DETERMINATION OF BACTERIAL COMPOSITION, HEAVY METAL CONTAMINATION AND PHYSICO-CHEMICAL PARAMETERS OF FISH POND WATER IN ABOTHUGUCHI CENTRAL, MERU COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other University or for any other award.

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DEDICATION

This research work is dedicated to my late grandmother who taught me the basic skills of life.

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ACRONYMS AND ABBREVIATONS

ANOVA	Analysis of Variance
APHA	American Public Health Association
CFU	Colony Forming Unit
CU	Copper
DCA	Deoxycholate citrate Agar
DO	Dissolved Oxygen
EC	Electrical Conductivity
EMA	Environmental Management Authority of Zimbabwe
EMBA	Eosin Methylene Blue Agar
ESP	Economic stimulus programme
FAAS	Flame Atomic Absorption Spectrometer
FE	Ferrum
FS	Faecal streptococci
KF	Kenner Faecal
Ml	Millilitre
MN	Manganese
MPN	Most Probable Number
pH	Potential Hydrogen
Pb	Lead
PMS	Plant and Microbial Sciences
SAS	Statistical Analysis System
Spp	Species
SSA	Salmonella – Shigella Agar
TCBS	Thiosulphate Citrate Bile Salts
UNEP	United Nations Environmental Programme
WHO	World Health Organization
Zn	Zinc

ABSTRACT

Fish farming has been practised all over the world for centuries. Throughout history, humans have used fish as a supplementary source of proteins and as a source of income. Fish performs all their body functions in water and therefore the quality of water is very important to their livelihood. Farmers have suffered losses due to the death and stunted growth of fish as a result of bacterial infections and diseases among other causes. The presence of these bacteria in fish pond water and their pathogenic effects varies with the quality of the pond water together with the variation in the frequency with which water is changed in the ponds. Physico-chemical parameters of pond water and heavy metals affect growth and productivity of fish. This research aimed at determining the contamination levels of both faecal and pathogenic bacteria namely *Salmonella* spp., faecal *Streptococcus,* faecal coliforms *Pseudomonas* spp., *Vibrio cholerae* and *E. coli* in fish ponds water, levels of heavy metal contamination and variability of physico-chemical parameters and their effects on fish. Isolation of faecal indicators and pathogens was carried out using standard laboratory methods. Some physico-chemical parameters were measured *in situ* using a portable Universal multiline P4 WTW (Wilheim Germany) meter while others were analysed in the laboratory. The determination of heavy metal presence and concentration in the water samples was carried out by the use of Flame Atomic Absorption Spectrometer (FAAS). The results indicated that the pond water was highly contaminated with faecal streptococci and faecal coliforms and they varied significantly in the sites at $p=0.0001$. Pearson correlation analysis showed positive correlation between prevalence of faecal streptococci and faecal coliforms ($r=0.732$ at $P< 0.01$). Potential pathogens namely *Vibrio* spp., *Salmonella* spp., *P. aeruginosa* and *E.coli* were isolated from the water samples with high population. Physico-chemical parameters namel pH and dissolved oxygen deviated from the permissible limits. The study has indicated that the fish ponds water was highly contaminated with both faecal and pathogenic bacteria with physico-chemical parameters varying significantly at $p=0.0001$. Heavy metals with the exception of iron were within the recommended limits hence no significant contamination of the fish pond water. The study recommends use of treated tap water, regular monitoring of fish pond water and sensitization of farmers on bacterial contamination of pond water. Further studies with the aid of molecular techniques should be used to characterize the bacteria. The finding of this study can, therefore, serve as an impetus to improve fish farming in Meru County, as a way of meeting the growing nutritional demands in the country.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Fish farming has been practised all over the world for centuries. Fish is one of the staple foods and contributes about 60 % of the world source of protein. However, 60 % of the developing countries derives more than 30 % of their animal protein from fish (Emikpe *et al*., 2011). Fish protein improves nutrition in that it has a high biological value in terms of high protein retention in the body, low cholesterol level and presence of essential amino acids (Emikpe *et al*., 2011). Fish farming takes place at a high degree in Mediterranean Sea, Red Sea, in Southern and Western Africa, in Israel, Japan and the countries bordering L. Victoria (Egbere *et al*., 2008).

Fish was introduced in Kenya in the early 1920's. Since then, fish have been limited to regions and areas with large masses of water mainly L. Victoria, L. Turkana among other fresh water lakes, rivers and in the coastal region of Indian Ocean. Fish from Lake Victoria represents 85 % of Kenya's fish supply and constitute 25 % of total catch from Africa's Inland fisheries (Gitonga, 2006).

Fish farming has previously not been well practised in Meru County though the region is an extensively agricultural zone. But as of now, fish farming is practised at small scale by farmers, organised groups and even institutions (Gitonga, 2006). The demand for fish as a source of food in Meru has escalated; this has led to an increase in fish farming. As more farmers have joined the field, fish farming has faced several challenges of infections and disease attack resulting to stunted growth and even death of fish (Egbere *et al*., 2008). Successful pond management requires an understanding of the role of nutrients and other water quality parameters, as well as regular monitoring of environmental conditions within the pond's ecosystem. Water quality is often overlooked in pond management, and this has led to common problems, such as excessive algal blooms, noxious smells and subsequent death of fish. In order to prevent these problems, an understanding of basic water chemistry and other physical parameters is necessary. The diseases and infections have been attributed to bacterial invasion. Bacterial diseases are responsible for heavy mortality in both wild and cultured fish (Egbere *et al*., 2008).

The main sources of contamination in water supplies are pathogenic microbes, heavy metal, organic substances and inorganic chemicals (Sosbey, 2002). To reduce the potential health risk resulting from the use of contaminated water, treatment methods are needed that are easy to use, effective, affordable, functional and sustainable (Sosbey, 2002). The health of fish is dependent on the quality of water. Enteric bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp. and *Vibrio* spp. are likely to accumulate in fish found in waters polluted with human wastes, located in polluted areas where hygienic standards are not maintained or in fish ponds supplied with water from polluted rivers. Therefore microbial quality of farmed fish is largely determined by the quality of water in which they are farmed (Fafioye, 2011).

Many studies have shown that the bacteria belonging mostly to genera *Aeromonas, Pseudomonas, Vibrio, Salmonella, Corynebacterium* and *Myxobacterium* cause infectious diseases in fish (Ampofo and Clerk, 2010). *E. coli* is a frequent contaminant of food and water and a well-recognised food borne pathogen (Dutta *et* *al*., 2010). Fish are susceptible to a wide variety of bacterial pathogens. The organisms especially bacteria are already present on the skin and in the alimentary tract of the living seafood's such as fish (Ampofo and Clerk, 2010). Bacteria have been found to cause disease subsequently leading to low production rate of fish. Most of these bacteria that cause diseases are considered to be saprophytic in nature. These bacteria only become pathogens when they are physiologically unbalanced, nutritionally deficient, or there are other stressors such as poor water quality and overstocking, which allow opportunistic bacterial infections to proceed (Egbere *et al*., 2008).

Previous studies have shown that different sources of water used for fish farming contribute differently to bacterial invasion of fish resulting to stunted growth and death (Egbere *et al*., 2008). Most aquaculture practices also favour disease occurrence, while high fish densities increase stress among stocks and the feeds provide abundant substrate for microbial growth and sub-optimal environment of inadequate water exchange predisposing infections (Ampofo and Clerk, 2010). Physical chemical properties of fish pond water are important in the growth and productivity of fish. Fish dependency on water is crucial hence the source, volume and the quality of physical chemical parameters such as dissolved oxygen, pH, temperature, conductivity, total alkalinity, total hardness, total solids, transparency values, carbon dioxide, nitrite-nitrogen, sulphates, carbonates and ammonia are some of the salient factors to consider in relation to fish health (Fafioye, 2011).

Agro based industries like sugar, paper, coffee, tea, dairy and fish tanneries discharge semi-treated effluents with high Biochemical Oxygen Demand (BOD) to the rivers

(Nzomo, 2005). Heavy metals such as cadmium, zinc, mercury, chromium, copper, cobalt, nickel, manganese, iron, vanadium and molybdenum cause heavy pollution particularly in the ponds, lakes and river systems in zones affected by effluents released from industries, sewage and agricultural drains (Ida, 2012). Some of the metals like copper, cobalt, zinc, nickel and molybdenum are naturally present in low concentrations in soils and water. The toxic metal flux into the ecosystem occurs through mining activities, discharge of raw and inadequately treated industrial and municipal effluent, vehicle service station or garage effluent and agricultural inputs like chemical fertilizers, herbicides and pesticides (Mutuku, 2010). The accumulation of these heavy metals over time in fish leads to the suppression of fish immunity hence allowing the normal pathogenic microbes to cause ulceration and possible septicaemia (bacteria in the blood stream) (Mutuku, 2010).

Most of the fish consumed in Kenya are obtained from the wild and partly through fish importation. The study of fish diseases is hindered by lack of adequate understanding of the ecological processes involving interactions between pathogens and their hosts in the aquatic ecosystem as well as the ill-understood physiological features of fish, characterized by their poikilothermic behavior in contrast with the better understood physiology of homoeothermic animals (Nyaku *et al*., 2007). The art of artificial fish culturing has not been adequately developed and the physical, chemical and biological environments of the fish ponds that are being used for fish farming have not been adequately studied. This work was therefore, carried out to assess the microbial contamination, heavy metal contamination and physico-chemical properties of selected fish ponds in Abothuguchi Central, Meru County and to ascertain the suitability of such ponds for artificial fish culture.

1.2 Problem statement and justification

The high demand for fish has resulted to the increase in the number of fish ponds in Abothuguchi Central, Meru County. Individual farmers, organised groups and institutions have developed, constructed fish ponds and started fish farming oblivious of the cost. Due to lack of knowledge and failure to consult the department of fisheries most of the farmers are carrying out fish farming in non-standard environment (Onyango *et al.*, 2009). Most of the farmers use large quantities of untreated waste water discharged from domestic effluents and other industrial sources (Onyango *et al.*, 2009). Most of these sources of water are highly contaminated with heavy metals which interfere with the general productivity of fish (Ida, 2012). According to Egbere *et al.* (2008) it is expected that the pH, total alkalinity, electrical conductivity and hardness as well as other physico-chemical parameters lie within acceptable ranges that would support fish productivity. The water quality parameters such as pH, temperature, dissolved oxygen may lead to outbreak of disease pathogens and parasites. Polluted waters contain vast amounts of organic matter that serve as an excellent nutritional source for growth and multiplication of the microorganisms (Egbere *et al.,* 2008).

Lack of knowledge on good hygiene practices has also directly contributed to the degradation of fish ponds water quality for habitats thereby resulting to death of fish. Lack of treated piped water is a major threat to fish farming in the region. Most of the farmers use water from rivers, streams, boreholes, wells, swamps and tap water. Most of the harvested fish from this region are small in size an indicator of stunted growth while some other farmers counted losses due to death of their fishes as a result of bacterial infection. Poor construction and maintenance of the ponds has resulted to unconducive physical chemical properties of the water thereby interfering with productivity (Eze and Ogbaran, 2010). Limited studies have been carried out to determine the bacteria that cause infections, diseases, stunted growth and death of fish in fish ponds. This study was therefore aimed at investigating the microbial load of bacteria pathogens that infects, cause diseases, stunted growth and death of fish cultured in ponds with different sources of water in addition to the physico-chemical parameters, the presence of heavy metals and their effects on fish.

1.3 Research questions

i) Is fish pond water in Abothuguchi Central significantly contaminated with faecal and pathogenic bacteria?

ii) What are the levels of heavy metal contamination of fish pond water in Abothuguchi Central?

iii) Is there any variability in physico-chemical parameters of fish pond water in Abothuguchi Central?

1.4 Research hypotheses

i) The fish ponds are not significantly contaminated with faecal and pathogenic bacteria in Abothuguchi Central.

ii) The fish ponds are significantly contaminated with heavy metals in Abothuguchi Central.

iii) There is no significant variation in the physico-chemical parameters of pond water in Abothuguchi Central.

1.5 Objectives

1.5.1 General objective

To determine the microbial load, physico-chemical parameters and heavy metal levels of fish ponds in Abothuguchi Central, Meru County.

1.5.2 Specific objectives

i) To determine the microbial quality and the level of pathogenic bacteria in pond water in Abothuguchi Central.

ii) To determine the levels of heavy metal contamination of fish pond water in Abothuguchi Central.

iii) To determine the variability of physico-chemical parameters of fish pond water in Abothuguchi Central.

1.6 Significance of the study

Fish are important sources of both food and income to many people in developing countries. For successful fish farming, the determination of microbial composition of pond water and its effects is indispensable. The findings on the microbial load of pond water will be used for the surveillance, prevention and control of many fish diseases and infections. The results will be used to educate the farmers on the variability of physic-chemical parameters and their effects on fish. The stake holders will be enlightened on the levels of heavy metal contamination of fish ponds and their effects on fish. There are limited studies on water quality of fish ponds in Abothuguchi Central, Meru County and Kenya at large. Therefore, this research work will be a contribution to data bank and stakeholders of fish farming. The public will be enlightened especially on the necessity to ensure that they carry out proper management of their fish ponds to avoid pathogens that could affect both the fish (tilapia) and humans.

The data collected will be used to identify bacteria pathogens common to most of the ponds in Abothuguchi Central region. In addition, data obtained on the quantity of heavy metal and the physical-chemical parameters will serve as an eye opener to the farmers on pond water contamination. The study will also sensitize the stake holders in the agro based industries and the municipal council on the impact of raw effluent discharge into the environment. The research work will create awareness among the stakeholders on the significance of environmental management and conservation. The research will enable farmers to make decisions on the source of their pond water, reduce fish farming cost, reduce the risk of fish diseases and infections, outbreak and at the same time reduce the death rate of fish.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fish farming

Fish farming is the principal form of aquaculture which involves raising fish commercially in ponds or tanks or enclosures, usually for food. There is an increasing demand for fish and fish protein, which has resulted in widespread overfishing in wild fisheries hence fish farming has offered fish marketers another source (Hastein *et al*., 2006). Over the past three decades, aquaculture has developed to become the fastest growing food-producing sector in the world (Hastein *et al*., 2006). A large proportion of fish products come from small-scale producers in developing countries or low income countries. More than 80 % of global aquaculture products are produced in fresh water (Hastein *et al*., 2006). From its early development in Asia, aquaculture has undergone huge development and is today highly diversified (Hastein *et al*., 2006).

Aquaculture consists of a broad spectrum of systems, from small ponds to large-scale, highly intensified commercial systems. The target of every farmer is to produce a wholesome fish of high quality and aesthetically pleasing to the eyes with high yield and economic value towards profit maximization. This therefore involves every means to increase yield by fertilizing the ponds that have low natural food productivity with organic manure to stimulate and improve the primary and natural food production (Ampofo and Clerk, 2010). Although organic manure play greater role in fish pond production, the potential health hazard associated with it should not be under estimated. Since fish are cold-blooded, every aspect of their physiology is controlled by temperature, which constitute to the infection of fish. Their metabolism, or the amount of energy they need to survive and grow, is also temperature-dependent. Every species of fish is adapted to live within a certain thermal range (Udeze *et al.,* 2012a). The art of artificial fish culturing has not been adequately developed and the physical, chemical and biological environments of the fish ponds that are being constructed have not been adequately studied or documented (Ayanwale *et al*., 2012). There are several species of tilapia which includes; *Oreochromis niloticus* (common tilapia), *Oreochromoismos sahibicus, Oreochrmois aureus, Oreochrmois spilurus, Oreochrmois andersonii*, *Tilapia zilii* and *Tilapia rendalli*. The other type of fishes commonly cultured in Kenya includes the common carp (*Cyprinus carpio*) and the African cat fish (*Clarias gariepinus*) (Onyuka *et al*., 2011).

2.2 Economic importance of fish

People in the world depend on fish as a supplement of their food nutrients. Fish has become an increasingly important source of protein and other elements necessary for the maintenance of a healthy body (Emikpe *et al*., 2011). Fish and seafood constitute an important food component for a large section of the world's population (Emikpe *et al*., 2011). Throughout history; humans have used fish as a source of protein (Adebayo-Tayo *et al*., 2012b). Fish is the third staple animal protein and forms a cheap source of protein. Fish and fish products constitute an important part in the international trade; more than 50 billion fish are eaten annually indicating increasing consumer interests in the commodity (Wafaa *et al*., 2011).

Fish has traditionally been a popular part of the diet in many parts of the world and in some countries constituted the main supply of animal protein. Today, even more people are turning to fish as a healthy alternative to red meat (Adebayo-Tayo *et al*., 2012a). The low fat content of many sea foods and the effect on coronary heart disease of the n-3 polyunsaturated fatty acids food in fatty pelagic fish species are extremely important aspects for health conscious people particularly in affluent countries where cardiovascular disease mortality is high (Adebayo-Tayo *et al*., 2012a). Although there is an argument regarding the concept of "fish for all" in India, initiated by the World Fish Centre since 30 to 50 % of the Indian population is predominantly vegetarian, there is no dispute that fish is very popular diet among the people of the states of West Bengal, Goa and Kerala (Sakthivel, 2003).

In Africa, as much as 5 % of the population depends partly or wholly on fish for their livelihood. Aquaculture has become one of the fastest developing source of animal protein to humans and animals due to dwindling wild fish stocks around the world and in particular Ghana (Al-Harbi and Uddin, 2005; Ashitey and Flake, 2010). In Nigeria, the rearing of African cat fish is proving to be a lucrative option for small scale inland fisheries (Egbere *et al*., 2008). Egbere *et al*. (2008) noted that fishes are responsible for about 55 % of protein intake of Nigerians. Unfortunately, most of these are obtained through naturally occurring fish from the wild and partly through fish importation. Generally, fish are good sources of vitamins B12 and B6 (Adebayo-Tayo *et al*., 2012). It is also a good source of fluorine and iodine which are needed for development of strong teeth and the prevention of goiter in man.

Economic studies have demonstrated that fish farming in Africa can be a good source of income. Fishery products are important not only from a nutritional point of view, but also as an item of international trade and foreign exchange earner for a number of countries in the world (Yagoub, 2009; Adebayo-Tayo *et al*., 2012a). Fish farming provides cash to families in addition to supplementing the diet of the farmers. Fish can be an important source of cash; even for farmers with limited resources (Egbere *et al*., 2008). Nigeria is the largest African aquaculture producer with over 600,000 metric tonnes a year. Egypt with over 400,000 metric tonnes follows Nigeria, and then there are only five other countries (Zambia, Madagascar, Togo, Kenya and Sudan) that each produce more than 100,000 metric tonnes (Egbere *et al*., 2008).

In Kenya, fish farming is practised both in small and large scale. In some cases, fish is a cash crop which fetches a lot of money. Fish farming is practised at a high degree around Lake Victoria which contributes 85 % of the country's fish. The most common fish which most of the farmers culture or grow in Kenya is tilapia (Gitonga, 2006). The annual average total production of fish in Kenya is estimated at 200,000 metric tonnes (MT) (Gitonga and Achoki, 2003). The fisheries sector contributes about 5 % to the country's gross domestic product (GDP). In 2004, the sector raised 8 billion Kenyan shillings, which supported the livelihood of about 500,000 people. The fisheries sector employs about 50,000 people who directly benefit from it, mainly from fishing, fish trading on local and international markets and employees in various sections of the fisheries industry (Gitonga and Achoki, 2003).

2.3 Bacterial infections and diseases

Fish are prone to various infections just like livestock that hamper their reproduction, growth and appearance and which affect their wholesomeness. Fish take a large number of bacteria into their gut from water, sediment and food (Emikpe *et al*., 2011). Fish are generally regarded as safe, nutritious and beneficial but aquaculture products have sometimes been associated with certain food safety issues (WHO, 2007). More than 140 invasive bacteria species have been identified in great lakes and other water bodies. All these have contributed negatively to the economic impact in various countries (Udeze *et al*., 2012). It has been well known that both fresh and brackish water fishes can harbor human pathogenic bacteria particularly the coliform group (Emikpe *et al.*, 2011).

Numerous group of bacteria particularly coliform group enters into the fish gut through water, sediment and food (Dutta *et al.*, 2010). Faecal coliform in fish demonstrates the level of pollution in their environment because coliform are not normal flora of bacteria in fish (Emikpe *et al.*, 2011). However, consumption of fish and shell fish may also cause diseases due to infection or intoxication, some of these diseases have been specifically associated with pathogens which are resistant to antibiotics (Adebayo-Tayo *et al*., 2012b). This resistance can further be passed on to human beings after the consumption of fish (Adebayo-Tayo *et al*., 2012a).

The microbiological flora in the intestines of sea foods such as finfish, shellfish and cephalopods is quite different being psychotrophic in nature and to some extent believed to be a reflection of general contamination in the aquatic environment. In filter feeding bivalve Mollusca shellfish (oyster) has shown that high accumulation and concentration of bacteria and viruses from the environment is generally taking place (Emikpe *et al.*, 2011). Worldwide, all fisheries are threatened by various factors such as pollution, which brings about the introduction of industrial waste which comprise of various inorganic and organic waste and invasive species of bacteria which are majorly enteric in nature, and their target point is the colonization of the intestine of fish and other sites in the fish (Udeze *et al*., 2012b).

The high density of bacteria in fish may be due to their consumption of bacteria for a long period of time through food and water (Shankar *et al*., 2009). The survival of these bacteria is dependent on the conditions prevailing in the aquatic environment and fish are often simply their hosts (Emikpe *et al*., 2011). Bacteria are prokaryotic, harmful bacteria can enter water through two main sources which are point sources or non-point sources of contamination. Point sources are those that are readily identifiable and typically discharge water through systems of pipes, but non-point sources originate from a wider area (USEPA, 2008). Successful invasion depends on the ability of the pathogen to outcompete the normal micro flora for nutrients (Ampofo and Clerk, 2010).

Bacteria can make fish sick and even kill them. In nature both salt water and fresh water fish can contract bacterial diseases, and aquarium fish are even more at risk. Bacterial infections often follow a parasitic infestation. Parasites, such as flukes, create microscopic holes in the skin of the fish and that allows bacteria (almost always present in even healthy ponds) enter and infect the fish. This type of bacterial problem usually manifests as red, infected sores called ulcers. Fish acts as an important food vehicle for some zoonotic pathogens such as *Salmonella* and *Vibrio*. Contamination of fish with pathogens is a major public health concern. The presence of *Salmonella* in seafood has been reported in Vietnam, India, Sri Lanka, Thailand, Taiwan, Japan and Nigeria (Adedeji *et al*., 2012).

Most of the organisms isolated in the study by Adebayo-Tayo *et al.,* (2012a) causes food poisoning such as shigellosis, salmonellosis caused by *Shigella* and *Salmonella* respectively. Infection also results when pathogenic bacteria, such as *Aeromonas* and *Pseudomonas*, build up and attack a fish whose immunity system is low. It can develop over time or be brought in by other fish. Bacterial diseases are responsible for heavy mortality in both wild and cultured fish. Fish are susceptible to a wide variety of bacterial pathogens. According to a research by Onyuka *et al.* (2011), out of 162 samples analyzed, 133 (82.1 %) were contaminated, with *S. typhimurium* as the most prevalent (49.6 %), followed by *E. coli* (46.6 %), and lastly *V. cholerae* (2.8 %).

Bacterial diseases are common in all fish and occur most often when environmental conditions, such as water quality, are not favorable. Inadequate oxygen levels in the pond can stress fish and make them susceptible to bacterial infections. Emikpe *et al*. (2011), notes that fish samples of different sources were contaminated with total aerobic bacteria as well as enterobacteria. Fish of good quality should have bacterial count less than 10^5 per gram but what was obtained from the fish samples examined in the study exceeded the acceptable limit recommended by Food and Agricultural Organization (1979) (Emikpe *et al*., 2011). These contaminations are often associated with spring die-offs in fish ponds. As the water warms during the spring, fish weakened by the winter months are often invaded by harmful bacterial that can cause death. This weakened condition can also be enhanced by frenzied spawning activity that further stresses the fish (Emikpe *et al.,* 2011).

A study carried out in Lake Victoria indicated that fish collected from the beaches were contaminated with enteric bacteria. This was found to be due to the use of contaminated water collected directly from the lake by local artisanal fish processors to clean the fish due to lack of piped water (Onyuka *et al.*, 2011). These organisms are likely to accumulate in fish in waters polluted with human wastes, located in polluted areas where low hygienic standards are maintained or in fish ponds supplied with water from polluted rivers (Onyuka *et al*., 2011). Bacteria are common and widely distributed in the aquatic environment in various parts of the world with the water temperature having selective effect on the growth of fish. This group includes the genus *Vibrio*. The psychotropic grows at low temperature below 20 ^{o}C while mesophillic grows at a temperature between 20 ^{o}C to 45 ^{o}C. Non-indigenous bacteria consist of *Salmonella* species, *Shigella* species, *E. Coli* and *Clostridium perfrengens* which may be due to polluted fish pond water (Onyuka *et al*., 2011).

Bacteria represent a major and important group of microorganisms because of their frequent occurrence and activities that may have a negative impact on fish quality (Hastein *et al*., 2006). These bacteria may enter the fish through the gills, penetration of egg membrane, ingestion, rupture of skin, wounds or through the digestive tract. Symptoms of bacterial infections include: loss of appetite, fin and tail rot, pale gills and fluid in the abdomen. Behavioural signs of diseases includes decreased feeding; weak, lazy or erratic swimming; floating on water with the belly up, gathering or crowding at the inlet. Physical signs include gaping mouth, cloudy or distended eyes, open sores, lesions, loss of scales, pale, eroded, swollen, bloody or brownish gills.

Previous studies have shown the presence of six genera (*E. Coli, Pseudomonas, Salmonella, Proteus, Klebsiela* and *Vibrio*) in the fish hence posing a threat to the fish industry (Udeze *et al*., 2012b). Bacterial diseases in aquaculture can be controlled by antibiotics; however continuous intensive use of antibiotics was undesirable as it leads to the development of undesirable resistance and thereby reducing the efficacy of the drugs (Alishmaa, 2007).

In addition, antibiotics accumulate in the environment and in the body of a fish, thus posing a potential hazard to consumers and to the environment ***(***Abutbul *et al*., 2004). The implications of the presence of these pathogens in the fish ponds examined are that they could cause mortality to the fish and could also cause several diseases to man on consumption of improperly heat-processed tilapia caught from these ponds (Egbere *et al.,* 2008).

2.3.1 *Escherichia coli*

Escherichia coli is a thermo-tolerant coliform that belongs to the total coliform group. Thermo-tolerant coliforms are capable of fermenting lactose at 44.5±0.2 ºC. *E. coli* are differentiated from this group by their ability to produce indole from tryptophan or by the production of enzyme ß-glocurinadase (Mwajuma, 2010). It is believed to be of faecal origin and has been found to be present in fresh faeces in concentrations as high as 10^9 per gram. *E. coli* is found in sewage, treated effluents, all natural waters and soils which are subject to recent faecal contamination, whether from humans, agriculture or wild animals and birds. It has been suggested that *E. coli* may be found or may even multiply in tropical waters that are not subject to human faecal contamination. *Escherichia coli* and other groups of coliforms may be present where there has been faecal contamination originating from warm-blooded animals (Chao *et al*., 2003).

E. coli is recognized as a good indicator of faecal contamination (Chao *et al*., 2003). It is identified as the only species in the coliform group found exclusively in the intestinal tract of human and other warm-blooded animals and subsequently excreted in large numbers in faeces, approximately 10^9 per gram (Geldreich, 1983; Onyuka *et al*., 2008). Invasion of fish muscle due to the breakage of immunological barrier of fish by pathogens is likely to occur, when the fish are raised in ponds with faecal coliforms, *E. coli* and *Salmonella* of greater than 10^3 per ml in pond water, respectively (Guzman *et al*., 2004). *Escherichia coli*, the predominant species of the faecal coliforms, has been found in the intestinal tract of fish on the gills, in the muscles and on the skin when sewage water has been used to rear fish (Ampofo and Clerk, 2010).

2.3.2 *Salmonella* species

Salmonella is a motile, non-spore forming, Gram-negative, rod-shaped bacterium in the family Enterobacteriaceae. *Salmonella* is facultatively anaerobic (can grow with or without oxygen), catalase positive and oxidase negative bacteria (Huss and Gram, 2003). *Salmonella* species can multiply and survive in the estuarine environments and tropical freshwater environments for weeks although open marine waters are free from *Salmonella* (Huss and Gram, 2003). These mesophilic organisms are distributed geographically all over the world, but principally occurring in the gastrointestinal tracts of mammals, reptiles, birds, and insects and environments polluted with human or animal excreta (Saeed and Naji, 2007).

Adebayo-Tayo *et al.*, (2012a), noted that high prevalence of *Salmonella* in catfish can be attributed to high temperatures in pond water, which promoted the growth of

Salmonella species as well as the cross contamination from viscera to flesh during processing. Heinitz *et al.* (2000) highlighted that the incidence of *Salmonella* in seafood is highest in the central Pacific and African countries and lowest in Europe including Russia, and North America (12 % versus 1.6 %). Salmonellosis is a food and water-borne bacterial infection of man and animals. The U.S. Food and Drug Administration's (FDA) data showed that *Salmonella* was the most common contaminant of fish and fishery products (Allshouse *et al*., 2004).

2.3.3 *Vibrio* species

Vibrio species are Gram-negative, facultatively anaerobic, motile curved rods with a single polar flagellum. The family Vibrionaceae is autochthonous to aquatic environments including estuarine, coastal waters and sediments worldwide, and some species are well known pathogens of marine organisms including fish and shellfish (Merwad *et al*., 2011). Vibriosis is one of the major disease problems in shellfish and finfish aquaculture. Vibriosis is caused by gram-negative bacteria in the family Vibrionaceae. Most of the species are mostly mesophilic, generally occurring in tropical waters and highest numbers are in temperate waters during summer months. Species such as *V. cholerae, V. parahaemolyticus, V. vulnificus, V. alginolyticus, V. mimicus, V. fluvialis, V. furnissii, V. metschnikovii, V. hollisae* and *V. damsel* are human pathogens (Adeleye *et al*., 2010). They account for a significant proportion of human infections such as gastroenteritis, usually associated with consumption of raw or undercooked seafood, wound infections, septicemia and ear infections (Adeleye *et al*., 2010).

Although the vast majority of environmental *V. parahaemolyticus* isolates are non-virulent, it is a leading cause of gastroenteritis linked to seafood consumption in the United States (Iwamoto *et al.* 2010; Wafaa *et al*., 2011). Seafood products harvested from contaminated waters or which have been improperly preserved after harvesting are known to play an important role in infections by *Vibrio* spp. especially crustaceans (Wafaa *et al.*, 2011). Water temperature can greatly affect the vibrio levels in seafood. *Vibrio* can multiply rapidly between 20 ºC and 40 °C (FEHD, 2005). *Vibrio* is acid sensitive and grows best at pH values slightly above neutrality that is 7.5 to 8.5 (FEHD, 2005).

2.3.4 Pseudomonas species

They are gram-negative, non-acid fast, non-sporulating rods with single polar flagellum, measuring about 2×0.4 μ. *Pseudomonas* spp. is found in soil, fresh water, sediments and sea water, and is known as plant and root colonizers (Hossain *et al.,* 2006). *Pseudomonas aeruginosa* is a gram negative pathogen, versatile and opportunistic in terms of its genetic, metabolic potential and mechanism of virulence. The bacteria belonging to the genus *Pseudomonas* are present in most natural waters and infect most species of fish. These parasites are considered opportunistic pathogens, causing disease when the host is subjected to some type of stress (Egbere *et al*., 2008).

Pseudomonas spp. has been credited with causing pseudomonad septicaemia, red spot disease, fin or tail rot, and others on fish. Fish diseases caused by *Aeromonas* and *Pseudomonas* species are considered to be the major bacterial problems facing aquaculture development causing mass mortalities, reduced production and low quality of aquatic organisms. Clinical symptoms include, haemorrhages in the mouth region, opercula, and ventral side of the body. Small petechial haemorrhages can occur throughout body cavity. Organs such as the liver and kidney may also be affected. Bacteria of the genus *Pseudomonas* also afflict fish with fin rot and internal ulcers. This bacterium effectively attaches itself to the tissue of the host fish by means of little hair like structures called fimbriae (Takyi *et al*., 2012).

2.3.5 Streptococcus species

Streptococcus species are Gram-positive bacteria that cause Streptococcal infection in fish due to intensification of aquaculture and causes significant economic losses in fish farm industry (Gun *et al*., 2006)**.** Most *Streptococcus* spp. has an optimum growth temperature of about 37 °C, but some will grow at temperature as low as 10 °C, a characteristic that supports their general appearance as pathogens of fishes (Alshimaa, 2007). Historically, *Streptococcus* species were not serious pathogens of fish, but recently this bacterium have become more prominent and cause high economic losses in wild and cultured fishes (Russo *et al*., 2006).

Alshimaa, (2007), noted that the external signs observed on infected fish by *Streptococus* species have hemorrhage in the anal and pectoral fins, and petechial on the abdomen with bilateral exophthalmia. Affected fish may exhibit one or more of the following clinical signs, depending on the fish species, which were erratic swimming such as spiraling or spinning, loss of buoyancy control, lethargy, darkening, uni- or bilateral exophthalmia "pop- eye", corneal opacity (whitish eye), hemorrhage in or around eye, base of fins, anus, over the heart, or elsewhere on the body, the gill plate, ascites and ulceration (Alishmaa, 2007)

2.3.6 Contamination of fish with other bacteria

Aeromonad bacteria are ubiquitous in the environment and several *Aeromonas* species have been reported to cause disease in fish, as well as being potential food-borne pathogens that may cause disease in humans (Hastein *et al*., 2006). *Hafnia alvei*, a Gram-negative, facultative anaerobic bacterium of the family Enterobacteriaceae, is found in natural environments, such as sewage, soil and water, but is also a gastro-intestinal commensal (Hastein *et al*., 2006). This bacterium is not usually considered pathogenic but has occasionally been reported to cause disease in fish as well as terrestrial animals and humans (Padilla *et al*., 2005; Hastein *et al*., 2006). *Streptococcus agalactiae* affects numerous wild and cultured fish species worldwide and causes disease involving septicemia and colonization of numerous organs. Mycobacteriosis (*Mycobacterium* species) is a chronic disease reported in seawater, brackish water and freshwater fish species, in aquaculture and aquariums as well as from the wild (Hastein *et al*., 2006).

2.4 Parasites

Parasites are capable of causing damage to its hosts by injuring tissues or organs while burrowing or consuming food. Despite the ubiquitous distribution of many of these parasites, infestation of a host is usually limited unless the host is subjected to increased stress. Increased stress may take the form of crowding, insufficient oxygen levels, or poor water quality (Adedeji and Adetunji, 2004; Adedeji, *et al*., 2012). The damage done to the host will usually be directly proportional to the level of infestation. The parasites can produce bloody lesions on scaled fish and erosion of fins and spines in all species. Gills can become swollen, hemorrhagic, and produce heavy mucus. Mortality can result with heavy infections. Several helminths inhabiting fish as larval stages are capable of causing diseases in human beings if they are ingested (Adedeji and Adetunji, 2004; Adedeji, *et al.,* 2012). Nematodes or "roundworms" infect many different species of farmed and wild fish. Small numbers of nematodes often occur in healthy fish, but high numbers cause illness or even death in fish (Adedeji and Adetunji, 2004; Adedeji, *et al*., 2012).

2.5 Fungi

Fungi are generally considered secondary symptoms in fish as they usually only occur where other trauma, such as injury or disease, have created an opportunity for fungal infection. The presence of *Aspergillus* spp can produce some toxins that are fatal (Adebayo- Tayo *et al.*, 2012a). This could become the possible source of aspergillosis transmission among the consumers. *Aspergillus* and related molds generally grow faster and are more resistant to high temperatures and low water activity than *Penicillium* spp. and tend to dominate spoilage in warmer climates (Doyle, 2007; Adebayo-Tayo *et al.,* 2012a). Many aspergilli produce mycotoxins: aflatoxins, ochratoxin, territrems, cyclopiazonic acid (Doyle, 2007; Adebayo Tayo *et al*., 2012a).

2.6 Zoonotic effects

Viruses, bacteria, fungi and parasites in fish may cause disease or food-borne infections in humans. Under certain conditions, bacteria which cause fish diseases may also infect humans, without necessarily being regarded as a major human health problem (Zakia *et al*., 2012). Several bacteria are, however, reported to cause infection and mortality in both fish and humans and these represent a particular hazard, caused either by handling infected fish on fish farms or in grocery stores or by the ingestion of raw or inadequately processed infected fish and/or contaminated fish products (Hastein *et al*., 2006).

The genus *Streptococcus* includes many species that can cause disease in different hosts, including fish in sea, brackish and fresh water as well as in mammals and humans (Hastein *et al*., 2006). *Streptococcus iniae* has been described as a cause of disease in both fish (mad fish disease) and people (Facklam *et al*., 2005; Hastein *et al*., 2006). Several *Mycobacterium* spp., such as *M. marinum, M. chelonei* and *M. fortuitum*, have been reported in both fish and humans (Hastein *et al*., 2006). Fish with mycobacteriosis pose a particularly significant threat of transmitting the infection to humans and thus may well become hazardous to human health (Hastein *et al*., 2006). These bacteria cause diseases and infections to humans such as salmonellosis (003) typhoid fever (001); paratyphoid fever (002); *Vibrio parahaemolyticus* infection (005.8); and shigellosis.

The presence of faecal coliform in fish intended for human consumption may constitute a potential danger in causing disease. It can also be a possible transfer of antibiotic resistance from aquatic bacteria to human infecting bacteria from nonaquatic sources (Ampofo and Clerk, 2010). Contamination of hands and surfaces during cleaning and evisceration of fish is a common route for pathogenic infection in humans (Adedeji *et al.*, 2011). Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on the season, patients' contact with fish and related environment, dietary habits and the immune system status of the exposed individual. They are often bacterial species facultatively pathogenic for both fish and man and may be isolated from fish without apparent symptoms of disease (Novotny *et al*., 2004).

Novotny *et al*. (2004), noted that humans get infected through contact with infected fish while handling them, water or other constituents of fish life environment and orally by consumption of infected fish or related products or food contaminated with water or other constituents of water environment. Mycobacteriosis is a disease caused by *Mycobacterium* spp. and it is particularly significant among infections transmissible from fish to human beings. Mycobacteriosis of fish is a chronic progressive disease spread all over the world (Novotny *et al*., 2004).

2.7 Sources of bacterial contamination

According to the U.S. Environmental protection Agency (USEPA), 90 % of the world's water is contaminated. The causes of contamination include: pathogenic microbes, heavy metals, organic substances and inorganic chemicals. Worldwide, all fisheries are threatened by various factors, such as pollution, which brings about the introduction of industrial waste which comprise of various inorganic and organic waste and invasive species of bacteria. These bacteria are majorly enteric in nature, and their target point is the colonization of the intestine of fish and other sites in the fish (Udeze *et al*., 2012a). Man, in his effort to get rid of his wastes has introduced into natural water bodies, noxious substances including organic wastes that promote the growth of pathogenic bacteria, fungal, viral and protozoan microbes (Adams and Kolo, 2006).

In resource poor countries, where waste disposal method is known to be poor, constituting a negative impact on the water bodies. This has ended up affecting aquatic life in water bodies and also contributing to the poor transportation system, which encourage the development of water weeds. These water weeds have an effect on fish whereby they reduce their life span as a result of the various toxins released into the water. Faecal source of pollution has contributed to the high level of fish disease which has been experienced in recent years (Udeze *et al.,* 2012a).

Public health must therefore be of prime concern when dealing with fish farming and its products. Countries with less restriction on release of waste into water bodies and those that use untreated wastewater for aquaculture must be more observant (Ampofo and Clerk, 2010). There is a correlation between organisms found in water and in fishes. These organisms could have gotten into water from normal sources such as faecal contamination by man and other animals (Udeze *et al*., 2012a). Fish cultured in various types of organic waste fertilized ponds are susceptible to infection with pathogenic bacteria. Fish from the non-fertilized pond had bacteria detected in the different tissues but at relatively low numbers (Ampofo and Clerk, 2010).

Atiribom *et al*. (2007) reported that high concentrations of bacteria and nitrates discharged into water can occur from animal husbandry operations like grazing and that this can result in health hazards to man due to the presence of pathogens. A major activity in aquaculture is the application of cow dung, poultry droppings to pond bottom as manure during pond fertilization. Such activities and the direct deposition of human faecal wastes in water bodies in man’s disposal effort, lead to contamination of water bodies with pathogens and other toxic substances Atiribom *et al*. (2007).

In intensive aquaculture, disease tends to spread relatively easily because of the high density of stocking and intensity of feeding in limited water areas. The proliferation of disease-causing agents through the common water source between ponds, farms and the stocking of fish, fingerling or brood stock transported from other fish farms without adequate precaution, can spread diseases (Akolisa and Okonji, 2005). Majority of water borne pathogenic microorganisms enter water courses as a result of faecal and waste water contamination (Adewoye and Adegunlola, 2010).

2.8 Quality of water

Just as farm land is to crops in terrestrial agriculture, so is water to fish farming. Fish performs all their body functions in water. The quality of the water in which fish are contained is therefore very important to their livelihood. Environmental conditions are some of the major factors that limit fish production. Such factors include water quality, relating to physical, chemical and biological properties of the ponds (Fafioye, 2011). The ponds and rivers that harbor the fish may be the source of contaminants. This may be due to indiscriminate deposition of human, animal excreta and other environmental wastes into natural water and land. This is mainly during the rainy season, as the faecal matters from various sources are washed from contaminated land into different water bodies (Emikpe *et al.,* 2011).

Physical factors that are important in domestic fish farming include, shape and size of fish pond, types of substrata material, temperature, turbidity and pond transparency. The chemical factors include: Dissolved Oxygen (DO), alkalinity, hardness of water, hydrogen ion concentration (pH), conductivity and mineral constituents such as nitrates and phosphates. In addition, there are biological factors which equally influence fish production; such factors include vegetation, predation and aquatic plants (Ayanwale *et al.*, 2012). The quality (bacteriological, physico-chemical and chemical) of water determines to a great extent the yield of fish in a fish farm.

Water quality parameters include turbidity, pH, electrical conductivity, temperature, total dissolved solids and anions like chlorides, nitrates, and fluorides and heavy metals. The heavy metals affect organisms especially when they build up in biological systems and become hazards to organisms (Osha, 2004). There are laid down international standards that the load of bacteria and physico-chemical quality of water used for fish farming must meet. Among these quality parameters are the standards specifying that water for fish farming should not contain more than 400 coliforms/100 ml and no pathogen should be present (Egbere *et al*., 2008). In pond ecosystem, water quality and soil properties play a significant role in the life of benthic organisms (Tabatabaie *et al*., 2009).

The suitability of water for the survival and growth of fish is governed by a myriad of water quality variables. According to Udo (2007), the quality of water used in a pond is affected by the chemical properties of the soils on which it runs. In the course of constructing fish ponds the upper horizons of the terrestrial soil is usually excavated, thereby exposing the subsoil to water when ponds are filled. The main requirement is that, soil for pond consists of a mixture of particles from within or outside the pond and the accumulation of sediments which have a direct contact with water can either enhance or hinder the growth of benthic organism (El-Marakby *et al*., 2006).

The increasing demands placed on the currently available water resources can raise the potential of contaminating surface water and ground water by enteric pathogens. Faecal contamination through untreated or inadequately treated sewage effluents entering lakes, rivers or ground waters that in turn serve as water supplies creates conditions for rapid dissemination of the pathogens (Mwajuma, 2010). This shows that different sources of water contribute to the bacterial load of fish pond and the effect of these pathogenic bacteria on fish.

2.8.1 Physico-chemical parameters

Fish population from various breeding systems may be affected by diseases that are as a result of physical and chemical ambient, nutritional and constitutional agents. Water chemistry is defined by a multitude of parameters, which are more important for fish life such as pH, dissolved gasses (oxygen and carbon dioxide), organic matter in suspension, its concentration in ammonia, nitrites, nitrates, phosphates, chlorides, sulfates, hydrogen, sulfides, detergents, pesticides and phenols (Vasile, 2008).

2.8.1.1 pH

The general state of water quality can be estimated by pH, which is a simple measure of acidity or alkalinity. Optimum pH for fish growth and health is between 6 and 9 (Stone and Thomforde, 2003). The pH of pond water is influenced by amount of carbon dioxide present which originates from animal and plant respiration (Udeze *et al*., 2012a). The pH value below 7 represents acidic conditions while that above 7 indicates alkalinity. Fish do not grow well in ponds with acidic water, which usually are located on acidic soils, but acidity in ponds can be corrected by liming (Silapajarn *et al*., 2004). Mortalities will occur when pH values are less than 4.5 or greater than

10. In addition to the direct effects pH can have on fish and other aquatic animals, it interacts with other water quality variables such as ammonia, hydrogen sulfide, and dissolved metals, affecting their aqueous equilibrium and toxicity as well (Rossana, 2012).

2.8.1.2 Conductivity

The electrical conductivity (specific conductance) of water is an expression of its capacity to conduct a current and is related to the concentration of free ions and to water temperature. These ions play a critical role in the lives of fish and their presence or absence in water determines the productivity and growth of fish (Mwaura, 2005). Conductivity provides a quick, convenient estimate of the ionic content and thus the quality of water, as well as a quick check on water suspected of having received ionic pollutants (Mwaura, 2005).

2.8.1.3 Dissolved Oxygen

Dissolved oxygen (DO) is one of the most important water quality factors for pond owners. Oxygen is needed by fish and other aquatic organisms, and levels of DO will determine the ability of ponds and other water bodies to support aquatic life (Thilza and Muhammad, 2010). Oxygen dissolves in water at very low concentrations measured in parts per million (ppm, which can be used interchangeably with milligrams per liter (mg/L). Ponds will rarely have more than 10 ppm DO. Most oxygen in water is produced by algae and green plants through photosynthesis and is also naturally incorporated into water from the atmosphere through surface diffusion and turbulence caused by wind (Rossana, 2012).

Warm water increases the metabolism of fish and therefore increases their consumption of oxygen. Fish exposed to low, nonlethal levels of DO over prolonged periods will be chronically stressed, stop eating, and be more susceptible to disease. The optimum range is 5.0-15.0 mg L^{-1} (Thilza and Muhammad, 2010). Low oxygen concentrations also increase the activity of anaerobic bacteria, which create methane and hydrogen sulfide gases during anaerobic decomposition. Whenever DO levels fall below 3 to 4 ppm, oxygen stress will occur. Lack of adequate dissolved oxygen is the leading cause of fish deaths. Normal oxygen content in a healthy pond will range from 5 to 15.0 mg L^{-1} (Rossana, 2012).

2.8.1.4 Ammonia

Adverse environmental parameters can have direct or indirect effects on fish. Direct effects are where tissues are damaged directly by a water quality problem, such as ammonia causing gill damage. In some cases such effects can be mitigated by other parameters. For example, the toxicity of ammonia is much reduced by low pH and low temperature (Thilza and Muhammad, 2010). Ammonia is a form of nitrogen found in organic materials and many fertilizers. It is the first form of nitrogen released when organic matter decays and is the main nitrogenous waste excreted by most fish and freshwater invertebrates. Fish exposed to sub-lethal levels (greater than 0.02 ppm) for extended periods of time can lead to reduced growth and increased susceptibility to disease (Thilza and Muhammad, 2010).

2.8.2 Heavy metals

A heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations (Irwandi and Farida, 2009) the heavy metals do not generally break down further into less harmful constituents and they accumulate where they are released, (Akan *et al*, 2009). The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years, because they are indestructible and most of them have toxic effects on organisms. Among environmental pollutants, metals are of particular concern; due to their potential toxic effect and ability to bio accumulate in aquatic ecosystems (Censi *et al.*, 2006). Due to high level of toxicity, persistence and tendency to accumulate in surface waters, heavy metals and metalloids are very dangerous for living organisms if their concentration is higher than allowed. Dissociation of heavy metals ions in water is a very slow process and they cannot be detoxicated by metabolic processes. Pollution of the aquatic environment by inorganic chemicals has been considered a major threat to the aquatic organisms including fishes. The agricultural drainage water containing pesticides and fertilizers and effluents of industrial activities and runoffs in addition to sewage effluents supply the water bodies and sediment with huge quantities of inorganic anions and heavy metals (ECDG, 2002).

Heavy metal concentrations in aquatic ecosystems are usually monitored by measuring their concentrations in water, sediments and biota, which generally exist in low levels in water and attain considerable concentration in sediments and biota. Heavy metals including both essential and non-essential elements have a particular significance in ecotoxicology, since they are highly persistent and all have the potential to be toxic to living organisms (Storelli *et al.*, 2005).

Sediments are important sinks for various pollutants like pesticides and heavy metals and also play a significant role in the remobilization of contaminants in aquatic systems under favorable conditions and in interactions between water and sediment. Fish samples can be considered as one of the most significant indicators in freshwater systems for the estimation of metal pollution (Ozortuk *et al.*, 2009). For the normal metabolism of the fish, the essential metals must be taken up from water, food or sediment. These essential metals can also produce toxic effects when the metal intake is excessively elevated (Ozortuk *et al.*, 2009).

Oyewale and Musa (2006) noted that the heavy metal status of the lower basin of Kainji dam (used for hydroelectricity generation), which includes Lakes Kainji/Jebba, Nigeria had the potential for human exposure to heavy metals from eating fish caught in the lakes. Water, sediments and fish from the lakes were sampled and evaluated for As, Cu, Co, Cr, Fe, Mn, Ni, Pb, Sb, Ti, V and Zn using the energy dispersive x-ray florescence technique (Oyewale and Musa, 2006). Fe and Mn were found to be present at high mean concentrations in the water (13 and 9µg g^{-1}), sediment (7,092 and 376µg g^{-1}) and fish (11.4 and 4.6µg g^{-1}) samples. Ti (4.1 µg g^{-1}), Cr (2.2 µg g^{-1}), Co (1.2 µg g^{-1}), Cu (1.3 µg g^{-1}) and Pb (1.2 µg g^{-1}) in the water samples and Ti (27 µg g-1), V (27 µg g-1), Cr (27 µg g-1), Co (40 µg g-1) Ni (33 µg g-1), Cu (25 µg g-1), Zn (59 µg g-1), and Pb (19 µg g-1) in the sediment samples were found to be of medium mean concentrations (Oyewale and Musa, 2006).

A research was carried out on the status of heavy metals in water of river Gomti, India. The metals were analysed using Flame Atomic Absorption Spectrophotometry (FAAS). They found the heavy metals in the river water to be in the range of 0.0013 to 0.0043 mg^{-1} for copper, 0.0791 to 0.3190 mg^{-1} for iron, 0.0144 to 0.0298 mg^{-1} for Zinc (Singh and Mohan, 2005). They concluded that the water was unpolluted with respect to copper, iron and zinc but highly polluted with respect to lead (Singh and Mohan, 2005). The metal concentrations in fish organs (muscle, gills and liver) of *Oreochromis niloticus* are closely associated with metal content of water and sediments (Samir and Ibrahim, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out in Abothuguchi Central, Meru Central Sub-County, Meru County (Fig 3.1). The area was chosen due to its high number of fish ponds which use diverse sources of water. Abothuguchi Central lies on the North Eastern side of Mt. Kenya and this is one of the geographical features that contribute to the climatic patterns experienced in the region. Abothuguchi central lies along the equator and within longitudes $37^{\circ}40'$E and latitude 0° $03'$N with an altitude of approximately 5199 meters and this explains the cool temperatures experienced all-round the year. It has a total population of 116,516 (KNBS, 2009).

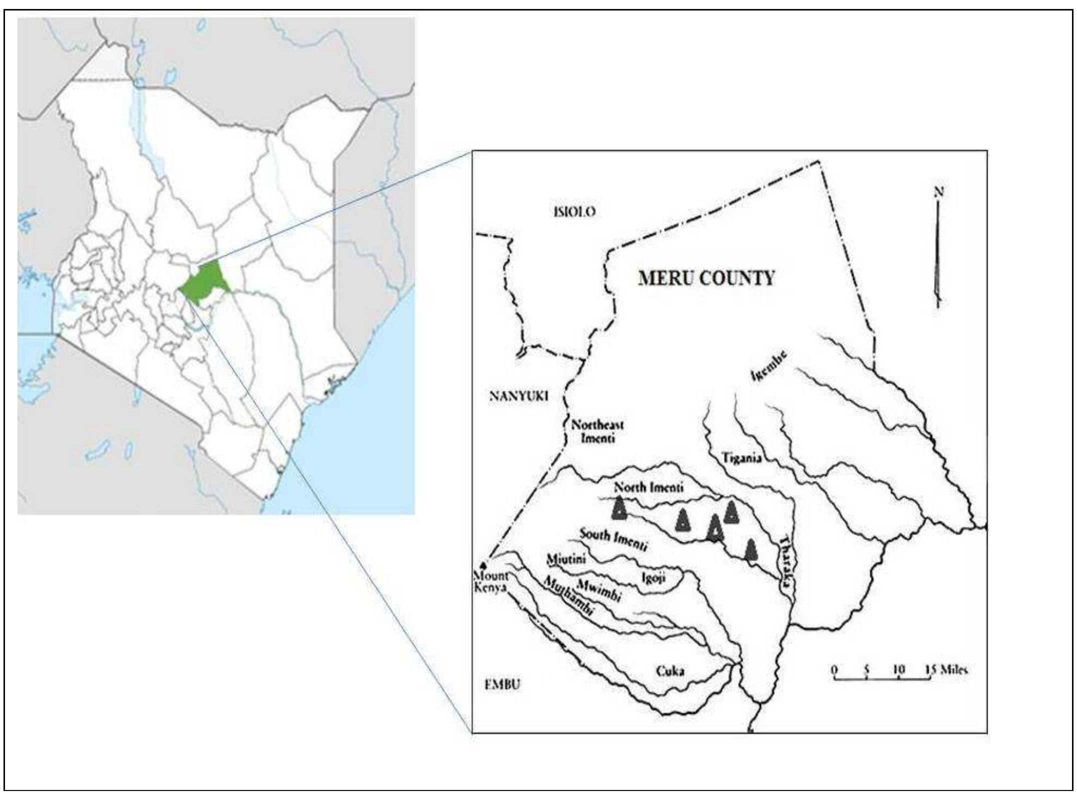


Figure 3.1. Map of Kenya showing Meru County from Google maps. Triangles indicate the sampling points.

It lies within the time zone of EAT (UTC+3) and it has many seasonal and permanent rivers hence water scarcity is not much of a problem in the area. The soils are volcanic and very rich in almost all the plant nutrients especially in the high areas. The precipitation experienced in the area is bimodal whereby the short rains are experienced between March and May while the long rains are experienced between October and December. This pattern enables the farmers to grow a wide range of crops for subsistence and commercial purposes. The other major crop in Abothuguchi Central is coffee as well as tea in areas near Mt. Kenya.

The average rainfall received in a year is 1300 mm in the highlands and 380 mm in the low lying areas of Abothuguchi Central. However the kind of crops grown varies depending on the ecological zones which vary in terms of precipitation, temperature and soils. Some of the crops grown in the highlands include cash crops such as coffee, tea, sugar, pyrethrum and food crops such as maize, sugarcane, bananas, sorghum, millet, yams and cassavas. Livestock reared include cattle, sheep, rabbits, chicken and goats.

3.2 Sampling design and sample size

This was a cross sectional study design. The sampling technique employed was stratified sampling in which the sampling zone was divided into five locations which were approximately two Kilometers apart and from each location, six sites (fish ponds) were identified. The samples were taken from every site twice. The sampling technique mainly targeted different fish ponds with different sources of water, geographical patterns and economic activities. The sites were Mbwinjeru (Mbu) whose main source of water was a river, it is purely an agricultural zone and its located on the lower sides of Abothuguchi Central hence it experiences high temperatures as compared to other site. The second site was Kithirune (Kit) whose source of water is mainly tap water and it is located around a market place; it is quite cold due to its altitude as compared to Mbwinjeru. The third site was Githongo which is within a market, the farmers use tap water and it is cold due to its altitude. The fourth site was Muri which next to Mt. Kenya forest, it is extremely cold due to its high altitude and the farmers uses River water. The fifth site was Kianthumbi (Thu) whose source of water is a swamp and it is on a lower altitude other than that of Mbwinjeru.

The samples were collected from each fish pond twice between November 2012 and May 2013. The samples were collected during the wet season and the dry season to cater for seasonal variations. With the confidence interval of 95 % and an error of 5 %, the sample size was determined using the formula by Fisher (Fisher, 1998).

$$n=\frac{Z^2PqD}{d^2}$$

Where n= sample size, p= anticipated prevalence which was 3 % (0.03) in this study, q= failure which was calculated as (100 % -3 %) giving 97 % (0.97), Z= is the appropriate value from normal distribution for the desired confidence level which was 1.96 in this study, d= allowable error (0.086) and D= design effect which was given a value of 2 because replication was carried out. Based on 3 % prevalence and Z value of 1.96, the sample size was:

$$n= \frac{1.96^2(0.03\times0.97\times2)}{(0.086)^2} = 30$$

At each fish pond, water samples were collected directly into pre-sterilized polypropylene (glass) bottles of 250 ml.

At the fish ponds, 250 ml bottles were opened aseptically, then held at their bases and submerged to a depth of about 20 cm with the mouth facing upwards. Samples were taken by filling the bottles to the top to exclude air. A total of 30 samples were collected which are consistent with Samie *et al*. (2011). The ponds were stirred before the samples were collected. The water samples were collected in 250 ml plastic sterile bottles and then placed in ice cool box at a temperature of 4 °C after which they were transported to Kenyatta University Microbiology laboratory for analysis. All the samples were analysed within 24 hours.

3.3 Bioassays

3.3.1 Detection of faecal streptococci (FS)

Azide dextrose broth was used for the detection of faecal streptococci in the fish pond waters. The single double strength broths were inoculated with 10 ml of the water samples with the use of a pipette and incubated at 37 °C for 24 hours. Presence of gas production and fermentation of sugars (indicated by the yellow colour change of the Bromthymol blue) were used as positive tests which were confirmed by presence of colonies on Kenner faecal (KF) (Mariita and Okemo, 2009). The presences of pinpoint colonies from the slant were Gram stained to confirm the presence of FS. All red and pink colonies were counted (using colony counter) (Mariita and Okemo, 2009).

3.3.2 Detection of faecal coliforms

The analysis of water for the presence of faecal coliforms was carried out using the multiple–tube fermentation technique (APHA, 2003). This was carried out in three steps; the presumptive, the confirmed and completed tests. Each batch was inoculated with the diluted (all dilutions were carried out using sterile water blanks) water samples. In the presumptive test a three series of five tubes each containing 10 ml, 1 ml and 0.1 ml portions of the sample were inoculated with lactose broth initially sterilized by autoclaving. Pure sterile lactose broth was inoculated with sterile distilled water in the same way and used as a control. Inoculated tubes were then incubated at 37 °C for 48 hours (Gyles, 2007). Sterile loop transfers was made from all tubes showing acid and gas production (of total coliform MPN) to tryptose bile broth (EC Broth) and incubated at 44 °C for 24 hours. Gas production in a fermentation tube within 24 hours or less was considered as a positive reaction.

The estimated number of faecal coliforms, present in 100 ml was read from a tabulated probability table using corresponding results of various combinations of positive and negative reactions from each of the three batches (APHA, 2003). For confirmation, samples that were considered to have a positive reaction from the tryptose broth were streaked on a plate of Eosin Methyl Blue (EMB) agar which gave well isolated colonies. Incubation was carried out at 37 °C for 48 hours. Development of the typical colonies on the plates was observed and a Gram staining carried out. Completed test was carried out by picking two colonies that were considered to be of faecal coliform and transferring them to nutrient agar slopes and fermentation tubes containing brilliant green lactose broth. Incubation was again was carried out at 44.5 °C for 48 hours. From the agar slope, a Gram stain was made to confirm the completed test. Brilliant green lactose broth was also observed for gas production (Gyles, 2007). Gas and turbidity in the tubes as well as metallic sheen or pink with dark centre colonies on EMB agar indicated positive for faecal coliforms. All isolates that produced gas at 44.5 °C, stained Gram negative and were non spore forming and rod-shaped were regarded as faecal coliform and the total counts calculated using a standard probability table (APHA, 1992).

3.3.3 Detection of *Salmonella*

Identification of *Salmonella* was carried out in three successive phases. The first phase of selective enrichment was carried out using the tetrathionate broth base as outlined by APHA (2003). One millilitre of each sample from different sites was mixed well with 10 ml of tetrathionate broth and the mixture was incubated for 24 hours at 35 °C. For selective growth, the phase of plating (pour plating method was carried out using 1 ml of the enriched with nutrient agar) samples. Streaking was carried out from the same enriched samples on Deoxycholate Citrate (DCA) agar, Salmonella-Shigella (SS) agar and MacConkey agar (Andrews and Hammack, 2003). Incubation at 37 °C was carried out for 24 hours. Enumeration of typical colonies (typical colonies clear to pale pink on DCA agar, pink on SS agar and white on MacConkey agar) was carried out using colony counter and Gram staining. Typical colonies were confirmed by biochemical tests: TSI, motility and urease tests according to the procedure described by Mariita and Okemo (2009).

3.3.4 Screening for *Vibrio*

Vibrio detection was carried out in three successive phases.

(i) Enrichment in a non-selective medium

One ml of the samples was enriched in sterile alkaline peptone water and dispensed in 10 ml tubes (HPA, 2003). Incubation was carried out for 18 hours at 35 °C.

(ii) Plating out on selective medium

The streaking of the enriched samples was carried out on Thiosulfate citrate bile salts (TCBS) agar. For quantitative analysis, pour plating of the samples was carried out. The agars were incubated at 35 °C for 24 hours. The presence of characteristic yellow colonies was suspected to be of *Vibrio cholerae* after streaking was carried out on nutrient agar (Mariita and Okemo, 2009).

(iii) Biochemical reactions

Gram staining was carried out and confirmed by biochemical tests such as such as TSI, Motility test, Citrate utilization test, urease test and cytochrome oxidase test among others (Mariita and Okemo, 2009).

3.3.5 Detection of *Escherichia coli*

In suspected case of *E. coli*, samples were inoculated into enrichment glucose peptone broth and incubated at 37 ^{o}C for 24 hours before being sub-cultured onto Sorbitol MacConkey agar (Bopp *et al*., 1999). After 24 hours of incubation, *Escherichia coli* were identified based on morphological and biochemical tests such as motility-indole-urease test, methyl-red-voges-proskauer (MRVP) test and Simmons citrate utilization (Alam *et al.,* 2010).

3.3.6 Detection of *Pseudomonas*

i) Isolation procedure and maintenance

Isolates were prepared from the samples collected by the use of membrane filter method together with a selective growth medium. *Pseudomonas* spp. was isolated using a qualitative method, after concentrating 100 ml of pond water through 0.45 μm membranes which were then pre-enriched in NKS Cetrimide plates (Sartorius AG, Germany) at 30 °C for 24 hours. Enrichment was followed by *Pseudomonas* selective medium (Oxoid, CFC-SR103) at 30 °C for 24 hours. Positive cultures were sub cultured on Nutrient Agar (NA) (Difco) for the isolation of a pure single colony for identification (Hossain *et al*., 2006).

ii) Morphological and biochemical characterization

Colony characteristics, including green pigment production were determined on Acumedia and NA (Difco). All isolates were characterized by the following classical tests according to Bergey's Manual of Systematic Bacteriology (Palleroni, 1984): Gram staining, cytochrome oxidase production, catalase production and growth on MacConkey agar at 42 °C.

iii) **Biochemical tests**

a) Oxidase test

On a strip of paper 2-3 drops of oxidase reagent were placed. With the use of a glass spreader a moderate amount of the organism was taken and streaked on the moist surface of the paper. A nichrome wire which could give false positive results was avoided. The presence of *Pseudomonas* was confirmed by the appearance of intense purple colouration (Hossain *et al*., 2006).

b) Nitrate reduction

On nitrate broth, an NBA plate (Nitrate blood agar) was dried at 37 ^{o} C for one hour after which the plate was seeded by stab inoculation with the sample under test and then it was incubated overnight at 37 ^{o}C. Formation of large greenish zone which was as a result of the reduction of nitrate to nitrite which subsequently caused the alteration of the haemoglobin to methaemoglobin due to bacterial growth confirmed the presence of *Pseudomonas.* On the use of nitrate broth, the reduction of nitrate was based on the presence of the bacteria which caused reduction of nitrates into nitrite, ammonia, nitrous oxide and nitrogen gas. The nitrate broth was made by combining nutrients broth, 5 g/litre KNO_3 or $NANO_3$. One tube of nitrate broth having a Durham tube was inoculated. One millilitre of naphthylamione and 1 ml of sulphanilamide reagent were added to the tube cultures. Reduction of nitrates was confirmed by the appearance of red colour within 80 seconds (Hossain *et al*., 2006).

3.4 Heavy metals quantification

The determination of heavy metal presence and concentration in the water samples was carried out by the use of Flame Atomic Absorption Spectrometer (FAAS no VAA 350) Contamination levels of manganese, lead, iron, zinc and copper were analysed.

3.4.1 Metal standard solutions

These were prepared by accurately weighing 1.00 g of the metal powder in a clean beaker and carefully adding 20 ml concentrated nitric acid. The resulting solution was then transferred into a 1 litre flask and made up to the mark with distilled water. The solution contained 1000 µg/ml of the specific metal. This was stored in labelled plastic containers as stock solution. Dilutions were made to obtain working solutions of varying concentrations for different metals which were later used with FAAS (APHA, 1992).

3.5 Physico-chemical parameters

Physical and chemical parameters that is water temperature, pH, salinity, DO, total alkalinity, transparency, sulphates, phosphates, nitrates, ammonia and electrical conductivity were measured in situ using a portable Universal multiline P4 WTW (Wilheim Germany) meter. The meter was calibrated and operated as per the manufacturer's instructions. At the sampling point, the measuring probes were lowered into the water and then allowed to settle for 1-2 minutes before the readings were taken (APHA, 1992).

3.6 Data analysis

The data was analysed by the use of Statistical Analysis System (SAS) computer software Version 9.3. Two way ANOVA was used to show the interactions between the sites and the seasons while One way ANOVA was used to determine the significant differences at P value $\leq$ 0.05. P value of <0.05 was considered as significant. For significance difference, Tukey`s Honest Significant Difference (HSD) test was used to separate the means. Correlation coefficient test was used to determine whether there was a relationship between faecal streptococci and faecal coliforms.

CHAPTER FOUR

RESULTS

4.1 Enumeration of faecal bacteria indicators and detection of pathogens

Faecal bacteria were quantified while pathogenic bacteria were detected and confirmed using biochemical procedures.

4.1.1 Enumeration of faecal bacteria indicators

There was significant interaction (p=0.0001) between season and site in determining the FS CFU/100ml populations (Table 4.1). The FS CFU/ 100ml during the dry season were not significantly different (P=0.6043) from the FS CFU/100ml during the wet season, however their population was higher during the wet season (Table 1). The FS CFU/100ml differed significantly (p=0.0001) in the various sites. Streptococcal species showed white colonies on streptococcal KF agar plates (Plate 4.1a), cream colonies on nutrient agar plates (Plate 4.1b) and confirmed by Gram stain (Plate 4.2 a).

There was no significant (0.9367) interaction between site and season in determining the MPN of faecal coliform in the fish pond water. The FC MPN /100 ml during the dry season did not differ significantly (p=0.0680) from the MPN during the wet season (Table 4.1). The MPN varied significantly (p=0.0001) in the various sites (Table 4.1). The wet season indicated a high population of FC as compared to the dry season. The FC MPN /100 differed significantly (p=0.0001) in the various sites when compared with the seasons. In addition the prevalence of FS from all sites showed a significant positive correlation with that of FC (r = 0.832 at $P < 0.01$ level).

Table 4.1: Populations of Faecal streptococci and faecal coliform

TREATMENT	POP FS CFU/100mL	POP FC MPN/100mL
<u>Season</u>		
Wet season	575466.67 ± 90950.51a	150.533 ±17.1043a
Dry season	546833.33 ± 61069.36a	115.600 ± 22.0154a
<u>Sites</u>		
Githongo	768166.67 ± 84811.09 b	189.750 ± 40.2319ab
Kithirune	423333.33 ± 28954.86 c	139.917 ± 7.8889bc
Mbwinjeru	1079166.67 ±109533.85a	248.583 ±19.6740a
Muri	396750.00 ± 100441.31c	70.417 ± 9.3909cd
Kianthumbi	138333.33 ± 18701.54d	16.667± 2.6949d
<u>P values</u>		
Season	0.6043	0.0680
Site	0.0001	0.0001
Season*site	0.0001	0.9367

Values (Means±SE) followed by dissimilar letter(s) along the columns are significantly different at P≤0.05 (Tukey's HSD test)

Key
Pop- Population (Each column represents the separation of means and the standard deviation)

FC-faecal coliform density expressed in MPN/100 mL (most probable number) and FS-faecal streptococci density expressed in CFU/100 mL (colony forming unit).

According to one way ANOVA, there was significant (p=0.0001) difference in FS CFU/100mL in all the sites during the dry and wet season (Table 4.2). Higher FS CFU/100mL populations were observed during the wet season (W) in all sites as compared to the dry season (D) (Table 4.2). The wet season indicated a high population of FS as compared to the dry season. The FS CFU/100ml differed significantly (p=0.0001) in the various sites with the highest population being observed in Mbwinjeru and the lowest in Kianthumbi (Table 4.2) in both dry and wet seasons. There was significant (p=0.0001) difference in FC MPN in all the sites during the wet and dry season (Table 4.2). The highest FC MPN populations were during the wet season as compared to the dry season in all sites (Table 4.2). The FC MPN /100 differed significantly (p=0.0001) in the various sites with the highest population being observed in Mbwinjeru and the lowest in Kianthumbi (Table 4.2) in both dry and wet seasons.

Table 4.2 Comparison of FS and FC populations during the wet and dry seasons

Site	Pop FS CFU /100mL	POP FC MPN/100mL
Githongo (D)	640833.33±142575.46bc	170.833±81.497abc
Githongo (W)	895500.00±69538.36ab	208.667±18.3588ab
Kithirune D	393333.33±34896.67cd	126.833±3.590bcde
Kithirune W	453333.33±45946.83cd	153.000±13.873abcd
Mbwinjeru D	890000.00±74565.41ab	223.333±34.988ab
Mbwinjeru W	1268333.33±181427.98a	273.833±14.963a
Muri D	701666.67±84829.90bc	44.000±9.295cde
Muri W	918333.33±91851.43ab	96.833±4.743bcde
Kianthumbi D	108333.33±23863.04d	13.000±1.789e
Kianthumbi W	168333.33±24686.93d	20.333±4.835ed
P. value	0.0001	0.0001

Values (Means ±SE) followed by dissimilar letters along the columns are significantly different at P≤0.05. (W): wet season, (D): dry season.

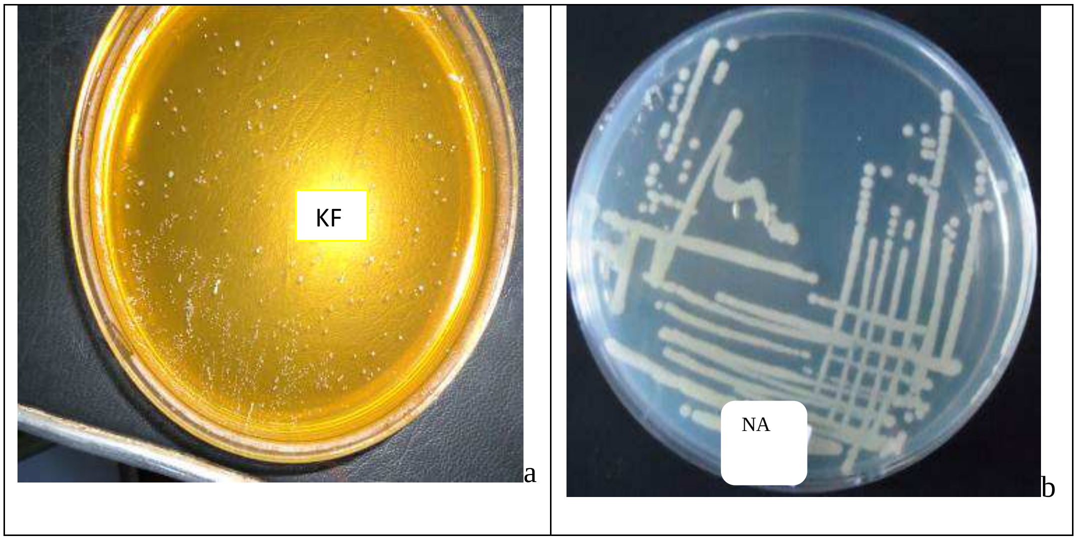


Plate 4.1.

KEY: a). Typical colonies of FS on KF agar.

b). Typical colonies of FS on nutrient agar.

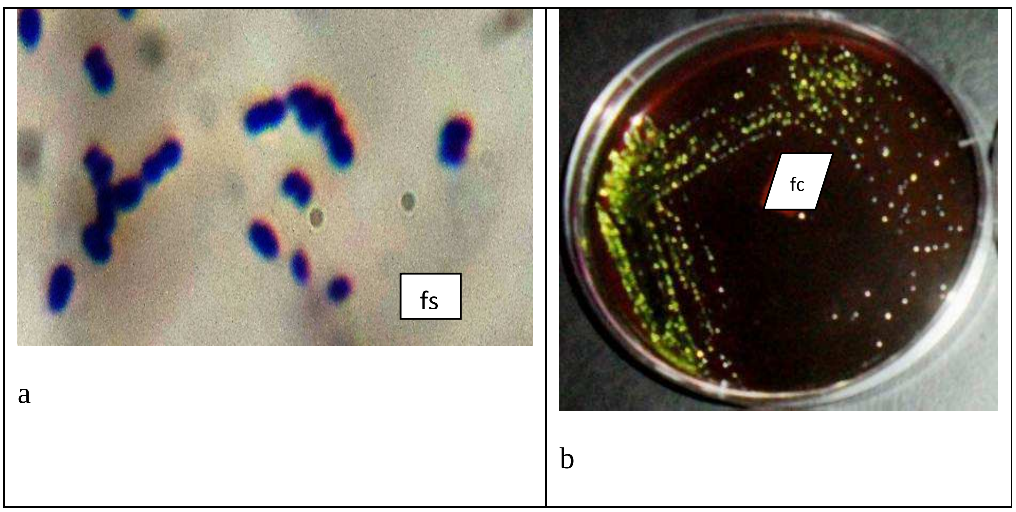


Plate 4.2

KEY: a). Streptococcal strains showing the characteristic of short to long chains following gram stain reaction.

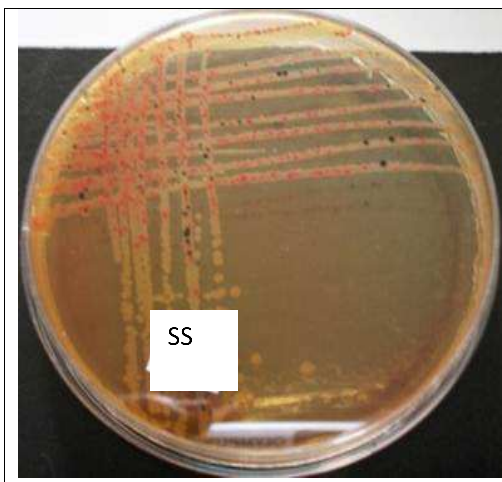
b). Typical colonies of faecal coliforms on EMB media.

4.1.2 Detection of pathogens

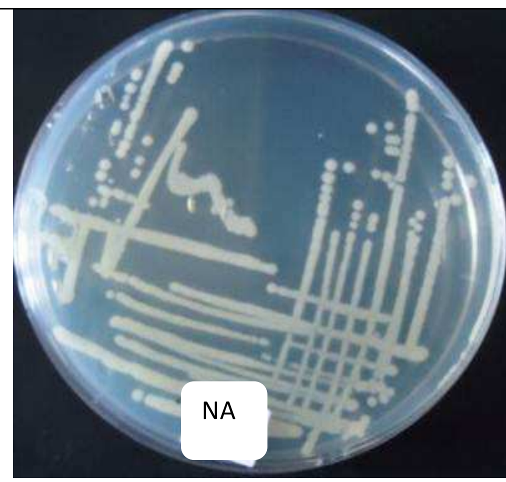
Over the study period, presence of the potentially pathogenic bacteria in water samples from various sources varied within the sampling sites. The pathogenic bacteria were detected with the use of the following biochemical tests: Simmons citrate agar test, Indole test, oxidase test, urease test, motility on SIM media, reaction on TSI, urease test and Gram stain. Several bacterial pathogens mainly *Vibrio*, *Salmonella*, *Escherichia coli* and *Pseudomonas* were isolated from most of the sites though their presence was significantly different.

4.1.2.1 *Salmonella* species

Salmonella spp were the most prevalent with (40 %), followed by *E. coli* found in (34 %), *Pseudomonas aeruginosa* (16 %) and *Vibrio* spp. (10 %) as the least (Appendix VII), (Plate 4.3a-c). The TSI agar slants inoculated with typical colonies showed yellow butts, red slants with some blackening and some with or without gas formation (Plate 4.3d-f). There was bacterial growth and colour change from green to blue on the Simmons citrate agar slant. The cytochrome oxidase test was positive as shown by the formation of a blue-purple colour. Biochemical tests were used to confirm whether the isolates were indeed *Salmonella* spp. (Appendix I). These included the utilization of citrate in Simmons citrate agar (positive), indole and motility test in SIM (both positive); reaction on TSI (positive) and urease test (negative) and oxidase test (negative).

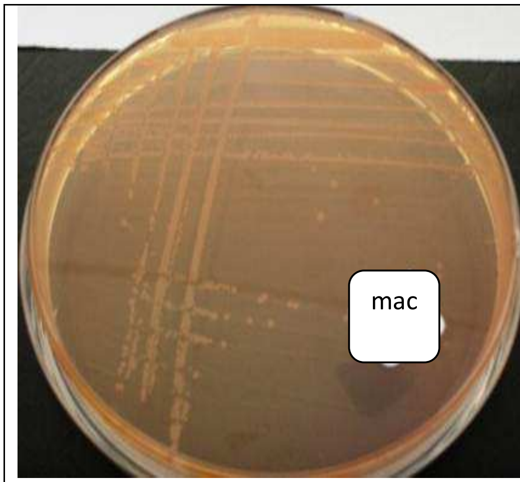


a

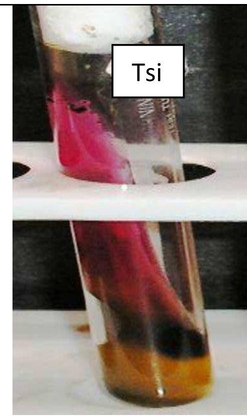


b

Plate 4.3
KEY: a): Black *Salmonella* colonies on SS agar.
b): Cream *Salmonella* colonies on nutrient agar plate.



c

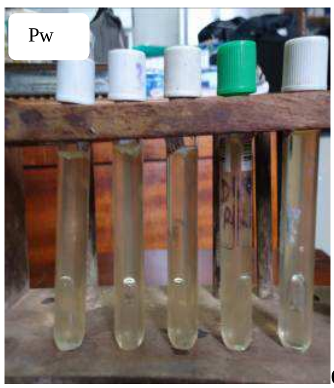


d

Plate 4.3

KEY: c): *Salmonella* colonies on MacConkey agar plate.

d): Yellow butt and red slant and some blackening on TSI slant showing the presence of *Salmonella* spp.



e


f

Plate 4.3

KEY: e): Gas production in peptone water as indicated by bubble formation in Durham inserted in tube.

F): Reaction in urea agar slants. Colour change shows absence of *Salmonella*

spp.

4.1.2.2 *Vibrio* species

Vibrio spp. showed characteristic golden yellow colonies on TCBS (Plate 4.4a) and constituted 10 % of the pathogens detected.

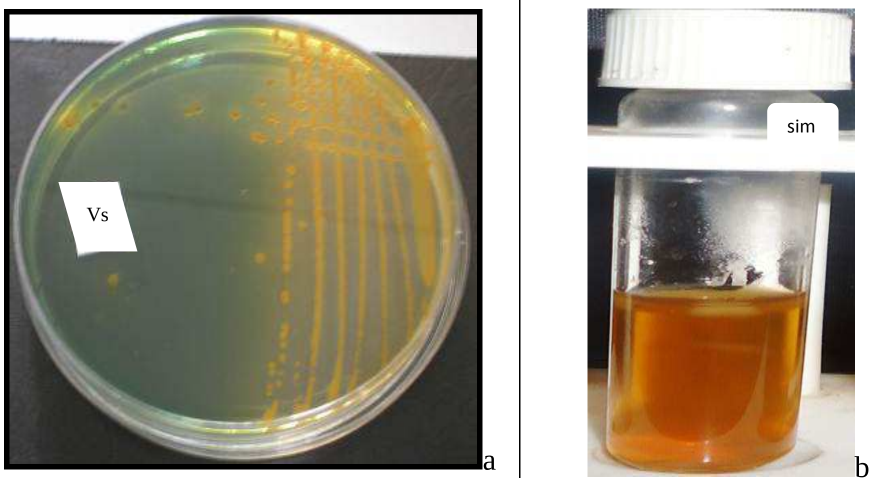


Plate 4.4

KEY: a): *Vibrio* spp. on TCBS.

b). *Vibrio* colonies showing motility on SIM media.

4.1.2.2.1 Biochemical analysis of *Vibrio spp.* isolated from the water samples collected from the various sampling sites.

The identity of the isolates as *Vibrio* spp. was confirmed by typical golden yellow colonies on TCBS and positive test for Oxidase test (Appendix II).

4.1.2.3 *Escherichia coli*

In total pathogens detected, 34 % were *E. coli*. These were confirmed by Enteropathogenic *Escherichia coli* (Appendix III).

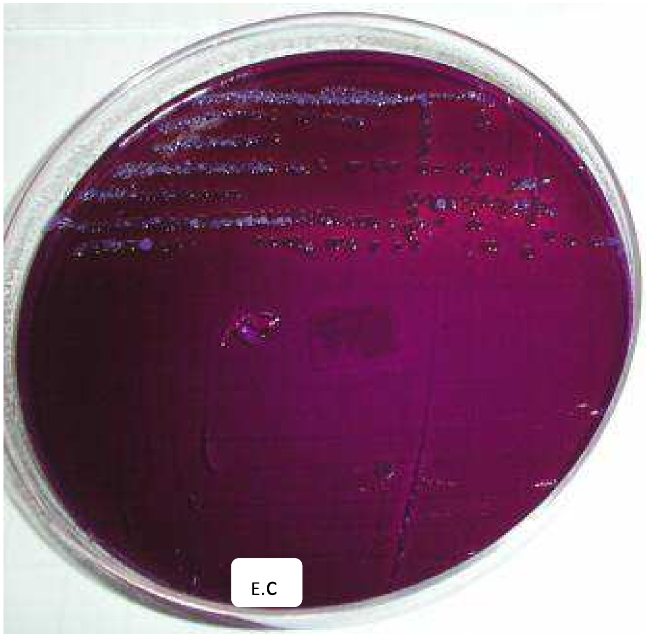


Plate 4.5: Colourless colonies of *E. coli* on MacConkey

4.1.2.4 *Pseudomonas* species

Of the total pathogenic bacteria detected (16%) of them were *Pseudomonas aeruginosa* (Plate 4.5, Appendix IV and VII).

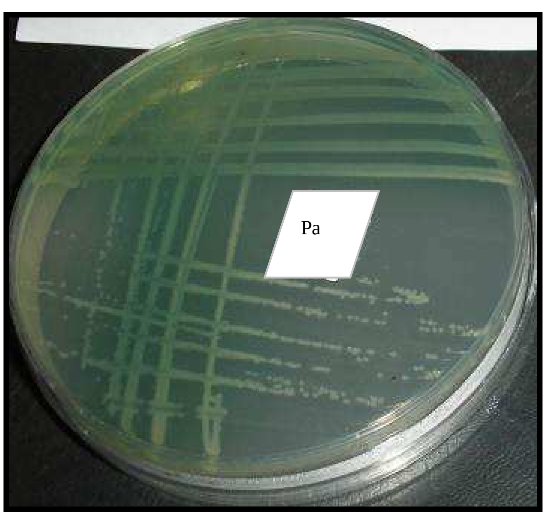


Plate 4.6: Green colonies of *Pseudomonas aeruginosa* on Nutrient agar.

4.2 Physico-chemical parameters

The season and site did not have significant interactions (p=0.1322) in determining the pond water temperature (Table 4.3). The temperature ranged between 18-29 ºC. There was significant (p=0.0001) difference in water temperature between the dry and wet season (Table 4.3). The temperature in the pond water varied significantly (p=0.0001) in all sites (Table 4.3). There was no significant (p=0.6043) interaction between site and season in determining the pond water pH. The water pH varied significantly (p=0.0058) between the dry and wet season (Table 4.3) in comparison with the sites. There was significant difference (p=0.001) in water pH in all the sites sampled (Table 4.3).

Water conductivity in the various sites varied significantly (p=0.0001), however there was no significant (p=0.4628) interaction between season and site determining the water conductivity (Table 4.3). There was significant difference (p=0.0004) in water conductivity between the dry and wet season (Table 4.3). The site and season did not have significant (p= 0.8138) interaction in determining the water turbidity (Table4.3). Pond water turbidity varied significantly (p=0.0247) between the dry and wet season. There was significant (p=0.0001) difference in water turbidity in the various sites (Table 4.3).

Site and season did not interact significantly (0.9995) in determining the phosphates concentration in the pond water. Phosphates concentration in the water did not vary significantly (p=0.7278) between the dry and wet season (Table 4.3). The phospates concentration did not vary significantly (p=0.0582). Sulphates concentration in the pond water did not differ significantly (0.0565) in the wet and dry season, however the sulphates concentrations varied significantly (p=0.0001) among the studied sites. No significant (p=0.6737) interaction was observed between site and season in determining the sulphates concentration in pond water (Table 4.3).

The nitrates concentrations were significantly different (p=0.0001) among the sites, however there was no significant interaction (p= 0.3048) between site and season in determining the nitrates concentration in pond water (Table 4.3). Nitrates concentrations differed significantly (p=0.0031) between the wet and dry season (Table 4.3). Based on two way ANOVA there was no significant (p= 0.9356) interaction between site and season in determining ammonia concentration in fish pond water (Table 4.3). Ammonia concentration differed significantly (p=0.0464) between the wet and dry season. Ammonia concentration in the various sites was significantly (p=0.0137) different. Based on the two way ANOVA there was no significant interaction between site and season in determining the amount of DO in pond water. The amount of dissolved oxygen did not differ significantly (p=0.0665) based on season (Table 4.3). High DO was recorded during the dry season. The DO in pond water from the various sites did not differ significantly (P=0.2903) (Table 4.3).

Table 4. 3: Interaction of Physico-chemical parameters between sites and seasons

Treatment	Temp °C	pH	E. C (µs/cm)	Turbidity	PO_4_MgL$^-$	SO_4_MgL$^-$	NO_3_MgL$^-$	NH_3_MgL$^-$	DO_MgL$^-$
Season									
Wet season	21.13±0.355b	5.953±0.1303b	95.96± 11.2b	94.72±10.16a	0.324±0.09a	16.57±2.65a	21.57± 3.06a	0.26± 0.049a	3.997±0 .08a
Dry season	24.25±0.4003a	6.343± 0.101a	128.11±13.88a	71.0± 9.184b	0.28±0.104a	12.38±2.53a	14.68±2.90b	0.148± 0.028b	4.23± 0.092a
Sites									
Githongo	23.58± 0.542b	6.19± 0.11ab	146.58±13.47b	95.93±11.2ab	0.445±0.15a	19.08±2.58b	26.7±3.81b	0.293± 0.07ab	4.075± 0.096a
Kithirune	21.79± 0.66c	6.408±0.15ab	114.3± 13.47b	118.58±12.6a	0.128±0.03a	8.93±1.003c	17.18±2.61b	0.20± 0.078ab	4.192± 0.11a
Mbwinjeru	25.8± 0.534a	5.942±0.156bc	203.23±12.55a	111.4±11.94ab	0.605±0.25a	35.1± 4.51a	39.22±3.55a	0.13± 0.032ab	3.858±0.155a
Muri	20.33± 0.482d	5.53± 0.210c	35.30± 3.56c	72.35± 15.63b	0.28±0.108a	5.66± 1.05c	6.64 ±1.47c	0.33± 0.075a	4.18± 0.123a
Kianthumbi	21.92± 0.378c	6.67± 0.157a	60.78± 3.68c	16.09± 2.977c	0.046±0.01a	3.63± 0.51c	0.93± 0.187c	0.059± 0.008b	4.25± 0.177a
P value									
Season	0.0001	0.0058	0.0004	0.0247	0.7278	0.0565	0.0031	0.0464	0.0665
Site	0.0001	0.0001	0.0001	0.0001	0.0582	0.0001	0.0001	0.0137	0.2903
Season×Site	0.1322	0.6043	0.4628	0.8138	0.9995	0.6537	0.3048	0.9356	0.9772

Values (Means ±SE) followed by dissimilar letters along the columns are significantly different at P≤0.05 (Tukey's HSD test)

Key:

E.C- Electrical conductivity; PO_4- phosphates; SO_4- sulphates; NO_3- Nitrates; NH_3- Ammonia; DO- Dissolved oxygen.

According to one away ANOVA, The pond water temperature was significantly ($p<0.0001$) different in all the sites during the dry and wet season. Significantly ($p< 0.0001$) higher temperatures were recorded during the dry seasons as compared to the wet seasons (Table 2). The highest temperature was recorded in Mbwinjeru while Muri had the lowest (Table 4.4) in both dry and wet season.There was no significant ($p=0.5742$) difference in pH in all sites during the dry and wet season (Table 4.4). The pH of the water in all the ponds was more acidic during the dry season as compared to the wet season (Table 4.4). The highest pH value recorded was in Kithirune while the lowest value recoded was in Muri during the dry season while Kianthumbi had the highest value with Muri having the lowest during the wet season. There was significant ($p= 0.0001$) difference in conductivity in all sites during the dry and wet season (Table 4.4). Significantly ($p= 0.0001$) high conductivity of the pond water was observed during the dry season in all ponds (Table 4.4). The highest electrical conductivity was recorded in Mbwinjeru while Muri had the lowest (Table 4.4) in both dry and the wet season.

Turbidity of the pond water was significantly ($p=0.0001$) different in all the sites during the dry and wet season (Table 4.4). Turbidity of the pond water was significantly ($p=0.0001$) higher during the wet season as compared to the dry season in all sites (Table 4.4). The pond water was significantly ($p=0.0001$) least turbid during the dry and wet season in site Thu (Table 4.4). The turbidity of the sampling sites varied between 16 and 118.0 NTU during the study period. The highest value of turbidity recorded was in Mbwinjeru while the lowest value recorded was in Kianthumbi in both seasons.

There was no significant (p=0.3740) difference in Phosphates concentration in all the sites during the dry and wet season. Mbwinjeru recorded the highest levels of phosphates while Kianthumbi recorded the lowest in both seasons (Table 4.4). Sulphates concentrations were significantly (p=0.0001) different in all the sites with the highest concentrations being recorded during the wet seasons as compared to the dry seasons (Table 4.4). The highest concentration was recorded in Mbwinjeru and the lowest in Kianthumbi in both seasons.

There was significant (p=0.0001) difference in nitrates concentrations in all the sites during the dry and wet season (Table 4.4). Significantly (p=0.0001) higher concentrations of nitrates were observed during the wet season as compared to the dry season (Table 4.4). Mbwinjeru had the highest levels while Kianthumbi recorded the lowest concentration in both seasons. Ammonia concentrations were not significantly (P=0.0466) different in all the sites during the dry and wet season (Table 4.4). The highest levels were recorded in Muri while the lowest in Kianthumbi. Dissolved oxygen (DO) concentration in the pond water was also not significantly (p= 0.4445) different in all the sites during the dry and wet season (Table 4.4). The highest value of DO was obtained in Muri while the lowest in Mbwinjeru in both seasons.

Table 4.4: Comparison of Physicochemical parameters between the wet and dry season

SITE	**Temp °C**	**PH**	**E. C** (µs/cm)	**TURBIDITY**	**PO_4_Mg_L^{-1}**	**SO_4_Mg_L^{-1}**	**NO_3_Mg_L^{-1}**	**NH_3_Mg_L^{-1}**	**DO_Mg_L^{-1}**
Git D	25.17± 0.48b	6.33± 0.163a	165.20± 19.55b	84.47±18.00abcd	0.398± 0.21a	16.17± 4.36bcd	20.73 ± 6.34bc	0.225± 0.07a	4.167 ± 0.17a
Git W	22.83± 0.31b	6.16± 0.166a	127.95±16.65bcd	107.39±13.17ab	0.4917±0.24a	22.00± 2.62bc	32.62± 3.09ab	0.362± 0.13a	3.98± 0.098a
Kit D	24.58±0.201b	6.60 ±0.126a	135.7±18.39bc	100.50±21.71ab	0.115± 0.039a	7.37± 0.63cd	12.07 ± 4.42cd	0.12± 0.028a	4.317± 0.191a
Kit W	22.67± 0.21cd	6.22 ±0.259a	92.9±16.65cde	136.67± 9.795a	0.142± 0.06a	10.50± 1.75cd	22.28± 0.24bc	0.277± 0.155 a	4.067± 0.112a
Mbu D	27.33± 0.42a	6.34± 0.307a	230.0±16.03a	105.50±1.432ab	0.568± 0.455a	30.13± 8.162ab	33.93± 6.47ab	0.087± 0.044a	3.917± 0.229a
Mbu W	24.00± 0.00bc	6.10± 0.25a	176.46±12.22ab	117.21±24.73ab	0.642± 0.248a	40.00 ±3.64a	44.50±1.53a	0.173± 0.043a	3.80± 0.228a
Mur D	21.83± 0.17ed	6.33± 0.29a	41.67± 3.073ef	52.32±20.94bcd	0.270± 0.163a	4.82± 0.764d	5.93± 2.19dc	0.257±0.093a	4.37±0.2155a
Mur W	19.17± 0.31f	5.932±0.36a	28.93±5.482f	92.38± 21.80abc	0.297± 0.16a	6.50±1.996cd	7.35± 2.13dc	0.403± 0.119a	4.00± 0.082a
Thu D	23.00± 0.37cd	6.617± 0.24a	68.00± 4.00def	12.22± 0.95d	0.042± 0.01a	3.42± 0.712d	0.75± 0.28d	0.047± 0.01a	4.367± 0.23a
Thu W	20.58± 0.20e	6.610± 0.267a	53.55±4.721ef	19.97±5.66cd	0.050± 0.010a	3.83± 0.79d	1.10±0.256d	0.072±0.012a	4.13±0.279a
P value	<.0001	0.5742	<.0001	<.0001	0.3740	<.0001	<.0001	0.0466	0.4445

Values (Means ±SE) followed by dissimilar letters along the columns are significantly different at P≤0.05. (W): wet season, (D): dry season.

Key:

E.C- Electrical conductivity; PO_4- phosphates; SO_4- sulphates; NO_3- Nitrates; NH_3- Ammonia; DO- Dissolved oxygen.

Localities: Mbu- Mbwinjeru location, Git- Githongo location, Kit- Kithirune location, Mur- Muri location, Thu- Kiathumbi location.

4.5 Heavy metals

The data has shown that, the order of occurrence of heavy metals in water is: Fe > Mn > Zn > Cu > Pb. Data on heavy metals was analyzed using two way ANOVA. There was no significant (p=0.3619) difference in the concentration of lead in the ponds between wet and dry season (Table 4.5). There was no significant difference (0.1929) between the lead concentrations in all the sites (Table 4.5). No significant (P= 0.9035) interaction was observed between the season and site in influencing the lead concentration in the ponds (Table 4.5). Githongo recorded the highest concentration of lead while Kianthumbi recorded the lowest. Data on copper concentration showed significant difference (P=0.0341) in Copper concentration between the dry and wet season (Table 4.5). No significant difference (p=0.1738) in Copper concentration was observed among the sites. Two way ANOVA showed no significant (p=0.7814) interaction between site and season in influencing Copper concentration in the ponds. Mbwinjeru had the highest concentration of copper while Kianthumbi had the lowest.

The study showed significant (p=0.0290) difference in Zinc concentration in the water between the dry and wet season (Table 4.5). There was no significant (p=0.1752) in Zinc concentration in pond water among the sites. Two way ANOVA showed no significant (p=0.8056) interaction between site and season in determining Zinc concentration in the pond water. The highest value of Zinc obtained from the sampling sites was in Mbwinjeru while the lowest value was in Muri.

Iron concentrations in the ponds did not differ significantly (P=0.7892) between the wet and dry season (Table 4.5). It was also observed that the Fe concentrations in the various sites did not differ significantly (p=0.5488). There was no significant (p=0.9998) interaction between season and site in determining the iron concentration in the pond water (Table 4.5). Kianthumbi had the highest concentration of iron while Muri had the lowest. Manganese concentration in the water varied significantly (p=0.0384) between the dry and wet season, however comparison of the sites showed no significant (p=0.2800) difference in Manganese concentration in the ponds (Table 4.5). There was no significant (p=0.8435) interaction between site and season in determining Manganese concentration in the pond water. Kianthumbi had the highest concentration of manganese while Muri had the lowest.

Table 4.5: Comparison of Heavy metals between seasons and the sites

TREATMENT	Lead_MgL^{-1}	Copper_MgL^{-1}	Zinc_MgL^{-1}	Iron_MgL^{-1}	Manganese_MgL^{-1}
Season					
Dry	0.035 ±0.02007a	0.0691± 0.01661b	0.0768±0.01478b	0.3174±0.09277a	0.1900±0.02851b
Wet	0.068± 0.031a	0.1272± 0.02101a	0.1397±0.02378a	0.3537±0.08965a	0.29667±0.04054a
Site					
Githongo	0.145± 0.0609a	0.10917± 0.0293a	0.1167±0.02401a	0.33417±0.04226a	0.19167±0.062107a
Kithirune	0.0993± 0.0614a	0.0967± 0.03192a	0.1025±0.01871a	0.3150±0.04384a	0.2083±0.064501a
Mbwinjeru	0.0101± 0.0032a	0.155± 0.0438a	0.1658±0.04498a	0.397±0.07995a	0.300±0.06963a
Muri	0.002± 0.00039a	0.0517± 0.017a	0.0533±0.00873a	0.1354±0.03053a	0.1917±0.03786a
Kianthumbi	0.0018± 0.0004a	0.07817± 0.022a	0.1029±0.04476a	0.4965±0.30404a	0.3250±0.04106a
P values					
Season	0.3619	0.0341	0.0290	0.7892	0.0384
Site	0.1929	0.1738	0.1752	0.5488	0.2800
Season×Site	0.9035	0.7814	0.8056	0.9998	0.8435

Values (Means ±SE) followed by dissimilar letters along the columns are significantly different at $P \leq 0.05$ (Tukey's HSD test).

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Microbial quality of fish pond water

Faecal bacteria (faecal streptococcal and faecal coliform) were detected in all the samples. This confirmed that the water used in the fish ponds was faecally contaminated (Ashbolt *et al*., 2001; Hunter *et al*., 2002; Emikpe *et al*., 2011). High faecal contamination detected in the present study is similar to the findings reported in Pakistan (Nahiduzzaman *et al*., 2000) and Italy (Maugeri *et al*., (2000). On the population of the faecal bacteria, there was significant variation in their populations across all the sites at p=0.0001. These levels in all the sites exceeded 1.0×10^1 CFU/100 ml recommended by FAO (1979) hence all the water samples collected showed high levels of contamination. On the population of the two faecal bacteria, Pearson correlation analysis indicated that prevalence of FS from all sites showed a significant positive correlation with that of FC ($r = 0.732$ at $P < 0.01$ level). These results are consistent with the findings of (Egbere *et al*., 2008) in Nigeria and (Fafioye, 2011) in Ghana.

A previous study has shown that the lack of animal waste management as well as wastewater could directly affect water quality as a result of surface run off (Ampofo and Clerk, 2010). Surface run off could be a plausible source of pond water contamination especially during the wet season (Ampofo and Clerk, 2010) in Ghana. Free roaming animals and pets especially dogs also contribute to faecal contamination of surface water. In addition, there are also cattle reared by farmers that too roam the region for green pastures. Along the rivers in this region, there is possible run-off from roads, parking lots and yards that could be carrying animal wastes into natural water course and ponds (Emikpe *et al.*, 2011). In the studied region of Meru, like any parts of Meru is densely populated, a factor that could contribute to pond contamination due to closeness of latrines to water points, washing and bathing in rivers which serves as the source of fish pond water (Doyle, 2007; Adebayo-Tayo *et al.*, (2012a).

Mbwinjeru had the highest population of both faecal streptococci and faecal coliform in both the dry and the wet season. This could be attributed to the agricultural activities that take place around the site. Manure and fertilizer used by the farmers may have found their way into the ponds. The high temperatures found in this area could be providing a conducive environment for the growth of the bacteria. The source of water used in this area is mainly from a river and therefore it might be the source of contamination. The farmers mainly use rivers as the source of their fish pond water which may harbour some bacteria. These findings are consistent with those of Ghana (Emikpe *et al.*, 2011).

Kianthumbi had the lowest population of faecal streptococci and faecal coliform in both the dry and the wet season which could be associated with its source of water which is mainly from a swamp and the zone is also relatively cold. These findings are consistent with those reported in Nigeria (Egbere *et al.*, 2008). Faecal coliform in fish demonstrates the level of pollution of their environment because coliforns are not the normal flora of bacteria in fish. Birds can be a significant source of faecal coliform bacteria. Swans, geese and other waterfowl can all elevate bacterial counts in ponds (Doyle and Ericson, 2006). The presence of faecal coliforms in all the sampling sites was an indication that water sources in the four sites were contaminated.

5.1.2 Pathogenic Organisms

From water analysed in these fish ponds, pathogenic bacteria were also detected (Appendix VII). *Salmonella* spp. were the most prevalent with 40 %, followed by *E. coli*, with 34 %, *Pseudomonas aeruginosa* 16 % and *Vibrio* spp. 10 % (Appendix VII). These findings are consistent with previous studies conducted in Kisumu County, Kenya by Onyango *et al*. (2009); in Nigeria Egbere *et al.* (2008); in India (Nabonita *et al*., 2011) and Cameroon (Kuitcha *et al*., 2010). These bacterial isolates are common intestinal bacteria of both animals and humans gut however this contamination may also have come from public untreated water or water taken by animals or cycling between the livestock and their environment or even contamination in feeds (Doyle, 2007; Adebayo-Tayo *et al.*, (2012a).

Potential sources of these pathogens in water include wastewater effluents, combined sewer overflows, run-off from urban land, animal waste, and municipal waste sludges disposed off on land or in water (Ram *et al.* 2008). Previous research has shown that, different kinds of livestock manure are contaminated with pathogenic bacteria such as *Salmonella*, *Shigella*, *Pseudomonas*, *Vibrio*, *Streptococcus*, and *E. coli* species (Abdelhamid *et al.,* 2006). This therefore shows that sources of contamination in the sites could have been as a result of human and livestock activities (Emikpe *et al.*, 2011). The bacteria from fish only become pathogens when fish are physiologically unbalanced, nutritionally deficient, or there are other stressors namely: poor water quality, overstocking, which allow opportunistic bacterial infections to prevail (Austin, 2011). In addition, Physico-chemical characteristics influence the growth and diversity of microbial populations. The study has indicated that the fish ponds were significantly contaminated with both faecal and pathogenic bacteria.

5.1.3 Physico-chemical parameters

The variations observed in the physico-chemical properties of the fish ponds carried out in the present study could be attributed to the influences of the micro-climatic, topographic and edaphic conditions of fish ponds in the area. In addition, human and animal or livestock activities could also be a factor. The findings were similar to those reported in Nigeria by Kumar (2004) and in Samburu County, Kenya by Mwaura (2005). The highest recorded temperature and the lowest (Table 4.2) were within the recommended limits of 20 ºC to 30 ºC Kumar (2004). The World Health Organization has recommended 27 ºC as the permissible limit for fish growth and productivity (WHO, 2006) while the Environmental Management Authority of Zimbabwe has set a limit of 35 ºC (EMA, 1997). The low temperature value could be attributed to the site of some of the ponds for example Muri and Kithirune which were in a region of high altitude bordering a forest. During the wet season the temperatures were low as compared to the dry season. These findings are similar to those reported in Samburu County, Kenya (Mwaura, 2005).

Water from Mbwinjeru and Githongo was found to be slightly warmer due to their geographical location being on a lower altitude. The warm temperature provides better growth and food conversion than low temperature (Chaudhari, 2003; Mwaura, 2005). These findings are in congruence with previous studies conducted in Kenya (Mwaura, 2005). The lowest value observed fall below acceptable range of between 24 °C and 34 °C for the tropics according to a previous study in Nigeria by Udo (2007). Ideally, increased temperature causes an increase in the metabolic activity of the fish while reducing the DO content in the system (Afzal *et al.*, 2007). WHO recommends a temperature range of between 25 °C and 32 °C for good performance of fish (WHO, 2006). High water temperature enhances the growth of thermo-tolerant microorganism. At temperatures below 15 °C, the growth of fish ceases and they may end up dying while at very high temperatures, there is less solubility of oxygen, stress and fish may eventually die. Aquatic organisms can tolerate a wider range of temperatures, provided that fluctuations are not severe, sudden and of long duration **(**Priyadarshini *et al.*, 2011).

The pH values obtained are consistent with the findings by Mwaura (2005) in Samburu County, Kenya; in Nigeria Ayanwale *et al.* (2012) in the USA (Sipaúba-Tavares *et al.*, 2007) and in Brazil Silapajarn *et al.* (2004). The desirable range for pond pH is 6.5 - 9.0 according to WHO (Appendix VI) and the acceptable range which is in between 5.5 to 10.0 (Stone and Thomforde, 2003). The mean value of the pH falls below the recommended pH range of 6.5 – 9.0 WHO (2006) for good fish performance which shows that all the fish ponds were acidic. It is only Kithirune (dry season) and Kianthumbi (wet season) which had a pH above 6.5. During the wet season, the pH was alkaline. The low pH could have been caused by acid sulphate run off (Silapajarn *et al.*, 2004). Low pH might also be attributed to high rates of carbon dioxide release by zooplanktons during respiration. This is a common phenomenon in aquaculture ponds (Silapajarn *et al.*, 2004).

Higher acidic levels observed in the present study could be due to the chemical additives applied to the aquaculture pond with an objective of better production, high stocking density or lack of buffering activities in the farms as indicated by pH values on. This observation was consistent with the findings in Nigeria by Thilza and Muhammad (2010). The values of electrical conductivity were within the permissible limit set by WHO of 1000 µS/cm (WHO, 2006). They are also consistent with the findings in Brazil (Sipaúba-Tavares *et al.,* 2007). The high values of electrical conductivity experienced in Mbwinjeru may be due to high temperatures where as those in Muri may be due to low temperatures associated with the altitudes. Electrical conductivity is the measure of the ability of water to conduct an electric current. Since the electric current is conducted through the movement of ions in solution, it also gives an indication of the concentration of ions or total dissolved salts in the water. The low levels during the wet season may be attributed to the rainy season in which the samples were collected. Previous studies have shown that dilution of water during the rainy seasons lowers the levels of electrical conductivity (Sipaúba-Tavares *et al.,* 2007). The electrical conductivity was high during the dry season which corresponds to a study in Samburu County, Kenya (Mwaura, 2005).

The values of turbidity varied significantly. The values were within the permissible limit set by WHO of 1000 mg/l (WHO, 2006). The results in the present study are consistent with the findings in Nigeria (Ehiagbonare and Ogunrinde, 2010) and Ghana (Takyi *et al.,* 2012). Turbidity affects the appearance of water. Water with high turbidity is normally associated with high microbiological contamination which is in congruence with the bacterial population in Mbwinjeru which had high values of turbidity. This High turbidity may be as a result of rains which cause flooding which may result to soil erosion and surface run off hence depositing nutrients, silt, and domestic wastes into the water. Kianthumbi had the lowest values of turbidity due to the source of water which was a swamp.

The values of phosphates varied significantly. These figures were consistent with those obtained by in Nigeria (Ehiagbonare and Ogunrinde, 2010). The values obtained from Mbwinjeru exceeded the permissible limit set by WHO of 0.5 mg/l (WHO, 2006). The High values are suggestive of possible pollution of the fish ponds under study which is supported by a previous study in USA (Wudtisn and Boyd, 2005). The source of water for Mbwinjeru was a river and it was an agricultural zone. The higher phosphorus concentration may be associated with the increase in phosphorus produced during the decomposition of organic fertilizer and also from the feed through fish excreta. Both soluble organic phosphorus and orthophosphate are released during the process of organic fertilizer decomposition under aerobic conditions (Wudtisn and Boyd, 2005). Kianthumbi had low levels of phosphates in both seasons which may be associated with the source of water and less agricultural activities.

The values of sulphates varied significantly which are consistent with the findings in the USA (Silapajarn *et al.,* 2004). The World Health Organisation has recommended 100 mg/l (WHO, 2006) and therefore they are within the limits. The values obtained are higher than those reported in Nigeria (0.66 to 1.09 mg/l) (Ehiagbonare and Ogunrinde, 2010). The high values found in Mbwinjeru could be associated with the high usage of detergent and soaps by people in the neighborhood or high usage of fertilizers in farming (Ehiagbonare and Ogunrinde, 2010). The values of nitrates obtained from this study varied significantly (Table 4.3). The values obtained were within the recommendations of WHO of 50.0 mg/l (WHO, 2006). These values are consistent with the findings reported in Nigeria by Ehiagbonare and Ogunrinde (2010). However, the values were lower than those reported in Brazil by Sipaúba-Tavares *et al.* (2007). The high value of nitrates in Mbwinjeru in both seasons suggested that there is the presence of pollutants like bacteria and pesticides (Nzunga, 2011). This can be remedied by water change and increase in plant density. The higher levels can be attributed to agricultural and domestic activities and surface run offs during the rainy season (Nzunga, 2011).

The values of ammonia were within the recommended values of less than 1 mg/l (Kumar, 2004). The mean value was within the permissible limit set by WHO of 0.5 mg/l (WHO, 2006). The high values obtained in Muri in both seasons could be due to low temperature and the high rate of feeding and low stocking density densities; hence the excess feed decomposes and pollutes the pond water (Thilza and Muhammad, 2010). Ammonia is introduced into the pond through dead phytoplankton, uneaten feeds, dead and decaying organic matter. It can be attributed to addition of manure to fertilize the pond or through the process of nitrogen fixation by algae and water plants (Edwards, 2008).

Ammonia in the range >0.1 mg L^{-1} tends to cause gill damage, destroy mucous producing membranes, effects like reduced growth, poor feed conversion and reduced disease resistance. Ammonia affect fish in different ways for example when they are poisoned by ammonia, fish congregates close to the water surface, gasp for air and are restless (Edwards, 2008). Their skin becomes light colored and covered with thick layer of mucus. In some cases hemorrhages occurs mainly at the base of the pectoral fins (Thilza and Muhammad, 2010).

The DO values varied significantly across the sites. However, these levels were below the WHO recommended values of 5.0 mg/l and above (WHO, 2006). The low DO obtained may be attributed to the small size of the ponds and eutrophication due to over fertilization with manure or fertilizer (Thilza and Muhammad, 2010). This may be due to the presence of microbes and plants as fish are not the only consumer of oxygen in aquaculture system. Low concentration of dissolved oxygen in water causes suffocation in fish while its super saturation may result into the gas bubble disease leading to the mass mortality of fish in both the cases. Low dissolved oxygen concentration increases the toxicity of ammonia to culture organisms.Oxygen depletion in water leads to poor feeding of fish, starvation, reduced growth and more fish mortality, either directly or indirectly Bacteria, phytoplankton and zooplankton consume high quantities of oxygen (Thilza and Muhammad, 2010). The solubility of oxygen in water decreases as the water temperature increases.The study has shown that the physico-chemical parameters varied significantly in all the study sites.

5.1.4 Heavy metals

Heavy metals are chemical elements with a specific gravity that is at least four to five times the specific gravity of water at the same temperature and pressure (Duruibe *et al.,* 2007). Fish and other aquatic organisms are constantly immersed in water containing pollutants. They absorb the pollutants through skin, gut (from food) and respiratory surfaces (Wegu and Akanimor, 2006). The heavy metals: copper, iron, zinc, lead and manganese concentrations in ponds water in all the sampling sites were compared with international standards. The obtained results showed that, with the exception of Fe, the heavy metal concentrations in water did not exceed WHO (World Health Organization, 1993), EC (Europian Community, 1998), EPA (Environment Protection Agency, 2002), CIW (Criterions of the Irrigation Water, 1997) and TSE-266 (Turkish Standards, 2005) guidelines (Appendix V). High concentration of metals in the fish ponds may be attributed to the increased cover of the aquatic and higher plants which absorb metals from water and sediments. Areas with high metal content had high bacteria counts which are consistent with previous study in Kenya (Mutuku, 2010) and Italy (Ozurtuk *et al.,* 2010).

The data has indicated that, the order of occurrence of heavy metals in the fish pond water is: iron > manganese > zinc > copper > lead. These findings are consistent with those reported in Egypt (Al-Afify *et al.*, 2010). The WHO has recommended a permissible limit of 0.01 mg/l (WHO, 1993). A previous study carried out in England shows that high level of lead in some pond water can be attributed to industrial and agricultural discharge (Mason, 2002). The pollution of aquatic environment by heavy metals affects aquatic organisms and poses considerable environmental risks and concerns (Amisah *et al*., 2009). Heavy metals pollutants after entering into aquatic environment accumulate in tissues and organs of aquatic organisms. The high levels in Githongo and Kithirune of lead in the present study could also be attributed to spill of leaded petrol from the combustion of petrol in automobile cars and the closeness of these ponds to the two markets.

Lead is a cumulative toxin and its other sources include automobile exhaust fumes, used dry-cell batteries, from sewage affluent, run off wastes and atmospheric deposition (Mason, 2002). Acute intoxication of fish with lead can be recognised by damaged gills epithelium, erythrocytes, leucocytes and nervous system. In human beings, it binds with SH group of proteins, apart from that, lead damages blood circulation, central nervous system, liver and kidneys (Mason, 2002). In addition, lead can Delay embryonic development, suppress reproduction, and inhibit growth, increase mucus formation, neurological problem, enzyme inhalation and kidney dysfunction (Kori-Siakpere and Ubogu, 2008).

The amount of copper found out by the present study did show interaction between the sites and the seasons which is consistent with the findings reported in the USA (Silapajarn *et al.*, 2004) and Turkey (Ozurtuk *et al.*, 2010). The results are also consistent with those in Egypt (Samir and Ibrahim, 2008). The results are within the permissible limits set by WHO of between 1 and 2 mg/l (WHO, 1993). Copper toxicity in natural water arising from pollutants may cause severe damage in gills and necrotic changes in the liver and kidneys. Long term exposure to Copper, higher than normal levels can cause nausea, vomiting, stomach cramps, or diarrhoea when ingested by humans from the fish (Javed and Usmani, 2013).

The amount of zinc recorded in this study varied significantly between the sites and the seasons. These values are within the set values by WHO of 3.0 mg/l (WHO, 1993). These values are consistent with the findings in USA by Silapajarn *et al.* (2004) and in Egypt (Samir and Ibrahim, 2008). The main source of zinc entering aquatic is dissolved zinc from zinc related appliances such as galvanized pipes. Low levels can be attributed to less zinc load from industrial, agricultural, domestic and urban waste waters (Ozortuk *et al*. 2009). Zinc accumulation result in several dysfunctions in fish. It exerts adverse effects by accruing structural damage which affects the growth, development and survival. Sub lethal levels adversely affect hatchability, survival and hematological parameters of fish (Kori-Siakpere and Ubogu, 2008).

The amount of iron detected did not show any significant difference across the sites. These results were consistent with previous studies in Egypt (Samir and Ibrahim, 2008) and Turkey (Ozurtuk *et al*., 2010). The high amount of iron which exceeded the limits (WHO, 1993) may be attributed to the high density of people with buildings having iron sheets roofs. Due to corrosion, the iron ions find their way into the fish ponds. The amount of manganese recorded in the present study revealed interaction between the sites and the seasons. The WHO has recommended 0.1 to 0.5 mg/l for optimal productivity of fish (WHO, 1993). These values are consistent with those reported in USA (Silapajarn *et al.*, 2004) and those by Samir and Ibrahim (2008) in Egypt.

The major sources for manganese in air and water are iron and steel manufacturing and the burning of diesel fuel in the motor cars (Samir and Ibrahim, 2008). High levels of Manganese can cause lung, liver and vascular disturbances, declines in blood pressure, brain damage in fish and failure in the development of animal foetuses (Javed and Usmani, 2013). Dissociation of heavy metals into ions in water is a very slow process and they cannot be detoxicated by metabolic processes. These metals are generally very toxic to the lives of the fish. The heavy metals in general in the study have indicated a low level of contamination with the exception of iron hence the fish ponds are not significantly contaminated with heavy metals.

5.2 Conclusions

i) The fish ponds were found to be significantly contaminated with faecal streptococci and faecal coliforms.

ii) The pathogenic bacteria: *Salmonella, Vibrio* spp. *E. coli* and *Pseudomonas aeruginosa* were detected indicating high contamination and a high risk of infection.

iii) Heavy metals with the exception of iron were found to be within the WHO standards.

iv) All the physico-chemical parameters varied from one site to another and the temperature, ammonia, sulphates, nitrates, phosphates, electrical conductivity and turbidity were found to be within the values of WHO (2006).

v) The pH and the dissolved oxygen were found to be below the limits provided by WHO (2006).

vi) The populations of both faecal and pathogenic bacteria, variability of physico-chemical parameters and levels of heavy metal contamination were influenced by the source of water, geographical patterns of the site, human and livestock activities.

5.3 Recommendations

Based on the findings from this study:-

i) In order to evaluate the ecological condition of freshwater fish, both fish inner organs and water reservoirs must be monitored regularly for water quality and fish.

ii) The pond water should be routinely examined to check on the microbial contamination shown.

iii) Water treatment campaigns should be organized and held in the area to enlighten the residents on the safety of fish.

iv) The farmers should use treated tap water.

v) Molecular techniques should be used to characterize bacteria from the environment for accurate identification in order to take care of the limitations involved during use of biochemical tests.

vi) More studies should be carried out involving the fish surface and body organs to determine the microbial load and heavy metal contamination.

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APPENDICES

APPENDIX I

Biochemical reactions of *Salmonella* spp. isolated from the water samples collected from the various sampling sites

	Results		
Test/substrate	**Positive**	**Negative**	***Salmonella* spp. reaction**
Glucose	Yellow butt	Red butt	+
Hydrogen sulphide	Blackening	No blackening	+
Urease	No color change	Change to pink	-
Simmon citrate	Growth; Blue color	No growth; No color change	+
Motility	Turbidity	No turbidity	+
Indole	Color change	No color change	+
Oxidase	Color change to blue	No color change	-

KEY: + Positive

- Negative

APPENDIX II

Biochemical reactions of *Vibrio* spp. isolated from the water samples collected from the various sampling sites

Test/substrate	Results		
	Positive	**Negative**	***Vibrio* spp. reaction**
Glucose	Yellow butt	Red butt	-
Hydrogen sulphide	Blackening	No blackening	-
Urease	No color change	Change to pink	-
Simmon citrate	Growth; Blue color	No growth; No color change	+
Motility	Turbidity	No turbidity	+
Indole	Color change	No color change	+
Oxidase	Color change to blue	No color change	-/+

KEY: + Positive

- Negative

APPENDIX III

Biochemical reactions of *E. coli* isolated from the water samples collected from the various sampling sites

Test/substrate	Results		
	Positive	**Negative**	***Escherichia coli* reaction**
Glucose	Yellow butt	Red butt	-
Hydrogen sulphide	Blackening	No blackening	-
Urease	No color change	Change to pink	-
Simmon citrate	Growth; Blue color	No growth; No color change	-
Motility	Turbidity	No turbidity	+
Indole	Color change	No color change	+
Oxidase	Color change to blue	No color change	+

KEY: + Positive

- Negative

APPENDIX IV

Biochemical reactions of *Pseudomonas aeruginosa* isolated from the water samples collected from the various sampling sites

Test/substrate	Results		
	Positive	**Negative**	***Pseudomonas aeruginosa* reaction**
Glucose	Yellow butt	Red butt	-
Hydrogen sulphide	Blackening	No blackening	-
Urease	No color change	Change to pink	+
Simmon citrate	Growth; Blue color	No growth; No color change	+
Motility	Turbidity	No turbidity	+
Indole	Color change	No color change	+
Oxidase	Color change to blue	No color change	+

KEY: + Positive

- Negative

APPENDIX V

Guidelines for heavy metals concentration (mg/l) in fish pond water

Guidelines	Copper	Iron	Lead	Zinc	Manganese
TSE-266 (2005)	2	0.2	0.01	**-**	**-**
WPCL (2004)	0.02	0.3	0.01	**-**	**-**
WHO (1993)	**2**	**0.3**	**0.05**	**3.0**	**0.1-0.5**
EPA (2002)	1.3	0.3	0.05	**-**	**-**
EC (1998)	2	0.2	10	**-**	**-**
RUSSIA	0.01	0.2	0.1	-	0.01

APPENDIX VI

Guidelines for physico-chemical parameters for fish pond water

Physico-chemical parameter	WHO (2006)	EMA (1997)
Temp °C	27 °C	35 °C
pH	6.5-9.0	6.0 - 9.0
Conductivity (µs/cm)	1000	1000
Turbidity (NTU)	1000	1000
Dissolved oxygen (mg/l)	5.0	5.0
Nitrates (mg/l)	50.0	10
Ammonia (mg/l)	0.5	0.5
Sulphates (mg/l)	100	-
Phosphates (mg/l)	0.5	0.5

APPENDIX VII

Population of pathogenic bacteria in percentage

Pathogenic bacteria	Number of samples (n= 30)	Population in percentage
Salmonella spp.	12	40 %
E. coli	10	34 %
Pseudomonas aeruginosa	5	16 %
Vibrio cholerae	3	10 %