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BACTERIOLOGICAL AND PHYSICOCHEMICAL CHARACTERISATION OF AORA LOKO STREAM WATER, MASENO, KENYA

Musyimi M. David[1+]
Wekulo K. John[2]
Jiveri Bonface[3]

[1,2,3]*Researchers in the Department of Botany, School of Physical and Biological Sciences, Maseno University, Private Bag, Maseno, Kenya.*

(+ Corresponding author)

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ABSTRACT

Safety and quality of drinking water is an important aspect in public health. Water pollution is one main cause of worldwide diseases and death. Human activities around water catchment areas contribute to some level of stream pollution. Aora Loko stream serve communities living around Mabungo and Maseno town as a source of domestic water. This study aimed at determining the bacteriological and physicochemical parameters of the stream water in order to determine its suitability for human domestic use. Water samples were collected at four different sites. The sites were site A – Mabungo near Bosnia, Site B- university football field, Site C- near university farm where bathing and swimming activities take place, and Site D- near car wash area next to Kisumu-Busia road. Bacterial analysis of the water samples was conducted using standard methods on Nutrient agar. Bacteriological analysis identified four genera of bacteria of medical importance comprising of Escherichia, Salmonella, Streptococcus and Staphylococcus. There were significant differences ($P \leq 0.05$) among the physicochemical parameters determined. Generally the physicochemical parameters showed variation along the stream. Temperature values within sites A and B were 23.6 0C and 23.8 0C respectively while temperature values within sites C and D were slightly lower 22.8 0C and 22.6 0C. pH values ranged between 9.0 - 9.2, and therefore were found to be above the WHO/FEPA permissive limits 6.5 - 8.5. Water conductivity values ranged between 77.3- 77.7 µs/cm. BOD values for sites C and D were 3.4 mg/l and 3.0 mg/l hence below the permissive limits. The results indicated that the Aora Loko stream water is slightly polluted due to presence of coliforms.

Contribution/ Originality: This study documents the quality of water of Aora Loko stream in Western region of Kenya. It is the first ever bacteriological and physicochemical study to have been undertaken in this important stream that supplies water to the communities living around Maseno and Mabungo area.

1. INTRODUCTION

Microbiological examination of stream or river water is obligatory for use-related purposes such as drinking water production, irrigation and recreation [1]. Fresh water is essential for agriculture, industry and even human existence, without fresh water of adequate quantity and quality, sustainable development will not be possible [2]. Safety and quality of drinking water are of important public health concern. Access to safe water is a fundamental need and basic human right [3]. Surface water resources are more vulnerable to pollution than ground water

resources [4]. Pollution of surface water occurs when too much of an undesirable or harmful substance flows into a body of water, exceeding the natural ability of that water body to remove the undesirable material [5]. About 20 % of the world's population does not have access to safe drinking water [6]. Water borne diseases are estimated to be responsible for about 3 million deaths and render sick to a billion of people [7]. About 1.7 million annual deaths are attributed to unsafe water supplies with most of these being due to diarrhoeal diseases which also affect 90% of children from developing countries to which majority of these cases are due to bacterial pathogens contamination [8]. Deterioration of water quality especially in developing countries poses tremendous effects and human risks [9]. Chemical analysis can however determine whether water is polluted and provides other useful information [10]. Changes in water parameters may disturb the quality of water [11]. Nutrient elements in water can lead to change in physicochemical conditions of water such as pH, Oxygen availability, turbidity, biological oxygen demand, electrical conductivity and dissolved solids. Fecal contamination from human fecal material is considered to be a greater risk to human health because it may contain human enteric pathogens [12, 13]. Water pollution caused by fecal contamination is a serious problem due to the potential of contracting diseases from pathogens. Fecal bacteria indicators of water pollution include: fecal coliforms, *Escherichia coli*, fecal streptococci, and Staphylococcus spp. All except *E. coli* are composed of a number of species of bacteria that share common characteristics such as shape, habitat, or behavior. Most diseases of water are of the gastro-intestinal tract which includes typhoid fever, Cholera, hepatitis; dysentery and paratyphoid [14]. Lack of ecological education and over exploitation of natural resources has severely affected the water resources by increasing the pressure on urban hydrology [15]. Constant monitoring of river systems is fundamental to evaluate the effects of environmental factors on water quality. There are no previous studies that have been undertaken on bacteriological and physicochemical characterisation of Aora Loko stream water. The study examined the safety of Aora Loko stream water for human consumption by evaluating the bacteriological and physicochemical parameters. It was hypothesised that the Aora Loko stream water contained various bacteria and there were significant variation of physicochemical parameters along different parts of the stream.

2. MATERIALS AND METHODS

2.1. Study Site

The study was conducted in Aora Loko stream which is a combination of two streams flowing through Maseno University from the adjacent Maragoli hills before meandering its way through Seme, into Lake Victoria in Kisumu County, Kenya. The stream is relatively narrow and deep, and as it flows southwards, it widens. The stream is lotic throughout the year and it is within the tropical rainforest. The stream lies between 24°, 30°, 45° East, 30°, 35°, 41° West longitude and 0°, 34°, 55° North and 0°, 20°, 31° latitudes. Aora Loko stream is a major water source in the community as most human activities depend on it. The study was conducted between May to July 2015. The area has both agricultural and human domestic practices that contribute to stream pollution.

2.2. Collection of Water Samples

Water samples were collected from four sites comprising the source of stream in Mabungo near Bosnia (Site A), university football field (Site B), near university farm where bathing and swimming activities take place (Site C) and car wash area next to Kisumu-Busia road (Site D). Water was collected manually using sterilized plastic bottles. Samples were collected carefully by dipping sterilized bottle below the water and covered immediately; the samples were then kept in the dark at 4°C according to Haller, et al. [16]. Three samples were collected at four different study sites along the stream, with three replicates.

2.2.1. Samples Processing

Serial dilution of the collected water samples were carried out using sterile distilled water. All the different dilutions were properly labeled and used for preparation of cultures.

2.3. Isolation, Biochemical Tests and Identification of Microbial Isolates:

Morphological and biochemical characteristics of the microbial isolates were used for the identification of the isolates according to Baron and Sydney [17]; Benson [18] and Bitton [19]. Bacterial analysis of the water samples was conducted using standard methods on Nutrient agar in a completely randomized design with three replicates. The bacterial isolates were subjected to various tests that included catalase test, carbohydrate fermentation test and gram staining. Enteric bacteria isolated on respective selective or differential media were identified on the basis of their colonial, morphological and biochemical properties followed by Bergey's Manual of Determination Bacteriology, 1994 to compare the characteristics with the results obtained.

2.3.1. Gram Staining Technique

Bacteria were stained with crystal violet stain. All bacteria were stained blue or purple. Iodine solution which is a mordant was added, a mordant is a substance that improves the staining property of the dye. In this case, Iodine favored more interaction between cell and the dye (crystal violet), so that cells were more strongly stained. Destaining was done using alcohol. Then the two results were used to categorize the bacteria either as positive or negative gram staining bacteria.

2.3.2. Carbohydrate Fermentation Test

The test was performed by inoculating 0.2 ml of nutrient agar culture of the isolated organisms into the tubes containing five basic sugars such as dextrose, maltose, lactose, sucrose and mannitol and incubated for 24 h at 37^{0}C. Acid production was indicated by color change from red to yellow and gas production was noted by the accumulation of gas bubbles in the inverted Durham's tube [20].

2.3.3. Catalase Test

A volume of 3 ml of 3% H_2O_2 (10 volume solution) catalase reagent was taken in a test tube. Single colony from the pure culture of *E. coli* was taken with a glass rod and merged in the reagent and observed for bubble formation which indicated positive test. Absence of bubble formation indicated negative result [20].

2.4. Physicochemical Analysis

2.4.1. Temperature

Temperature was taken at all sampling sites, using a thermometer. The bulb was dipped into the stream and allowed to stand for one minute before recording the reading directly.

2.4.2. PH

The pH meter was calibrated by inserting its probe in a standard pH solution of 7.0 then be rinsed with distilled water and inserted in water samples. The pH levels were read off above the temperature level that was displayed on the screen.

2.4.3. Water Conductivity

The cell was rinsed with more portions of the sample (water). The temperature was adjusted to 25^{0}C then was immersed in sample, sample level above vent holes. The conductivity of water samples was read and recorded.

2.4.4. Biological Oxygen Demand

Biological oxygen demand was calculated by the use of values obtained from dissolved oxygen demand.

2.4.5. Dissolved Oxygen

The dissolved oxygen demand was measured in-situ by use of dissolved oxygen (DO) meter. The values were recorded in (mg/l).

The general equation for BOD determination value was indicated below

$BOD\ (mg/l) = D_1 - D_2$

D_1= initial DO (dissolved oxygen demand) of samples

D_2= final DO of the samples.

2.5. Statistical Analysis of the Data

Data collected from this study was subjected to analysis of variance (ANOVA) using SAS statistical package and least significance difference (LSD) was used to separate the means at $p<0.05$.

3. RESULTS

3.1. Isolation, Biochemical and Identification of Bacteria from Maseno University Stream Water

The isolation of bacteria in Maseno University stream water samples in different sites. Four bacteria isolates were observed (Table 1), and identified as the *Escherichia coli*, Salmonella species Staphylococcus species and Streptococcus species (Table 2). Bacteria isolates of *E. coli*. Salmonella and Streptococcus were isolated in all the sites but isolate of Staphylococcus was on isolated in site B.

The bacteria were isolated and identified according to their morphological appearance; biochemical tests that involved Gram's staining technique, catalase test and carbohydrate fermentation test (Table 2).

3.1.1. Isolate A

Morphologically, the isolate was rod shaped and flagellated. In Gram's staining technique, the isolate was Gram negative. Isolates fermented dextrose, maltose, lactose, sucrose and mannitol. Isolates of *E coli* responded positive to Catalase test and this was confirmed by the production of vigorous bubbles (Table 2).

3. 1.2. Isolate B

Morphologically the isolate had rod like shape. Gram's staining technique revealed the isolate as gram negative. The isolates fermented dextrose, maltose and mannitol but did not ferment lactose and sucrose (Table 2). Acid production was indicated by the color change from reddish to yellow. Catalase test was positive confirmed by the production of vigorous bubbles.

3.1.3. Isolate C

Morphologically it was cocci shaped that occurred in chain. The isolate was tested Gram positive, while Carbohydrate fermentation test and catalase test were negative (Table 2).

3.1.4. Isolate D

Morphologically appeared cocci shaped that formed grape like clusters. The isolate was Gram positive and fermented maltose, sucrose, lactose and dextrose with mannitol changing from yellow to red the isolate was catalase test positive (Table 2).

Table-1. Bacteria isolated at different sites from Aora Loko stream water

Name of Isolate	Sites			
	A	B	C	D
Isolate A	✓	✓	✓	✓
Isolate B	✓	✓	✓	✓
Isolate C	✓	✓	✓	✓
Isolate D	×	✓	×	×

KEY:

✓ Indicates the presence of the isolate

× Indicates the absence of the isolate

Table-1. Bacteria isolated from the water collected from Maseno Stream water and its biochemical characteristics

Isolate	Biochemical tests				Identification of bacteria
	Gram staining		Catalase test	Carbohydrate fermentation test	
	Positive	Negative			*Escherichia coli*
Isolate A		-	+	+	*Salmonella*
Isolate B		-	+	+	Streptococcus
Isolate C	+		-	-	*Staphylococcus*
Isolate D	+		+	+	*Escherichia coli*

KEY- (+) indicates Gram positive bacteria

(-) indicates Gram negative bacteria

Catalase and Carbohydrate fermentation test

(+) indicates positive results

(-) indicates negative results

3.2. Physicochemical Parameters Analysis

3.2.1. Water Temperature

Site A and site B were not significantly ($p \geq 0.05$) different in temperature levels (Tables 3 and 4). However there were significant differences in water temperatures between sites C and D respectively. The temperature values for site A and B were within the permissive WHO/FEPA limits (23-30^{0}C) while for Site C and Site D were slightly lower.

3.2.2. Water pH

The analysis of variance indicated that there was a significant difference ($p \leq 0.05$) in pH among different sites (Tables 3 and 4). Site D differed significantly with the other sites in pH levels. The pH values for all sites were above the permissive WHO/FEPA limits.

3.2.3. Water Conductivity

There was no significant difference ($p \geq 0.05$) in water conductivity values among the different sites (Tables 3 and 4). The water conductivity of site A and site B were relatively higher (Table 3). The water conductivity levels for all the sites were within the WHO/FEPA permissive limits (30-100µs/cm).

3.2.4. Water Biological Oxygen Demand

There was a significant difference ($p \leq 0.05$) in biological oxygen demand levels among different sites (Table 3 and 4).

Table-2. Physicochemical parameters of Aora Loko stream water at four sites

Physicochemical parameters	Sites	Value measured	WHO/FEPA Permissive limit
Temperature ^{0}C	A	23.6±0.03a	23-30
	B	23.8±0.09a	
	C	22.8±0.06b	
	D	22.6±0.06b	
	LSD (P=0.05)	0.20	
pH	A	9.0±0.00b	6.5-8.5
	B	9.0±0.01b	
	C	9.0±0.01b	
	D	9.2±0.03a	
	LSD (P=0.05)	0.06	
Water conductivity µs/cm	A	76.7±0.33a	30-100
	B	77.7±0.33a	
	C	77.6±0.31a	
	D	77.3±0.33a	
	LSD (P=0.05)	1.07	
Biological Oxygen Demand (mg/l)	A	5.1±0.09a	4-5
	B	4.2±0.10b	
	C	3.4±0.00c	
	D	3.0±0.03d	
	LSD (P=0.05)	0.22	

Means with the same letter down the column are not significantly different at (p=0.05). Mean values of three replicates±S.E.

Table-3. Analysis of variance for physicochemical parameters of Aora Loko stream water

Parameter	Source	DF	Sum of squares	Mean of square	F Value	Pr>F
Temperature	Model	3	2.92	0.97	83.52	<.0001
	Error	8	0.09	0.01		
	Site	3	2.92	0.97	83.52	<.0001
pH	Model	3	0.12	0.04	43.36	<.0001
	Error	8	0.01	0.001		
	Site	3	0.12	0.04	43.36	<.0001
Water conductivity	Model	3	1.88	0.63	1.95	0.1994
	Error	8	2.56	0.32		
	Site	3	1.88	0.63	1.95	0.1994
Biological oxygen Demand	Model	3	8.06	2.69	189.57	<.0001
	Error	8	0.11	0.04		
	Site	3	8.06	2.69	189.57	<.0001

4. DISCUSSION

Water is one abundant natural resource required by all living things. Quality drinking water is important to human physiology and continued human existence [21]. The bacteriological analysis of Aora Loko stream water recorded a total of 4 bacterial genera which included Escherichia species, Streptococcus species, Salmonella species and Staphylococcus species, which are bacteria of medical importance [22, 23]. Similar findings were reported by Omondi, et al. [8]. The occurrence of these organisms at the various sites of the stream may be associated with human activities like washing, swimming, bathing and defecation. Faecal indicator bacteria like total coliforms, faecal coliforms (thermotolerant coliforms), *E. coli* and intestinal enterococci (faecal streptococci) are excreted by humans and warm-blooded animals [24]. Coliform organisms can provide basic information on sources of water quality [25-27]. *E. coli* can be found in faeces and drinking water containing high concentrations of nutrients. Some strains of *E. coli* cause diseases like diarrhea or diseases in infants that result to death. *E. coli* is a predominant coliform that was found in all the four sites in this stream.

Streptococci species are more resistant to heat [27]. Staphylococcus species cause superficial skin lesions [26-28] and serious infections such as pneumonia [26]. The isolation of Staphylococcus spp. at only one site (B) could be attributed to variation in temperature and the level of organic matter at the site. Slightly high temperature and increased level of organic matter could favour its occurrence at site B.

Salmonella is one of the primary bacterial food borne pathogens to humans that is commonly present in raw water [26, 27]. High pH thus alkaline environment will favor Salmonella growth. The isolation of Salmonella at different sites indicates that Aora Loko stream water is contaminated with human faeces and animal wastes. Streptococci spp. are normally associated with fecal material from human, and their presence in water indicates the potential incidence of enteric pathogens [27, 29]. The presence of these pathogenic microorganisms predisposes the residents to diseases such as typhoid, cholera and dysentery [21]. Presence of any bacterial cell in drinking water indicates that the water is contaminated with faeces, and therefore not suitable for drinking. The presence of faecal coliform bacteria is an indicator that a potential health hazard exists for individuals exposed to the source of water. The bacteriological quality of most of the stream water in the Maseno- Mabungo area is poor, mainly due to pollution from widespread and indiscriminate human and animal defecation and very poor waste disposal practices.

The physical and chemical properties are great important in aquatic systems through their influence in determining the quality of good water [30]. Temperature values of Aora Loko stream water varied among the sampled sites. Temperature values for site A and site B were significantly higher than the temperatures values of site C and site D. Temperatures for sites A and B were within the permissive WHO/FEPA limits 23-30^{0}C [12]. The variation in temperature along Aora Loko stream water could be due to variation in sunlight intensity as reported also by Agbabiaka and Oyeyiola [5]. This variation could be attributed to shading effects by vegetation cover for instance the site C had some trees growing nearby and hence the place was cold. Temperatures govern the existence of aquatic organisms. High temperatures affect organisms by denaturing their enzymes, leading to death of these organisms. Low temperatures also inactivate the organisms hence leading to its death.

pH is a measure of the concentration of hydrogen ions in a diluted solution [31]. The drinking water standards recommend a pH within the values 6.5-8.5, therefore pH above or low than the recommended standards clearly imply that water is unsuitable for consumption [13]. The desirable pH of fresh water is in the range of 6.5-8.5. Moreover, pH could control the pathogenic microorganism growth [27]. From the Aora Loko stream water pH values indicate that the stream water is unfit for human consumption. The pH value of stream water was above the permissive WHO/FEPA limits [12]. The high pH levels could be due to high concentration of magnesium ions and calcium ions [32]. The higher value of alkalinity indicates presence of bicarbonates, carbonates and hydroxide in water body. Low pH indicates acidic conditions of water that cannot support normal life conditions. Any alteration in pH leads to change in other physicochemical parameters [31, 33].

Water conductivity is a measure of ability of water to conduct electrical current. Conductivity is considered to be a good indicator for evaluating total dissolved solid materials in water and nature of the purity of water [13]. Values for water conductivity of Aora Loko stream water were within the WHO/FEPA permissive limits (30-100µs/cm) [7, 12]. This indicates that the level of total dissolved solid materials is within the required standards. The low conductivity values of the samples imply that the dissolved salts are minimal [34]. High dissolved solids generally raise the water conductivity level making it unsuitable for consumption.

Biological Oxygen Demand (BOD) is a measure of oxygen in water required by microbes to degrade the organic matter under aerobic conditions [24, 35]. The stream water BOD values were significantly different among the sites and only sites A and B had BOD values within the permissive limits between the ranges of 4-6mg/l. The lower BOD values indicated that there was less or little oxygen and hence this could hinder the microbial activities. The low BOD values may have been due to low temperature, and increased organic wastes [5, 26]. High BOD depletes oxygen level to a critical condition thus indicating the organic pollution status of water [36]. The main sources of organic pollution include untreated domestic sewage, agricultural runoff and residual fertilizers. In

natural course, the organic matters on oxidation enter into biogeochemical cycles [31]. The BOD of Aora Loko stream water may be influenced through ways such as disposition of domestic effluents and human wastes into the stream. Unpolluted waters typically have BOD values of 2 mg/l or less, while those receiving wastewaters may have value up to 10 mg/l [37].

5. CONCLUSIONS

The study has clearly shown that Aora Loko stream water is slightly polluted. Four important medical bacteria were isolated and identified during microbial analysis of the stream water, which included *E. coli*, Salmonella species, Streptococcus species as well as Staphylococcus species. Physicochemical parameters clearly indicated that the Aora Loko stream water is slightly polluted and not fit for direct human consumption. Necessary treatment procedures can however, be applied to raise the quality of the stream water to the WHO standards for safe drinking water. There is need to educate the residents around Mabungo and Maseno township on better ways of disposing organic wastes to help in maintaining the levels of physicochemical parameters within the WHO/FEPA permissive limits. The findings from this study indicate that stream waters can cause an environmental health hazard due to the high concentrations of chemical and bacteriological pollution. The observed bacteriological and physicochemical characteristics of Aora Loko stream water necessitate immediate actions to be taken to minimize pollution of the stream.

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