MICROBIAL AND PHYSICOCHEMICAL PARAMETERS OF RIVER KUYWA WATER IN BUNGOMA COUNTY, KENYA

BY

WEKULO JOHN KEYA

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MASENO UNIVERSITY

DECLARATION

This thesis is my own original work and has not been previously presented in this or any other University. All sources of information have been specifically acknowledged by means of references.

Signature_____ Date _____

Wekulo John Keya MSC/SC/00004/016

Supervisors

This thesis has been submitted for examination with our approval as University supervisors

Prof. David Mutisya Musyimi, PhD.

Signature _____Date_____
Department of Botany
School of Physical and Biological Sciences
Maseno University

Prof. Godfrey Wafula Netondo, PhD.

Signature _____Date_____
Department of Botany
School of Physical and Biological Sciences
Maseno University

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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. Physicochemical and biological factors influence microbial community and quality of water. Residents in Bungoma County depend on river Kuywa water for domestic and agricultural use. The river water is currently experiencing contamination due to human activities such as farming and disposal of industrial and domestic wastes. The human and physical factors may affect the microbial, physicochemical parameters and quality of the water. Information on the current status of pollution of river Kuywa water is required. The purpose of this study was to investigate microbial and physicochemical parameters of river Kuywa water. The specific objectives were to determine: - the types of bacteria and fungi, physicochemical parameters influencing water quality, microbial counts in river Kuywa and the relationship between microbiological and physicochemical parameters of river Kuywa. Water samples were randomly collected from one meter along the shoreline from four sites of the river in triplicates: 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 botany laboratory for analysis. Sites were selected based on accessibility, human activities and health problems reported in the region. Bacteria were cultured using Nutrient agar, MacConkey agar, Salmonella Shigella agar while fungi using Potato dextrose agar. The identification of bacteria and fungi were then carried out using the standard microbiological techniques. Physicochemical parameters such as Temperature, pH, Turbidity and dissolved oxygen were measured *in-situ* using portable meters. Biological oxygen demand was calculated from DO values. Chemical oxygen demand, phosphates and nitrates were determined using dichromate reflex and spectrophotometric method respectively. Data on physicochemical parameters and microbial count were subjected to analysis of variance. Means were separated and compared using Fisher's Least Significance Difference at P = 0.05. In this study 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. Physicochemical parameters levels such as pH 7.68-11.47, turbidity 10.03-13.42 NTU, COD 0.31-0.48 mg/l, NO^-_3 1.09-1.47 mg/l and PO^-_4 0.48-0.81 mg/l exceeded WHO permissible limits while temperature 23.83-25.88^{O}C and DO 7.73-12.52 mg/l were within WHO permissible limits. Microbial and physicochemical parameters varied between seasons. Wet season recorded highest bacterial and fungal counts (6.61-7.66 and 3.83-6.75 cfu/ml) respectively. The pH ranged between 8.68-9.02, turbidity 10.88-11.62, COD 0.36-0.42, NO^-_3 0.69-1.94 and PO^-_4, DO 8.98-10.23 and temperature between 23.32-27.04^{o}C. Spearman rank correlation showed a positive relationship between some physicochemical parameters and microbial counts. Bacterial counts were positively correlated with temperature (r=0.85) and nitrates (r = 0.95) and negatively correlated with pH (r = - 0.04), Turbidity (r = - 0.89), DO (r = - 0.85), and BOD (r = - 0.89). Fungal count showed negative correlation with temperature and nitrates (r= -0.50), (r=-1.00). Bacterial and fungal counts were not within WHO permissible limits. Presence of *Salmonella, Shigella* and *E. coli* and *Penicilium* spp. indicate the poor quality of river Kuywa water and may pose serious health problems. The results provide baseline information that can be used by researchers and government authorities such as National Environmental Management Authority and Kenya Bureau standards to improve on the quality of river Kuywa water.

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

Ag_2SO_4	:	Silver Sulfate
ANOVA	:	Analysis of Variance
BOD	:	Biochemical Oxygen Demand
Cfu/ml	:	Colony forming units per millimeter
COD	:	Chemical Oxygen Demand
DO	:	Dissolved Oxygen
FAS	:	Ferrous Ammonia Sulfate
H_2O_2	:	Hydrogen Peroxide
H_2SO_4	:	Sulfuric acid
$K_2Cr_2O_7$	:	Potassium Dichromate
KOH	:	Potassium Hydroxide
LPCB	:	Lactophenol Cotton Blue
LSD	:	Least Significance Difference
MR-VP	:	Methyl Red- Voges Proskauer
NaOH	:	Sodium hydroxide
NEMA	:	National Environmental Management Authority
NO_3^-	:	Nitrates
NTU	:	Nephelometric Turbidity Unit
PDA	:	Potato Dextrose Agar
PO^-_4	:	Phosphates
WHO	:	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Water is considered as one of the essential components of diet that support all forms of life (Harikumar *et al*., 2017). According to Yadav *et al.* (2018), water covers about 70.9% of the earth's surface. Freshwater, which is vital for sustainable development, covers less than 2% of earth's surface. Surface and underground water are major sources of fresh water but surface water is vulnerable to contamination as contaminants can easily flow into it.

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). Miime *et al.* (2011) reported that worldwide approximately 1.7 million deaths recorded annually are attributed to unsafe water supplies. Most of these deaths are due to diarrheal diseases which mostly affect about 90% of children. In Africa and Asia, access to potable water is a major problem with above 800 million people affected (Karamage *et al*., 2016). Approximately half a million children die annually due to diarrheal diseases (Mathew *et al*., 2017). Water resources particularly rivers in the world are degraded by discharge of untreated sewage, untreated industrial wastes for example solvent chemicals, papers and sludge; leaching of agricultural chemicals (fertilizers and pesticides) due to increased human activities. For instance, assessment of surface water quality in India by Islam *et al*. (2015) reported that human activities contributed to water pollution. 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 (Chen *et al*., 2017). Worldwide, indicator microbes like *E. coli, Coliform bacteria* and *Faecal streptococci* have been used to assess water faecal contamination (Chen *et al*., 2017).

In Kenya, approximately 17 million people (43%), lack access to potable water, due to contamination of water sources. This leads to waterborne diseases which are mostly of gastro-intestinal tract (GIT) such as typhoid, paratyphoid, cholera, allergy and dysentery (Olaulo *et al*., 2014 and Mazumder, 2017). Water sources in Kenya particularly rivers are becoming contaminated by both point and nonpoint sources attributed to human activities which lead to organic, inorganic and aesthetic pollution of water (Aywa, 2017). The contaminated river water is a habitat for various microbes which pose a health hazard to humans, animals and plants. Various studies have been done in Kenya and established presence of microbes in river water. For instance, studies on Nairobi river (Musyoki *et al*., 2013) reported the presence of pathogenic microbes like; *E. coli, Shigella flexneri, Salmonella paratyphi, Klebsiella aeroginosa* and *Enterococcus faecalis*. A study by Waithaka *et al*. (2015) on water running in community taps and River Kandutura in Nakuru, established the presence of *E. coli, Shigella spp* and *Salmonella spp.*

People in developed and developing countries use untreated water for both domestic and irrigation purposes (Lüneberg *et al*., 2018). Untreated water for crop irrigation reduces crop productivity and risks health of approximately a billion people worldwide (Wafula, 2014). 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 and physicochemical 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. Reports on heavy metals present, the

influence of selected physicochemical parameters on macroinvertebrates and the effect of agronomic activities in the river Kuywa have been reported (Omwoma, 2011; Oruta, 2017 and Wasike, 2015). However, data on season variation of microbiological and physicochemical parameters of river Kuywa and how it affects water quality is lacking. Documentation of this would add to the body of knowledge on types of microbes existing and health risks associated with river Kuywa water.

Microbes have been isolated and identified using various methods to various levels of success. For instance, Musyimi *et al*. (2017) studied bacteria of river Aora at Maseno, in Kenya, using morphological and biochemical characteristics. Similarly, Menya *et al*. (2018) used morphological and biochemical characteristics and identified *Escherichia coli, Enterobacter spp., Citrobacter spp., Proteus spp., Serratia spp., Shigella spp., Providencia spp., Morganella spp., Salmonellae spp.* and *Klebsiella spp*. in water obtained from Omubhira stream in Kakamega county, Kenya. These studies reveal that indeed water bodies, particularly rivers are contaminated with microbes (Zuma, 2010). However, the extent of microbial contamination varies from one water body to another and that some contamination may be above the recommended World Health Organization (WHO) limits. A study by Oruta *et al*. (2017) in river Kuywa water recorded different types of macroinvertebrate. However, the only few studies conducted on river Kuywa have been on the aquatic biology of the river (Oruta *et al*., 2017), which have reported different types of macroinvertebrates in the river. There are no studies that have investigated on the physicochemical and microbiological parameters that can indicate the quality of the water. Therefore, a study on different types of bacteria and fungi in river Kuywa water would add to the body of knowledge on types of microbes (bacteria and fungi) existing in the water and enable us understand the health risks associated with river Kuywa water.

Physicochemical parameters such as pH, temperature, turbidity, dissolved oxygen, biological oxygen demand and chemical oxygen demand are vital in defining the quality of potable water and they are utilized to predict the pollution level and framing of suitable strategies for restoration and remediation measures. They are influenced by anthropogenic inputs originating from agricultural, domestic and industrial activities (Nshimiyimana *et al*., 2016). Changes in physicochemical parameter levels of rivers or other water bodies provide congenial conditions for microbes to thrive which are detrimental to human, plant and animal health (Kiema, 2014; Ford *et al*., 2017). Tenge *et al*. (2015) assessed drinking water quality on river Malakisi in Kenya and established that turbidity and BOD levels were on higher side of WHO permissible limits. A study by Oyoo (2017) on river Kuja, in Kenya established variation of physicochemical levels for instance pH levels were within WHO permissible limits while biological oxygen demand, chemical oxygen demand and turbidity were above WHO permissible limits. Reports on influence of selected physicochemical parameters on metals, the effect of agronomic activities in the river (Kuywa) and the relationship between water quality and macroinvertebrate assemblages in river Kuywa have been reported (Omwoma, 2011; Omwoma *et al*., 2014 and Oruta *et al*., 2017). A study on the physicochemical parameters and how they affect microbiological (bacteria and fungi) available in river Kuywa is of interest, particularly bearing in mind that this river is the major source of water for domestic, agriculture, and industrial purposes across a vast area of its catchments.

Nutrients are important in assessing water quality and they are influenced by agricultural and industrial activities. Farmer (2018) showed that, storm water runoff and discharge of sewage into rivers are two major ways that various nutrients enter the aquatic ecosystems. Nitrates (inorganic nitrogen and organic/soluble phosphate) are important in microbial growth and distribution.

Ouma (2015) established that high microbial content in both seasons (dry and wet) were attributable to low nitrates and high phosphorous in five catchment areas of Lake Victoria. Similarly, Chatterjee (2010) revealed that nitrates increased microbial population in river Damodar, in India. Omwoma *et al*. (2014) studied the effect of agronomic on the levels of physicochemical parameters in river Kuywa within the Nzoia sugar factory nuclear plantations. This study (Omwoma *et al*., 2014) failed to relate the physicochemical parameters to microbes (bacteria and fungi) which is a focus of the current study.

Water bodies are areas of intense microbial activity and for microbial interaction as nutrients and levels of physicochemical parameters in water bodies are the main source of food and driving force 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). Sogbanmu *et al*. (2018) assessed water quality in rural and urban areas of Ibeju-Lekki, in Nigeria and established high bacterial count compared to WHO permissible limits. A study by Onyango *et al*. (2018) on water quality in Isiolo, in Kenya, found high microbial count compared to WHO permissible limits. There is need to explore the microbial count in river Kuywa water and establish whether they are within WHO permissible limits.

Seasonal variation is a contributing factor to levels of microbes and physicochemical parameters of a river (Akbarimehr *et al*., 2016). Contrasting results have been reported by various researchers on assessing the distribution of microbes by seasons and concentration of various physicochemical parameters. For instance, Aywa (2017) assessed the suitability of river Athi water for irrigation use, in Athi river town, Kenya and established seasonal variation of bacteria between seasons with a high bacterial count during wet season compared to dry season. Tenge *et al*. (2015) on the other hand studied river Malakisi, in Malakisi, Kenya and found no

variations in microbial distribution between the wet and dry seasons. A study by Achieng *et al*. (2017) on river Sosiani showed that the quality of water did not vary in physicochemical parameters based on the seasons. Therefore studies are needed to be conducted to establish seasonal variations of microbiological and physicochemical parameters in river Kuywa and this will help us understand if the water quality meets WHO permissible limits in both seasons.

1.2. Statement of the Problem

Globally, water pollution is a major cause of diseases and death (Musyimi *et al*., 2017), and accounts for the death of about 1.7 million people annually (WHO, 2017). Contaminated river water is a habitat for various microbes which pose a health hazard to humans, animals and plants. Various studies in Kenya have established the presence of pathogenic microbes in river waters, such studies have not been conducted in river Kuywa. River Kuywa is an important water body which runs from mt. Elgon and drains into river Nzoia. The quality of Kuywa river water has been affected by waste discharge, agricultural and industrial activities as evidenced by recent health statistics indicating more than 40,000 cases of typhoid and allergy recorded in nearby health facilities (Wasike, 2015). Locally, river Kuywa provides water for consumption, agricultural and recreational activities. However studies conducted in river Kuywa have been on the aquatic biology of the river. For instance, Oruta *et al*. (2017) reported different types of macro invertebrates in the river. There are no studies that have investigated on the physicochemical and microbiological parameters that can indicate the quality of the water. Therefore, a study on different types of microbes in river Kuywa water would add to the body of knowledge on types of microbes (bacteria and fungi) existing in the water and enable us understand the health risks associated with river Kuywa water.

The presence and distribution of microbes in water bodies is influenced by physicochemical parameters (Zuma, 2010). Reports on influence of selected physicochemical parameters on the effect of agronomic activities in the river Kuywa and the relationship between water quality and macroinvertebrate assemblages in river Kuywa have been reported (Omwoma, 2011). Therefore, a study on physicochemical parameters and how they relate to bacteria and fungi in river Kuywa is of interest particularly bearing in mind that this river is the major source of water for domestic, agriculture and industrial purpose across a vast area of its catchment. However, it is not known whether the bacterial and fungal count in Kuywa river water lies within the WHO permissible limits for domestic water use.

1.3. Justification

Kuywa river water serves a large population of Bungoma County with water for use in domestic, agricultural and recreational activities. The river is therefore an important resource to the people in the county. Documentation of microbiological and physicochemical parameters of Kuywa river water may establish whether the water meets WHO permissible limits. Water quality has specific criteria for identifying levels of microbiological and physicochemical pollutants to determine whether it is fit for human consumption. This study helps to identify the possible sources of pollution of river Kuywa.

Microbiological and physicochemical parameters that are properly monitored can be analyzed for trends over time and help come up with suitable methods and policies to control contamination problem (Makinde and Benjamin, 2016). Establishing levels of microbiological and physicochemical parameters and comparing the same with recommended values by WHO permissible limits may serve as an early warning of potential pollution problems of river Kuywa. The study will inform policy makers such as the National environmental management authority

(NEMA) on the status of quality of river Kuywa contamination so as to guide relevant stakeholders on the management of the environment surrounding the river.

1.4. Objectives of the Study

1.4.1. General Objective

The general objective of this study was to investigate on the microbial and physicochemical parameters of river Kuywa water in Bungoma County, Kenya with a view of establishing the quality and safety of the water for human consumption.

1.4.2. Specific Objectives

1. To determine the types of bacteria and fungi present in river Kuywa water using biochemical and morphological characteristics.
2. To determine the levels of physicochemical parameters (pH, temperature, turbidity, dissolved oxygen, biological oxygen demand, chemical oxygen demand, nitrates and phosphates) in river Kuywa water.
3. To determine bacterial and fungal counts in river Kuywa water.
4. To determine the relationship between physicochemical parameters (pH, temperature, turbidity, dissolved oxygen, biological oxygen demand, chemical oxygen demand, nitrates and phosphates), and bacterial and fungal counts in river Kuywa water.

1.5. Hypotheses

1. There are different types of bacteria and fungi present in river Kuywa water.
2. There are significant differences in the levels of pH, temperature, turbidity, dissolved oxygen, biological oxygen demand, chemical oxygen demand, nitrates and phosphates in river Kuywa water.
3. There are significant differences in bacterial and fungal count in river Kuywa water.

4. There are positive relationships between pH, temperature, turbidity, dissolved oxygen, biological oxygen demand, chemical oxygen demand, nitrates, phosphates and bacterial and fungal counts in river Kuywa water.

CHAPTER TWO

LITERATURE REVIEW

2.1 Microbial Contaminants of River Waters

Contamination of water bodies microbiologically is one major cause of waterborne and food-borne diseases globally. Microbiological water body contamination results from human feacal and warm blooded animal faeces and can lead to serious health problems (Megan *et al*., 2013; Rodrigues and Cuhna, 2017). The major microbial pathogens associated with water contamination are bacteria, fungi, viruses and protozoan parasites. Bacterial pathogens are mostly associated with faeces and their presence in water is due to feacal contamination (Olaolu *et al*., 2014). Poor domestic waste disposal and wastewater release into environment have negative impact on human and animal health (Megan *et al*., 2013). Microbiologically contaminated water serves as a medium for several waterborne diseases such as typhoid fever, cholera, campylobacteriosis, giardiasis, shigelloisis, Paratyphoid, dysentery, allegy and Hepatitis A (Megan *et al*., 2013).

According to WHO (2006), no indicator bacteria should be present in 100 ml of drinking water (Ouma, 2015). In most studies, indicator organisms have been used to assess water quality microbiologically as it is hard to detect all potential pathogenic bacteria and fungi (Hussam, 2016). Owour *et al*. (2016) used morphological, chemical and molecular techniques on stools and water samples to identify *V. vulnificus* and *V. cholera,* while Rutanga (2014) employed morphological and biochemical techniques on microbial analysis of ground water of Gikonda industrial Area Park and identified *E. coli, Staphylococcus spp, Klebsiella and Enterobacter spp*. Oliveira *et al*. (2016), carried out fungal analysis of ground water, in Brazil, using morphological approach and identified, *Penicilium spp, Aspergilus spp* and yeast. From these studies, it is

evident that most of water bodies particularly rivers, are microbial contaminated though contamination levels may differ from one river to the other. Conservation and sustainable use of fresh water has been considered of great importance and has been receiving attention in recent years (Haque *et al*., 2019). Reports on influence of selected physicochemical parameters on metals, the effect of agronomic activities (Omwoma, 2011 and Omwoma *et al*., 2014) and the relationship between water quality and macroinvertebrate assemblages in river Kuywa, respectively have been reported (Oruta *et al*., 2017). Despite the known crucial role of microbial communities in waterborne diseases transmission, few studies have been conducted on river Kuywa to establish the microbiological water quality and if within the WHO limits or not.

2.2. Indicators of Contaminated Water

Surface water is commonly associated with pathogenic microbes which when present in large amount, pose tremendous effects to human and animal health (Augustyn *et al*., 2016). In most studies, indicator organisms have been used to assess water quality microbiologically as they provide evidence of presence or absence of pathogenic microbes (Ontumbi *et al*., 2015).

According to Hussam (2016) density of indicator organisms is determined by health hazards and sources of contamination. Kemboi and Mwangi (2016) lists the characteristics of indicator microbes thus; they must not multiply in the environment, be non-pathogenic, easily detectable and present in greater numbers than pathogens.

Bacterial indicators mostly comprise of members of Enterobacteriaceae family (Coliforms) such as *Faecal coliforms, E. coli, Faecal streptococci* and *Clostridium* (Olaolu *et al*., 2014) and are known to cause diseases in human and animals. Choudhury *et al*. (2016) employed multiple tube and biochemical techniques and found *Coliforms, E. coli, Klebsiella spp, Bacillus spp,*

Enterobacter spp and *Pseudomonas spp* as indicator microorganisms in Bahini River, in India. A study by Kemboi and Mwangi (2016) on water quality used by residents of Kabianga, Kericho County, in Kenya, identified *E. coli*, filamentous fungi and yeasts. Furthermore, a study by Oliveira *et al*. (2016) identified *Penicilium spp, Aspergilus spp* and yeast as common fungal indicators in ground water in Brazil. Aywa (2017) observed that microbial pathogens are one of the potential irrigation water quality parameters but are often neglected and *E. coli* is the most preferred indicator of microbial contamination. Indicator microbes in river Kuywa are yet to be established, although they have been reported in other rivers by previous studies (Tenge *et al*., 2015; Oliveira *et al*. (2016) and Aywa, 2017). The microbial limit of indicator organisms in drinking water in Kenya are shown in table 2.1.

Table 2.1: WHO limits for potable water

Parameter	Unit	Maximum permitted levels	Health impacts
Total coliform counts	Cfu/100ml	10	Indication of faecal contamination
Thermo-tolerant coliforms or *Escherichia coli*	Cfu/100ml	0	Urinary tract infection, bacteraemia, meningitis, diarrhea, renal failure and haemolytic anaemia
Faecal streptococcus	Cfu/100ml	0	Indication of recent faecal contamination
Clostridium perfringes spores	Cfu/100ml	0	Index of intermitted faecal contamination

2.3. Morphological Identification of Bacteria in River Water

Bacteria are microscopic microorganism which live under diverse environmental conditions and differ on morphological aspects which depict their physical features (Yang *et al*., 2016). Bacteria are categorized in three forms: spherical, rod and spiral forms and they may be identified through culturing by separating cultures based on morphological characteristics like, colour, growth rate

and colony texture and through microscopy technique (Petersen and McLaughlin, 2016). The main bacteria of concern in contaminated water include; *Salmonella spp, Shigella sp, E. coli* and *Vibrio cholera* and the presence of coliforms has been widely used (Abila *et al*., 2012).

Water contamination by bacteria causes waterborne diseases like typhoid, cholera, shigelloisis Paratyphoid and dysentery (Musyimi *et al*., 2017). Contrasting results have been reported by various researchers while assessing the distribution and concentration of bacteria in water (Shafi *et al*., 2017). Abila *et al*. (2012) used morphology technique such as colour, shape, microscopic features and Gram staining features on borehole water in Kitui County, Kenya and identified 9 pathogenic genera including, *Salmonella, Escherichia, Vibrio, Listeria, Staphylococcus, Enterobacter, Klebsiella* and *Pseudomonas.* Nyongesa *et al*. (2016) and Augustyn *et al*. (2016) employed morphological approach-membrane filter technique, that is, bacterial size (generally 0.45 um), colour and shape to identify *E. coli.* From these studies, it is evident that *Escherichia coli* are dominant in river water while other microbes like *Salmonella, Vibrio, Staphylococcus* and *Klebsiella* are not found in most of river water.

Identification of bacteria basing on morphological characteristics on culture media in water is a common practice (Abila *et al*., 2012). However, similar studies are lacking for river Kuywa water to determine the presence of these microbes and how they affect water quality.

2.4. Morphological Identification of Fungi in Water

Fungi range from unicellular, microscopic and multicellular organisms found in diverse environments. Aquatic fungi show morphological and physiological adaptations such as release of spores as well as floating conidiospores. Most aquatic fungi are able to cause human health risks (Oliveira *et al*., 2016).

Fungal contamination of water causes waterborne diseases like Ringworm, Aspergillosis, Candidiasis, fungal eye infection and allergy (Solo-Gabriele *et al*., 2016). Previous studies have identified fungi basing on morphological characteristics (Oliveira *et al*., 2016). Makinde and Benjamin (2016) studied waste water and water sources of a cocoa processing company in Ghana which revealed the presence of *Aspergillus spp.* These studies were done using growth morphology approach and microscopic aspect for identification that included black powdery, filamentous, non-septate, conidia and opaque. A study on ground water by Oliveira *et al*., (2016) used morphological characters such as colour, septation and shape of conidium and identified *Penicilium spp* as dark green, Brown, filamentous, septate, conidium flask-shaped and opaque.

Most researchers have mostly relied on growth morphology to identify fungi up to species level (Oliveira *et al*., 2016). However, data on various fungi responsible for water contamination in river Kuywa is lacking, particularly bearing in mind that this river is the major source of water for domestic, agriculture, and industrial purposes across a vast area of its catchments.

2.5 Biochemical Identification of Bacteria in Water

Biochemical approach identifies microbes basing on their metabolic activities (Nannipieri *et al*., 2017). Biochemical approach serve as a preliminary characterization of microbes and it gives an idea of what these microbes can do. Possibly it may be used to discriminate different strains of the same species basing on biochemical profiles and differences in enzymatic activities unlike molecular technique which identifies microbes to strain level.

Various studies have been done using biochemical identification approach, for instance, Behera (2012) employed biochemical test in identification of unknown microbes in river Rourkela which were revealed as *Enterobacter spp*. The study used methyl red which resulted as negative and

urease test which resulted positive. Kipyegon and Raymondi (2016) used catalase and carbohydrate fermentation tests to study microbes in Ponds, which yielded positive results for the presence of *E.coli*. The same study also identified *Salmonella spp* as Gram staining negative, Catalase positive and carbohydrate fermentation positive. Similar studies by Waithaka *et al*. (2015) identified *Salmonella spp* as Gram negative, *E. coli* as catalase positive in river Nakuru, in Kenya.

The biochemical identification of bacteria in river Kuywa water is yet to be performed, although biochemical identification of bacteria have been reported in other rivers in Kenya by (Tenge *et al*., 2015; Waithaka *et al*. 2015 and Kipyegon and Raymondi 2016).

2.6 Physicochemical Characterization of Contaminated River Water

Physicochemical parameters are key indicators in assessing environmental fate such as; determining the phase equilibrium of a substance in a closed system and monitoring water quality (Ligawa, 2011). Some general water quality parameters like turbidity, temperature, pH and conductivity usually serve as general indicators of water quality across the board (Mwaura, 2006).

Water pollution is brought about by industrialization, urbanization and agricultural activities and it affects physicochemical and microbiological aspects of water bodies (Oduor, 2017). The presence and distribution of microbes in water bodies is influenced by physicochemical parameters (Masters, 2015). Ibrahim and Bologun (2009) studied water quality of Kontagora reservoir, Nigeria, and established that, physicochemical properties of water immensely influence the use of water.

Several studies have been done on water bodies in relation to physicochemical aspects in various parts of the world with contrasting results. Gichana *et al*. (2014) studied water quality of Nyangores stream, in Kenya and found that, temperature, dissolved oxygen (DO), potential hydroxide (pH) and biological oxygen demand (BOD) varied from site to site with only pH and DO were within WHO permissible limits. A study by Aywa (2017) on river Athi, in Kenya, found that pH, TDS and Electrical conductivity (EC) respectively were higher than WHO permissible limits. Nyairo *et al*. (2015) while studying river Mara water in Kenya, found that pH, NO_4 and PO_4 were within WHO permissible limits and only pH was slightly below WHO permissible limits.

These previous studies have shown that, levels of various physicochemical parameters vary among rivers and between seasons. Reports on the influence of agronomic activities on physicochemical parameters (Omwoma *et al*., 2014) and the relationship between physicochemical parameters and macroinvertebrate have been documented (Oruta *et al*., 2017) in river Kuywa. However, the extent to which physicochemical parameters of river Kuywa water vary between seasons and their contribution to microbiological water quality and distribution has not been studied. The WHO recommended standards for physicochemical levels for drinking water are shown in table 2.2.

Table 2.2: Recommended standards for physicochemical parameters in drinking water

S/NO	Parameter	WHO limits	References
1	Temperature (^{O}C)	23-30	Mgbemena *et al*., 2012
2	pH	6.5-8.5	
3	Chemical Oxygen Demand (mg/l)	Above 0.250	Sahoo *et al*., 2016
4	Biological Oxygen Demand (mg/l) (BOD)	4-6	Kumar *et al*., 2013
5	Dissolved Oxygen (mg/l) (DO)	Above 6	Mgbemena *et al*., 2012
6	Nitrates (mg/l) (NO_3^-)	<45	
7	Phosphate (mg/l) (PO^-_4)	0-1	
8	Turbidity (NTU)	0-5	

2.6.1. Water Temperature

Water temperature as a physical property depends on seasons, geographical location and meteorological conditions such as rainfall, humidity, cloud cover, wind velocity and turbidity (Mathew *et al*., 2017). The guideline levels recommended for drinking water by WHO are between 23- 30^{o}C (Mgbemena *et al*., 2012).

The growth rate of microorganisms' increases by increasing the temperature as high temperature accelerates the chemical and biological processes in the water resulting in reduction of its ability to hold the essential dissolved gases like oxygen (Raju *et al*., 2012). For instance mesophiles grow at 20-45^{o}C is suitable for microorganisms proliferation, Psychrophiles grow at 10-15^{o}C and thermophiles grow at 45-122^{o}C (Wafula, 2014 and Stevenson *et al*., 2015). High temperature level reduces dissolved oxygen and hence put microorganisms under stress (Wafula, 2014). A study by Ouma (2015) on five catchment areas of Lake Victoria basin, in Kenya, recorded temperature levels between 22-27^{o}C in wet season and 24-30^{o}C in the dry season. Musyimi *et al*. (2017) found that the temperature varied from site to site along Aora river. For instance one site

had 23.8^{o}C while other sites had lower temperature levels of between 22.8 and 22.6^{o}C which were below WHO permissible limits.

Variation in temperature levels in both dry and wet season could be due to higher rainfall with intense cloud covers which reduced intensity of sunrays hence leading to low temperature levels. Higher temperature levels may be attributed to deforestation which exposes river water directly to sunlight and this raises water temperature (Wafula, 2014). Water temperature levels play a crucial role in ecosystem and agricultural use. Establishing a relationship between microbes and temperature in river Kuywa water would help to understand how temperature affects availability of microbes in the water.

2.6.2. Water pH

Drinking water is often slightly basic (between 7 and 8.5) due to the presence of hard-water minerals (Sewe, 2013). pH is affected not only by the reaction of carbon dioxide but also by organic and inorganic solutes present in water (Dwivedi *et al*., 2014). Changes in pH can be indicative of water pollution (Ouma, 2015). The WHO (2006) standards recommend that drinking water be in the pH range of 6.5-8.5.

pH controls the growth of pathogenic microorganism by affecting shape of enzymes, alters ionic charges on the molecule (Johnson *et al*., 2014). Most microbes grow at neutral pH (6.5-7) while others can grow at pH 1.0 acidophilic to alkaline conditions (Johnson *et al*., 2014). Increase in pH level affects microbial abundance where basic pH leads to a net decrease in in survival of microbes (Mounjid *et al*., 2014). Studies have been done with contrasting results of pH levels, for instance. Wafula (2014) on river Mara, in Kenya, established that pH levels ranged between 6.5-6.9 which were within the WHO set permissible limits. Sindani (2013) revealed that, pH

levels of water were slightly acidic and below the WHO permissible limits within two seasons of study. For instance wet season recorded 4.6 while dry season had 5.2. Omwoma *et al*. (2014) studied water quality of river Kuywa in Kenya, within dry and wet season and recorded lower pH levels which ranged between 5.6- 6.2 and were below WHO permissible limits.

Variation in pH levels has been attributed to concentration of carbon dioxide augmented into a water body from; respiration activities by aquatic organisms, release from microbes and atmospheric carbon dioxide (Omwoma *et al*., 2014). The dissolved carbon dioxide forms a weak carbonic acid which then lowers the pH of water (Omwoma *et al*., 2014). Rutanga (2014) indicated that, continuous use of water with low pH level for irrigation purposes renders the soil unfit for further cultivation as the soil will be acidic and this will hamper crop growth.

Omwoma *et al*. (2014), though finding level of pH, did not link it to microbial growth. Such a relationship between microbes and pH levels which may help us understand how pH influence water quality and microbial availability.

2.6.3 Water Turbidity

Turbidity of water depends on runoff, human activities, phytoplankton and storm water pollution (Wilson, 2016). The WHO standard for drinking water has a turbidity level of 0-5 Nephelometric Turbidity Unit (NTU) (Gunnarsdottir *et al*., 2016). Excessive turbidity in water interferes with water purification processes such as flocculation and filtration, which may increase treatment cost. Elevated turbid water is often associated with the possibility of micro-biological contamination as high turbidity makes it difficult to disinfect water properly and provides spaces for microbes to attach and proliferate (Heibati *et al*., 2017).

Failure to meet turbidity recommended limits, indicate possible presence of pathogens in water and elevated turbidity indicate high microbial load while low turbidity levels indicate safe water

(WHO, 2017). Kakoi *et al*. (2016) studied turbidity level in river Nairobi, Kenya and recorded high turbidity levels than WHO permissible limits between both seasons. Obed (2012) studied turbidity level in river Asakawkaw in volta region, Ghana where they established high turbidity levels than WHO permissible limits. A study by Idowu *et al*. (2016) on biodegradation of pollutants in waste water, Nigeria, revealed, varied turbidity levels for instance one site had 4.5 while other sites had 5.6 and 6.1 respectively.

The studies attributed high turbidity levels to increased human/anthropogenic activities along the river. These activities discharge suspended matter in water and displace the settled matter (Obed, 2012). The high turbidity levels increased microbial communities in these rivers by creating room for microbial attachments. Previous studies (Obed, 2012 and Kakoi *et al*., 2016) did not link turbidity to microbial growth. There is a need to establish the relationship between microbes and turbidity levels in order to understand how turbidity levels affects water quality and microbial diversity.

2.6.4 Water Dissolved Oxygen

Dissolved oxygen (DO) change rapidly under the influence of different environmental factors such as temperature elevation, speed of flow of water, aeration of the water as it tumbles over rocks, the chemical nature of bottom sediments and as a product of photosynthesis by submerged aquatic plants and microbial life (Ouma, 2015; Cronk and Fennessy, 2016).

The guidelines level recommended for drinking water by WHO is above 6 mg/l. Dissolved oxygen is important for the survival of aquatic organisms (Ouma, 2015). Wafula (2014) found that, adequate DO levels are important to provide for aerobic life forms which carry on natural stream purification.

Sufficient amount of oxygen in the water is shelter for bacteria and other pathogens, which are anaerobic and injurious to human health (Raju *et al*., 2012). DO is very important factor for the aquatic organisms, because they affect their biological process. For the oxidation of the organic matters and the sediments, the complex organic substances are converted to simple dissolved inorganic salts which could be utilized by the microbes (Mounjid *et al*., 2014).

The studies further indicated that, when DO levels fall below 5.0mg/l, aquatic life is put under stress. Kilonzo *et al*. (2014) found DO levels within WHO permissible limits in Mara river, in Kenya. Bora and Goswami (2017) on Kolong River, in India, found that, DO levels varied from site to site. For instance one site had 4.2 while other sites had 6.0 and 6.5 respectively.

Bora and Goswami (2017) attributed low DO levels to higher temperature, increased organic matter in rivers and increased microbial communities in river. Despite the crucial role of DO level in aquatic ecosystem and agricultural use, the DO level in Kuywa river water and how it affects the water quality is not known.

2.6.5 Water Biological Oxygen Demand

Biological oxygen demand (BOD) is the amount of oxygen required for the biological decomposition (oxidation) of dissolved organic matter by microbes under standard condition at a standardized time and temperature. Usually, the time is taken as 5 days and the temperature is 20°C. BOD is also used to evaluate the amount of organic material in water bodies that support microbial growth and the high BOD levels in water samples suggest that aerobic microbes are oxidizing the organic matter present in contaminated water (Ligawa, 2011).

The guideline levels recommended for drinking water by WHO is 4-6 mg/l. High BOD level is associated with high organic matter in water which indicates high presence of microorganisms

(Ligawa, 2011). According to Ligawa (2011), certain microorganisms oxidize organic pollutants into carbon dioxide and oxygen leading to a negative correlation between BOD and DO. Organic decomposition elevates BOD levels hence creating tension on oxygen availability by depleting DO which is important for aquatic life (Oyoo, 2017).

Amde *et al*. (2016) established BOD levels within WHO permissible limits for river Modjo in Ethiopia. For instance one site had 4.0 mg/l while other sites had 4.4 mg/l, 4.3 mg/l, and 6.0 mg/l respectively. Study by Oyoo (2017) on river Kuja and South Kanyikele community in Homa Bay County, in Kenya, recorded BOD level of 720mg/l which was above the WHO permissible limits 4-6 mg/l.

High BOD levels was attributed to increased organic and inorganic pollutants in rivers indicating high microbial communities in water while low BOD levels may be due to reduced organic and inorganic materials in river indicating low microbial presence in river water (Oyoo, 2017). Reports on BOD levels and how they relate with microbial count in other rivers in Kenya have been reported by (Ligawa, 2011 and Oyoo, 2017). However, data on BOD level of river Kuywa water and how it affects microbiological growth and diversity is lacking.

2.6.6 Water Chemical Oxygen Demand

Chemical oxygen demand (COD) is the amount of dissolved oxygen required to cause chemical oxidation of the organic material in water. The presence of biologically resistant organic materials in aquatic system is indicated by COD (Oyoo, 2017). The guideline level recommended for drinking water by WHO is 0.250 mg/l (Sahoo *et al*., 2016).

COD has a negative correlation with DO, and elevated levels of COD, lowers DO levels because digestion of organic substances use a lot of oxygen as to be oxidized in water. Studies have been

done on COD levels worldwide with contrasting results, for instance, Chabukdhara *et al*. (2017) on physicochemical analysis, in Ghaziabad district, India established that, COD levels were within the WHO permissible limits for all sites with levels ranging between 0.1-0.24 mg/l. Naveen *et al*. (2017) showed variation of COD levels from site to site. For instance one site had 0.25 mg/l while others had 0.27 mg/l and 0.30 mg/l.

Higher COD levels were attributed to increased organic matter and high nutrients level. Rutanga (2014) found that, high COD level in water is responsible for a heavy depletion of oxygen. However, similar studies are lacking in river Kuywa water to help us understand how COD level affect water quality.

2.6.7 Water Nitrates

Nitrates is the most highly oxidized form of nitrogen compound commonly present in natural waters and it is a vital nutrient for growth, reproduction and the survival of microorganisms because of its presence in the molecules of nucleic acids and proteins (Tyagi *et al*., 2018). Nitrates enters water through numerous ways that include; surface runoff from surrounding catchment areas, effluent from point and non-point sources, animal excreta, dead animal cells, agricultural and industrial activities. Cyanobacteria are responsible for most nitrates fixation in freshwater systems due to the heterocysts (Zuma, 2010 and Ouma 2015). Nitrate (NO_3^-) is highly soluble in water and is stable over a wide range of environmental conditions. It is easily transported in streams and groundwater (Sewe, 2013).

Zuma (2010) and Amde *et al* (2016) reported that, high nitrates level (>1mg1^{-1}) results in eutrophication and nitrates >10 mgl^{-1} can result in significant loss of species diversity and lead to water becoming toxic to animals and humans. According to Ouma (2015), excessive nitrates can

result in restriction of oxygen transport in the bloodstream and infants under the age of four months.

A study by Amde *et al*. (2016) reported high nitrates levels ranging 0.4-2.0 $mg1^{-1}$ above standard limits for both dry and wet seasons. Similarly, Akubuenyi *et al*. (2013) found nitrates level in the range of 1.30-4.50 $mg1^{-1}$ for both seasons. These studies attributed availability of nitrates in river waters to; runoffs from near-surface soils and nitrates reduction in rainy seasons were attributed to high solubility. The studies further revealed that, high microbial load in studied rivers was due to high nitrates level. River Kuywa being in an agricultural area may be receiving a lot of the nitrate nutrients. This has however not been documented.

2.6.8. Water Phosphates

Phosphorus is important in cell metabolism, reproduction, genetic composition and energy transfer and hence is regarded as an essential element for the development of all living organisms. Phosphates availability in water bodies depends on human sewage, agricultural runoff from fields, sewage from animal feedlots, chemical and fertilizers industries and detergents. Phosphates is taken up by algae, cyanobacteria, heterotrophic bacteria and larger aquatic plants and used for growth (Zuma, 2010 and Ouma, 2015). Sewe (2013) reported that, in the case where phosphates is a growth limiting nutrient, the discharge of raw or treated wastewater or industrial waste as well as non-point source runoff to a body of water may result in the stimulation of growth of photosynthetic aquatic macro and micro-organisms in nuisance quantities.

Akubuenyi *et al*. (2013) assessed water quality and found phosphates level in the range of 0.5-2.69$mg1^{-1}$ for both seasons (dry and wet). A study by Amde *et al*. (2016) reported high

phosphates levels ranging from 0.18-19.5 $mg1^{-1}$ which were above standard limits for both dry and wet seasons. High phosphates level was attributed to firm rocks deposit, run off from surface catchment and interaction between the water and sediment from dead plant and animals at the bottom of rivers. Similarly, the studies further attributed high microbial presence in rivers to phosphates availability as it the source of carbon and energy needed by microbes for their reproduction, survival and growth. A study carried out in a different environment such as river Kuywa would further enhance understanding of the growth and diversity of microbes in this river.

CHAPTER THREE

MATERIALS AND METHODS

3.1. 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 has upper and lower part, upper part ends at Bungoma-Kimilili Bridge and lower part covers the way to river Nzoia. The river lies between latitude 34^{o} 00' E and 35^{o} 00" E and longitude 0^{o} 47' 24" N and 0^{o} 43' 40" N and covers an area of about 110 Km^{2} long (Wasike, 2015). The study area receives annual rainfall of 1500 mm with above 850 mm in wet and less than 170 mm during dry season (Juma and Kelonye, 2016). The study was based in the lower part of the river where most human activities took place. Water samples were collected from four sites namely:- Matisi (Site A), Ngwelo (Site B), Nzoia water pump (Site C) and Chalicha (Site D) (Fig 1) (Fig 1).

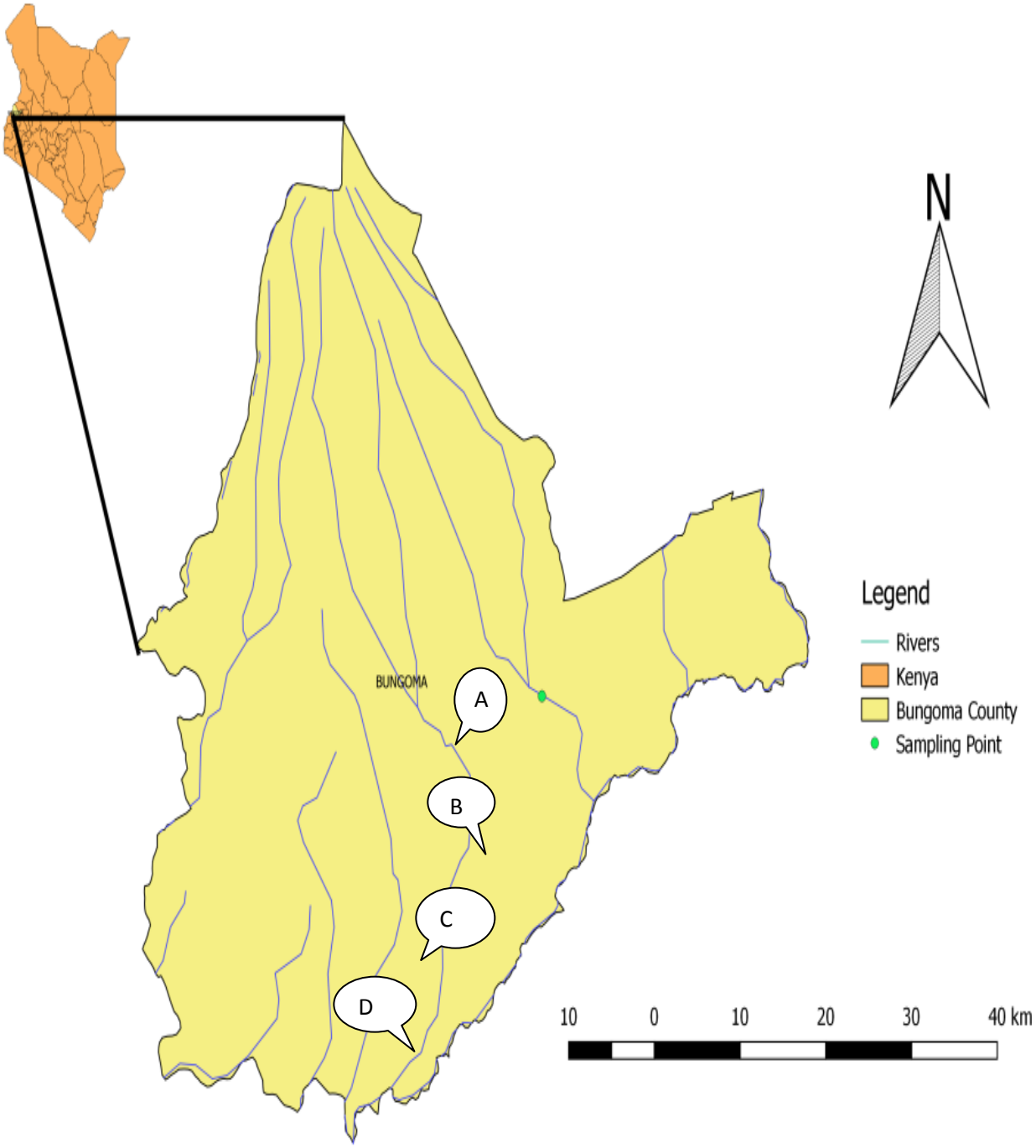


Fig 1: The sampling sites of river Kuywa (A = Matisi, B = Ngwelo, C = Nzoia water pump and D = Chailicha). (Source: Google map September, 14th, 2018).

3.2. 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 3.1, see also appendix 3), using clean sterilized 250 ml bottles from 20 to 30 cm depth (to avoid floating materials) according to Mgbemena *et al*. (2012). Water for dissolved oxygen (DO) and biological oxygen demand (BOD) determination were collected in 250 ml dark glass containers. The bottles were carefully closed and transported on ice and stored at 4^0C in a refrigerator until the analysis of microbiological and physicochemical parameters. Water samples were collected one meter from the shoreline 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. Water quality does not change drastically and therefore sampling once in dry and wet season was sufficient to indicate the quality of water. This ensured that both the rainy and dry periods were captured so as to investigate the effect of seasons on microbial (bacteria and fungi) and physicochemical parameters in river Kuywa. The sampling distance was about five kilometers between the sites. Characteristics of different sampling sites are represented on table 3.1

3.1 Table of characteristics of 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	A gricultural, effluents released from sugar factory

3.3. Bacterial Analysis of Water Collected from River Kuywa

3.3.1. Media Preparation

Nutrient agar (NA), MacConkey agar and Salmonalla shigella agar were prepared as described by Mgbemena *et al.* (2012); Izuchukwu *et al*. (2016) respectively. A clean spatula was used to measure 28 g of nutrient agar powder in a weighing balance and suspended into one litre of distilled water and allowed to boil to dissolve completely. It was sterilized by autoclaving at 121^{o}C for 15 minutes to remove suspension and bacteria by plugging with cotton and covering with aluminum foils.

Forty nine point five three (49.53) grams of MacConkey agar powder was suspended into one litre of distilled water and allowed to boil to dissolve completely. It was sterilized by autoclaving at 121^{o}C for 15 minutes and cooled to 45^{o}C.

Sixty three (63) grams of *salmonella shigella* powder was suspended into one litre of distilled water and allowed to boil to dissolve completely. The mixture was sterilized by autoclaving at 121^{o}C for 15 minutes to remove suspension and bacteria by plugging with cotton and covering with aluminum foils.

3.3.2. Isolation of Pure Bacterial Culture

The water samples from different sites were handled separately. 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^{o}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.

3.3.3. Morphological identification of bacteria isolates from water samples from river Kuywa

The pure isolated bacterial colonies were morphologically identified according to procedure described by Magesha *et al*. (2017). 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. A glass slide smear was prepared by placing deionized water drop on the slide then microorganisms obtained from the pure cultured colonies as explained in section 3.3.2 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.

3.3.4. Culturing and determination of total bacteria count

This was carried out as previously described by Makinde and Benjamin (2016). 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^{o}C. Total

bacteria count was done by adopting procedure of Magesha *et al*. (2017) 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).

3.4. Fungal analysis from water samples collected from river Kuywa

3.4.1. Media

Twenty and a half (20.5) grams of Potato dextrose agar (PDA) was suspended in one litre of distilled water, boiled to dissolve medium completely in a conical flasks. It was autoclaved for 15 minutes at 121^{o}C to sterilize the medium to remove suspension and fungi by plugging them with cotton and covering them with aluminum foil. One percent tetracycline solution was added to the medium 15 ml that was just above setting temperature before pouring into Petri dishes to prevent bacterial growth.

3.4.2. Isolation of pure fungi colonies

The water samples from different sites were handled separately. The culture techniques adopted from Chaudhuni *et al*. (2016) was employed. 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, biochemical and molecular characteristics. The pure culture isolates were sub-cultured in Potato dextrose agar and incubated at room temperature for 3-7 days for fungal enumeration.

3.4.3. 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 as described by Juthi (2016) and Leonardi *et al*. (2017). The slide was mounted and observed under magnification x400. Identity was confirmed with the help of mycology manual version 1.1 and the literature of (Alexopoulos *et al*., 2002; Juma, 2016).

3.4.4. Culturing and Determination of total Fungi Count

This was done as described by Lee and Hong (2015). Serial dilution of water samples was carried out using distilled water to a dilution factor of 10^5. 0.1 ml 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^oC. Fungi colonies were counted on the 3^{rd} 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 3^{rd} and 7^{th} day.

3.5. Bacteria Biochemical Tests of Water Samples from River Kuywa

3.5.1. Gram Staining Technique

Gram staining technique was performed according to the procedures described by Chauhan *et al*. (2017) where bacteria were separated into Gram positive and Gram negative. A glass slide smear was prepared by placing deionized water drop on the slide then microorganisms obtained from the pure cultured colonies as explained in section 3.3.2 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.

3.5.2. Indole Test

Indole test procedures was done as described by Islam (2018). This determined the ability of bacteria to split amino acid tryptophan to form compound indole. One percent tryptophan 10 ml broth was taken in test tubes and inoculated by fresh pure culture obtained from pure colonies as explained in section 3.3.2. After 48 hours of incubation period at 37^{o}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.

3.5.3. Citrate Test

This test was performed according to procedure described by Aligwekwe (2018) by inoculating the bacteria into Simmon's citrate medium obtained from pure colonies as explained in section 3.3.2. 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.

3.5.4. Methyl Red Test

Methyl red test was performed by the method described by Behera (2012) to detect production of acids formed during metabolism using mixed acid fermentation pathway using pyruvate as a substrate. Tubes of MR-VP broth were inoculated by fresh pure culture obtained from pure colonies as explained in section 3.3.2 for 18 hours. 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^{o}C after which, one milliliter (1 ml) of the initial 30 ml 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.

3.5.5. Voges Proskauer Test

This test was performed according to the procedure described by Abu and Wondikom (2018) to test for the presence of acetylmethylcarbinol (acetoin), an intermediate of the 2,3-butanediol fermentation pathway. A tube of MR-VP broth was inoculated with a pure culture of the test organism as explained in section 3.3.2 and incubated for 24 hours at 35^{o}C. One ml of broth was transferred to clean test tubes; 0.6 ml of 5% alpha naphthol was added, followed by 0.2 ml 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.

3.5.6. Carbohydrate Fermentation Test

Carbohydrate fermentation test was performed according to the procedure described by Musyimi *et al*. (2017). The test was performed by inoculating 0.2 ml 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^{o}C. Four percent of basic sugars were obtained by dissolving four grams of basic sugars in 100 ml of distilled water. 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.

3.5.7. Catalase test

Catalase test was done according to the procedure described by Ahmed *et al*. (2017) to determine aerobic and anaerobic bacteria and it was important in differentiating morphologically similar Enterococcus *Staphylococcus (catalase positive)* and *Streptococcus* spp (catalase negative). Three ml 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.

3.5.8. Oxidase test

This test was performed as previously described by Ahmed *et al*. (2017). 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.

3.6. Determination of physicochemical parameters

3.6.1. Water temperature

This was done according to Keupers and Wilems (2017) and WHO (2017) where temperature was determined *in situ* at all sampling sites, by suspending a thermometer about 10 cm below water surface for at least 2 minutes before taking the readings.

3.6.2. Water pH

The pH was determined *in situ* using a combined meter (model MI 806) as described by Keupers and Wilems (2017). The pH meter was calibrated by inserting its probe in standard buffer solutions with pH 4.0 and 7.0 then being rinsed with distilled water and inserted in water samples for 2 minutes before taking the readings. It was done in triplicates per site.

3.6.3. Water turbidity

Turbidity was determined *in situ* according to Keupers and Wilems (2017) and WHO (2017) at different sites using turbidimeter (Nephelometer) (model HACH 2100P). Before analysis, the turbidimeter was calibrated using prepared standards in the desired range for accuracy as indicated in the manufacturer's operating instruction. After calibration, standardized readings were taken in Nephelometric turbidity units (NTU).

3.6.4. Water Dissolved Oxygen

This was done as described by Vaidya and Labh (2017). The dissolved oxygen was determined *in situ* by use of dissolved oxygen (DO) meter (model MW-600). During determination, pre-rinsed probe was immersed approximately 1.25 cm into water samples and stabilized readings taken.

3.6.5. Water Biological Oxygen Demand

Biological oxygen demand (BOD) was calculated by the use of values obtained from dissolved oxygen according to Obed (2012) as follows;

BOD (mg/l) = D_1-D_2, Where:

D_1= initial DO measurement (dissolved oxygen demand) of water samples on the first day was done *in situ*

D_2= final DO measurement of the samples at the 5^{th} day was taken *ex situ* after storing water samples in dark place for five days, D_2 which was the value of DO taken.

3.6.6. Water Chemical Oxygen Demand

Chemical oxygen demand (COD) was determined according to procedure by Reece (2017), using dichromate reflux method. Ten ml of 0.25 M of potassium dichromate $K_2Cr_2O_7$ and 30 ml of H_2SO_4+ Ag_2SO_4 reagent were added into 20 ml of diluted water samples diluted by distilled water. The mixture were refluxed for 2 hours and cooled to room temperature. The solutions were diluted to 150 ml using distilled water and excess $K_2Cr_2O_7$ was titrated with ferrous ammonia sulfate (FAS). Ferrous ammonia sulfate was first used in blank (A) and finally used in sample. The difference between (A-B) was then multiplied by the normality of ferrous ammonia

sulfate (N). The samples from different sites were treated independently. The COD values were determined using the equation:

COD= ((A-B) * N*100*8)/ Volume of sample, where:

A- is the ml of FAS used as blank

B- is the ml of FAS used for sample

N- is the normality of FAS

8- is milli equivalent of oxygen

3.6.7. Analysis of water nitrates

The procedure described by Obed (2012) was employed in nitrates determination. Ten ml of the water samples obtained from collected water samples was pipetted into test tubes and one ml of 1.3 M NaOH was added and gently mixed, followed by one ml of reducing mixture and gently mixed. The mixture was heated for ten minutes at 60°C in a water bath and allowed to cool at room temperature. One ml of colour developing reagent was added to the mixture and shaken and the absorbance read at 520 nm using spectrophotometer (UV-1650 PC-UV-VIS, Shimadzu).

3.6.8. Determination of water Phosphates

This was carried out as previously described by Ouma (2015). Phosphates (PO_4^-) were analyzed by use of ascorbic acid followed by turbidimetric analysis. Twenty five (25) ml aliquot of the water sample was measured in a 50 ml graduated tube. Four (4) ml of combined reagent prepared according to manufacturers' specification consisting of ascorbic acid, ammonium molybdate, potassium antimonyl tartarate and sulphuric acid was added. The tube was covered with parafilm, shaken well and left to stand for 10-30 minutes to develop stable blue colour

thereafter reading was taken at 880 nm absorbance using spectrophotometer. The photometer was zeroed with a blank solution.

3.7. Data Analysis

Data obtained from this study was subjected to Statistical Analysis System (SAS) version 9.1. Data on physicochemical parameters of river Kuywa water, bacterial count and fungal count were obtained in triplicate, descriptive statistics including the mean, standard deviation and standard error were calculated at ($p \leq 0.05$) confidence interval. Analysis of variance (ANOVA) was used to test for any significant differences between the means of physicochemical parameters, bacterial and fungal counts during dry and wet seasons. Means that were considered significantly

Different at ($P = 0.05$) were separated using Fisher's LSD. Spearman's correlation coefficient was used to determine the relationship between water physicochemical parameters (temperature, pH, turbidity, DO, BOD, COD, NO^-_3 and PO^-_4) and bacterial and fungal count.

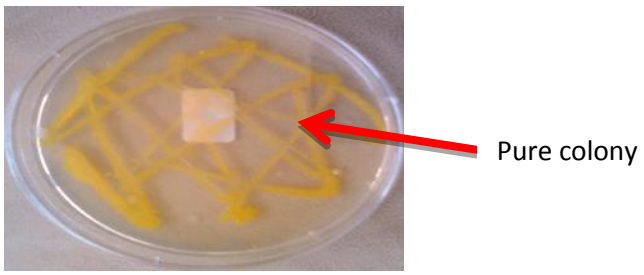
CHAPTER FOUR

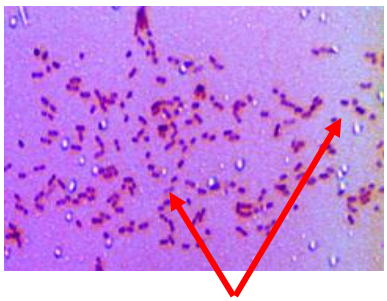
RESULTS

4.1. Morphological and Biochemical Characterization of Microbes from River Kuywa.

4.1.1. Morphological characterization of bacteria from river Kuywa water

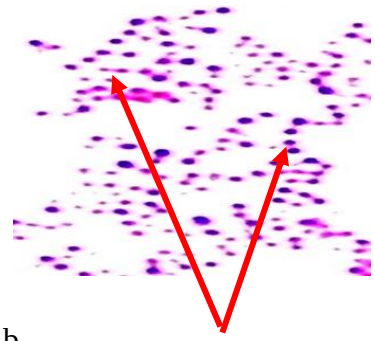
Nine pure bacterial isolates were tentatively identified as A – I (Plates 1-9). The isolates were found to be morphologically different based on colony size, shape, surface, margin, colour, elevation and opacity and cell shape (Table 4.1). **Isolate A** had circular shape, smooth surface, irregular margins, 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.





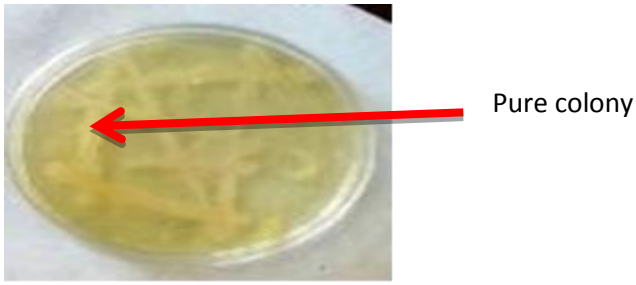
a b

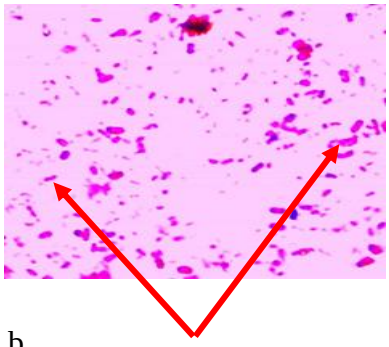
Plate 1: Pure bacterial isolate A on NA medium (1a) and the cells mg. ×1000 (1b) showing morphological characteristics.



a b Purple cocci shaped cells

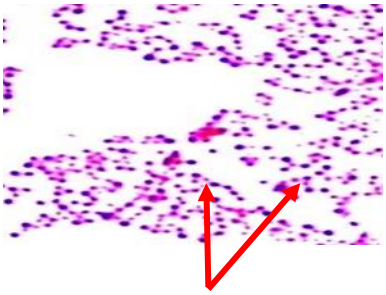
Plate 2: Pure bacterial isolate B on NA medium (2a) and the cells mg. ×1000 (2b) showing morphological characteristics.





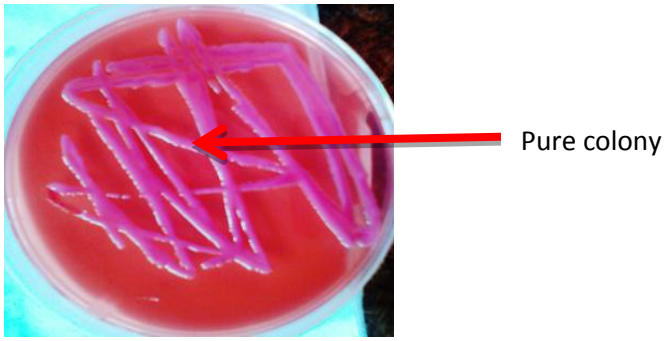
a b Pink rod shaped cells

Plate 3: Pure bacterial isolate C on NA medium (3a) and the cells mg. ×1000 (3b) showing morphological characteristics.

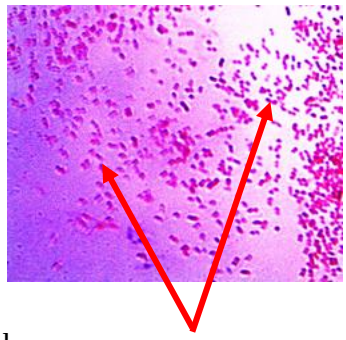


a b Purple cocci shaped

Plate 4: Pure bacterial isolate D on NA medium (4a) and the cells mg. ×1000 (4b) showing morphological characteristics.

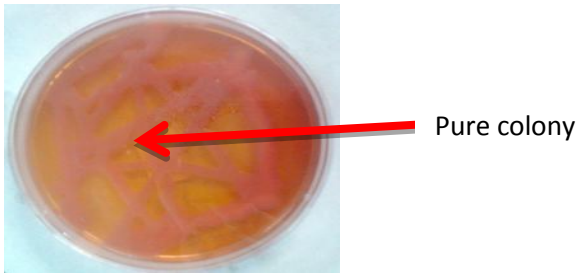


a

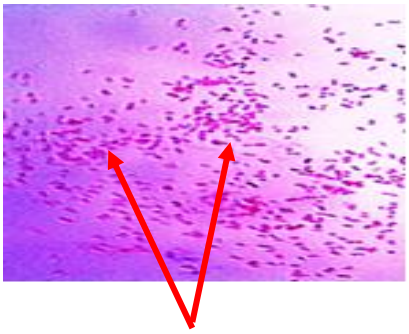


b

Plate 5: Pure bacterial isolate E on MacConkey medium (5a) and the cells mg. ×1000 (5b) showing morphological characteristics

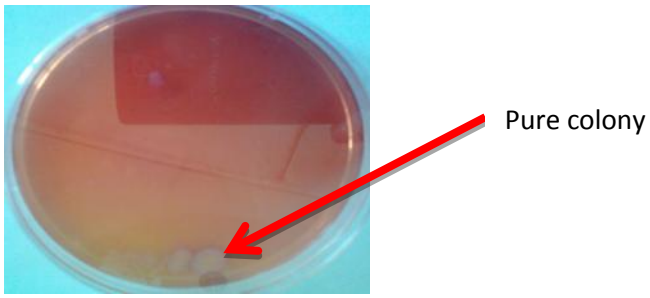


a

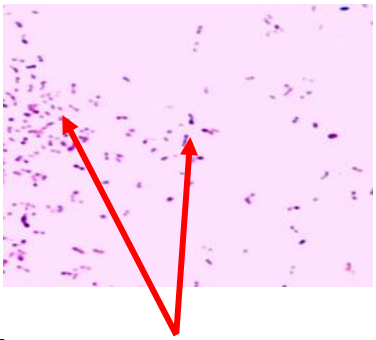


b

Plate 6: Pure bacterial isolate F on MacConkey medium (6a) and the cells mg. ×1000 (6b) showing morphological characteristics.

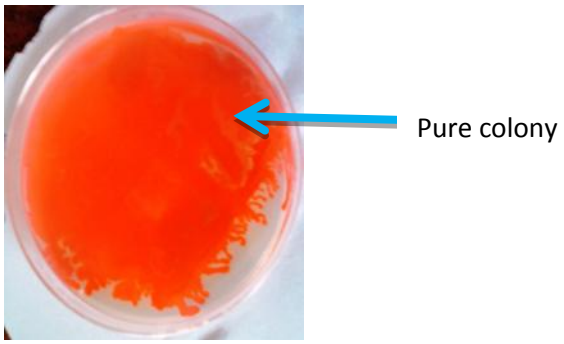


a

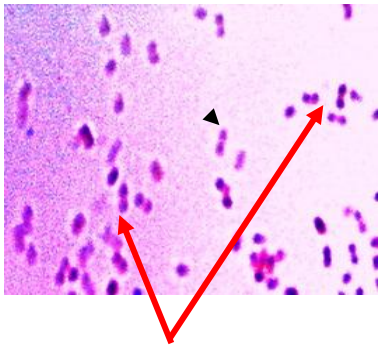


b

Plate 7: Pure bacterial isolate G on Salmonella shigella medium (7a) and the cells mg. ×1000 (7b) showing morphological characteristics.

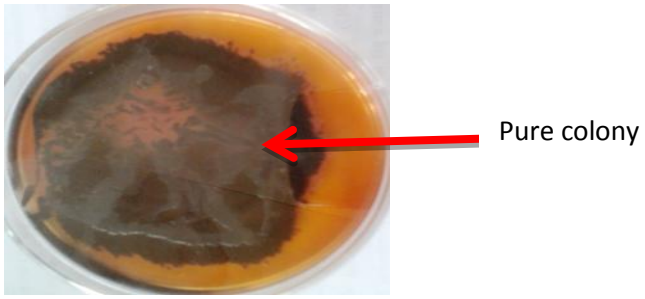


a

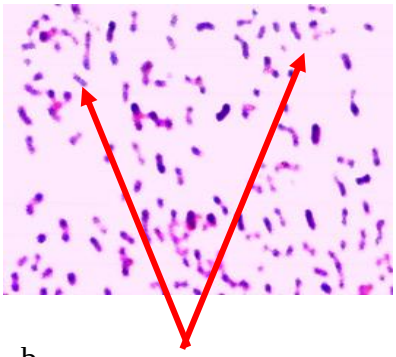


b

Plate 8: Pure bacterial isolate H on nutrient agar medium (8a) and the cells mg. ×1000 (8b) showing morphological characteristics.



a



b

Plate 9: Pure bacterial isolate I on *Salmonella shigella* medium (9a) and the cells mg. ×1000 (9b) showing morphological characteristics (Photo by John Wekulo)

Table 4.1: Morphological growth and appearance of bacteria isolates from water collected from river Kuywa

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

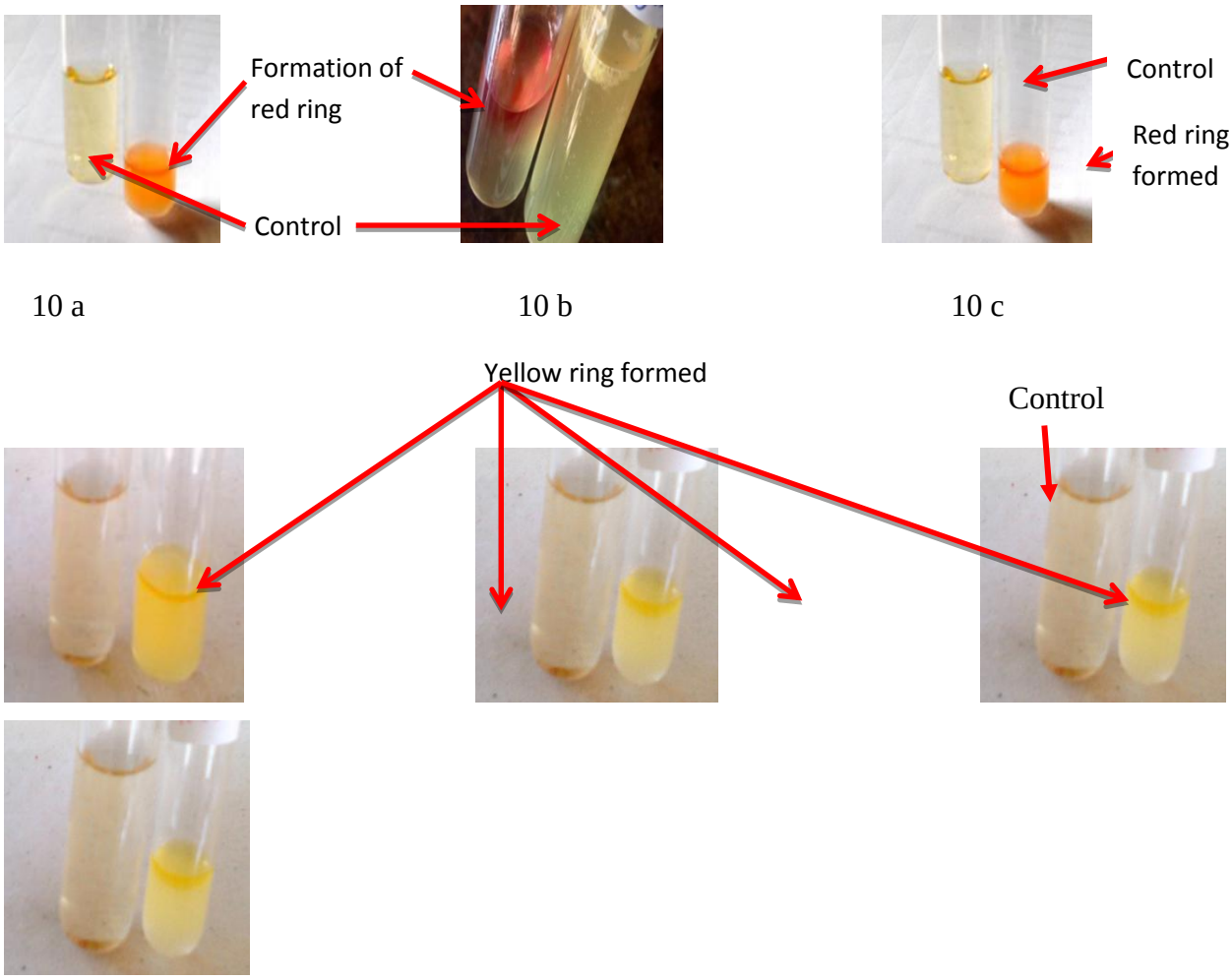
4.1.2. Biochemical characterization of bacteria isolated from river Kuywa

4.1.2.1 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 4.2).

4.1.2.2 Indole test

Indole test of bacteria isolates **A**, **D** and **E** showed a development of a red ring at top layer of the medium (Plate 10, Table 4.2) 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 (Plate 10) an indication of indole negative bacteria.



10 a 10 b 10 c

10 d **10 e** **10 f** **10 g**

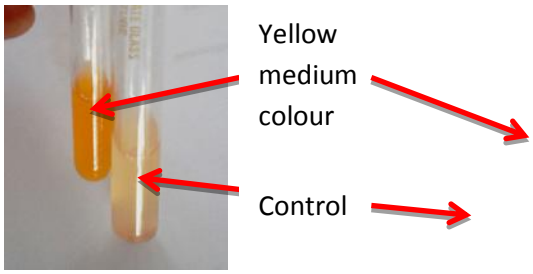
Plate 10: Indole positive bacterial isolates A (10a), D (10b) and E (10c), indole negative bacterial isolates B (11d), D (11e), E (11f) and I (10g)

4.1.2.3 Citrate test

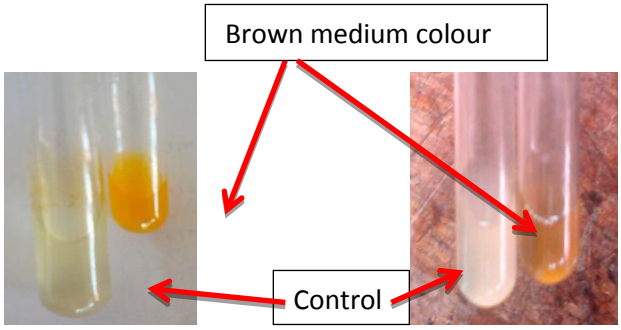
Isolates **A**, **B**, **C**, **G** and **I** were able to utilize citrate as sole carbon source because the colour of media changed from green to blue (Tables 4.2) indicating that the bacteria were citrate positive. Bacterial isolates **D**, **E**, **F** and **H** were unable to utilize the citrate as sole carbon source since the media retained the green colour after incubation period confirming the isolates were citrate negative (Table 4.2).

4.1.2.4. Methyl red test

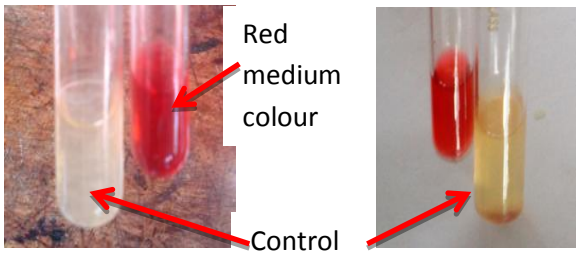
Bacterial isolates A and H after incubation, changed to yellow (Plate 10 and Table 4.2) indicating that the isolates were methyl red negative. Medium incubated with bacterial isolates C and isolate F changed to brown (Plate 11) indication of methyl red negative. Bacterial isolates B, D, E, I and G on addition of methyl red indicator, the medium changed to red from whitish (Plate 11). This was an indication of methyl red positive bacteria.



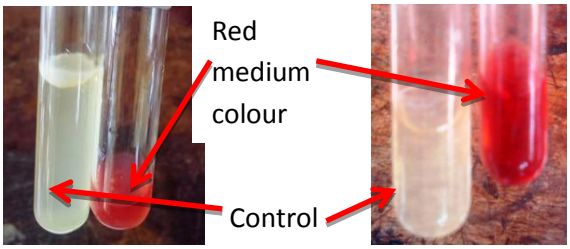
11a



11b 11c 11d



11e 11f

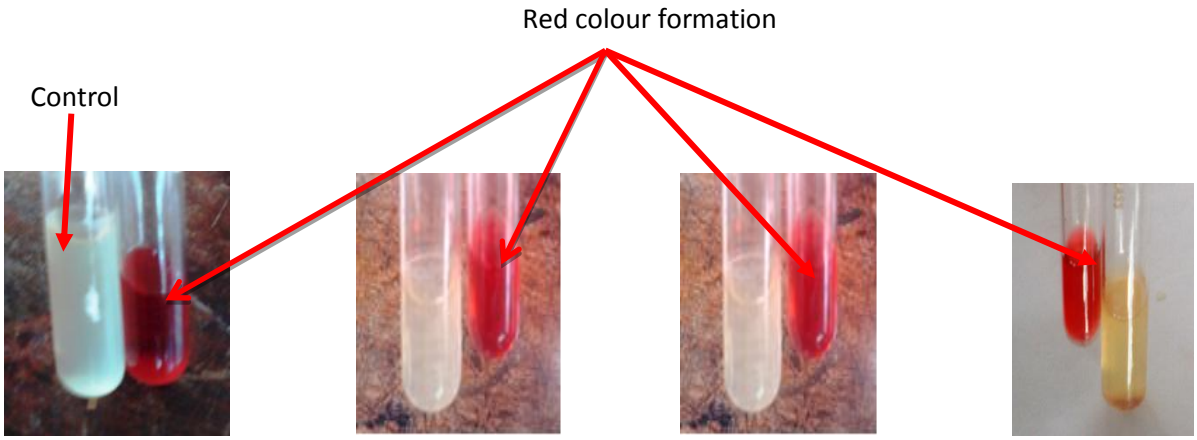


11g 11i

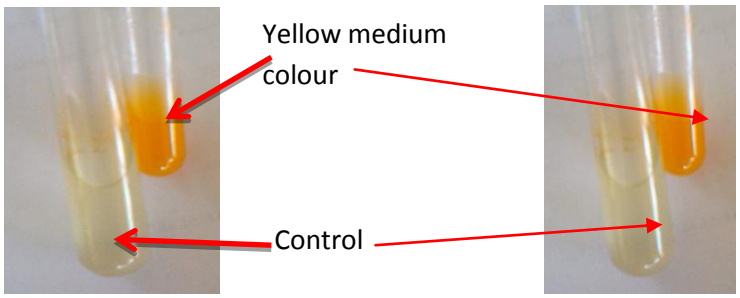
Plate 11: Methyl red negative A (11a), H (11b), C (11c) and F (11d), methyl red positive B (11e), D (11f), E (11g) and G (11i)

4.1.2.5. Voges- Praskauer test

The medium incubated with bacterial isolates A, B, D and G changed to red (Table 4.2 and Plate 12) indicating the presence of diacetyl, the oxidation product of acetoin. Bacterial isolates **E** and **H** incubated medium turned yellow (Plate 12), an indication of the absence of diacetyl, the oxidation product of acetoin.



12a 12b 12c 12d



12e 12f

Plate 12: Voges Praskauer positive bacterial isolates A (12a), B (12b), D (12c) and G (12d), Voges Praskauer negative bacterial isolates E (12e) and H (12f) (Photo by John Wekulo)

4.1.2.6. 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 4.2).

4.1.2.7. Catalase test

The bacterial isolates **D**, **C**, **E**, **F**, **H**, **G** and **I** produced bubbles during these tests (Table 4.2). 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 (Table 4.2) indicating that they were anaerobic bacteria.

4.1.2.8. 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 4.2) indicating the presence of cytochrome c oxidase enzyme.

4.1.2.1.1. 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 4.2).

Table 4.2: 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.

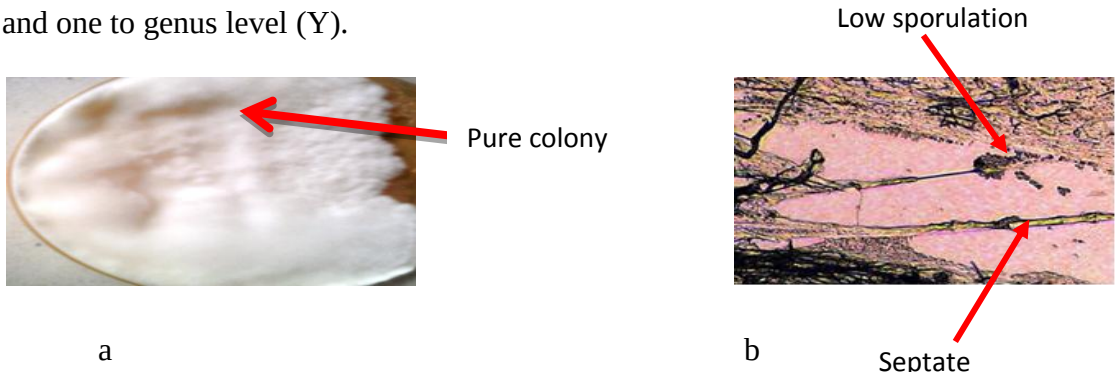
Legend: + =Positive reaction

- = 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

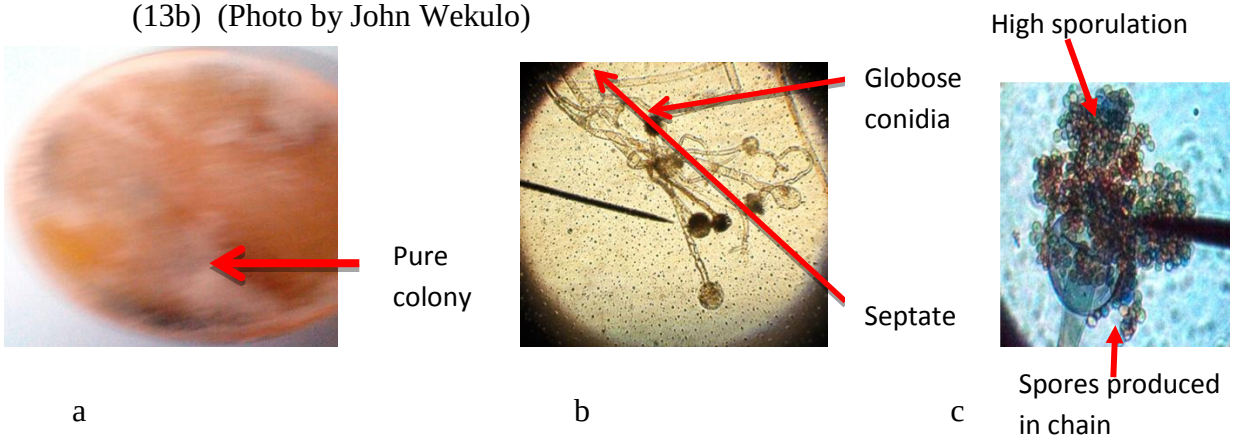
4.1.2. Morphological characterization of fungi from water collected from river Kuywa

Three pure fungal isolates were tentatively differentiated based on morphological and microscopic characters as X, Y and Z (Plates 13, 14 and 15). Isolate X was white, floccose, 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 and Alexopolous *et al*. (2002), the three isolates were identified to be X-*Fusarium oxysporum,* Y-*Aspergillus flavus complex* and Z- *Penicilium spp.* as shown in table 4.3. Two of the fungal isolates were identified to species level (X and Y) and one to genus level (Y).



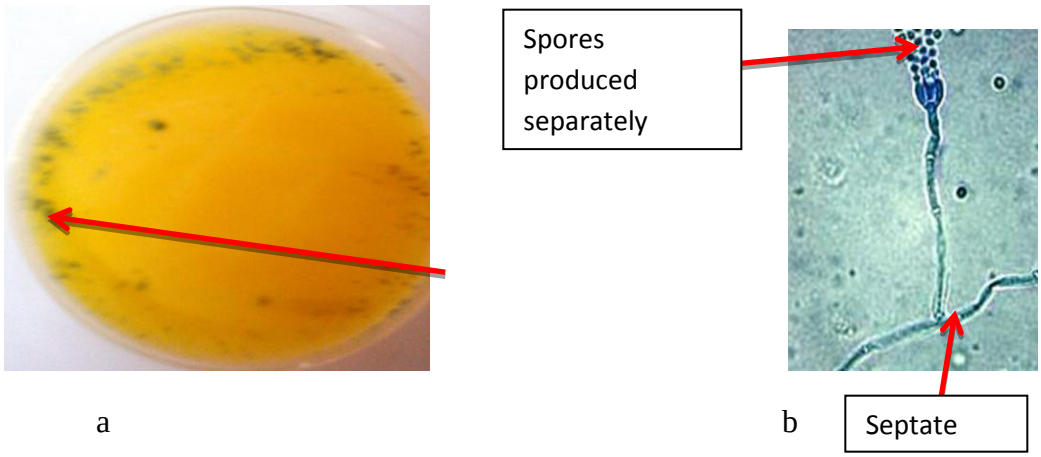
a b

Plate 13: Pure fungal isolates X on PDA medium on 7th day (13a) and the mycelia tip mg. ×400 (13b) (Photo by John Wekulo)



a b c

Plate 14: Pure fungal isolate Y on PDA medium on 7th day (14a) and the mycelia tip mg. ×400 (14b and 14c) (Photo by John Wekulo)



a b

Plate 15: Pure fungal isolate Z on PDA medium on 7th day (15a) and the mycelia tip mg. ×400 (15b) (Photos by John Wekulo)

Table 4.3: 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.

4.2. Physicochemical parameters influencing the water quality

Physicochemical parameters varied considerably among the four sampling sites and between dry and wet seasons.

4.2.1. Water temperature

The results obtained from level of temperature shows that sites had significant influence on temperature levels because temperature levels varied from site to site (Table 4.4 and P=0, Appendix 1). Site C- Nzoia water pump recorded highest temperature 25.60 ^{o}C which was significantly different from other sites. However, temperature levels in all sites were within the WHO permissible limits (23-30^{O}C). On the other hand, seasons had significant influence on temperature levels (Table 4.5 and P=0, Appendix 1). The highest water temperature level was obtained during the dry season which was significantly different from wet season. However, temperature levels in both dry and wet seasons were within the WHO permissible limits (23-30^{O}C).

4.2.2. Water pH

Sites had no significant influence on pH level in river Kuywa (Table 4.4 and P=42, Appendix 1). The highest pH level was obtained in site B- Ngwelo 11.47 though it was not significantly different from pH level obtained other sites. pH level in all sites except site A- Matisi were above WHO permissible limits (6.5-8.5). Seasons had no significant influence on pH level in river Kuywa (Table 4.5 and P=85, Appendix 1). The highest pH level was obtained in wet season though it was not significantly different from pH level obtained in dry season. pH level in both seasons (dry and wet) were above WHO permissible limits (6.5-8.5).

4.2.3. Water turbidity

The results obtained from turbidity level in Kuywa river indicated that sites had no significant influence on river Kuywa turbidity level (Table 4.4 and P=44, Appendix 1). The highest turbidity level was obtained in site B- Ngwelo however this was not significantly different from other sites. In all sites, turbidity level was above WHO permissible limits (0-5 NTU). Turbidity level in Kuywa river indicated that seasons had no significant influence on river Kuywa turbidity level (Table 4.5 and P=63, Appendix 1). The highest turbidity level was obtained in wet season however this was not significantly different from dry season. For both seasons, turbidity level was above WHO permissible limits (0-5 NTU).

4.2.4. Water DO

Sites had no significant influence on DO level in river Kuywa (Table 4.4 and P=0.19, Appendix 1). The highest DO level was obtained in site B- Ngwelo though it was not significantly different from other sites A, C and D. DO level in all sites was within WHO permissible limits (above 6 mg/l). On the other hand, seasons had no significant influence on DO level in river Kuywa (Table 4.5 and P=45, Appendix 1). The highest DO level was obtained in wet season though it was not significantly different from dry season. DO level in river Kuywa for both seasons were within WHO permissible limits (above 6 mg/l).

4.2.5. Water BOD

The results obtained from BOD level in river Kuywa shows that sites had significant influence on BOD level (Table 4.4 and P=0, Appendix 1). Site A- Matisi recorded highest BOD level 5.75 mg/l in river Kuywa which was significantly different from other sites. Sites C and D recorded BOD level within WHO permissible limits (4-6 mg/l)while sites A and B, BOD level was slightly above WHO permissible limits. The results obtained from BOD level in river Kuywa

shows that seasons had significant influence on BOD level (Table 4.5 and P=0, Appendix 1). Wet season recorded highest BOD level in river Kuywa which was significantly different from dry season which had low BOD level. For both dry and wet seasons, BOD level in the river was within WHO permissible limits (4-6 mg/l).

4.2.6. Water COD

Sites had significant influence on COD level in river Kuywa because COD levels varied from site to site (Table 4.4 and P=0, Appendix 1). The highest COD level was obtained in site D-Chalicha 0.48 mg/l. Water COD levels in all sites were above WHO permissible limits (above 0.250 mg/l). Seasons had significant influence on COD level in river Kuywa (Table 4.5 and P=0 see Appendix 2). COD levels were significantly different among seasons. The highest values were obtained in wet season. Water COD level for both dry and wet season was above WHO permissible limits (above 0.250 mg/l).

4.2.7. Water nitrates

The results obtained from nitrates level in river Kuywa shows that sites had significant influence on nitrates level because nitrates levels varied from site to site (Table 4.4 and P=0, Appendix 1). Site A- Matisi recorded highest nitrates level in river Kuywa which was significantly different from other sites B, C and D. In all sites, nitrates level in river Kuywa was below WHO permissible limits (above 45 mg/l). The results obtained from nitrates level in river Kuywa shows that seasons had significant influence on nitrates level (Table 4.5 and P=0, Appendix 1). Wet season recorded highest nitrates level in river Kuywa which was significantly different from dry season which had low nitrates level. For both dry and wet seasons, nitrates level in river Kuywa was below WHO permissible limits (above 45 mg/l).

4.2.8. Water phosphates

The results obtained from level of phosphates shows that sites had significant influence on phosphates levels because phosphates levels varied from site to site (Table 4.4 and P=0, Appendix 1). The highest water phosphates level was obtained in site A-Matisi. This was significantly different from other sites B, C and D. However, phosphates levels in all sites were below WHO permissible limit (1 mg/l). The results obtained from level of phosphates shows that seasons had significant influence on phosphates levels (Table 4.5 and P=0, Appendix 1). The highest water phosphates level was obtained in wet season. This was significantly different from dry season. However, phosphates levels in both dry and wet seasons were below WHO permissible limit (>1 mg/l).

Table 4 .4: Influence of sites on physicochemical parameters of river Kuywa

Sites	Means of physicochemical parameters							
	Temp ^{O}C	**pH**	**Turbidity**	**DO mg/l**	**BOD mg/l**	**COD mg/l**	**NO_3^-**	**PO_4^-**
Matisi	23.83±0.36d	7.68±0.25a	10.03±0.02a	10.00±0.11a	5.75±0.27a	0.31±0.00d	1.47±0.20a	0.81±0.18a
Ngwelo	25.88±1.13a	11.47±3.41a	13.42±3.02a	12.52±3.20a	5.03±0.10b	0.38±0.02c	1.32±0.21b	0.63±0.16b
Nzoia water pump	25.60±0.67b	8.10±0.35a	10.60±0.19a	8.18±0.18a	4.67±0.10c	0.42±0.02b	1.09±0.42b	0.60±0.14c
Chalicha	25.38±1.19c	8.17±0.43a	10.95±0.37a	7.73±0.18a	3.73±0.14d	0.48±0.02a	1.37±0.36a	0.48±0.16d
LSD	**0.12**	**5.27**	**4.61**	**4.89**	**0.22**	**0.01**	**0.25**	**0.01**

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.

Table 4.5: Influence of seasons on physicochemical parameters of river Kuywa

Seasons	Means of physicochemical parameters							
	Temp ^{O}C	**pH**	**Turbidity**	**DO mg/l**	**BOD mg/l**	**COD mg/l**	**NO_3^-**	**PO_4^-**
Dry	27.04±0.44a	8.68±0.11a	10.88±0.18a	8.98±0.17a	4.64±0.17b	0.36±0.06b	0.69±0.11b	0.28±0.03b
Wet	23.31±0.16b	9.02±1.77a	11.62±1.53a	10.23±0.03a	4.95±0.09a	0.42±0.10a	1.94±0.91a	0.98±0.05a
LSD	**0.08**	**3.73**	**3.26**	**3.45**	**0.15**	**0.01**	**0.18**	**0.01**

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.

4.2.1.1. Seasonal effect on microbial counts in river Kuywa water

Seasons had no significant influence on bacterial count in river Kuywa (Table 4.6 and P=26, Appendix2). The highest bacterial count was obtained in wet season though it was not significantly different from dry season. Bacterial count in river Kuywa for both seasons exceeded WHO permissible limits (0/100 ml).

The results obtained from fungal count shows that seasons had significant influence on fungal count (Table 4.6 and P=0, Appendix 2). The highest fungal count was obtained in wet season which was significantly different from wet season. However, fungal count in both dry and wet seasons exceeded WHO permissible limit (0/100 ml).

Table 4.6: Influence of seasons on bacterial and fungal count of river Kuywa

Sites / Seasons	Microbial count		WHO permissible limits (0/100 ml)
	Bacterial count cfu/ml	**Fungal count cfu/ml**	
Dry	$6.61 \pm 8.69 \times 10^5$a	$3.83 \pm 3.44 \times 10^5$a	
Wet	$7.66 \pm 3.58 \times 10^5$a	$6.75 \pm 3.50 \times 10^5$b	
LSD	**1.9×10^6**	**7.70**	

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

4.3. Microbial counts of bacteria and fungi in river Kuywa water

4.3.1. 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 4.7 and P=30, Appendix 2). 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 (0/100 ml) (see appendix 2).

4.3.2. 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 4.7 and P=0, Appendix 2). 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 (see appendix 2).

Table 4.7: Influence of sites on bacterial and fungal count of river Kuywa

Sites	Microbial count		WHO permissible limits (0/100 ml)
	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	
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 ($P\leq0.05$). Mean values of three replicates ±S.E

4.4. Relationships between microbes (bacteria and fungi) and physicochemical parameters

Bacterial count in river Kuywa water at site A- Matisi had strong positive correlation with temperature and nitrates while had weakly negatively correlation with pH (r=-0.04, P=0.97), Turbidity (r=-0.89, P=0.31), DO (r=-0.85, P=0.36), BOD (r=-0.89, P=0.31), COD (r=-0.46, P=0.69) and phosphate (r=-0.19, P=0.88) at ($P\leq0.05$) (See table 4.8). At site B- Ngwelo, bacterial count had weakly positively correlation with pH, turbidity and phosphates while had strongly negatively correlation with temperature (r=-0.20, P=0.87), DO (r=-0.66, P=0.54) and COD (r=-0.98, P=0.13). At site C- Nzoia water pump, bacterial count strongly positively correlated with all of the physicochemical parameters while at site D- Chalicha, bacterial count

had weakly negatively correlation with all physicochemical parameters except nitrates (Table 4.8).

Fungal count in river Kuywa at site A-Matisi had strongly negatively correlation with temperature and nitrates (r= -0.50, P=0.67), (r=-1.00, P=0) respectively while had strongly negatively correlation with pH, turbidity, DO, BOD, COD and phosphates. At site B- Ngwelo, fungal count had weakly negative correlations with physicochemical parameters except for temperature and turbidity which had positive correlation while at site C-Nzoia water pump, fungal count strongly negatively correlated with turbidity, DO, BOD and COD (r=-1.00, P= 0). At site D- Chalicha, fungal count had a strong negatively correlation with temperature, turbidity (r=-1.00, P=0) and nitrates (r= -0.50, P=0.67) (Table 4.7)

Table 4.8: Correlation coefficient values and P values between bacterial and fungal count and physicochemical parameters at various sampling sites

Sites	Microbes		Temp	pH	Tur	DO	BOD	COD	NO^-_3	PO^-_4
A	Bacteria	R	0.85	-0.04	-0.89	-0.85	-0.89	-0.46	0.95	-0.19
		P	0.36	0.97	0.31	0.36	0.31	0.69	0.21	0.88
	Fungi	R	-0.50	0.50	1.00	0.50	1.00	0.00	-1.00	0.50
		P	0.67	0.67	0.00	0.67	0.00	1.00	0.00	0.67
B	Bacteria	R	-0.20	0.01	0.32	-0.66	-0.66	-0.98	-0.94	0.15
		P	0.87	0.99	0.79	0.54	0.54	0.13	0.22	0.91
	Fungi	R	0.00	-0.19	0.50	-0.50	-0.50	-1.00	-1.00	-0.50
		P	1.00	1.88	1.67	1.67	1.67	0.00	0.00	0.67
C	Bacteria	R	0.20	0.99	0.66	0.32	0.32	0.32	0.95	0.95
		P	0.87	0.09	0.54	0.79	0.79	0.79	0.21	0.21
	Fungi	R	0.87	0.19	-1.00	-1.00	-1.00	-1.00	1.00	0.87
		P	0.33	0.88	0.00	0.00	0.00	0.00	0.00	0.33
D	Bacteria	R	-0.18	-0.10	-0.11	-0.43	-0.76	-0.95	0.76	-0.98
		P	0.40	0.67	0.62	0.72	0.45	0.22	0.12	0.12
	Fungi	r	-1.00	0.00	-1.00	0.12	0.50	1.00	-0.50	0.87
		p	0.00	1.00	0.00	0.93	0.67	0.00	0.67	0.33

Where A= Matisi, B= Ngwelo C=Nzoia water pump D=Chalicha

CHAPTER FIVE

DISCUSSION

5.1. Types of microbes (bacteria and fungi) isolated from river Kuywa water

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) and Thathana *et al*. (2017).

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 (Bhatnagar-Mathur *et al*., 2015).

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).

This study could not ascertain the specific strain of bacteria and fungi and therefore further work should be done on genetic identification of bacteria and fungi using 16SrRNA and rDNA markers in order to identify the microbes to strains level.

5.2. Influence of physicochemical parameters on water quality

Quality of water for drinking is determined by its physical, chemical and biological properties which include a host of natural and human factors. The natural factors are geological, hydrological and climatological while human factors include polluting activities such as discharge of domestic, industrial, urban and other waste waters (Bartam and Balance, 1996). Water temperature levels were within WHO permissible limits (23-30^{o}C) in all sampling sites. The least water temperature level was obtained at site A-Matisi and highest at site D-Chalicha. This could be attributed to direct insolation as a result of sparse and less dense riparian vegetation cover observed along river Kuywa water. The findings from this study are in agreement with those of Obed (2012). Seasonal variations indicated that water temperature values were within WHO permissible limits in both seasons though values were high in dry season. The relatively high water temperatures in dry season may partially be attributed to

reduction in rainfall with reduced cloud cover which allowed direct sunlight to reach river water and this raises water temperature values (Musyimi *et al*., 2017). The low temperature level obtained in wet season may be perceived as a result of higher rainfall accompanied by intense cloud cover which reduced intensity of sunlight rays.

Water pH levels were above WHO permissible limits (6.5-8.5) in all sampled sites. The high pH could be attributed to increased floods and human activities. The findings from this study disagree with those reported by Mulanda *et al*. (2011) who found pH levels in all sites within WHO permissible limits. Seasonal variations indicated that water pH values were above WHO permissible limits in both seasons. The deviation of pH in both dry and wet seasons to alkalinity could be due to increased storm and agricultural runoffs from point and nonpoint sources of pollution. Human activities such as accidental spills, sewer overflows and discharge of chemicals by communities and industries can possibly have significant effect on pH levels (Sewe, 2013). It is known that most organisms have adapted to life in water of a specific pH and may die when there is even a slight shift (Ouma, 2015).

In all sites, water turbidity levels were above WHO permissible limits (0-5 NTU). The least water turbidity levels were obtained at site A-Matisi and highest at site B-Ngwelo. This can be due to intense agricultural, soil erosion and livestock activities in the region. The findings from this study agree with those of Ontumbi *et al*. (2015). On the other hand, seasonal variations showed that turbidity levels were above WHO permissible limits (0-5 NTU). The findings could be attributed to sediment loading resulting from the land use practices such as increased land tillage from agricultural farms, livestock herding along river Kuywa. Similar results have been documented by Ontumbi *et al*. (2015). High level of turbidity is associated with higher levels of

microbes availability because high turbidity levels indicate presence of sediments whose particles provide high surface ratio, hence attachment of microbes (Ouma, 2015).

DO values in all sites were within WHO permissible limits (above 6 mg/l). The least DO level was obtained in site D while highest in site B. The variation in DO levels amongst sites could be attributed to high decomposition of organic matter (Masese *et al*., 2017), which indicates a high contamination load in the river Kuywa water. Similar findings were reported by Musyimi *et al.* (2017). Seasonal variations showed that DO values were within the permissible limit (above 6 mg/l) in both seasons. Musyimi *et al*. (2017) attributed the normal DO levels in water to the high photosynthetic activities of algae, low oxygen demand by microbial breakdown of allochthonous inputs, increment in atmospheric diffusion of O_2 and reduced temperatures in wet season.

BOD values in all sites were within WHO permissible limits (above 4-6 mg/l) except at site D which was below the permissible limits. The variation in BOD levels amongst sites could be due to acidification of water by elevated microbial degradation of organic debris and concentrated dissolved solids in sampled sites in river Kuywa. The findings from this study disagree with those of Tenge *et al*. (2015) who reported BOD levels 13 mg/l above WHO permissible limits. Seasonal variations showed BOD levels in this study within WHO permissible limits (4-6 mg/l) in both seasons. The results could be due to, low organic matter in river Kuywa which demands low DO. The high BOD levels leads to more and rapid oxygen depletion in water bodies hence less oxygen is available to aquatic organisms.

COD values in all sites were above WHO permissible limits (above 0.250 mg/l). The variation in COD levels amongst sites could be attributed to microbial activities and high organic contents in

river Kuywa in different sites. The findings from this study are in agreement with those of Kithia *et al*. (2007) who reported COD higher values of COD above WHO permissible limits. On the other hand, COD levels were above WHO permissible limits (above 0.250 mg/l) in both seasons. These could be attributed to high amount of organic or nutrients materials in river Kuywa water. Similar results were obtained by Kithiia (2007) on river Athi in Kenya, who reported higher COD levels in range of 2.14-17.01mg/l which were within WHO permissible limits. The high COD levels reduce DO levels in water bodies creating anaerobic condition which is deleterious to aquatic life.

In all sampling sites, nitrates were below WHO permissible limits (above 45 mg/l). Variation in nitrates levels among sites could be attributed to agricultural and livestock activities. The findings of this study are in agreement with those of Suzanne *et al*. (2017). For both dry and wet seasons, nitrates levels in river Kuywa were below WHO permissible limits (above 45 mg/l). The availability of nitrates in river Kuywa is attributed to increased agricultural runoffs in studied rivers. Nitrates in water create a number of human health problems such as 'methemoglobinaemia' or blue baby syndrome, nitrosamine is carcinogenic in nature and it affects the esophagus and pharynx, prostate and gastrointestinal cancer and osteoporosis (Ouma, 2015).

In all sampling sites, phosphates were below WHO permissible limits (> 1mg/l). Variation in phosphates level in sites could be attributed to differences in discharges of agricultural runoff, human sewage and livestock activities in the region. The findings of this study are in agreement with those of Chatterjee *et al*. (2010). Phosphates values below WHO permissible limits (>1mg/l) in both dry and wet seasons. The high phosphate level may be due to human sewage, agricultural runoff from crops and sewage. Higher phosphate concentrations contribute microbial

growth such as *Acinetobacter spp* (Zuma, 2010) thus contributing to the high microbial cell counts.

5.3. Microbial count of bacteria and fungi in river Kuywa water

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 (0/100 ml). Both bacterial and fungal counts in all sampling sites exceeded WHO permissible limits (0/100 ml). 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 (0/100 ml) 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) and Udosen and Umana (2018). 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).

Bacteria and fungi were strongly and positively correlated with water temperature. The strong positive association between bacteria and fungi and water temperature indicates the strong effect temperature has on microbial growth in that moderate water temperature enhances the growth of microorganisms. Similar results were reported by Prest *et al*. (2016).

Bacteria and fungi were strongly and positively correlated with water pH at sites B and D. These results were in accordance with the results obtained by Mounjid *et al*. (2014) which showed that the increase in pH levels affects the abundance of microorganisms where basic pH leads to a net decrease in survival of microbes.

Bacteria and fungi were strongly and positively correlated with turbidity levels in all sites except at site A-Matisi. Similar results were reported by Mounjid *et al*. (2014) who indicated that high turbidity levels indicate presence of sediments whose particles provide high surface volume ratio, hence attachment of microbes. On the other hand, bacteria and fungi were strongly and negatively correlated to DO, BOD and COD levels in sites A and C while sites B and D had positive correlation. The positive correlation indicates the strong effects of DO, BOD and COD on microbial growth as they provide shelter for bacteria and other pathogenic microbes in water. Similar results were obtained by Mounjid *et al*. (2014).

Bacteria and fungi were strongly and negatively correlated with nitrates levels in sites A, B and D. This could be attributed to facts that high nitrates levels are often accompanied by bacterial contamination. These results are similar to those obtained by Peuler *et al*. (1999). On the other hand, bacteria and fungi were strongly and positively correlated with phosphates levels in sites in river Kuywa. The positive correlation between microbes (bacteria and fungi) and phosphates indicates the strong effect of phosphates in bacteria and fungi growth. High phosphates concentrations contribute to its ions into microbes thus contributing to high microbial cell counts (Zuma, 2010).

CHAPTER SIX

CONCLUSION, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1. Conclusion

1. 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. This finding indicates that river Kuywa is contaminated with disease causing microorganisms.

2. The levels of physicochemical parameters varied among sites and some were high than WHO permissible limits e.g. temperature 23.83-25.88^{o}C, pH 7.68-11.47, turbidity 10.03-13.42 NTU, DO 7.73-12.52mg/l, BOD 3.75-5.75mg/l and COD 0.31-0.48 mg/l. Some physicochemical parameter values varied between seasons indicating that river Kuywa is contaminated in both seasons e.g. temperature 27.04-23.31 ^{o}C, BOD 4.64-4.95 mg/l, COD 0.36-0.42 mg/l, NO-3 0.69-1.94 mg/l and 0.28-0.98 mg/l.

3. 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. The levels of physicochemical parameters influenced bacterial and fungal count in river Kuywa. For instance, the strong positive association between bacteria and fungi and water temperature indicates the strong effect temperature has on microbial growth in that moderate water temperature enhances the growth of microorganisms. Turbidity levels indicates presence of sediments whose particles provide high surface volume ratio, hence attachment of microbes

6.2 Recommendations

1. The existence of different types of bacteria and fungi in river Kuywa water is an indication that river Kuywa is contaminated and hence pose a health risk to human and animal in the catchment area of Bungoma County. Therefore, the water should be treated before human consumption.
2. Present study indicates varied levels of physiochemical parameters with association to bacterial and fungal counts, it is therefore important to continue monitoring the water quality of river Kuywa so as to assess trends in contamination using physicochemical parameters as indicator of pollution in river Kuywa.
3. The high bacterial and fungal count in river Kuywa water is an indication that river Kuywa is highly contaminated and this pose health risks to human and animals, hence water from river Kuywa need to be treated before human consumption.

6.3. Suggestions for further research

The following are the suggestion for further research:

1. The current study could not ascertain the specific strain of bacteria and fungi therefore further work should be done on genetic identification of bacteria and fungi using 16SrRNA and rDNA markers in order to identify the microbes to strains level.
2. This study has provided data on microbiological and physicochemical characters of river Kuywa water. The study, however only focused on bacteria and fungi. Further research should focus on other possible microbial contaminants such algae, viruses and protozoa that may be present in river Kuywa water.

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APPENDICES

Appendix 1: Analysis of variance with reference to the effect of sites and seasonal influence on physicochemical parameters of river Kuywa water

Parameter	Sources of variation	DF	Sum of squares	Mean of squares	F value	Pr>f
Temp ^{O}C	Sites	3	15.16	5.05	527.13	<.0001
	Season	1	83.63	83.63	8726.26	<.0001
	Sites*Season	3	13.85	4.62	481.74	<.0001
	Coef Var		0.39			
pH	Sites	3	55.42	18.47	0.99	0.4204
	Season	1	0.70	0.70	0.04	0.8485
	Sites*Season	3	63.05	21.02	1.13	0.3659
	Coef Var		48.67			
Turbidity	Sites	3	40.22	13.41	0.94	0.4422
	Season	1	3.35	3.35	0.24	0.6339
	Sites*Season	3	48.27	16.09	1.13	0.3651
	Coef Var		33.49			
DO	Sites	3	84.95	28.32	1.78	0.1912
	Season	1	9.38	9.38	0.59	0.4537
	Sites*Season	3	45.50	15.17	0.95	0.4381
	Coef var		41.49			
BOD	Sites	3	12.67	4.22	133.42	<.0001
	Season	1	0.57	0.57	18.01	0.0006
	Sites*Season	3	2.36	0.79	24.82	<.0001
	Coef var		3.71			
COD	Sites	3	0.08	0.03	260.15	<.0001
	Season	1	0.03	0.03	234.00	<.0001
	Sites*Season	3	0.01	0.00	22.62	<.0001
	Coef var		2.62			
NO_3^-	Sites	3	0.46	0.15	3.68	0.0345
	Season	1	9.40	9.40	225.62	<.0001
	Sites*Season	3	1.49	0.50	11.91	0.0002
	Coef var		15.52			
PO_4^-	Sites	3	0.34	0.11	984.42	<.0001
	Season	1	2.92	2.92	25020.3	<.0001
	Sites*Season	3	0.02	0.01	66.99	<.0001
	Coef var		1.71			

Appendix 2: Analysis of variance with reference to the effect of sites and seasonal influence on bacterial and fungal of river Kuywa water

Parameter	Sources of variation	DF	Sum of squares	Mean of squares	F value	Pr>f
Bacteria	Sites	3	2.02	6.75	1.40	0.28
	Season	1	6.51	6.51	1.35	0.26
	Sites*Season	3	1.92	6.41	1.33	0.3003
	Coef Var		30.82			
Fungi	Sites	3	18.79	62.64	7.91	0.0018
	Season	1	51.04	51.04	64.47	<.0001
	Sites*Season	3	45.83	15.28	0.19	0.8996
	Coef Var		16.81			

Appendix 3: The sampling sites along river Kuywa water

Site A- Matisi

Site B-Ngwelo

Site C- Nzoia water pump

Site D-Chalicha

Photos by Wekulo Keya John 14th January 2018