broth and agar slants. The production of acid and gas in the lactose broth and microscopic confirmed the identity of Gram –negative on-spore-forming rods from slants, this constituted a positive completed test.

Isolation and Identification of Coliforms.

The media was prepared aseptically and dispensed in the plates. Samples from the positive completed test were cultured on the EMB. The colonies that grew on EMB appeared pink mucoid and entire. Green metallic sheen. Pink colonies with dark center and pink/white colonies. The colonies from EMB were further sub-cultured in MacConkey, *Salmonella shigella* media and Xylose Lysine Deoxycholate agar (XLD) and observations made.

Isolation using MacConkey

The colonies that grew in EMB were further sub-cultured in MacConkey. These colonies were further sub-cultured in *Salmonella shigella* media.

125 **Xylose Lysine Deoxicholate (XLD) Agar**

126 This was a selective media for isolation of *Salmonella and shigella* species.

127 **Motility**

128 The colonies from EMB, were picked and stabbed into the media. Non -motile colonies only
129 grew on the stab. The motile grew along the stab and were swum out away from the stabbed
130 area.

131 **Morphological and Biochemical Tests**

132 Microorganisms' morphologies were classified by direct examination with the light microscope
133 and the staining characteristics by gram stain, which classified microorganisms into Gram
134 negative and morphologically as *Bacilli*.

135 **Oxidase Test**

136 Oxidise discs were moistened using distilled water then a colony was picked from EMB plate
137 those that were appearing pink dry and from MacConkey plate that appeared pink and those
138 from XLD plate were smeared on the discs. After 3min an observation was done.

139 **RESULTS AND DISCUSSION**

140 **Presumptive Test**

141 The results of the presumptive tests are as in Table 1. There was growth in almost all the tubes
142 which was a positive presumptive test with higher MPN of 460. This corresponds to cfu of
143 lactose fermentation. This bacteria were presumed to be fecal coliform. During the rainy seasons,
144 all the tubes had higher MPN with highest being 1100 (Table 2).

145 **Table 1: Presumptive Test dry season**

Sample points	Number of positive tubes	3 tubes BSLB 10 mL	3 tubes SSLB 1 mL	3 tubes SSLB0.1mL	MPN
Sample1	T1	3	3	1	460
	T2	3	3	1	460
	T3	3	2	1	150
Sample 2	T1	3	3	0	240
	T2	3	2	0	93
	T3	3	2	1	150
Sample 3	3	3	3	1	460
	3	3	3	1	460
	3	3	2	1	150

146 **Table 2: Presumptive test Rainy season**

Sample points	Number of positive tubes	3 tubes BSLB 10 mL	3 tubes SSLB 1 mL	3 tubes SSLB 0.1 mL	MPN
Sample1	T1	3	2	2	210
	T2	3	3	2	1100
	T3	3	2	2	150
Sample 2	T1	3	3	2	1100
	T2	3	2	0	93
	T3	3	2	2	210
Sample 3	3	3	3	2	1100
	3	3	3	2	1100
	3	3	3	2	1100

147 **Confirmed Test**

148 In the confirmed test one transfers material from the highest dilution of those lactose broth tubes
 149 that showed growth and gas production were streaked onto Eosin methylene blue (EMB) agar
 150 plates. EMB prevents growth of Gram-positive organisms. Coliforms which were Gram-
 151 negative produced acid; under acidic conditions the Eosin and methylene blue dyes were
 152 absorbed by the organisms of a colony.

153 **Completed Test**

154 In the completed test, the colonies formed were inoculated into lactose broth and agar slants. The
 155 production of acid and gas in the lactose broth and microscopic indicated the presence of Gram -
 156 negative, non-spore-forming rods from slants constitute a positive completed test.

157 **Eosin Methylene Blue Agar (EMB)**

158 There was growth of different colonies in the media .The colonies were pink with dark center
 159 (Figure 1), mucoid and entire margin, and greenish with metallic sheen and pink mucoid with
 160 entire margin. Since EMB is a selective media for gram negative bacteria the bacteria which
 161 fermented lactose in the medium formed colored colonies, while those that did not ferment
 162 glucose appeared as colorless colonies. EMB agar was used to distinguish coliforms and fecal
 163 coliforms that signal the possible pathogenic microorganism contamination in water samples.
 164 EMB was also used to differentiate in the colon-typhoid -dysentery group: *Escherichia coli*
 165 colonies grew with metallic sheen, with dark Centre and non-lactose fermenting gram negative
 166 bacteria appearing pink.

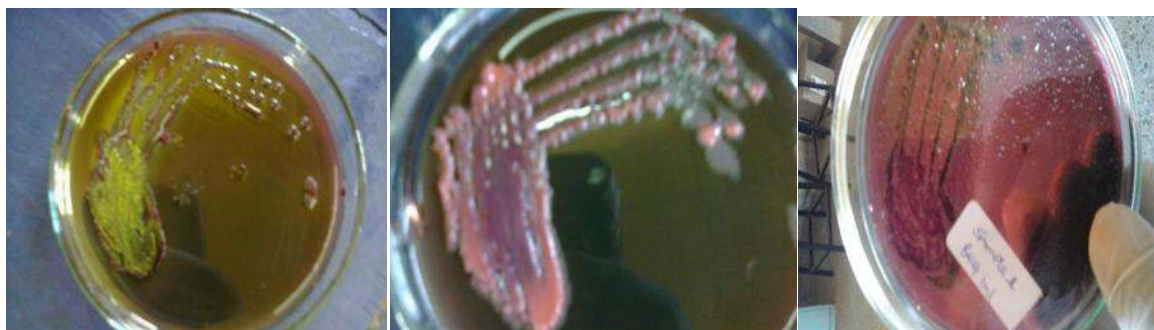


Figure 1: Growth of different media

Using MacConkey Results

The colonies that were transferred from EMB were grayish small mucoid with entire margin, pink large mucoid with entire margin, dry rough pink colonies, however in another plate the pink colonies were small and large with the same characteristics, and since MacConkey is a selective media for isolating gram-negative bacteria and other enteric bacilli, The agar distinguishes those gram negative bacteria that can ferment glucose from those that cannot ferment glucose. Lac bacteria such as *Escherichia coli* and *klebsiella* produced acid which lowered the pH of the agar to 6.8 and resulted in appearance of pink colonies. Lac-Non-lactose fermenting bacteria *salmonella*, *proteus* species, *pseudomonas eurogenosa* and *Shigella* did not use lactose but used peptone water. This forms ammonia, which raises the PH of the agar and leads to formation of white/colorless colonies on the plate. Some organisms, especially *klebsiella* produced mucoid colonies.

Xylose Lysine Deoxycholate Results

The pink dark centered colonies from EMB plate, appeared white mucoid with entire margin, white mucoid pulvinate with an entire margin. The media turned yellow. XLD is a selective medium used in the isolation of salmonella and *Shigella* species in water. It had a PH of 7.4 leaving it with a bright pink or red appearance due to the indicator phenol red, sugar fermentation. *Salmonella* fermented the sugar xylose to produce acid *Shigella* colonies which remained red. Salmonella metabolized thiosulphate to produce hydrogen sulphide which lead to the formation of colonies with black centers. This differentiated them from similarly colored *Shigella* colonies.

Salmonella Shigella Results

The colonies from XLD plate that appeared white were transparent large and entire colonies in SS media. Dark centered pink colonies in XLD plate were transparent/ white mucoid colonies. The pink colonies in XLD plate were pink and dry (Figure 2).



Figure 2: Salmonella Shigella plates showing growth of microorganisms.

The colonies that appeared pink in EMB, turned black raised mucoid colonies indicated presence of *Salmonella* and the pink colonies indicated the presence of *Shigella*. The colonies in MacConkey were pink with a characteristic smell. *Salmonella Shigella* agar is selective and differential media widely was thus used to isolate it. Inhibition of gram positive microorganisms was obtained by bile salt mixture. Members of the genus *Salmonella* did not ferment lactose but produced hydrogen sulphide gas. The resulting bacteria colonies appeared colorless with black centers. *Shigella* did not ferment lactose or produced hydrogen sulphide gas the resulting colonies were colorless (Figure 3).



Figure 3: Plates with growth of *Salmonella*

Sulphide and some pink colonies in the plate indicates presence of *Shigella*, they do not produce hydrogen sulphide.

Motility

The positive samples from presumptive test, were cultured in motility media. The samples from points 1 and 3 showed positive results for motility. Motile organisms extended from the stab line and produced turbidity throughout the media. Non-motile organism from sample point 2 grew only along the stab line and leave the surrounding media clear. The two samples that showed presence of motile organisms were from sample point 1 and 3 during rainy season. This was attributed to the fact that more runoffs enter the river at these points and that the population around these point are high compared to sample point 2. The isolated organism was *Proteus* spp.

Morphological and Biochemical Tests

Microorganisms displayed diversity of cell arrangements. Microorganisms' morphologies were classified by direct examination with the light microscope and the staining characteristics by gram stain, which classified microorganisms into gram negative and morphologically as *Bacilli*.

Biochemical tests

These tests was used to test the ability of microorganisms to ferment sugar present in the media or its ability to use other components like citrate in Simmons citrate media as a source of carbon and energy. Positive tests were reported. The tubes which were inoculated with colonies from SS had a blue slant. The small mucoid colonies was obtained from XLD plate. The only organism that utilizes the citrate and not the sugar in TSI is *Pseudomonas aeurogenosa*. Simmons citrate blue was a positive citrate test while green was a negative/no growth (Figure 4 and 5). The increase in pH caused the color change in the bromothymol blue indicator turning it to blue. Under neutral conditions the medium remained green color, the color change to blue is useful because the growth on Simmons citrate agar is often limited and would be hard to observe if it were not for the color change. Bacteria that develop in such media are *Salmonella* and *Providencia*.



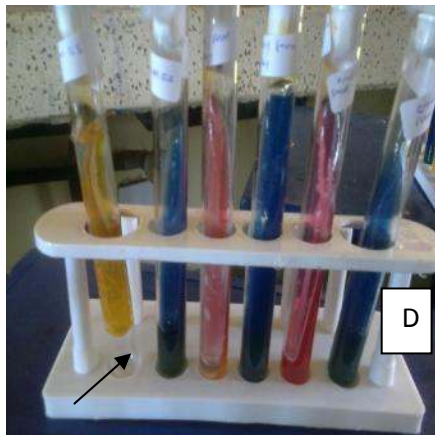
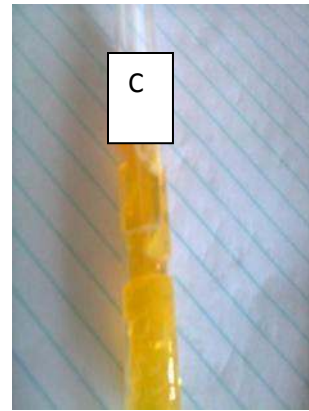
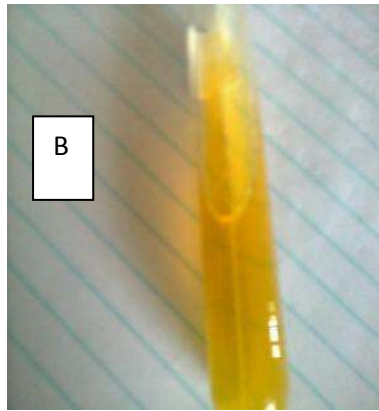
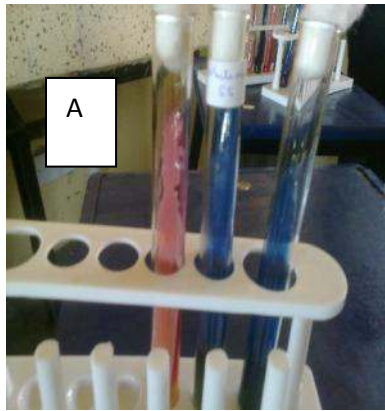
Figure 4: Positive tube



Figure 5: Negative tubes

Triple Sugar Iron Tests

The colonies that were transferred from XLD plate which were small pink colonies, appeared red indicating that there was no utilization of the sugars. The only organism that do not utilize sugars is *pseudomonas aeurogenosa*. Others, were able to utilize either glucose or sucrose appearing as red slant and yellow butt or yellow slant with yellow butt. Triple sugar iron test, tests the ability of microorganisms to ferment sugar and produce hydrogen sulfide. It is used as selective identification of enteric bacteria including salmonella and *Shigella*. Blackening of the butt was due to production of hydrogen sulfide due to utilization of glucose only. Carbon dioxide production was recognized as bubbles of gas between the agar and the wall of the tube or within agar itself. The carbon dioxide production is sufficient to split the agar into two or more sections. The microorganisms that were isolated, none of them produced hydrogen sulphide.



KEY

A- Tube 1 from left red slant yellow butt, no gas production only glucose utilized -organisms *Salmolla and Shigella*.

B-Yellow slant yellow but no gas production all sugars utilized organism-*serratia*

C-Yellow butt red slant there was gas production only glucose utilized organism-*Escherichia, Shigella*

D-Tube 1 from left Yellow butt yellow slant gas production-organism *Enterobacter, Klebsiella*.

Tube 3 and 5 from left-No sugar was utilized entire tube red that is red slant red butt-organism *Pseudomonas aeruginosa*.

Oxidase Test

The small dry pink colonies from SS plate were oxidase positive. There was a color change to dark blue/purple. This means the bacterium contains cytochrome producing intracellular oxidase enzyme. This oxidase enzyme catalyzes the oxidation of cytochrome c turning reagent color to blue/purple. Organisms lacking cytochrome as part of their respiratory chain do not oxidize the reagent, leaving it colorless within the limit of the test, and are oxidase negative. There was

high concentration of *E.coli* upstream and downstream than midstream during the dry season while there was low concentration of *Klebsiella* during the same season (Table 3). The concentration of microorganisms increased substantially during the rainy season (Table 4).

Table 3: Bacterial types in Kabianga River during dry season

Bacterial type	Upstream	Midstream	Downstream
<i>Escherichia coli</i>	6.8x $10^3 \pm 1.2 \times 10^3$	5.8x $10^3 \pm 1.2 \times 10^3$	$6.8 \times 10^3 \pm 1.2 \times 10^3$
<i>Salmonella spp</i>	$1.3 \times 10^3 \pm 1.1 \times 10^3$	$1.3 \times 10^3 \pm 1.1 \times 10^3$	$1.3 \times 10^3 \pm 1.1 \times 10^3$
<i>Shigella spp</i>	$1.3 \times 10^2 \pm 1.1 \times 10^2$	$1.1 \times 10^2 \pm 1.1 \times 10^2$	$1.3 \times 10^2 \pm 1.1 \times 10^2$
<i>Pseudomona aeruginosa</i>	$1.2 \times 10^2 \pm 1.1 \times 10^2$	$1.1 \times 10^2 \pm 1.1 \times 10^2$	$1.2 \times 10^2 \pm 1.1 \times 10^2$
<i>Proteus mirabilis</i>	$1.1 \times 10^2 \pm 1.1 \times 10^2$	$0.9 \times 10^2 \pm 1.1 \times 10^2$	$1.1 \times 10^2 \pm 1.1 \times 10^2$
<i>Klebsiella</i>	$1.1 \times 10^2 \pm 1.1 \times 10^2$	$0.9 \times 10^2 \pm 1.1 \times 10^2$	$1.1 \times 10^2 \pm 1.1 \times 10^2$
<i>Enterobacter</i>	$1.1 \times 10^2 \pm 1.1 \times 10^2$	$0.9 \times 10^2 \pm 1.1 \times 10^2$	$1.1 \times 10^2 \pm 1.1 \times 10^2$

Table 4: Bacterial types in Kabianga River during Rainy season

Bacterial type	upstream	Midstream	Downstream
<i>Escherichia</i>	9.8x $10^3 \pm 1.2 \times 10^3$	5.8x $10^3 \pm 1.2 \times 10^3$	$9.8 \times 10^3 \pm 1.2 \times 10^3$
<i>Salmonella spp</i>	$2.3 \times 10^3 \pm 1.1 \times 10^3$	$1.3 \times 10^3 \pm 1.1 \times 10^3$	$2.3 \times 10^3 \pm 1.1 \times 10^3$
<i>Shigella spp</i>	$2.3 \times 10^2 \pm 1.1 \times 10^2$	$1.1 \times 10^2 \pm 1.1 \times 10^2$	$2.3 \times 10^2 \pm 1.1 \times 10^2$
<i>Pseudomona aeruginosa</i>	$2.2 \times 10^2 \pm 1.1 \times 10^2$	$1.1 \times 10^2 \pm 1.1 \times 10^2$	$2.2 \times 10^2 \pm 1.1 \times 10^2$
<i>Proteus mirabilis</i>	$1.1 \times 10^2 \pm 1.1 \times 10^2$	$0.9 \times 10^2 \pm 1.1 \times 10^2$	$1.1 \times 10^2 \pm 1.1 \times 10^2$
<i>Klebsiella</i>	$1.1 \times 10^2 \pm 1.1 \times 10^2$	$0.9 \times 10^2 \pm 1.1 \times 10^2$	$1.1 \times 10^2 \pm 1.1 \times 10^2$
<i>Enterobacter</i>	$1.1 \times 10^2 \pm 1.1 \times 10^2$	$0.9 \times 10^2 \pm 1.1 \times 10^2$	$1.1 \times 10^2 \pm 1.1 \times 10^2$

CONCLUSION

Microbiological quality of the surface water was high above the compliance level of National standards, and the World Health Organization (WHO) guidelines for drinking water. The water from this river is not potable, and poses a health risk to communities that rely on the river as the primary source of domestic use. This is indicated by higher concentration of bacteria during dry and rainy seasons. Mitigation need to be undertaken the treat the water before use or advice people on the dangers of using the water.

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