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Full Length Research Paper

Removal of *Escherichia coli* in treated wastewater used for food production in Morogoro, Tanzania

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The aim of this study was to assess the removal efficiency of *Escherichia coli* at Mafisa and Mzumbe domestic wastewater treatment ponds in Morogoro, Tanzania. The study was done from October, 2013 to April, 2014. A total of 125 water samples from inlets and subsequent anaerobic, facultative and maturation ponds as well as treated wastewater were collected and analysed for *E. coli*. The estimated retention times of the wastewater treatment units were 19 and 22 days in Mafisa and Mzumbe ponds, respectively. The concentration of *E. coli* ranged from 4.70 to 5.60 log cfu/mL in untreated wastewater and was reduced to <1.00 to 2.00 log cfu/mL in the treated wastewater. During rainy and cold seasons, the effluent discharged out at Mafisa during August 2013; and March and April, 2014 was about 2 log cfu/mL while at Mzumbe *E. coli* concentration in effluent discharged out was up to 1.23 log cfu/mL. The concentration of *E. coli* in untreated and treated wastewater from the two wastewater treatment ponds study sites were comparable (P<0.05). Reduction of *E. coli* concentration in wastewater treatment ponds study sites was significant with less reduction seen at Mafisa, during rainy and cold seasons in March, April and August. To conclude, the simple wastewater treatment ponds in the study sites were effective and demonstrated potential for reduction of public health risks associated with use of treated wastewater in agricultural irrigation and aquaculture.

Key words: Agricultural irrigation, aquaculture, retention time, wastewater treatment.

INTRODUCTION

Water is an essential resource in supporting life of humans, animals, plants and other living organisms.

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In Sub-Saharan Africa many people struggle for access to a limited clean and safe drinking water. Currently, wastewater is widely used in food production systems such as in agricultural irrigation and aquaculture which has been compelled by the growing scarcity of clean water (WHO, 2006a; WHO, 2006b). Globally, households and commercial business points in urban and peri-urban areas increasingly produce wastewater from toilets, bathrooms, laundries and kitchens. Wastewater generated from these facilities may constitute major source of pollution of water bodies and environment (Senzia et al., 2009; Akpor and Muchie, 2011; Mkali et al., 2014). About 98% (Mateo-sagasta et al., 2015) to 99.9% (Pescod, 1992) of domestic wastewater constitutes of water and the remaining percent include organic matters and faecal pathogens of major public health concern such as *E. coli, Salmonella* and *Shigella*. For instances the *E. coli* pathotypes (Diarrheagenic *E. coli*) are among the leading bacteria causing infections in human and animals including sepsis/meningitis, urinary tract infection (UTI) and diarrhoea. These pathotypes *E. coli* include enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvassive (EIEC), enterohaemorhagic (EHEC), enteroaggregative (EAEC) and diffusely adherent *E. coli* (DAEC) (Hussain, 2015; Jafari et al., 2012; Kaper et al., 2004).

Wastewater and sludge in developing countries including Sub-Saharan Africa is mainly collected from pit latrines, septic tanks and a limited amount is collected through centralized piped networks. Pit latrines are common in rural and unplanned urban and peri-urban areas (Mara, 2013). Feacal sludge from poorly constructed pit latrines and wastewater disposal system in areas with high water table or lowland contaminates ground water and often predisposing to human, animals and environmental health risks (Jiménez et al. 2010; Mwang'onde et al., 2013). In urban areas, full pit latrines and septic tanks are emptied; the sludge is collected by sanitation trucks and delivered to the designated areas on land or in ponds (Tilley et al., 2014; Mateo-sagasta et al., 2015). However, wastewater can either be treated or not treated prior to discharge to receiving water bodies (Mateo-sagasta et al., 2015). Generally, in areas where wastewater treatment ponds are not in place, untreated wastewater or sludge is applied on bare or agricultural land (Jiménez et al., 2010).

Centralized wastewater treatment systems include natural biological treatment systems such as man-made wastewater treatment (stabilization) ponds which are common in tropical and subtropical countries where land is not a compromising factor (Mara, 2003; Naddafi et al., 2009). The stabilization ponds are characterized by a primary treatment including screening solid wastes, in anaerobic pond (s) for ≥1 day, in facultative pond (s) for ≥7 days and in maturation pond (s) for ≥12 days and sometimes supplemented with storage or treatment reservoirs (Mara, 2013). Total retention time in well designed treatment ponds are about 20 to 40 days (Mara, 2000). The anaerobic and facultative ponds are designed to ensure the removal of biological oxygen demand (BOD5) for five days, while maturation ponds are designed to remove the excretal pathogenic bacteria from 3 to 6 log units (Mara, 2013; Hwang, 2012). In addition wetlands systems are as well used to supplement for further reduction of pathogens (Mthembu et al., 2013; Kipasika et al., 2016) and or disinfection of treated wastewater (Silva et al. 2013).

The advantages of stabilization ponds include limited technological investment, low cost, cheap/unskilled labour, and minimal maintenance costs (Mara, 2003; Jiménez et al., 2010). However, the main disadvantage is the limitation of land availability in urban areas (Jiménez, 2006). In developing countries, Sub-Saharan Africa, data on generation, treatment, maintenance and wastewater use are limited (Jiménez, 2006; Sato et al., 2013). Sato et al. (2013) reported that only 3 countries (Senegal, Seychelles and South Africa) out of 48 countries had complete data on generation, treatment and use of wastewater; while 13 countries had incomplete data and the other 32 countries including Tanzania had no data. On the other hand, farmers from urban and peri-urban areas in developing countries depend on low quality irrigation water (LQIW) which is often in form of untreated wastewater, partially treated and haphazardly blended wastewater/ polluted surface water, ground water and well water (Mateo-Sagasta et al., 2013).

A driver for wastewater use is that; wastewater contains valuable resources such as nutrients (for example, nitrogen and phosphorus), organic matters and energy (Mateo-sagasta et al., 2015). It is available all year round and usually is free or available at very low cost (Jiménezet al., 2008). Furthermore, if treated wastewater or sludge is applied in agriculture, it provides nutrients required for growth of the plant (Kołodziej et al., 2016; Antonkiewicz, 2014).

The value of wastewater use has been recognized by farmers worldwide. For example irrigation agriculture plays a dominant role in increasing crop yields and sustainability of production throughout the year (Babayan et al., 2012). For instance, in Tanzania wastewater is used for horticulture production, though the data on the generation, treatment and use are not available (Sato et al., 2013). Wastewater has been used in many parts of Tanzania such as Kilimanjaro and Arusha (Senzia et al., 2009; Mkali et al., 2014) as well as in Morogoro urban and peri- urban areas. However, use of untreated wastewater, partially or even treated wastewater in food production may pose health risks to farmers, traders and consumers (Jiménez et al., 2010).

Although faecal coliforms (FC) count is used to monitor faecal pollution regardless of drawbacks like the growth of other thermotolerant non-faecal organisms at the same temperature of 44°C, but also the *E. coli* remains a good indicator bacterium for faecal pollution (Edberg et al.,

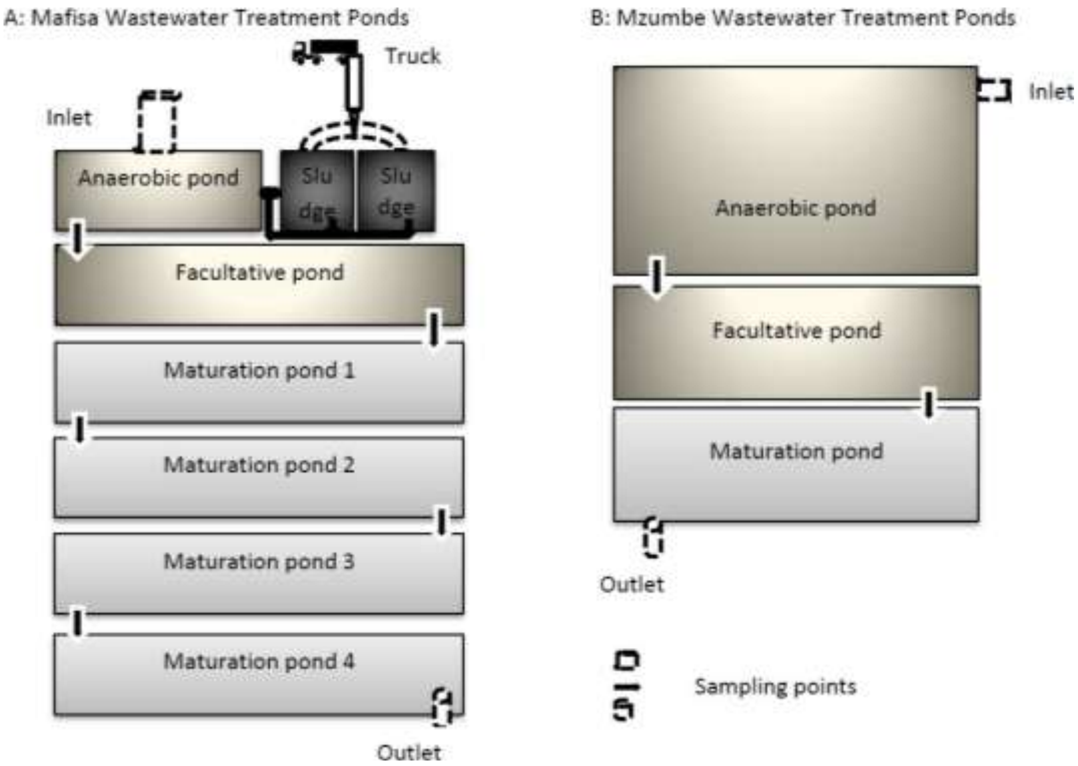

Figure 1. Schematic steps for Mafisa and Mzumbe wastewater treatment ponds.

2000; Okoh et al., 2007). Therefore, the present study was intended to assess the performance of wastewater treatment ponds in reducing the concentration of *E. coli* in treated wastewater to comply with the current WHO guidelines (WHO, 2006a; WHO, 2006b) and the Tanzania water quality standards (URT, 2007) for agricultural irrigation or aquaculture in urban and peri-urban areas of Morogoro.

MATERIALS AND METHODS

Study area

This study was conducted at Morogoro municipality, located at 06°49′20″S 037°39′55″E and Mzumbe is located at 06°53′29″S 37°33′37″E peri-urban of Mvomero district. According to the United Republic of Tanzania census of 2012 the Morogoro urban had about 320,000 inhabitants and Mvomero district had about 310,000 inhabitants. In Morogoro region, during March to August is usually the rainy and cold season with fewer daily hours of sunshine as compared to the dry season from September to February which is likely explaining the factors that may influence wastewater treatment ponds.

Study sites

Two wastewater treatment ponds units were selected; Morogoro urban water and sanitation authority (MORUWASA) - domestic wastewater treatment ponds unit (Mafisa) and Mzumbe wastewater treatment ponds (Figure1). Mafisa wastewater treatment unit consists of two ponds receiving faecal sludge delivered by sanitation trucks, a 9,000 m^3 anaerobic pond, a 12,000 m^3 facultative pond and four maturation ponds (10,000 m^3), all serial connected. Mafisa receives wastewater from residential areas, business areas, institutions and hospitals (Figure 1A). The sludge from sanitation trucks is pumped into the two small sludge ponds and then enters into an anaerobic pond which also receives wastewater from the municipality main sewer canal. The retention time in the Mafisa wastewater treatment unit is about 19 days. Treated wastewater is discharged through a concrete piped canal and passes through a wetland with about 20 acres of rice fields and is further used downstream for vegetable irrigation.

Mzumbe wastewater treatment system consists of an anaerobic pond (6,800 m^3), a facultative pond (2,400 m^3), and a maturation pond (1,600 m^3) serial connected with a retention time of about 22 days (Figure 1B). It serves about 10,000 people from university campus facilities, staff quarters and hospital. The quantity of received wastewater varies depending on the population of students and workers e.g. limited wastewater is generated during the periods of vacation and dry months (September to February) seasons. Treated wastewater is discharged through an earth canal and used downstream for vegetable irrigation. During dry season farmers compete on the limited available treated wastewater for irrigation and some farmers have to suspend growing vegetables.

Sample collection and handling

The purposive sampling technique was used. Samples were collected from the inlet and inlets-outlets of the anaerobic, facultative and maturation (final treated wastewater) ponds at the two study sites (Figure 1). In addition faecal sludge samples were collected from the trucks during emptying/delivering of the sludge from the trucks (Mafisa; Figure 1A). Sampling was done from

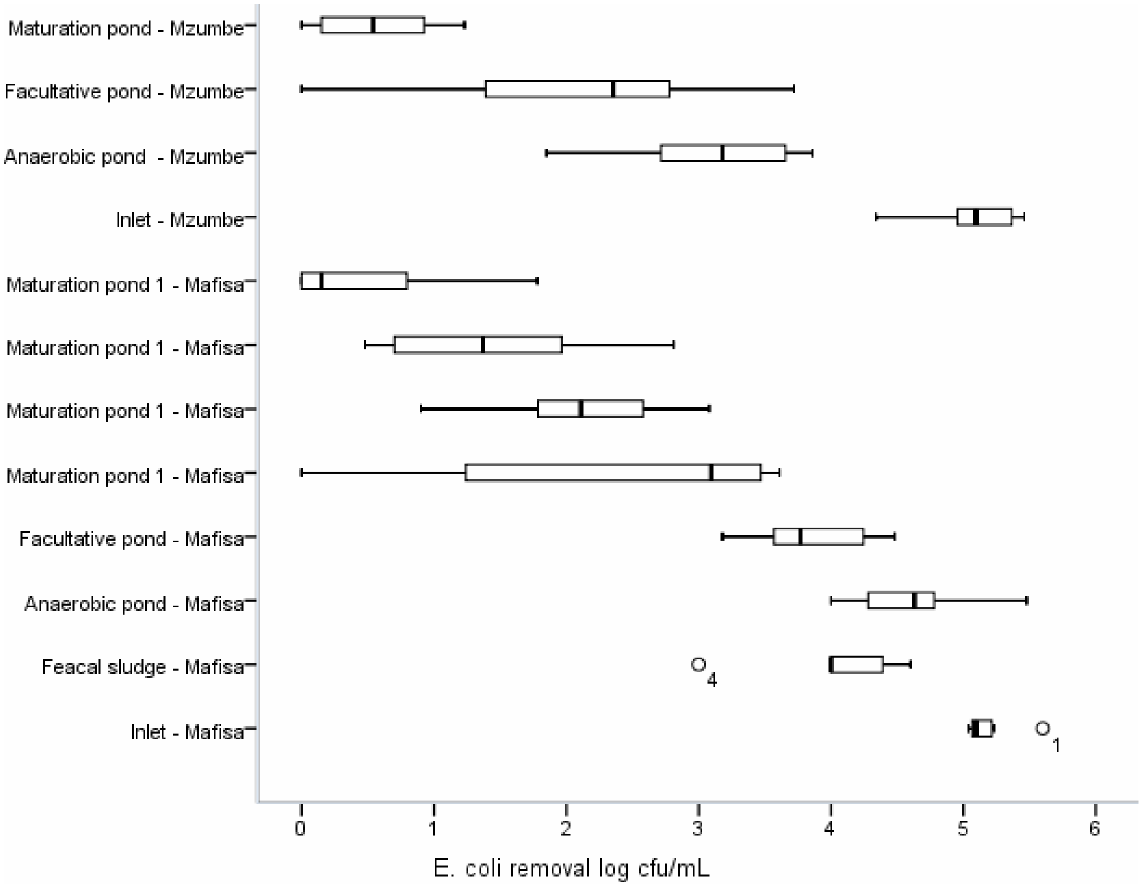

Figure 2. Reduction of concentration of *E. coli* at Mzumbe and Mafisa wastewater treatment ponds (O4 - and O1 - outliers).

August, 2013 to June, 2014 during dry (September to February) and rainy season (March to May). A total of 125 wastewater samples were collected during eleven sampling times. Eighty five wastewater samples were collected from Mafisa and 40 wastewater samples from Mzumbe wastewater treatment units. Wastewater samples were collected using sterile 250 mL glass bottles tied up with a rope. Sampling was done either in the morning or in the evening hours. Samples were immediately placed in an insulated box with cooling elements and transported to the Sokoine University of Agriculture in Morogoro for laboratory analysis on the same day, those were samples collected in the morning. While water samples collected during the evening were kept overnight in a refrigerator at 2 to 8°C prior the analysis on the following day.

Enumeration of *Escherichia coli*

Enumeration and isolation of *E. coli* was done using Petrifilm Select *E. coli* (SEC) plates (3M Microbiology Products, St. Paul, USA) as per manufacturer's instructions. In brief, one milliliter of sample from an appropriate serial 10 - fold dilutions in 0.1% buffered peptone water (BPW) (Oxoid Ltd, Hampshire, England) was inoculated onto SEC plates and incubated at 44°C for 24 h. All blue *E. coli* colonies on the SEC plates with entrapped gas, regardless of size or intensity of colour were counted and interpreted as *E. coli*. If there was no colony on the SEC plates, it was reported as less than 1 cfu/mL (detection limit) equivalent to 0 log cfu/mL.

Data analysis

The concentration of *E. coli* was calculated as colony forming units (cfu) per mL and transformed to $\log_{10}$ cfu/mL (log cfu/mL). Paired samples means of *E. coli* concentration in log cfu/mL between Mafisa and Mzumbe wastewater treatment units were analyzed by t-test using SPSS statistics 20.0 (IBM, California, USA) at $p<0.05$. Reduction of *E. coli* concentration in the different ponds was compared between the two study sites to ascertain if there were differences in removal efficiency of *E. coli*.

RESULTS AND DISCUSSION

Reduction of *E. coli*

The mean concentration of *E. coli* in untreated wastewater was 5.12 log cfu/mL and 5.08 log cfu/mL, which were reduced to 0.65 log cfu/mL and 0.55 log cfu/mL in treated wastewater at Mafisa and Mzumbe, respectively (Figure 2). The mean concentration of *E. coli* in faecal sludge from trucks at Mafisa was 4.05 log cfu/mL (Figure 2). The overall reduction of *E. coli* concentration in wastewater treatment ponds at the two

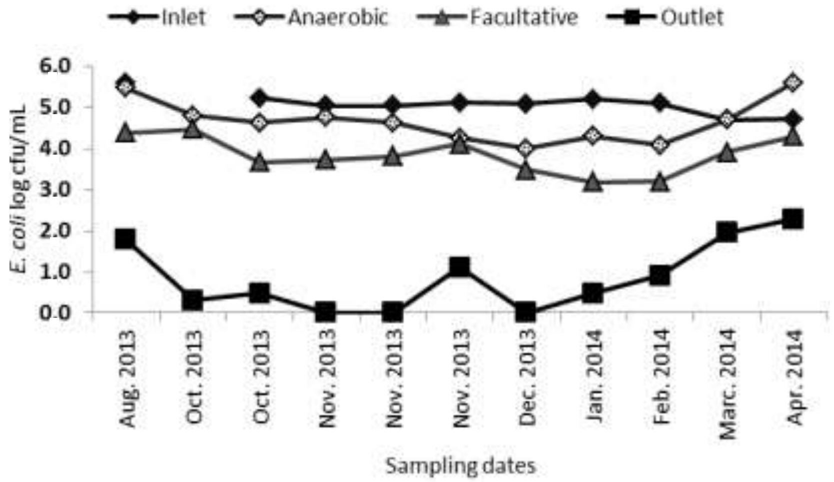

Figure 3. Effect of trend in concentration of *E. coli* at Mafisa wastewater treatment ponds.

study sites was approximately 4-log cfu/mL. The concentration of *E. coli* of about 5.0 log cfu/mL in untreated wastewater has been documented elsewhere (Hendricks and Pool, 2012; Farasat et al., 2012; George et al., 2007). Likewise a similar 4-log reduction of faecal indicators and pathogens was seen in a comparable wastewater treatment ponds systems treating municipal wastewater in Nigeria (Mohammed, 2006) and India (Tyagi et al., 2008). The removal of pathogenic bacteria in the biological wastewater treatment / wastewater stabilization- ponds has been reported as 3 to 6 log units (Jiménez et al., 2008).

Generally, wastewater stabilization ponds are low cost wastewater treatment systems and achieve high enteric pathogen removal in tropical and sub-tropical regions (Mara, 2003; Morris 2003). These systems are well-suited for developing tropical countries as they comprise of a simple technology which is easy to operate, maintain and often water is transported by gravity only. In contrast, modern or secondary and tertiary treatment technologies are unaffordable and complex to operate satisfactorily, thus requiring trained staff to operate, use chemicals, as well as maintenance and electricity is needed to pump and transport wastewater (Tilley et al., 2014). Consequently, such advanced treatment (Tertiary treatment) systems often break down and fail to remove faecal pathogens to acceptable levels allowing a safe use of wastewater in agriculture (Hwang, 2012; Jiménez et al., 2008).

The mean concentration of *E. coli* in untreated wastewater and treated wastewater at the two treatment systems was not different (P>0.05), while the E. coli reduction in the anaerobic and facultative ponds effluent was significantly higher at Mzumbe (P<0.01) (Figure 2). This was most likely due to longer retention times in the anaerobic pond (10 days), the facultative pond (6 days) and the maturation pond (6 days) at Mzumbe as compared to Mafisa anaerobic pond (2 days), facultative pond (4 days) and four maturation ponds (3, 3, 3 and 4 days). These findings are in agreement with the comparable studies elsewhere (Hwang, 2012; Mara, 2000). It is also well-established that retention time and pathogens (for example, *E. coli*) reductions in stabilization ponds are positively correlated (U. S. EPA, 2002). A similar reduction in *E. coli* concentration (<1 log cfu/mL) in effluent from the two study sites was due to an approximately 2-log reduction occurring in the four maturation ponds at Mafisa (Figure 2).

Maturation ponds generally show a high faecal indicator and pathogens reduction (Pescod, 1992; Hwang, 2012). A similar *E. coli* reduction (3.85±4.32 to 1.11 ±1.12- log *E. coli*) as seen in the present study was reported in a comparable wastewater treatment system in Thailand (Kantachote et al., 2009) consisting of an anaerobic pond (6 days), a facultative pond (9 days) and a maturation pond (4 days).

At Mafisa, the *E. coli* concentration in the wastewater treatment ponds was high in March, April and August (Figure 3). It should also be noted that rain events are so severe with extraordinary volumes of storm- and surface run-off water. However, no such seasonal variation was observed for *E. coli* concentration at Mzumbe which receives domestic wastewater and rain water only. There were no storm- and surface run-off water, contaminated with animal and human faeces entering to the wastewater treatment ponds (Figure 4). Generally, it is possible to increase environmental health risk if the receiving water bodies, flooding lowland crop/vegetables fields may be contaminated with faecal pathogens and may lead to the diarrheal diseases, such as, Cholerae outbreaks and typhoid fevers (Mwang'onde et al., 2013); and skin diseases (Trang et al., 2007), which is common when

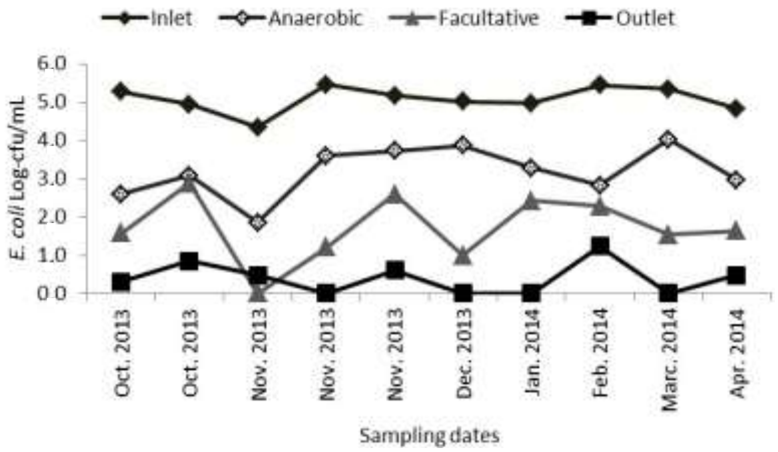

Figure 4. Effect of trend in concentration of *E. coli* at Mzumbe wastewater treatment ponds.

using untreated wastewater.

Use of treated wastewater for food production

The Mafisa and Mzumbe wastewater treatment units were able to reduce the *E. coli* concentration to less than 1 log cfu/mL a guideline value that is according to FAO (Pescod, 1992) and WHO guidelines (2006) allowing the treated wastewater to be used for unrestricted agricultural irrigation. Thus, the current agricultural use practices of the treated wastewater by the farmers at the two study sites would according to these guidelines not pose significant human health risks. Several cities and municipalities in Tanzania as well as other Sub-Saharan African countries have constructed wastewater treatment facilities similar to Mafisa and Mzumbe. Findings in this study thus demonstrate that, if the wastewater treatment ponds are well operated and maintained they have the capacity to effectively reduce faecal pathogens to a level where urban and peri-urban farmers can safely use treated wastewater to irrigate their crops that can feed the urban consumers (Senzia et al., 2009; Sato et al., 2013; Kulkarni, 2014). However, the *E. coli* concentrations seen at Mafisa in rainy and cold season during March, April and August where severe rain events occur with extraordinary volumes of storm - and surface runoff-water showed less reduction. This suggests that, even the treated wastewater may not be safe to use for unrestricted irrigation purposes, in particular if such events coincides with infectious disease outbreaks like dysentery or cholera. Specific health risk impact assessments are therefore, needed for such worst-case scenarios.

Conclusion

The findings from this study allow wastewater stakeholders to make informed decisions about the use of treated wastewater and the risk of contamination of downstream receiving water bodies and for use in food production. The performance of the two simple wastewater treatment units was satisfactory in reducing *E. coli* and potential risk from low quality irrigation water (e.g. treated wastewater) use during dry season. However, there were good estimated retention times of 19 and 22 days on both wastewater treatment ponds study sites. The concentration of *E. coli* was reduced to <1log cfu/mL, the level that is recommended by the current WHO guidelines (2006) for safe use in agricultural irrigation and aquaculture. Basing to the findings from the study sites on the quality of treated wastewater, it may be used for agricultural irrigation and aquaculture. The data generated from this study may contribute to the national and international policy and guidelines (e.g. FAO / WHO guidelines) with regard to the reduction of *E. coli* in wastewater and safe use of treated wastewater in agricultural irrigation and aquaculture.

Conflict of Interests

The authors have not declared any conflict of interests.

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