
Bacteriological Quality of Tilapia Fish from Treated Wastewater in Peri-Urban Areas, Morogoro, Tanzania

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Abstract: The aim of this study was to assess faecal bacterial contamination in tilapia fish from wastewater treatment ponds at Mzumbe and in pristine water in Mindu dam. Tilapia fish (fish flesh and fish intestines) and water samples were analysed for *Escherichia coli* and total plate count. The concentration of *E. coli* in fish intestines ranged from about 1 - 3.5 log cfu/g and <1 log cfu/g in fish flesh. Total plate count ranged from 1 - 3 log cfu/g in fish flesh and 2 - 6 log cfu/g in fish intestines. The concentration of *E. coli* in pristine water in Mindu dam ranged from 0 to 15 cfu/mL. From the inlet to outlet of Mzumbe wastewater treatment pond, there was a significant reduction ($p < 0.05$) of *E. coli* concentration along the gradient from 2.05 to 0.55 log cfu/mL, respectively. These findings demonstrated that tilapia fish from the two study sites were of good quality for human consumption based on microbial faecal contamination. On the other hand treated wastewater of the quality found in this study may be used for aquaculture. However, continuous monitoring for bacteriological quality and safety parameters including *E. coli* and total plate count is required.

Keywords: *Escherichia coli*, Fish Culture, Fish Flesh, Fish Intestines, Water

1. Introduction

The tilapia fish such as *Oreochromis niloticus*, is one of tropical species that inhabits in shallow water within temperature range of 11°C to 42°C [1]. Tilapia fish feeds on phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus and bacterial films associated with detritus [1, 2]. Worldwide, wastewater has been used in aquaculture farming though in most of Sub-Saharan African countries including Tanzania, there are no policies and data to support wastewater fed aquaculture [3-5]. The driving forces to grow fish in wastewater and utilize them include the economical, scarcity of clean water and limited land for fish culture. Others include urbanization and population increase; and low cost or free available wastewater resource [6, 7]. In tropical countries both forms

of economic and physical water scarcity predominates depending on hydrologic conditions [8]. Not only that, but also, predominantly, stable surface water supply is during wet season and decreases during dry season [9]. Use of treated wastewater allows a constant supply of water in year round. The management of wastewater is important for sustainable development of aquatic resources in the world [10]. Wastewater or other low quality water sources - fed fish culture is among the productive sectors that enhances better nutrition and improves household food security and income generation [1]. It has been estimated by FAO that about 1 billion people rely on fish as source of protein [7]. Moreover, fish are rich source of omega 3 polyunsaturated fatty acids as well as minerals and vitamins [11].

Wastewater fed fish-culture may contain excreta related bacterial hazards which may lead to diseases such as

gastroenteritis and Typhoid fever caused by pathogens like *E. coli* and *Salmonella typhi*, respectively. The *E. coli* and *Salmonella* have been reported to survive and multiply in the gut of fish cultured in wastewater fed ponds [12]. Fish grown in wastewater fed ponds are likely to be contaminated with pathogens since they feed on phytoplankton web-based and filters water contaminated with human and animal waste materials. It is also important to note that even fish that grows in a wild environment may directly feed on human or animal excreta [13], which may increase bacterial load in the gut. The occurrence of pathogenic bacteria in the gut of fish meant for human consumption may pose potential health risks to the handlers and consumers [14]. In Tanzania there is no food safety policy to support wastewater fed fish culture while fish grown in wastewater treatment ponds are consumed. Moreover, data on pathogenic bacterial contamination in fish grown in wastewater treatment ponds are required in order to ascertain the associated human health risks [15]. Customarily, pathogenic bacterial contamination has been monitored by assessing indicator bacteria such as total coliforms, *E. coli* and thermotolerant / faecal coliforms [16]. *Escherichia coli* is typically found in the intestines of warm-blooded animals including human and animals. It is thus a good indicator for monitoring faecal pathogenic bacterial contamination in water and foodstuffs including fish [17]. The hypothesis tested in this study was that fish from treated wastewater are of the same bacterial quality with those from pristine water. This study therefore assessed the occurrence of *E. coli* in tilapia fish grown in Mzumbe wastewater treatment ponds and Mindu dam which supplies water for residents in Morogoro urban and peri-urban areas.

2. Materials and Methods

This study was carried out during October, 2013 to February 2015 at Mzumbe wastewater treatment ponds and Mindu dam that serves as a source of fish from pristine environment. Mzumbe wastewater treatment ponds consist of anaerobic pond, facultative pond and maturation pond arranged in series. Man-made Mindu dam, pristine (clean) water reservoir is the main source of water for residents in Morogoro Urban and Peri-urban areas. It receives water from Mzingu, Ngerengere, Mlali, Lukulunge and Mgera rivers that flow from Uluguru Mountains into the dam and drains out through Ngerengere river. The dam is located about 10 km south west of Morogoro Municipality along Tanzania Zambia Highway towards Iringa Region. A total of 102 samples including 41 water and 61 tilapia fish were collected. Out of 41 water samples, 21 were from Mindu and 20 from Mzumbe wastewater treatment ponds. Out of 61 Tilapia fish samples, 41 were from Mzumbe wastewater maturation pond and 20 from Mindu dam. Samples were individually put in a polythene bags, then stored in a cool box with ice cubes. Immediately transported to Pest Management Centre laboratory, Sokoine University of Agriculture and analyzed in the same day.

2.1. Microbiological Analysis

To obtain fish flesh and intestines for laboratory analysis, in brief, the whole fish was placed on the sterile chopping board, then wiped using moistened cotton wool with 70% alcohol to decontaminate bacterial load on fish skin. Using hot knife, fish flesh was removed and placed on the chopping board with fish flesh placed upside. Aseptically, by using a sterile blade, fish flesh was chopped in small pieces. About 15 g was weighed into 120 mL of diluent (buffered peptone water-BPW, Oxoid Ltd, England) in 500 mL bottle and thoroughly mixed by hand. Fish intestines were removed using sterile forceps and then weighed to about 10 g into 90 mL of BPW in 250 mL bottle and thoroughly mixed by hand. Serial dilutions were prepared up to 10:10000 as per sample requirement. For enumeration of *E. coli*: one (1) mL of selected sample homogenate (fish flesh, fish intestines and water) was inoculated on petrifilm selective *E. coli* (SEC) plates (3M Microbiology Products, St Paul, USA) and incubated at 44°C for 24 hours. For total plate count: one (1) mL from the sample homogenate was inoculated into petri dish and pour plated with a molted Nutrient agar (NA), (Oxoid limited, Hampshire, England) and incubated at 37°C for 24 hours. All colonies from SEC plates and NA were counted and reported as *E. coli*/TPC in cfu/ g or cfu per mL for fish and water samples, respectively.

2.2. Data Analysis

Data were entered in Microsoft excel and further analyzed using SPSS statistics 20.0 of 2011 (IBM, California, USA). The concentration of *E. coli* and total plate count was reported in colony forming units (cfu) per mL and transformed to log₁₀ cfu per mL (log cfu/mL). Paired sample means of *E. coli* concentration in log cfu/mL between Mzumbe and Mindu were tested by Student's t-test. Concentration of *E. coli* and total plate counts for paired sample means was compared between the two study sites to ascertain if there were differences. The differences between groups were reported at P<0.05. The mean concentration of *E. coli* and TPC were presented in Boxplots.

3. Results and Discussion

3.1. Escherichia Coli Concentration in Fish Flesh and Intestines

These findings give the basis for the bacteriological quality of fish grown in domestic wastewater treatment ponds. Although fish flesh is sterile [18], it may become contaminated with faecal pathogenic bacteria, if it is grown in polluted environment [7, 19]. These findings demonstrated the risk of faecal bacterial contamination in tilapia fish in the two study sites. Occurrence of *E. coli* in fish flesh from Mzumbe wastewater treatment ponds and Mindu dam was 0.05% (n=41) and 0% (n=20), while in fish intestines was 68% (n=41) and 40% (n=20), respectively. The concentration of *E. coli* in fish flesh in the two study sites was <1 log cfu/g

(detection limit) (Fig. 1) and in fish intestines was about 2 log cfu/g and 3 log cfu/g for fish from Mindu dam and Mzumbe water, respectively (Fig. 2). Concentration of *E. coli* in fish intestines was significantly higher ($P < 0.001$) in Mzumbe wastewater than pristine water at Mindu dam. A comparable study in Brazil [20] found zero counts total and faecal coliforms equivalent to < 1 log cfu/g in fish flesh and 3.0 & 2.8 log cfu/g in fish intestines, respectively. Likewise in Vietnam [21] pathogenic bacteria were found in fish intestines and in intraperitoneal fluid without being found in fish flesh. However, the risk of pathogens invasion into fish flesh may increase with the duration of exposure to the polluted water. The contamination of *E. coli* in fish intestines was previously documented in comparable studies in Israel, Scotland, Norway, The Netherlands as well as in Tanzania [12, 22–25]. It was further found in Israel, Vietnam and Bangladesh that fish were contaminated with pathogens of faecal origin during culturing period, postharvest handling and processing [19, 26]. A study in The Netherlands, reported contamination in different fish organs in the order of the intestine $>$ gills $>$ skin $>$ liver [23]. Microflora of fish intestines often reflects to the bacterial quality of aquatic environment [27, 28]. Thus, it implies that wastewater fed aquaculture may pose health risks to human and the aquatic organisms.

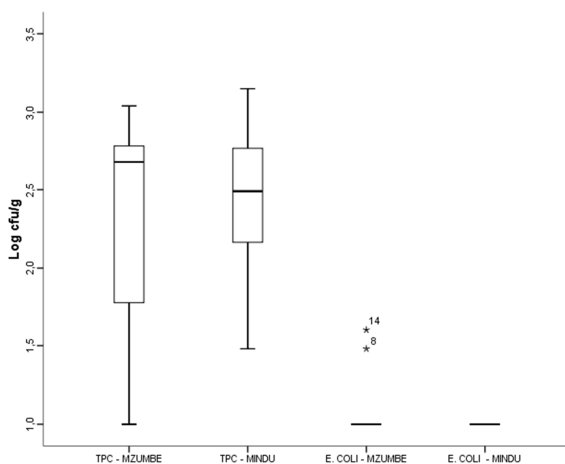


Figure 1. Microbial concentration in Fish flesh at the two study sites (* = outlier samples).

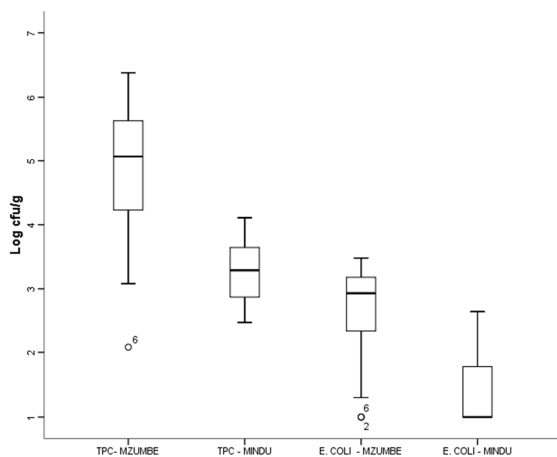


Figure 2. Microbial concentration in fish intestines (O = outlier samples).

3.2. Total Plate Count in Fish Flesh and Intestines

Total plate count (TPC) in fish flesh (Fig. 1) from the two study sites were comparable ($P > 0.05$). The mean density of TPC in fish flesh was < 3 log cfu/g. Studies on cultured freshwater fish in Kerala, India and Lagos, Nigeria found microbial counts ranging from 1-2 log cfu/g [29] and 4 log cfu/g [30], respectively. Findings from this study indicate that concentration of TPC was below the ICMSF criteria of 5.60–7.00 log cfu/g. The TPC of < 5 log cfu/g in fish flesh was reported earlier to indicate good quality of fish freshness [26]. Based on the density of TPC (< 3 log cfu/g) in fish flesh, it implies that fish from the two study sites were of good quality. The TPC in fish intestines from Mzumbe and Mindu dam was > 4 log cfu/g and < 4 log cfu/g, respectively (Fig. 2). Total plate count in fish intestines from Mzumbe was significantly higher ($P < 0.001$) than those from Mindu dam. The concentration of *E. coli* in intermediate and treated wastewater in Mzumbe was higher (2.05 to 0.55 cfu/mL) compared to about 1 log cfu/mL in Mindu dam water. This implies that fish from Mzumbe are feeding on contaminated benthic fauna and aquatic plants [2]. The TPC in fish intestines ranged from 2-6 log cfu/g which was a low count compared to previous findings reported elsewhere [27, 30–32]. The TPC found were in the range of 7-8 log cfu/g implying that fish were not of good quality for processing or human consumption.

3.3. Escherichia Coli in Water

The occurrence of *E. coli* in intermediate treated wastewater entering into maturation pond and in treated wastewater at outlet was 80% ($n=10$) and 70% ($n=10$), respectively. There was significant reduction of *E. coli* concentration between two points of inlet and outlet of the maturation pond being 2.05 to 0.55 log cfu/mL, respectively. The occurrence of *E. coli* was 66% ($n=9$) for samples from onshore and river catchments, 33% ($n=3$) off shore and 22% ($n=9$) inside the dam. The concentration of *E. coli* in both wastewater and pristine water in the present study were about 1 log cfu/mL and it was in congruence with findings from a previous study in Brazil [20]. The concentration of *E. coli* was 1 log cfu/mL in treated effluent from wastewater fed fish culture. The TPC is recognized as an acceptable measure for the general degree of bacterial quality and hygiene [19, 22]. The *E. coli* from onshore water was 15 cfu/mL followed by Mzingira and Tangeni rivers catchments, off-shore water (about 200 m) and inside the dam was 1-3 cfu/mL. These results were comparable with a study in Saudi Arabia [27], which documented TPC in the range of 0.45 to 1.20 log units. Therefore, during the period of this study, water from the two study sites conformed to the WHO guidelines 2006.

3.4. Food safety and Health Risks

The presence of bacterial pathogens in fish intestines may lead to a food safety and health risks through cross contamination in fish flesh and transmission to consumers if

good hygiene practices during fish preparation are inadequate [19]. Since *E. coli* is generic to intestinal tract of fish, it may lead to cross contamination on fish flesh during preparation if gut contents are exposed and ruptured. The risk for bacterial contamination in wastewater fed fish-culture may be reduced based on the previous documented control measures that include: use of good quality treated wastewater; withholding and depuration of fish prior harvesting; good hygienic postharvest handling practices during processing and preparation; obligatory observation of personnel hygiene and use of protective gears; provision of clean water to fishers, processors, during kitchen preparation, satisfactory cooking of fish and fish products, and monitoring bacterial quality and safety indicator parameters in water and fish [12, 19, 23, 33].

4. Conclusion

Findings from the present study show that tilapia fish grown in wastewater treatment (maturation) pond conform to the bacteriological quality set by ICMSF/FAO. Overall, they were of good quality as those grown in the dam. The bacteriological quality of treated wastewater from the study sites conformed to the WHO guidelines (2006), thus it may be used for fish culture. Fish from wastewater treatment ponds in the study sites are consumed by the neighborhood community. So, health risks to handlers and consumers as well as other stakeholders can be reduced given that good hygiene handling practices during pre- and post- harvest of fish are adequately implemented. In Tanzania, neither a policy nor data are in place for wastewater fed fish-culture practices. These findings therefore may contribute data to the national and international bodies such as WHO/FAO on wastewater use in aquaculture. Further research for identification and characterization of faecal pathogenic bacteria including the pathotypes *E. coli* and *Salmonella* spp. in fish and water as well as health risk analysis is recommended.

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