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Determination of bacterial composition, heavy metal pollution and physicochemical parameters of fish pond water in Abothuguchi Central, Meru County, Kenya

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Abstract. *Laibu PK, Maingi J, Kebira A. 2018. Determination of bacterial composition, heavy metal contamination and physicochemical parameters of fish pond water in Abothuguchi Central, Meru County, Kenya. Bioteknologi 15: 66-79.* Humans have consumed fish as a supplementary source of proteins and as a source of income. Because fish performs all their body functions in water, the quality of water is essential to their livelihood. Fish farmers have encountered losses due to the death and stunted growth of fish due to bacterial infections and diseases among other causes. Bacteria found in fish pond water and their pathogenic effects differ with the quality of the pond water along with the variation in the frequency with which water is changed in the ponds. Physico-chemical parameters of pond water and heavy metals influence the growth and productivity of fish. This study aimed at determining the contamination levels of both fecal and pathogenic bacteria namely *Salmonella* spp., fecal *Streptococcus*, fecal coliforms *Pseudomonas* spp., *Vibrio cholerae* and *E. coli* in fish ponds water, the concentration of heavy metal contamination and variability of physicochemical parameters and their effects on fish. Isolation of fecal indicators and pathogens was carried out using standard laboratory methods. Some physicochemical parameters were measured in situ using a portable Universal multiline P4 WTW meter while others were analyzed in the laboratory. The determination of the heavy metal presence and concentration in the water samples was carried out by employing of Flame Atomic Absorption Spectrometer. The results indicated that the pond water was heavily contaminated with fecal streptococci and fecal coliforms and they varied significantly in the sites. Pearson correlation analysis showed a positive correlation between the prevalence of fecal streptococci and fecal coliforms. Potential pathogens such as *Vibrio* spp., *Salmonella* spp., *P. aeruginosa*, and *E.coli* were taken from the water samples with high population. Physico-chemical parameters namely pH and dissolved oxygen deviated from the permissible limits according to international standard. The study has shown that the fish ponds water was highly contaminated with both fecal and pathogenic bacteria with physicochemical parameters varying significantly. Heavy metals except for iron were within the recommended limits hence no significant contamination of the fish pond water. The study suggests the use of treated tap water, routine monitoring of fish pond water and sensitization of farmers on bacterial contamination of pond water. More studies with the aid of molecular techniques should be employed to characterize the bacteria. The finding of this study can, thereby, serve as an impetus to improve fish farming in Meru County, as a way of meeting the growing nutritional demands in the country.

Keywords: Heavy metal, physicochemical, pond water

INTRODUCTION

Fish is one of the essential foods and contributes about 60% of the world source of protein. Most developing countries consumed more than 30% of their animal protein from fish (Emikpe et al. 2011). Fish farming has previously not been well practiced in Meru County through the area is an extensively agricultural zone. Now, fish farming is practiced at a small scale by farmers, organized groups and even institutions (Gitonga 2006). The demand for fish in Meru has increased; this has led to a growing interest in fish farming. Fish farming has faced several challenges of infections and disease occurrence resulting in stunted growth and even death of fish (Egbere et al. 2008). Successful pond management demands an understanding of the role of nutrients and other water quality parameters, as well as regular monitoring of environmental parameters within the pond's ecosystem. Water quality is often not prioritized in pond management, and this has led to common problems, such as noxious smells, excessive algal blooms, and subsequent death of fish. An understanding of primary water chemistry and other physical parameters is necessary to prevent these problems.

The primary sources of contamination in water supplies are pathogenic microbes, organic substances and inorganic chemicals, and heavy metal (Sosbey 2002). Treatment methods that are easy to use, effective, affordable, functional and sustainable are necessary (Sosbey 2002). Enteric bacteria such as *Escherichia coli*, *Salmonella* spp. and *Vibrio* spp, Staphylococcus aureus, are likely to accumulate in fish live in waters contaminated with human wastes, where sanitary standards are improper in the residential area or fish ponds supplied with water from polluted rivers. Therefore, the microbial quality of farmed fish is primarily determined by the quality of water in which they are cultivated (Fafioye 2011).

Bacteria from genera *Aeromonas*, *Pseudomonas*, *Vibrio*, *Salmonella*, *Corynebacterium* and *Myxobacterium* cause infectious diseases in fish (Ampofo and Clerk 2010). *E. coli* is a common contaminant of food and water and a

well-recognized foodborne pathogen (Dutta et al. 2010). Bacteria are known to cause disease subsequently leading to a low production rate of fish.

Different sources of water utilized for fish farming affect bacterial invasion of fish in many ways, causing stunted growth or even death (Egbere et al. 2008). Most aquaculture practices favor disease occurrence. While high fish densities increase stress among stocks and the feeds provide an abundant substrate for microbial growth, the sub-optimal environment of poor water exchange predisposing infections (Ampofo and Clerk 2010). Physical and chemical properties of fish pond water are essential in the growth and productivity of fish. Fish dependency on water is crucial thereby the source, volume and the quality of physical-chemical parameters such as dissolved oxygen (DO), pH, temperature, conductivity, total alkalinity, total hardness, total solids, transparency values, carbon dioxide, nitrite-nitrogen, carbonates, sulphates, and ammonia are some of the salient factors to consider in relation to fish health (Fafioye 2011).

Agro-based industries, for example, paper, sugar, coffee, dairy, tea, and fish tanneries discharge semi-treated effluents with high Biochemical Oxygen Demand (BOD) to the rivers (Nzomo 2005). Heavy metals like cadmium, zinc, mercury, chromium, cobalt, copper, nickel, manganese, iron, vanadium and molybdenum cause heavy pollution particularly in the ponds, lakes and river systems in zones affected by effluents discharged from industries, sewage as well as agricultural drains (Ida 2012). The accumulation of these heavy metals in fish leads to the suppression of fish immunity; thus, allowing the normal pathogenic microbes to develop ulceration and possible bacteria in the bloodstream (Mutuku 2010).

Most of the fish consumed in Kenya come from the wild and partly through fish importation. The research of fish diseases is hampered by poor understanding of the ecological intervention involving interactions between pathogens-hosts in the aquatic ecosystem as well as the lack of knowledge on physiological features of fish, characterized by their poikilothermic behavior in contrast with the better understanding of physiology of homoeothermic animals (Nyaku et al. 2007). The art of artificial fish culturing has not been sufficiently developed. Furthermore, the physical, chemical and biological environments of the fish ponds that are being used for fish farming have not been adequately studied. This work, therefore, assess the microbial contamination, heavy metal contamination and physicochemical properties of selected fish ponds in Abothuguchi Central, Meru County, Kenya and to ensure the suitability of such ponds for artificial fish culture.

The objectives of this study was: (i) To determine the microbial quality and the level of pathogenic bacteria in pond water in Abothuguchi Central. (ii) To determine the concentration of heavy metal contamination of fish pond water in Abothuguchi Central. (iii) To assess the variability of physicochemical parameters of fish pond water in Abothuguchi Central.

MATERIALS AND METHODS

Study area

The study was done in Abothuguchi Central, Meru Central Sub-County, Meru County (Figure 1). The location was chosen due to its high number of fish ponds which use a variety of sources of water. It lies on the North Eastern side of Mt. Kenya, and this is one of the geographical features that leads to the climatic patterns experienced in the region. Abothuguchi central lies adjacent to the equator, within longitudes 37°40ˈE and latitude 0° 03ˈN with an altitude around 5199 meters and this explains the cold temperatures experienced the whole year. The total population of Abothuguchi is 116,516 (KNBS 2009).

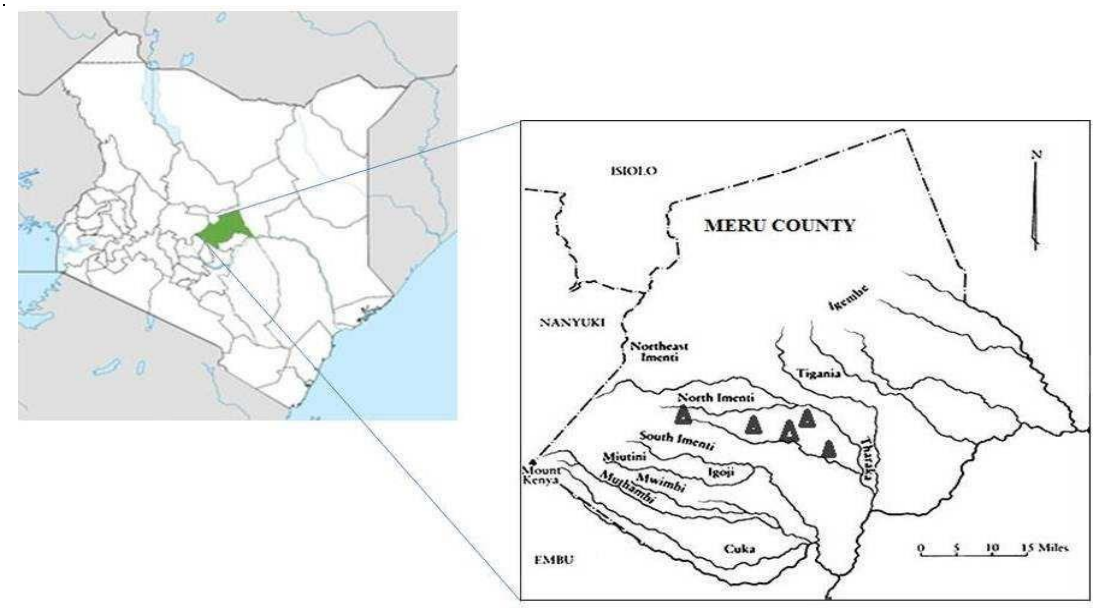


Figure 1. Map of Kenya showing Meru County from Google maps. Triangles show the sampling points.

Abothuguchi lies within the time zone of EAT (UTC+3), and it has many seasonal and permanent rivers; hence water scarcity is not a big problem in the area. The volcanic soils are very rich in almost all the plant nutrients, especially in the high regions. The city experienced bimodal precipitation whereby the short rains last between March and May while the long showers come between October and December. This pattern enables farmers to grow a wide range of crops for subsistence and commercial purposes. However, the kind of crops grown varies based on the ecological zones which differ in terms of precipitation, temperature, and soils. Some of the plants grown in the highlands include cash crops such as coffee, sugar, tea, pyrethrum and food crops such as maize, bananas, sugarcane, sorghum, yams, cassavas, and millet. Livestock reared include cattle, sheep, rabbits, chicken, and goats.

Sampling design and sample size

This study used a cross-sectional study design. We applied stratified sampling in which the sampling zone was divided into five locations each approximately two Kilometers apart. From each area, six sites (fish ponds) were identified. The sampling mainly targeted different fish ponds with different sources of water, geographic patterns and economic activities. The first sites (Mbwinjeru) has a river as the primary source of water. It is purely an agricultural zone, and it is located on the lower sides of Abothuguchi Central; hence it experiences higher temperatures as compared to the other site. The second site was Kithirune (Kit), located in the marketplace whose source of water mainly taps water. This place is quite cold due to its altitude as compared to Mbwinjeru. The third site was Githongo, located within a market. The farmers use tap water, and Gitjongo is cold because of its altitude. The fourth site was Muri which is next to Mt. Kenya forest, it is freezing due to its high altitude, and the farmers use river water. The fifth site was Kianthumbi (Thu) which use swamp as the water source, and it is on a lower altitude.

The samples were taken from each fish pond twice between November 2012 and May 2013 during the wet and the dry season to provide for seasonal variations. The sample size was determined using the equation by Fisher with the confidence interval of 95% and an error of 5%, t (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 measured 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 since replication was done. Based on 3% prevalence and Z value of 1.96, the sample size was:

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

Water samples were collected directly into 250 mL of pre-sterilized polypropylene (glass) bottles. The ponds were stirred before sample collection. The bottles were opened aseptically, held at their bases and submerged to about 20 cm deep with the mouth facing upwards. Samples were collected by filling the bottles to the top to exclude air. A total of 30 samples were collected (Samie et al. 2011). The water samples were placed in ice cool box after which they were transported to Kenyatta University Microbiology laboratory for analysis. All the samples were analyzed within 24 hours.

Bioassays

Detection of faecal streptococci (FS)

Azide dextrose broth detects fecal streptococci in the fish pond waters. The single, double strength broths were inoculated with water samples (10 mL) using a pipette and incubated at 37 °C for 24 hours. Positive test which confirmed the presence of gas production and fermentation of sugars (indicated by the yellow color change of the Bromthymol blue) showed the presence of colonies on Kenner fecal (KF) (Mariita and Okemo 2009). The occurrences of pinpoint colonies from the slant were Gram stained to confirm the presence of FS. All red and pink colonies were counted (Mariita and Okemo 2009).

Detection of faecal coliforms

The presence of fecal coliforms in the water was carried out using the multiple-tube fermentation technique (APHA 2003). The steps include the likely, the confirmed and the completed tests. Each batch was inoculated with the sterile diluted water samples. In the possible test, three series of five tubes each containing 10 mL, 1 mL, and 0.1 mL portions of the sample was inoculated with sterilized lactose broth. Pure, sterile lactose broth was inoculated with sterilized distilled water and served as a control. Inoculated tubes were incubated at 37°C for 48 hours (Gyles 2007). Sterile loop transfers were created from all tubes showing acid and gas production to tryptose bile broth (EC Broth) and then incubated at 44°C for 24 hours. A positive reaction will produce gas in a fermentation tube within 24 hours or less.

The estimated number of fecal coliforms found in 100 mL was determined from a tabulated probability table using the corresponding results of various combinations of positive and negative reactions from each of the three batches (APHA 2003). For further confirmation, suspected positive samples from the tryptose broth were streaked on a plate of Eosin Methyl Blue (EMB) agar to gave well-isolated colonies. Incubation was done at 37°C for 48 hours. Growth of the typical colonies on the plates was seen and a Gram staining performed. The completed test was done by picking two colonies that were considered to be fecal coliform, followed by transferring them to nutrient agar slopes and fermentation tubes containing brilliant green lactose broth. Incubation was carried out at 44.5°C for 48 hours. A Gram stain was conducted to confirm the completed test from the agar slope. Brilliant green lactose broth was also seen for gas production (Gyles 2007). Gas

and turbidity in the tubes, color of metallic sheen or pink with dark center colonies on EMB agar indicated positive for fecal coliforms. Fecal coliform was identified from all isolates that produced gas at 44.5°C, stained Gram-negative and were non-spore forming and rod-shaped where the total counts calculated using a standard probability table (APHA 1992).

Detection of Salmonella

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

Screening for Vibrio

Vibrio detection was conducted in three successive phases.

Enrichment in a non-selective medium. One mL of the samples were enriched in sterile alkaline peptone water, dispensed in 10 mL tubes, and incubated at 35 °C for 18 hours (HPA 2003).

Plating out on selective medium. The streaking of the enriched samples was performed on Thiosulfate citrate bile salts (TCBS) agar, then incubated at 35 °C for 24 hours. The existence of characteristic yellow colonies after streaking was suspected of being of *Vibrio cholerae* (Mariita and Okemo 2009).

Biochemical reactions. Biochemical tests such as TSI, Motility test, Citrate utilization test, urease test, and cytochrome oxidase test was applied to confirm gram staining (Mariita and Okemo 2009).

Detection of Escherichia coli

In a suspected case of *E. coli*, enrichment glucose peptone broth was used for inoculation of the samples, and the broth was incubated at 37 ºC for 24 hours before being sub-cultured onto Sorbitol MacConkey agar (Bopp et al. 1999). *Escherichia coli* were identified according to morphological and biochemical tests (motility-indole-urease test, methyl-red-voges-Proskauer (MRVP) test, and Simmons citrate utilization) (Alam et al. 2010).

Detection of Pseudomonas

Isolation procedure and maintenance. Isolates were collected by using a membrane filter together with a selective growth medium. *Pseudomonas* spp. was examined using a qualitative method, after collecting 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 subcultured on Nutrient Agar (NA) (Difco) to isolate a single pure colony for identification (Hossain et al. 2006).

Morphological and biochemical characterization. Colony characteristics, including green pigment production, were evaluated on Acumedia and NA (Difco). The following classical tests characterized all isolates according to Bergey's Manual of Systematic Bacteriology (Palleroni 1984): Gram staining, cytochrome oxidase production, catalase production and the growth on MacConkey agar.

Biochemical tests--Oxidase test. Two to three drops of oxidase reagent were placed on a strip of paper. A moderate amount of the organism was taken and streaked on the wet surface of the paper with the use of a glass spreader. We avoid a nichrome wire which could give false positive results. The presence of *Pseudomonas* appears as deep purple coloration (Hossain et al. 2006).

Biochemical tests--Nitrate reduction. On nitrate broth, a Nitrate blood agar was dried at 37ºC for one hour after which the plate was seeded by stab inoculation with the sample; then it was incubated at 37ºC overnight. Formation of a sizeable greenish zone which was as a result of the reduction of nitrate to nitrite caused the alteration of the hemoglobin to methemoglobin due to bacterial growth confirmed the presence of *Pseudomonas.* The reduction of nitrate by bacteria was based on the presence of the bacteria which caused the decline of nitrates into nitrite, nitrous oxide, ammonia, and nitrogen gas. The nitrate broth was made by mixing nutrients broth, 5 g/liter KNO_3 or $NANO_3$. One tube of nitrate broth was inoculated. One milliliter of naphthylamine and 1 mL of sulphanilamide reagent were added to the tube cultures. Reduction of nitrates will show the appearance of red color within 80 seconds (Hossain et al. 2006).

Heavy metals quantification

The determination of the heavy metal presence and concentration in the water samples was done by using Flame Atomic Absorption Spectrometer (FAAS no VAA 350) and the contamination amount of manganese, lead, iron, zinc, and copper were analyzed.

Metal standard solutions

Metal powder (1 g) in a clean beaker was added with 20 mL concentrated nitric acid. The mixed solution was then transferred into a 1-liter flask and made up to the mark with distilled water. The solution contained 1000 µg/mL of the specific metal, then it was kept in labeled plastic containers as a stock solution. Working solutions were diluted into varying concentrations of different metals which were later used with FAAS (APHA 1992).

Physico-chemical parameters

Physical and chemical parameters that are water temperature, pH, salinity, DO, total alkalinity, transparency, sulfates, 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).

Data analysis

The data were analyzed by the Statistical Analysis System (SAS) computer software Version 9.3. Two way ANOVA shows the interactions between the sites and the seasons while One way ANOVA calculates the significant differences at P value ≤ 0.05. The p-value of <0.05 was considered significant. In cases where data are significantly different, Tukey`s Honest Significant Difference (HSD) test was used to separate the means. Correlation coefficient test was used to examine whether there was a relationship between fecal streptococci and fecal coliforms.

RESULTS AND DISCUSSION

Enumeration of faecal bacteria indicators and detection of pathogens

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

Enumeration of faecal bacteria indicators

There was a significant interaction between season and site in determining the FS CFU/100 mL populations (Table 1). The FS CFU/ 100 mL dry season was not significantly different from the FS CFU/100 mL during the wet season. Nevertheless, their population was higher during the wet season. The FS CFU/100 mL differed significantly in the various sites. Streptococcal species displayed white colonies on streptococcal KF agar plates, cream colonies on nutrient agar plates, and confirmed by Gram stain (Figure 2-4).

Interaction between site and season in determining the MPN of fecal coliform in the fish pond water was not significant. The FC MPN /100 mL during the dry season remain similar from the MPN wet season (Table 1). The MPN varied considerably in the various sites (Table 1). The wet season indicated a higher population of FC. The FC MPN /100 differed significantly in the various sites when compared with the seasons. Also, the prevalence of FS from all sites exhibited a significant positive correlation with that of FC= 0.832 at P < 0.01 level).

According to one way ANOVA, there was a significant difference in FS CFU/100 mL in all the sites during the dry and wet season (Table 2). Higher FS CFU/100 mL populations were shown during the wet season (W) in all locations as compared to the dry season (D) (Table 2). The wet season indicated a higher population of FS. The FS CFU/100 mL differed significantly in the various sites with the highest population being observed in Mbwinjeru and the lowest in Kianthumbi (Table 2) in both dry and wet seasons. There was a significant difference in FC MPN in all the sites during the wet and dry season (Table 2). The highest FC MPN populations were seen during the wet season (Table 2). The FC MPN /100 differed significantly in the various locations with the highest population being observed in Mbwinjeru and the lowest in Kianthumbi (Table 2) in both dry and wet seasons.

Detection of pathogens

The presence of the potentially pathogenic bacteria in water samples from various water sources varied within the sampling sites. The following biochemical tests; Indole test, Simmons citrate agar, Indole test, oxidase test, urease test, motility on SIM media, the reaction on TSI, urease test and Gram stain confirmed pathogenic bacteria over the study period. From most of the sites, *Vibrio*, *Salmonella*, *Escherichia coli*, and *Pseudomonas* were mainly isolated although their abundance was different.

Table 1. Populations of Faecal streptococci and faecal coliform

Treatment	Pop FS CFU/100 mL	Pop FC MPN/100mL
Season		
Wet season	575466.67 ± 90950.51a	150.533 ±17.1043a
Dry season	546833.33 ± 61069.36a	115.600 ± 22.0154a
Sites 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
P values		
Season	0.6043	0.0680
Site	0.0001	0.0001
Season*site	0.0001	0.9367

Note: Values (Means±SE) followed by a dissimilar letter (s) along the columns are significantly different at P≤0.05 (Tukey's HSD test). Pop-Population (Each column indicates the separation of means and the standard deviation). FC-fecal coliform density expressed in MPN/100 mL (most probable number) and FS-faecal streptococci density expressed in CFU/100 mL (colony forming unit).

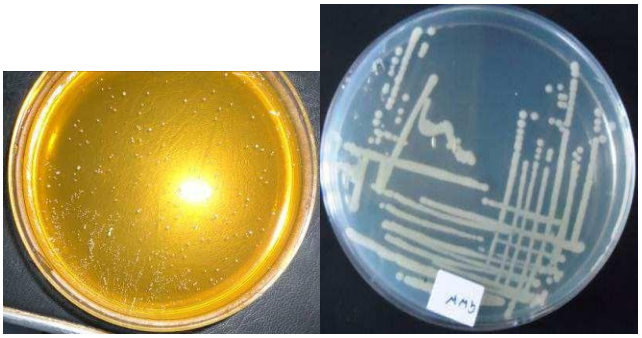
Table 2. Comparison of FS and FC populations during the wet and dry seasons

Site	Pop FS CFU /100 mL	POP FC MPN/100 mL
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 different letters along the columns are significantly different at P≤0.05. (W): wet season, (D): dry season.

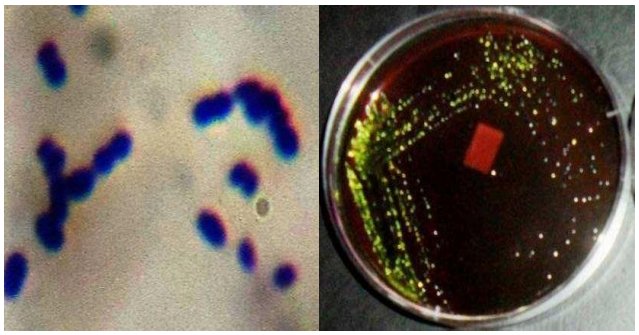
Salmonella species

Salmonella spp were the most prevalent (40%), followed by *E. coli* (34%), *Pseudomonas aeruginosa* (16%) and *Vibrio* spp. (10%) as the least, (Figure 2-9). The TSI agar slants inoculated with typical colonies formed yellow butts, red slants with some blackening and some with or without formation of gas (Figure 5-6). Bacterial growth and color change from green to blue are evident on the Simmons citrate agar slant. The formation of blue-purple color indicated a positive result for cytochrome oxidase test. Further confirmation was also performed using a series of biochemical tests (data not shown).



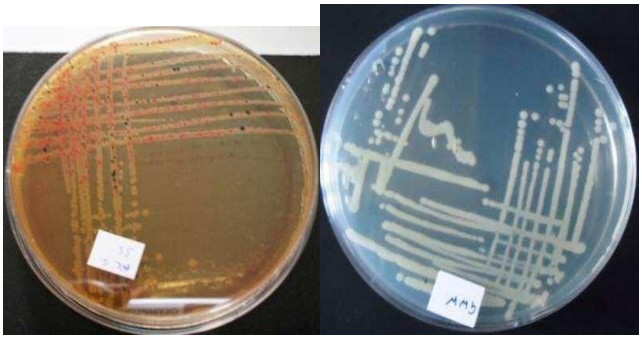
A B

Figure 2.A. Typical colonies of FS on KF agar, B.. Typical colonies of FS on nutrient agar



A B

Figure 3.A. Streptococcal strains showing the characteristic of short to long chains following gram stain reaction, B.. Typical colonies of faecal coliforms on EMB media



A B

Figure 4.A. Black *Salmonella* colonies on SS agar, B. Cream *Salmonella* colonies on nutrient agar plate.

Vibrio species

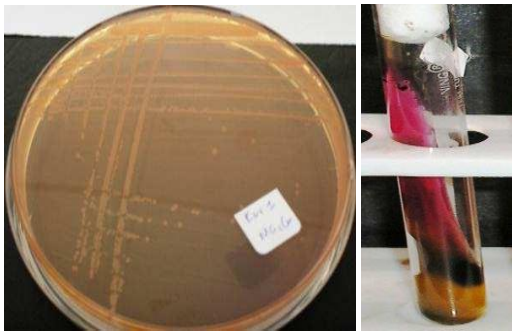
Vibrio spp. exhibited characteristic golden yellow colonies on TCBS (Figure 7.a) and constituted 10% of the pathogens detected. The identity of the isolates as *Vibrio* spp. was also confirmed positive test for Oxidase test.

Escherichia coli

Among total pathogens detected, 34% were confirmed Enteropathogenic *Escherichia coli* (Figure 8).

Pseudomonas species

Of the total pathogenic bacteria identified, 16% of them were *Pseudomonas aeruginosa* (Figure 9).

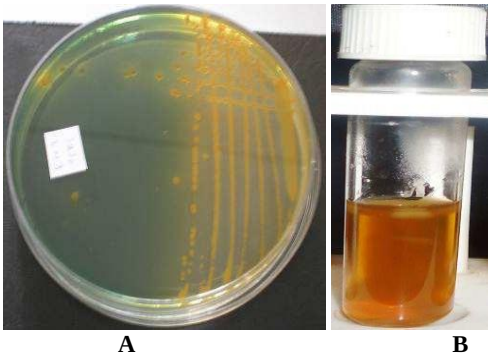


A B

Figure 5..A. *Salmonella* colonies on MacConkey agar plate, B. Yellow butt and red slant and some blackening on TSI slant showing the presence of *Salmonella* spp.

A B

Figure 6.A. Gas production in peptone water as indicated by bubble formation in Durham inserted in tube, B. Reaction in urea agar slants. Color change shows absence of *Salmonella* spp.



A B

Figure 7.A. *Vibrio* spp. on TCBS, B. *Vibrio* colonies showing motility on SIM media.

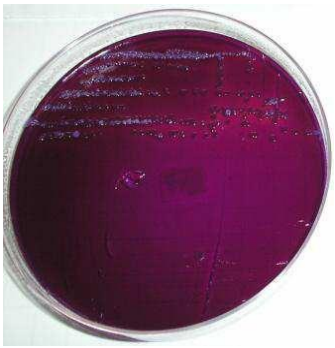


Figure 8. Colorless colonies of *E. coli* on MacConkey

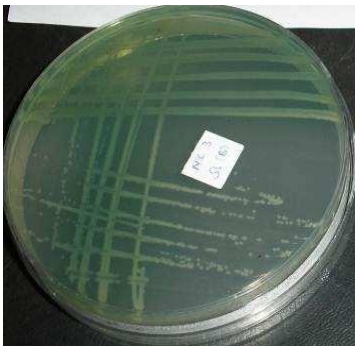


Figure 9. Green colonies of *Pseudomonas aeruginosa* on Nutrient agar

Physico-chemical parameters

The season and site did significant interactions in determining the pond water temperature (Table 3). The temperature ranged between 18-29 ºC. Their water temperature differed significantly between dry and wet season and varied greatly in all sites (Table 3). There was no significant interaction between location and season in determining the pond water pH. The water pH differed significantly (p=0.0058) between the dry and wet season in comparison with the sites. There was a significant difference in water pH in all the sites sampled (Table 3).

Water conductivity in the varied significantly between sampling sites. However, there was no significant interaction between season and location determining the water conductivity (Table 3). There was a considerable difference in water conductivity between the dry and wet season (Table 3). The site and season did not have strong interaction in determining the water turbidity (Table 3). Pond water turbidity varied significantly between the dry and wet season. There was a significant difference in water turbidity in the various sites (Table 3).

Site and season did not interact significantly in determining the phosphates concentration in the pond water. Phosphates concentration in the water fell in the narrow range between two seasons (Table 3). The phosphates concentration did not differ significantly. Sulfates level in the pond water did not change dramatically in the wet and dry season. However, the sulfates concentrations ranged considerably among the studied sites. No significant interaction was observed between location and season in determining the sulfates concentration in pond water (Table 3).

The nitrates concentrations were significantly different among the sites. However, there was no significant interaction between location and season in determining the nitrates concentration in pond water (Table 3). Nitrates concentrations differed significantly between the wet and dry season (Table 3). There was no significant interaction between site and season in determining ammonia concentration in fish pond water (Table 3). Ammonia concentration differed significantly between the wet and dry season. Ammonia concentration was significantly different depending on the location. Based on the two way ANOVA there was no significant interaction between location and season in determining the amount of DO in pond water. The amount of DO did not differ significantly based on the season. High DO was recorded during the dry season significantly (Table 3).

The pond water temperature was significantly different in all the sites during the dry and wet season. Higher temperatures were recorded during the dry seasons, with the most top record being in Mbwinjeru and the lowest was in Muri both dry and wet season. There was no significant difference in pH in all sites during the dry and wet season. The pH of the water in all the ponds was more acidic during the dry season. The highest pH value recorded was in Kithirune while the lowest value recorded was in Muri during the dry season. Meanwhile, Kianthumbi had the highest value with Muri having the lowest during the wet season. There was a significant difference in conductivity in all sites during the dry and wet season. The high conductivity of the pond water was observed during the dry season in all ponds. The highest electrical conductivity was observed in Mbwinjeru while Muri had the lowest in both dry and the wet season (Table 4).

The turbidity of the pond water was significantly different in all the sites during the dry and wet season (Table 4). The turbidity of the pond water was substantially higher during the wet season (Table 4). The pond water was significantly least turbid during the dry and wet season in location Thu (Table 4). The turbidity of the sampling sites varied between 16 and 118.0 NTU during the study period. The highest value of turbidity examined was in Mbwinjeru while the lowest value recorded was in Kianthumbi in both seasons.

There was no significant difference in Phosphates concentration in all the sites between two seasons. Mbwinjeru recorded the highest levels of phosphates while Kianthumbi showed the lowest in both seasons. Sulfates concentrations were significantly different in all the sites with the highest levels being recorded during the wet seasons. The highest level was recorded in Mbwinjeru and the lowest in Kianthumbi in both seasons (Table 4).

There was a significant difference in nitrates concentrations in all the sites during the dry and wet season. Higher concentrations of nitrates were observed during the wet season. Mbwinjeru had the highest levels whereas Kianthumbi recorded the lowest nitrates concentration in both seasons. Ammonia concentrations were not significantly different in all the sites during the dry and wet season. The highest levels were recorded in Muri while the lowest in Kianthumbi. Dissolved oxygen

(DO) concentration in the pond water remain the same in all the sites during the dry and wet season. The highest DO value was obtained in Muri while the lowest in Mbwinjeru in both seasons (Table 4).

Heavy metals

The order of occurrence of heavy metals in water is: Fe > Mn > Zn > Cu > Pb. Data on heavy metals were analyzed using two way ANOVA. There was no significant difference in the concentration of lead in the ponds between wet and dry season. There was no significant difference between the lead concentrations in all the sites. No significant interaction was observed between the season and location in influencing the lead concentration in the ponds. Githongo recorded the highest level of lead while Kianthumbi recorded the lowest. Data on copper concentration showed a significant difference in Copper concentration between the dry and wet season (Table 5). No significant difference in Copper concentration was observed among the sites. Two way ANOVA showed no significant interaction between location and season in influencing Copper concentration in the ponds. Mbwinjeru had the highest level of copper while Kianthumbi had the lowest.

Table 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)

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

Table 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.

Note: E.C-Electrical conductivity; PO_4-phosphates; SO_4-sulfates; 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

Table 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≤0.05 (Tukey's HSD test).

The study showed a significant difference in Zinc concentration in the water between the dry and wet season (Table 5). There was no notable in Zinc concentration in pond water among the sites. Two way ANOVA showed no significant interaction between location and season in determining Zinc concentration in the pond water. The highest value of Zinc was seen in Mbwinjeru while the lowest value was in Muri.

Iron concentrations were similar between the wet and dry season. Also, the Fe concentrations in the various sites did not change dramatically. There was no significant interaction between season and location in determining the iron concentration in the pond water. Kianthumbi had the highest level of iron while Muri had the lowest. Manganese concentration in the water varied significantly between the dry and wet season; however comparison of the sites showed no significant difference in Manganese concentration in the ponds (Table 5). There was no significant interaction between location and season in determining Manganese concentration in the pond water. Kianthumbi had the highest level of manganese while Muri had the lowest.

Discussion

Microbial quality of fish pond water

Fecal bacteria (fecal streptococcal and fecal coliform) were found in all the samples; thus, confirming the previous report (Ashbolt et al. 2001; Hunter et al. 2002; Emikpe et al. 2011). High fecal contamination detected in the present study is consistent to the findings reported in Pakistan (Nahiduzzaman et al. 2000) and in Italy (Maugeri et al. (2000). On the population of the fecal bacteria, there was significant variation in their populations across all the sites. The levels of fecal bacteria in all the locations exceeded 1.0x10^{1} CFU/100 (FAO 1979); hence all the water samples collected showed high levels of contamination. On the population of the two fecal bacteria, Pearson correlation analysis indicated that prevalence of FS from all sites showed a considerable positive correlation with that of FC. These results are consistent with the findings in Nigeria (Egbere et al. 2008) and Ghana (Fafioye 2011).

Lack of animal waste management, as well as wastewater, could directly affect water quality as a result of surface runoff (Ampofo and Clerk 2010). Surface runoff could be a plausible source of pond water contamination particularly during the wet season (Ampofo and Clerk 2010) in Ghana. Free roaming animals and pets especially dogs might also contribute to fecal contamination of surface water. Also, there are cattle reared by farmers that too walk the region for green pastures. Along the rivers in this location, there is possible run-off from roads, parking lots and yards that could be carrying animal wastes into a natural watercourse and ponds (Emikpe et al. 2011). In the densely populated region of Meru, a factor that could contribute to pond contamination due to the closeness of latrines to water points, washing and bathing in rivers that serves as the source of fish pond water (Doyle 2007; Adebayo-Tayo et al. (2012a).

Mbwinjeru had the highest population of fecal streptococci and fecal coliform during the dry and the wet season which could be attributed agricultural activities that take place around the site. Fertilizer and manure used by the farmers may have found their way into the ponds. The high temperatures in this area is a favorable environment for the growth of the bacteria. The source of water used in this area is primarily from a river, and therefore it might be the source of contamination. The farmers mostly use streams as of their fish pond water which may harbor some bacteria. These data are consistent with those of Ghana (Emikpe et al. 2011).

Kianthumbi showed the lowest population of fecal streptococci and fecal coliform in the dry and the wet season which could be associated with its source of water that is mainly from a swamp and the zone is also relatively cold. These data are consistent with those reported in

Nigeria (Egbere et al. 2008). Fecal coliform in fish demonstrates the level of pollution of their environment because coliforms are not the normal flora of bacteria in fish. Birds can be a significant source of fecal coliform bacteria. Swans, geese and other waterfowl can elevate bacterial counts in ponds (Doyle and Ericson 2006). The presence of fecal coliforms in all the sampling sites was an indication that water sources in the four locations were contaminated.

Pathogenic organisms

From water analyzed in these fish ponds, pathogenic bacteria were also detected. *Salmonella* spp. were the most prevalent, followed by *E. coli*, *Pseudomonas aeruginosa*, and *Vibrio* spp. These findings are consistent with previous studies in Kisumu performed in County, Kenya (Onyango et al. 2009); in Nigeria (Egbere et al. 2008); in India (Nabonita et al. 2011) and Cameroon (Kuitcha et al. 2010). These aforementioned bacterial isolates are common intestinal bacteria of both animals and humans gut; however, this contamination may also have come from untreated public 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, run-off from urban land, combined sewer overflows, animal waste, and municipal waste sludges disposed of on ground or in water. Because different kinds of livestock manure are contaminated with pathogenic bacteria such as *Streptococcus*, *Salmonella*, *Shigella*, *Pseudomonas*, *Vibrio*, and *E. coli* species (Abdelhamid et al. 2006), it 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 will develop into pathogenic 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). Besides, Physico-chemical characteristics influence the growth and diversity of microbial populations. The study has indicated that the fish ponds were significantly contaminated with both fecal and pathogenic bacteria.

Physico-chemical parameters

The variations observed in the physicochemical properties of the fish ponds in the present study are similar to the earlier findings in Nigeria by Kumar (2004) and in Samburu County, Kenya by Mwaura (2005). The variations could be attributed to the influences of the micro-climatic, topographic and edaphic conditions of fish ponds in the area. Also, human and animal or livestock activities could another be a factor. The highest recorded temperature and the lowest (Table 4.2) were within the recommended limits of 20ºC to 30ºC Kumar (2004). The World Health Organization has recommended 27ºC as the acceptable limit for fish growth and productivity (WHO 2006) whereas the Environmental Management Authority of Zimbabwe has set a limit of 35ºC. In a region of high altitude bordering a forest such as in Muri and Kithirune, we measured a lower temperature. During the wet season, the temperatures were lower than to the dry season as observed some years ago in Samburu County, Kenya (Mwaura 2005).

We observed that ater from Mbwinjeru and Githongo was found to be slightly warmer due to their lower altitude, in congruence with previous studies conducted in Kenya (Mwaura 2005). The provides better growth and food conversion than low temperature (Chaudhari 2003; Mwaura 2005). The lowest value observed fall below the acceptable range between 24°C and 34°C for the tropics according to a previous study in Nigeria by Udo (2007). 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 the proper performance of fish (WHO 2006). High water temperature enhances the growth of the 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 broader range of temperatures, provided that fluctuations are not so dramatic, sudden and of long duration **(**Priyadarshini et al. 2011).

The pH values measured 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). According to WHO, the desirable range for pond pH is 6.5-9.0 and range which is in between 5.5 to 10.0 (Stone and Thomforde 2003). The average value of the pH falls below the recommended pH range of 6.5-9.0 (WHO 2006) for better 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 might be caused by acid sulfate runoff (Silapajarn et al. 2004). Low pH might also be caused by high rates of carbon dioxide release by zooplankton during respiration. This is a common phenomenon in aquaculture ponds (Silapajarn et al. 2004).

Higher acidic levels in the present study could be due to the chemical additives applied to the aquaculture pond for better production, high stocking density or lack of buffering activities in the farms as indicated by pH values. 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 the WHO of 1000 µS/cm (WHO 2006). The data are also consistent with the findings in Brazil (Sipaúba-Tavares et al. 2007). The higher values of electrical conductivity in Mbwinjeru may be due to high temperatures. Likewise, those in Muri may be due to low temperatures associated with the altitudes. Electrical conductivity is measurement of the ability of water to conduct an electric current. Since the electric current is done through the movement of ions in solution, it also indicates the amount of ions or total dissolved salts in the water. The low levels during the wet

season might be caused by the rainy season in which the samples were collected. Dilution of water during the rainy seasons lowers the levels of electrical conductivity (Sipaúba-Tavares et al. 2007). During the dry season, the electrical conductivity was which corresponds to a study in Samburu County, Kenya (Mwaura 2005).

The values of turbidity varied significantly. The values were read within the permissible limit set by the 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 usually is associated with high microbiological contamination which is in congruence with the bacterial population in Mbwinjeru which had high values of turbidity. High turbidity may be as a result of rains which cause flooding that caused soil erosion and surface runoff hence depositing nutrients, silt, and domestic wastes into the water. Kianthumbi showed the lowest values of turbidity due to the source of water which was a swamp.

The values of phosphates differ significantly, consistent with those obtained by in Nigeria (Ehiagbonare and Ogunrinde 2010). The values obtained from Mbwinjeru exceeded the permissible limit set by the 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 previous research in the 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 increase in phosphorus produced during the decomposition of organic fertilizer and the feedthrough fish excreta. Both soluble organic phosphorus is 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 water source and less agricultural activities.

The values of sulfates hugely varied, 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 still fall within limits. The values obtained here are higher than those reported in Nigeria (0.66 to 1.09 mg/l) (Ehiagbonare and Ogunrinde 2010). The high amounts found in Mbwinjeru could be associated with the frequent use of detergent and soaps by people in the neighborhood or frequent usage of fertilizers in farming (Ehiagbonare and Ogunrinde 2010). The values of nitrates obtained from this study differ significantly (Table 4.3), yet fell within the recommendations (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 the presence of pollutants like bacteria and pesticides (Nzunga 2011) and can be remedied by water change and plant density. The higher levels can be attributed to agricultural and domestic activities, and surface runoffs during the rainy season (Nzunga 2011).

The values of ammonia were within the recommended amounts of less than 1 mg/l (Kumar 2004). The mean value was within the permissible limit set by the 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 densities; hence the excess feed decomposes and pollutes the pond water (Thilza and Muhammad 2010). Ammonia is introduced into through dead phytoplankton, uneaten feeds, dead and decaying organic matter. It can be attributed to the addition of manure to fertilize the pond or through the process of nitrogen fixation by algae and water plants (Edwards 2008).

Ammonia at concentration >0.1 mg L^{-1} tends to cause gill damage, destroy mucous producing membranes, poor feed conversion, effects like reduced growth and reduced disease resistance. Ammonia affects 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 a thick layer of mucus. In some cases, hemorrhages occur mainly at the base of the pectoral fins (Thilza and Muhammad 2010).

The DO values differ significantly across the sites. However, these levels were below the WHO recommended values of 5.0 mg/l (WHO 2006). The low DO may be attributed to the small size of the ponds and eutrophication as a result of 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 oxygen consumer in an aquaculture system. Low concentration of DO in water causes suffocation in fish while its supersaturation may result in the gas bubble disease leading to the mass mortality of fish in both cases. Low DO increases the toxicity of ammonia to culture organisms. Oxygen depletion in water can cause poor feeding of fish, reduced growth, starvation, and higher 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 when the water temperature increases. The study has shown that the physicochemical parameters varied significantly in all the study sites.

Heavy metals

Heavy metals are chemical elements with a specific gravity which 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 always immersed in water containing pollutants. They absorb the contaminants through the skin, gut (from food) and respiratory surfaces (Wegu and Akanimor 2006). Except for Fe, the heavy metal concentrations in the tested water here 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. High amount of metals in the fish ponds may be attributed to the increased coverage of the aquatic and higher plants which absorb minerals from

water and sediments. Areas with high metal content had high bacteria counts which are consistent with a previous study in Kenya (Mutuku 2010) and Italy (Ozurtuk et al. 2010).

The data has indicated that the order of heavy metals amount in the fish pond water is: iron > manganese > zinc > copper > lead which is 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 high level of lead in some pond water in England has been attributed to industrial and agricultural discharge (Mason 2002). The pollution of the aquatic environment by heavy metals affects marine organisms and poses considerable environmental risks and concerns (Amisah et al. 2009). Heavy metals pollutants accumulate in tissues and organs of marine organisms. The high levels of lead in Githongo and Kithirune in the present study could also be attributed to a spill of leaded petrol from the combustion of gasoline in automobile cars and the closeness of these ponds to the two markets.

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

The amount of copper in the present study showed interaction between the sites and the seasons which is consistent with the findings reported in the USA (Silapajarn et al. 2004), Turkey (Ozuturk et al. 2010), and in Egypt (Samir and Ibrahim 2008). The data 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 lead to severe damage in gills and necrotic changes in the kidney and liver. Long term high exposure to copper can cause nausea, vomiting, stomach cramps, or diarrhea when ingested by humans from the fish (Javed and Usmani 2013).

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

The amount of iron detected was similar across the sites, consistent with previous studies in Egypt (Samir and Ibrahim 2008) and Turkey (Ozurtuk et al. 2010). The great amount of iron may be attributed to the high density of people settled in 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 the interaction between the sites and the seasons. The WHO has recommended 0.1 to 0.5 mg/l of manganese for optimal productivity of fish (WHO 1993). These values are consistent with those reported in the USA (Silapajarn et al. 2004) and those by Samir and Ibrahim (2008) in Egypt.

The primary sources for manganese in the 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 disturb lung, liver and vascular, lowering blood pressure, causing brain damage in fish and failure in the development of animal fetuses (Javed and Usmani 2013). Dissociation of heavy metals into ions in water is a prolonged process, and metabolic processes can not detoxicate them. 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 except for iron; hence the fish ponds are not significantly contaminated with heavy metals.

Conclusion

The fish ponds were found to be significantly contaminated with fecal streptococci and fecal coliforms. The pathogenic bacteria detected in this study, *Salmonella*, *Vibrio* spp. *E. coli* and *Pseudomonas aeruginosa* indicate high contamination and a high risk of infection. Heavy metals were found to be within the WHO standards, except for iron. All the physicochemical parameters in the tested sites varied and the temperature, ammonia, sulfates, nitrates, phosphates, electrical conductivity, and turbidity were found to be within the values of WHO (2006). The pH and the dissolved oxygen fell below the limits provided by WHO (2006). The populations of fecal and pathogenic bacteria, the variability of physicochemical parameters, and concentration of heavy metal contamination were affected by the source of water, geographical patterns of the site, human and livestock activities.

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