International Journal of Biomolecules and Biomedicine (IJBB)
ISSN: 2221-1063 (Print), 2222-503X (Online)
http://www.innspub.net
Vol. 11, No. 2, p. 1-11, 2020

INNSPUB

RESEARCH PAPER OPEN ACCESS

Microbial characterisation and identification, and potability of River Kuywa Water, Bungoma, Kenya

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Key words: Bacterial count, Fungal count, Kuywa River, Unsafe water

Article Published: 23 October 2020

Abstract

Water contamination is one of the major causes of water borne diseases worldwide. In Kenya, approximately 43% of people lack access to potable water due to human contamination. River Kuywa water is currently experiencing contamination due to human activities. Its water is widely used for domestic, agricultural, industrial and recreational purposes. This study aimed at characterizing bacteria and fungi in river Kuywa water. Water samples were randomly collected from four sites of the river: site A (Matisi), site B (Ngwelo), site C (Nzoia water pump) and site D (Chalicha), during the dry season (January-March 2018) and wet season (April-July 2018) and were transported to Maseno University Microbiology and plant pathology laboratory for analysis. The characterization and identification of bacteria and fungi were carried out using standard microbiological techniques. Nine bacterial genera and three fungi were identified from Kuywa river water. *Clostridium* spp., *Staphylococcus* spp., *Enterobacter* spp., *Streptococcus* spp., *E. coli, Klebsiella* spp., *Shigella* spp., *Proteus* spp. and *Salmonella* spp. Fungi were *Fusarium oxysporum, Aspergillus flavus complex* and *Penicillium* species. Wet season recorded highest bacterial and fungal counts (6.61-7.66 and 3.83-6.75cfu/ml) respectively. The results indicated that the river Kuywa water is polluted and therefore unsafe for human consumption before treatment. It is therefore recommended that the communities to ensure that they boil water especially for drinking.

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Introduction

Surface and underground water are major sources of fresh water but surface water is vulnerable to contamination as contaminants can easily flow into it (Harikumar *et al*., 2017). Water resources particularly rivers in the world are degraded by discharge of untreated sewage and untreated industrial wastes. Free suspended bacteria in the water and bacteria associated with the suspended materials are quoted amongst pollutants (Noble *et al*., 1997).

Many people lack access to potable water and about 22% of the world's population per year are affected by water borne diseases (Akubuenyi *et al*., 2013; Karamage *et al*., 2016). Miime *et al*. (2011) reported that worldwide approximately 1.7 million deaths recorded annually are attributed to unsafe water supplies. Contaminated water serves as a medium of transmitting dangerous pathogens into humans, animals and plants and about 80% of human diseases are caused by water (Akubuenyi *et al*., 2013; Chen *et al*., 2017). Microbiological contamination with faecal bacteria subsequent to anthropogenic activity is considered a crucial issue throughout the rivers in Kenya.

In Kenya, approximately 17 million people (43%), lack access to potable water, due to contamination of water sources. The main problems that affect water quality of the river are the high pollution following different human activities and population explosion. Water sources in Kenya particularly rivers are becoming contaminated by both point and nonpoint sources attributed to human activities (Aywa, 2017).

River Kuywa flows southwards through Bungoma County (Wasike, 2015). Its water is widely used for domestic, agricultural, industrial and recreational purposes (Omwoma, 2011). This water body is feared to be contaminated microbiologically due to effluents from coffee and sugar factories, domestic wastes, livestock excrements and agricultural activities. These may alter the microbiological parameters which eventually affects water quality of the river. More than 40,000 cases of typhoid and allergy in the region are reported annually (Wasike, 2015) which could be attributed to water contamination.

Water bodies are areas of intense microbial activity and microbial interaction as nutrients and levels of physicochemical parameters in water bodies are the main source of food and driving forces for their proliferation and activities (Walker *et al*., 2014). The microbial count in river water differs quantitatively and qualitatively depending on level of contamination (Verani *et al*., 2019). There is very limited, or no published information, on river water quality assessment in Bungoma region yet disturbances from urban and agricultural development contribute to an overall decrease the biological integrity of a river. There are no previous studies that have been undertaken on characterization and identification of microbes in river Kuywa water. This study aimed at determining the the safety of river Kuywa water for human consumption.

Materials and methods

Study Site

The study was done on river Kuywa in Bungoma County. The river source is Mt. Elgon and it flows southwards through Bungoma County. It is a tributary of river Nzoia, relatively wide, deep and it is lotic throughout the year. The river lies between latitude 34° 00' E and 35° 00" E and longitude 0° 47' 24" N and 0° 43' 40" N and covers an area of about 110Km² long (Wasike, 2015). The study area receives annual rainfall of 1500mm with above 850 mm in wet and less than 170 mm during dry season.

Sample Collection

Water samples were collected from four sites; Matisi (Site A), Ngwelo (Site B), Nzoia water pump (Site C) and Chalicha (Site D) (table 1) using clean sterilized 250ml bottles from 20 to 30cm depth (to avoid floating materials) according to Mgbemena *et al*. (2012). The bottles were carefully closed and transported on ice and stored at 4^0C in a refrigerator until the analysis of microbiological parameters. Water samples were collected in triplicates once per season from the four different sites chosen based on accessibility in terms of plants and animals, slope angle, human activities and health problems reported in the regions during the dry season (January-March) and wet season (April-July) in

2018 respectively. The sampling distance was about five kilometers between the sites.

Table 1. Human activities around the sampling sites.

Sampling sites	Geographical position		Characteristic
	Latitudes	Longitudes	
Matisi (Site A)	00°37.186'N	034°42.094'E	Bathing, Clothes washing, swimming
Ngwelo (Site B)	00°34.412'N	034°40.825'E	fishing, livestock, agricultural activities
Nzoia water pump (Site C)	00°35.720'N	034°41.094'E	Agricultural activities, waste disposal in water and swimming.
Chalicha (Site D)	00°35.708'N	034°41.080'E	Agricultural, effluents released from sugar factory

Isolation of Pure Bacterial Culture

The culture techniques were adopted from Mgbemena *et al.* (2012). Water culture samples were prepared by streak and spread plate techniques. A sterile wire loop was used to collect a loop full of each of undiluted water sample and inoculated on the surface of nutrient agar, MacConkey agar and *Salmonella Shigella* agar, respectively. The inoculated culture were sub-cultured on fresh nutrient agar, MacConkey agar and *Salmonella Shigella* agar plates to obtain pure cultures which were used to further study their morphological and biochemical characteristics. The pure culture isolates were sub-cultured in nutrient agar and incubated at 37°C for 24 hours for bacterial enumeration. The samples were processed and analyzed to determine heterotrophic and coliforms using nutrient agar, MacConkey agar and *Salmonella Shigella* media.

Morphological identification of bacteria isolates from water samples from river Kuywa

The morphological characteristics of bacterial colonies such as shape, surface appearance, colour, margin, opacity and elevations were studied following the procedures of Bergey's manual of determinative bacteriology. The cell morphology was identified by placing pure bacterial colonies on the glass slide and observed under light microscope for cell shape.

Culturing and determination of total bacteria count

Serial dilution of water samples was carried out using distilled water to a dilution factor 10^5 and 0.1ml aliquot from each dilution was well labelled and used for total plate count. One mililitre aliquot was spread on nutrient agar (NA) by pour plate technique and incubated for 24 hours at 37°C. Total bacteria count was done by counting visible and distinct colonies on the media using digital colony counter model SUNTEX colony counter 570 and expressed as colony forming unit (cfu/ml).

Isolation of pure fungi colonies

Twenty four water samples were subjected to culture technique using streak plate and spread plate techniques. A sterile wire loop was used to collect a loop full of each of undiluted water sample and inoculated on the surface of Potato dextrose agar. The inoculated culture were sub-cultured on fresh media Potato dextrose agar plates to obtain pure cultures which were used to further study their morphological characteristics. The pure culture isolates were sub-cultured in Potato dextrose agar and incubated at room temperature for 3-7 days for fungal enumeration.

Identification of fungal isolates

Identification of fungal isolates was done according to Oliveira *et al.* (2016). Visual observation of sub-cultured colonies characteristics (colour, shape, margin, elevation and presence of aerial mycelium) was done. A phase contrast microscope was used to pre identify colonies of fungi which were emulsified on to glass slides using wet mount technique. Distilled water and lactophenol cotton blue staining (LPCB) were utilized as mountants. The mycelium was teased (picked) out with the needles and covered with clean cover slip carefully avoiding air bubbles and observed under the microscope for shape, conidia, conidiophore, arrangement of spores and septation. The slide was mounted and observed under magnification x400. Identity was confirmed with the help of mycology manual version 1.1.

Culturing and Determination of total Fungi Count

Serial dilution of water samples was carried out using distilled water to a dilution factor of 10^5. 0.1ml aliquot

from each dilution was labelled and used for total plate count. Samples of fungi were cultured on potato dextrose agar (PDA). 0.1ml aliquot was spread on potato dextrose agar by pour plate technique and incubated for 72 hours at 22°C. Fungi colonies were counted on the 3rd day using colour maker to spot on the lid. After 7 days, fungi colony were counted using a different colour maker to spot only the colony that grew on the plate and sum up the total colony number counted on 3rd and 7th day.

Gram Staining Technique

A glass slide smear was prepared by placing deionized water drop on the slide then microorganisms obtained from the pure cultured colonies were aseptically transferred to the water drop using a sterilized wire loop and were spread with inoculating loop. The smear was air dried; crystal violet was added to the smear then gently washed with tap water. Iodine solution which is a mordant was added and allowed to stand for 2 minutes. Smear was then decolourized by using 95% ethanol, washed with tap water, counterstained with safranin for 45 seconds and then washed with tap water. The smear was then dried using filter paper and examined under oil immersion for gram stain.

Indole Test

This determined the ability of bacteria to split amino acid tryptophan to form compound indole. One percent tryptophan 10ml broth was taken in test tubes and inoculated by fresh pure culture obtained from pure colonies. After 48 hours of incubation period at 37°C, one militre (1ml) of chloroform was added to the broth. The test tubes were shaken gently. Five drops of Kovács reagent was added directly to the tubes. These were also shaken gently and allowed to stand for twenty (20) minutes. Two test tubes were used per isolate with one being a control. Control test tube contained one percent tryptophan broth and inoculated by fresh pure culture obtained from pure colonies. Formations of red colouration at the top layer indicated positive while yellow colouration indicated negative results, respectively.

Citrate Test

This was employed in determining the ability of bacteria to utilize sodium citrate as its only carbon and energy source. The inoculated medium was incubated for 48 to 72 hours to allow complete utilization of Simmon's citrate medium by microorganisms. The colour of the medium indicated the result. If the colour of media changed from green to blue then the bacteria was citrate positive while if the media retained the green colour after incubation period it indicated citrate negative bacteria.

Methyl Red Test

Tubes of MR-VP broth were inoculated by fresh pure culture obtained from pure colonies. The inoculated MR-VP broth were incubated for 48-72 hours to allow microorganisms to fully utilize the supplied glucose, peptone and phosphate as explained by Behera (2012) buffer at 37°C after which, one milliliter (1ml) of the initial 30ml was transferred into test tubes. Three drops of methyl red were added to them. Two test tubes were used per isolate, one tube was the control containing MR-VP broth inoculated with pure culture and methyl red was not added after incubation period. Another tube after incubation period, methyl red drops was added. Change in colour to red indicated positive results while change in colour to yellow indicated negative results.

Voges Proskauer Test

A tube of MR-VP broth was inoculated with a pure culture of the test organism and incubated for 24 hours at 35°C. Oneml of broth was transferred to clean test tubes; 0.6ml of 5% alpha naphthol was added, followed by 0.2ml of 40% KOH. Test tubes were gently shaken to expose the medium to atmospheric oxygen and then allowed to remain undisturbed for 15 minutes. Two test tubes were used per isolate. Control test tubes contained MR-VP and pure culture obtained from pure colonies. Formation of red colour indicated positive bacteria while brown or yellow colour indicated negative bacteria.

Carbohydrate Fermentation Test

The test was performed by inoculating 0.2ml of nutrient agar, MacConkey agar and *Salmonella Shigella* agar culture of the isolated organisms into the tubes containing 4% basic sugars; dextrose,

maltose, lactose and sucrose and incubated for 24 hours at 37°C. Acid production was indicated by the colour change from red to yellow which showed bacteria fermenting the carbohydrate. Gas production was noted by the presence of bubbles in inverted (Durham) tubes due to fermentation.

Catalase test

Catalase test was done to determine aerobic and anaerobic bacteria and it was important in differentiating morphologically similar Enterococcus *Staphylococcus (catalase positive)* and *Streptococcus spp* (catalase negative). Threeml of catalase reagent (3% H_2O_2) was put on a glass slide. Single colony from the pure culture of bacteria from each sampled site was scooped with a glass rod and submerged in the reagent and observed for bubble formation which indicated positive test while absence of bubbles formation indicated negative results.

Oxidase test

The test was used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. A filter paper was soaked in 1% kovacs oxidase reagents and dried. A single colony from pure culture of bacterial was rubbed into paper using a wire loop. The colour change was timed using a stop watch whereby, if colour changed to dark purple within less than 10 seconds it indicated positive and if colour took more than four minutes to change it indicated negative results.

Data Analysis

Data on bacterial count and fungal count were subjected to Analysis of variance (ANOVA) using (SAS) version 9.1. Descriptive statistics including the mean, standard deviation and standard error were calculated at p = 0.05 confidence interval. Means that were considered significantly different at (P = 0.05) were separated using Fisher's LSD.

Results

Morphological characterization of bacteria from river Kuywa water

Nine pure bacterial isolates were tentatively identified as A – I. The isolates were found to be morphologically different based on colony size, shape, surface, margin, colour, elevation and opacity and cell shape (Table 2). Isolate A had circular shape, smooth surface, irregular margins, yellow colony colour, opaque and rods shaped. Isolate B was circular, rough surface, irregular margins, white colony colour, translucent and cocci formed in chains. Isolate C had irregular shape, mucoid surface, irregular margins, cream in colour, opaque and rods shaped. Isolate D had a smooth surface, entire margin, orange colony colour, opaque and cocci formed separately. Isolate E was circular, mucoid surface, entire margins, bright pink colony colour, raised, opaque and rods shaped. Isolate F was circular, mucoid surface, entire margins, pale yellow, flat, translucent and cocci formed in chain. Isolate G was circular in shape, had smooth surface, entire margins, colourless colony colour, raised, opaque and rods. Isolate H had irregular shape, smooth surface, filamentous, brick red colony colour, raised, opaque and rods. Isolate I had mucoid surface, entire margins, black colony colour, raised, opaque and rods shaped.

Gram staining

The bacterial isolates A, B and D remained purple after decolourizing with alcohol confirming the isolates as Gram positive while bacterial isolates C, D, E, F, H and I were pink after decolourizing with alcohol. This was an indication of Gram negative bacteria (Table 3).

Indole test

Indole test of bacteria isolates A, D and E showed a development of a red ring at top layer of the medium (Table 3) respectively. This was an indication of indole positive bacteria. On the other hand, bacterial isolates B, C, F, H, G and I after incubation period and on addition of Kovacs reagent formed a yellow ring an indication of indole negative bacteria.

Citrate test

Isolates A, B, C, G and I were able to utilize citrate as sole carbon source because the colour of media changed from green to blue indicating that the bacteria were citrate positive. Bacterial isolates D, E,

F and H were unable to utilize the citrate as sole carbon source since the media retained the green colour after incubation period confirming the isolates were citrate negative (Table 3).

Table 2. Morphological growth and appearance of bacteria isolates of river Kuywa water.

Isolate	Colony characteristics							Cell morphology
	Size	Shape	Surface	Margin	Colour	Eleva Tion	Opacity	
A	Large 4-5mm	Circular	Smooth	Entire	Yellow	Convex	Opaque	Rods
B	Large 4-5mm	Circular	Rough	Irregular	White	Convex	Translucent	Cocci forming chains
C	Large 4-5mm	Irregular	Mucoid	Filamentous	Cream	Flat	Opaque	Rods
D	Large 4-5mm	Circular	Smooth	Entire	Orange	Convex	Opaque	Cocci forming separately
E	Small 4-5mm	Circular	Mucoid	Entire	Bright pink	Raised	Opaque	Rods
F	Large 4-5mm	Circular	Mucoid	Entire	Pale yellow	Flat	Translucent	Cocci forming chain
G	Small 2-3mm	Circular	Smooth	Entire	Colourless	Raised	Opaque	Rods
H	Large 4-5mm	Irregular	Smooth	Filamentous	Brick red	Raised	Opaque	Rods
I	Small 2-3mm	Irregular	Mucoid	Entire	Black	Raised	Opaque	Rods

Methyl red test

Bacterial isolates A and H after incubation, changed to yellow (Table 3) indicating that the isolates were methyl red negative. Medium incubated with bacterial isolates C and isolate F changed to brown indication of methyl red negative. Bacterial isolates B, D, E, I and G on addition of methyl red indicator, the medium changed to red from whitish. This was an indication of methyl red positive bacteria.

Voges- Praskauer test

The medium incubated with bacterial isolates A, B, D and G changed to red (Table 3) indicating the presence of diacetyl, the oxidation product of acetoin. Bacterial isolates E and H incubated medium turned yellow, an indication of the absence of diacetyl, the oxidation product of acetoin.

Carbohydrate fermentation test

Carbohydrate fermentation test showed that all the isolates were able to utilize sucrose, maltose, lactose or dextrose. This was indicated by the colour change from red to yellow which showed bacteria fermenting given carbohydrate (Table 3).

Catalase test

The bacterial isolates D, C, E, F, H, G and I produced bubbles during these tests (Table 3). This was an indication that the bacteria isolates are aerobic bacteria. On the other hand, isolate A and B did not produce bubbles during these tests indicating that they were anaerobic bacteria.

Oxidase test

Bacteria isolates A, B, C and D changed colour to dark purple after four minutes indication of oxidase negative while bacterial isolates, E, F, G, H and I changed to dark purple within less than ten seconds (Tables 3) indicating the presence of cytochrome c oxidase enzyme.

Confirmation of identity

Based on morphological descriptions, biochemical tests and Bergey's manual of determinative bacteriology, the nine bacterial isolates were identified as isolate A - *Clostidium* spp., B- *Staphylococcus* spp., C- *Enterobacter* spp., D-*Streptococcus* spp., E- *E. coli, F- Klebsiella* spp., *G-Shigella* spp., *H- Proteus* spp., *I- Salmonella* spp (Table 3).

Table 3. Biochemical characterization of bacteria from water collected from river Kuywa.

Isolate	Colony characteristics												
	Colour	Gram's staining Test	I	Ci	MR	VP	Carbohydrate Test				G	Ca	O	Confirmation of identity
							S	L	Dex	Mal				
A	Yellow	+ve	+	+	-	+	-	-	+	-	+	-	-	*Clostridium* spp.
B	White	+ve	-	+	+	+	+	-	+	-	-	+	-	*Staphylococcus* spp.
C	Cream	-ve	-	+	-	-	+	+	+	-	+	+	-	*Enterobacter* spp.
D	Orange	+ve	+	-	+	+	+	-	-	+	-	-	-	*Streptococcus* spp.
E	Bright pink	-ve	+	-	+	-	-	+	+	-	+	-	-	*E. coli*
F	Pale yellow	-ve	-	-	-	-	+	+	-	+	+	+	+	*Klebsiella* spp.
G	Colourless	-ve	-	+	+	+	-	-	-	+	+	+	+	*Shigella* spp.
H	Brick red	-ve	-	-	-	-	-	-	+	-	+	+	+	*Proteus* spp.
I	Black	-ve	-	+	+	-	-	-	+	+	+	+	-	*Salmonella* spp.

Legend: + =Positive reaction - = Negative

MR- Methyl red; **I**- Indole; **Ci**- Citrate; **VP**- Voges Proskauer; **Ca**- Catalase; **O**- Oxidase; Gram- Gram's staining; **S**- Sucrose; **L**- lactose; **D**- dextrose; **M**- Maltose and **G**- Gas production

Morphological characterization of fungi from water collected from river Kuywa

Three pure fungal isolates were tentatively differentiated based on morphological and microscopic characters as X, Y and Z (table 4). Isolate X was white, floccose, and septate, and had a globose conidia. Isolate Y was cream in colour and velvety while isolate Z was dark green, velvety, septate and had a globose conidia. Based on the above descriptions and reference made using mycology manual vol. 1.1, the three isolates were identified to be X-*Fusarium oxysporum,* Y-*Aspergillus flavus complex* and Z- *Penicilium spp.* as shown in (table 4). Two of the fungal isolates were identified to species level (X and Y) and one to genus level (Y).

Table 4. Morphological characteristics of fungi from water collected from river Kuywa.

Isolate	Colony characteristics							Identification
	Texture	Surface colour	Mycelium/ Forms	Hyphae	Conidia and shape of conidiophores	Sporulation	Spores formation	
X	Fluccose	White	Filamentous	Septate	Globose	Low	Forms in chain exogenously	*Fusarium oxysporum*
Y	Velvety	Cream	Filamentous	Septate	Globose	High	Forming in chains exogenously	*Aspergillus flavus complex*
Z	Velvety	Dark green	Filamentous	Septate	Globose	Moderate	Forms separately	*Penicilium* spp.

Microbial counts of bacteria and fungi in river Kuywa water

Bacteria count

Sites had no significant influence on bacterial count though the bacterial count had different levels in each sampled site, significantly they were not different (P=28) in river Kuywa (Table 5). The highest bacterial count 8.50 * 10^5 cfu/ml was obtained at site B- Ngwelo which was not significantly different from sites A- Matisi, C- Nzoia water pump and D- Chalicha. Site D- chalicha had the least bacterial count 6.00 * 10^5 cfu/ml. Bacterial count in river Kuywa in all sites exceeded WHO permissible limits (0/100ml).

Fungal count

The results obtained from fungal count shows that sites had significant influence on fungal count whereby sites recorded varying levels of fungal counts. All sites were not significantly different except Ngwelo (Table 5).

The highest fungal count was obtained at site D- chalicha 6.00 * 10^5 cfu/ml which was not significantly different from site C- Nzoia water pump and site A- Matisi. The least fungal count 3.85 * 10^5 cfu/ml was obtained at site B- Ngwelo and it was significantly different from other sites A, C, and D.

Table 5. Influence of sites on bacterial and fungal count of river Kuywa.

Sites	Microbial count		
	Bacterial count cfu/ml	Fungal count cfu/ml	
Matisi	$7.32\pm6.47 \times 10^5$a	$5.33\pm8.03 \times 10^5$a	WHO permissible limits (0/100ml)
Ngwelo	$8.50\pm1.22 \times 10^5$a	$3.83\pm7.03 \times 10^5$b	
Nzoia water pump	$6.70\pm7.43 \times 10^5$a	$6.00\pm6.83 \times 10^5$a	
Chalicha	$6.00\pm9.85 \times 10^5$a	$6.00\pm7.30 \times 10^5$a	
LSD	2.7×10^6	1.09	

Means followed by the same letter (s) in the same column are not significantly different (P≤0.05). Mean values of three replicates ±S.E

Discussion

Eight pathogenic bacteria were isolated from river Kuywa water, namely, *Clostidium* spp, *Enterobacter* spp, *Staphylococcus* spp, *Klebsiella* spp, *Shigella* spp, *E. coli, Proteus* spp and *Salmonella* spp. This is an indication of some contamination of the river water. The bacterial count isolated were also found to be above WHO permissible limits (0/100ml). Similar findings have been reported by Hassanein *et al.* (2013) and Karikari and Ansa-Asare (2005).

The availability of these bacteria in river Kuywa water can be associated with the activities like washing, agricultural, livestock, soil erosion, swimming and waste discharge going on in these sites. The bacteria isolates mainly belonged to the family Enterobacteriaceae that is known to consist of several pathogenic bacteria (Karikari and Ansa-Asare, 2005). *Salmonella* spp and *E. coli* are considered as food and waterborne pathogens and *E. coli* is a good indicator of fecal contamination of water (Aishvarya *et al.*, 2018).

The isolation of *Klebiesiella* spp, *Clostridium* spp, and *Enterobacter* spp. from the different sites can be attributed to soil erosion. Microorganisms such as *Klebiesiella* spp, *Clostridium* spp, and *Enterobacter* spp colonize rhizosphere of trees such as Pine; this might have contributed to the presence of the bacteria in the river water (Mounjid *et al.*, 2014). Some species from respective genera are known to be pathogenic e.g. *Clostristridium botulum* is associated with botulism in food and tetanus (Perez and Carmen, 2017). Pathogenic *Staphylococcus* is a member of family Staphylococcaceae and is associated with inflammation and suppuration (Hassanein *et al.*, 2013).

The species of *Streptococcus* is known to be non-pathogenic and form part of the commensal human microbiota of the mouth, skin, intestine and upper respiratory tract (Mounjid *et al.*, 2014). *Shigella species* is closely related to *E. coli* (Mwembi, 2016). The presence of *Shigella* spp in river Kuywa indicates continued fecal contamination because *Shigella* survives up to four days in river water (Mounjid *et al.*, 2014). It is improbable that *Shigella* can be recovered from an environmental source, unless there is a continuous source of contamination such as waste water seepage (Hassanein *et al.*, 2013). *Shigella* spp is the causative agent of human shigellosis, dysentery and diarrhea (The *et al.*, 2016). Three fungal isolates were all pathogenic namely, *Fusarium oxysporum, Aspergillus flavus complex* and *Penicillium species*. The fungal count isolated were above WHO permissible limits (0/100ml). The availability of these fungi in river Kuywa water might be due to flooding and presence of organic matter in river Kuywa. Similar findings were reported by Chen *et al.* (2016).

Members of fungi isolated were ascomycetes that are known to consist of several pathogenic fungi. *Aspergillus flavus complex* is both a saprophytic and opportunistic pathogen and has a wide range of survival conditions. It grows at temperature of 12-48^{O}C and at optimum temperature of 28-48 ^{O}C (Medina *et al.*, 2014). *A. flavus complex* invades and infects crops e.g. maize, corns, peanuts, cotton and nut trees while in field and in storage and this leads to both human and animal aflatoxicosis due to aflatoxin induced in crops (Medina *et al.*, 2014).

Fusarium oxysporum is a pathogen and mostly found in soil. It causes fusarium wilts, a deadly vascular wilting syndrome in plants. *Fusarium oxysporum*

spores remain dormant in soil for over 30 years and spread easily in water and infect plants of family solenaceae (tomatoes, pepper, potatoes, eggplant), watermelon, legumes, lettuce, beets, basils, strawberries, sugarcane and bananas (Osman, 2016). *Penicillium* spp prefers cool and moderate environmental conditions and they are present where organic materials occur. They produce mycotoxins including; ochratoxin, Penicillic acid, Penicillic expansum which affects seeds under storage, fruits, bulbs of plants like apples and bears, citrus, garlic and pathogen to animals (Kotun, 2017).

The findings of this study suggest that the general sanitary qualities of river Kuywa water as indicated by total bacterial and fungal counts were undesirable for domestic and agricultural usage. For water to be considered as no risk to human and animal health, the total bacterial and fungal counts need to meet WHO permissible limits (0/100ml). Both bacterial and fungal counts in all sampling sites exceeded WHO permissible limits (0/100ml). This could be attributed to discharge of domestic and agricultural wastes as well as human excreta/wastes into river Kuywa. Similar findings have been reported by Raju *et al.* (2012). Bacterial count was above WHO permissible limits (0/100ml) from site A- Matisi to site D- Chalicha this suggests that there was pollution of the water by organic materials. This may be attributed to materials deposited from human and animal wastes and drained from runoff and seepages from agricultural activities and industrial wastes. The mesophilic (moderate) temperatures found in this area could be providing a congenial environment for the growth of the bacteria. The findings of this study are in agreement with the findings by Nienie *et al* (2017). Zuma (2010) reported that microbes attach themselves to suspended matter in the water columns hence creating conditions for microbes to grow and proliferate.

The fungal count in all sampling sites was above WHO permissible limits (0/100ml). The variation in fungal count in sampled sites may be attributed to activities in the sites for instance increased livestock activities, runoffs and residents of organic matter in river water. Similar results have been reported by Levi *et al.*, 2017 who reported high fungal counts above WHO permissible limits. The significant difference in bacterial and fungal counts among the four sampling points along river Kuywa water could have been as a result of moderate temperatures. Microbial growth rate increases with increase in temperature. Moderate temperature is known to accelerate the chemical and biological processes in the water and leads in reduction of its ability to hold the essential dissolved gases like oxygen (Raju *et al.*, 2012).

Conclusions

Nine bacteria namely; *Clostidium* spp., *Staphylococcus* spp., *Enterobacter* spp., *Streptococcus* spp., *Proteus* spp., *E. coli, Klebsiella* spp., *Shigella* spp. and *Salmonella* spp. were isolated and identified and three fungi namely; *Fusarium oxysporum, Aspergillus flavus complex* and *Penicillium species* were isolated and identified in river Kuywa water. The bacterial and fungal counts in river Kuywa were above WHO permissible limits (0/100ml) indicating that river Kuywa is microbiologically contaminated and unsafe for direct human usage. It is therefore recommended that the communities using this water for drinking should boil the water.

Acknowledgement

Many thanks to the department of botany for supporting this research work

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