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Using Constructed Wetlands to Remove Pathogenic Parasites and Fecal Coliforms from Wastewater in Dar es Salaam and Iringa, Tanzania

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Abstract

Wastewater treatment is a widely used health protection measure that can be applied to control the transmission of wastewater-related infectious diseases to communities exposed to wastewater. This study determined the efficiency of three full-scale constructed wetlands (CWs) in removing pathogenic parasites and fecal coliform (FC) bacteria from wastewater. Wastewater samples were collected from three CW systems located in the Dar es Salaam and Iringa regions of Tanzania. The modified Bailenger and modified Ziehl-Neelsen stain techniques were used to detect and quantify parasites. The membrane filtration method was used to detect and quantify FC bacteria. Data were analysed using IBM SPSS version 20. Helminth (*Ascaris lumbricoides*, hookworm, and *Taenia* spp.) eggs were completely removed by two CW systems. In all the systems, the removal of protozoa ranged from 99.8% to 100%. The mean concentrations of FCs in effluents ranged from 5 to 6 log units/100 mL. Effluents of all CW systems met the recommended parasitological quality requirements of the World Health Organization for the safe reuse of wastewater. FC effluents concentrations did not meet the local discharge standards of the Tanzania Bureau of Standards. Therefore, improvement to the CWs' design, operation, and maintenance are required for the efficient removal of bacteria.

Keywords: helminth, protozoa, fecal coliform, wastewater treatment, constructed wetland.

Introduction

Reuse or discharge of wastewater that is of low quality in terms of parasitological and microbiological parameters poses serious health risks to humans and animals (Sharafi et al. 2012). Apart from the transmission of pathogenic bacteria, another major public health concern about the use of wastewater is the transmission of parasitic diseases (Stott 2003). Tanzania has experienced sanitation- and health-related problems such as cholera outbreaks caused by poor sanitation services (Controller and Auditor General 2018). Wastewater treatment is a widely used measure to control the risks of disease transmission to people and animals exposed to wastewater and wastewater products (Pescod 1992).

Constructed wetlands (CWs) are an emerging technology that has been employed to treat wastewater from both point sources (domestic, municipal, and industrial sources) and nonpoint sources (agriculture, mines, and storm runoff) (Kimwaga et al. 2013, Mahmood et al. 2013). Different types of CWs are effectively used as an additional step in the treatment of primary, secondary, or tertiary treated wastewater (Kayombo et al. 2004). In Tanzania, CW technology began to be used in 1998, and applications have tended to increase over the years. Horizontal subsurface flow (HSS) and free water surface flow (FWS) are types of CW systems used in Tanzania, with HSS being the most commonly applied (Ahmada et al. 2018). CW systems are usually integrated with other treatment systems such as waste stabilization ponds (WSPs) and septic tanks, and therefore are most often used as secondary treatment systems. Effluents from these systems are used for various purposes such as agricultural irrigation and brickmaking, and as water sources for livestock (Outwater et al. 2013, Kihila et al. 2014). According to the World Health Organization (WHO) guidelines on the safe reuse of wastewater, monitoring of wastewater treatment is important to ensure that the system achieves specified standards and ultimately the health-based target. The following effluent standards have to be attained by the treatment system for safe reuse: ≤ 1 egg/L for helminths and 2–4 log reduction for other pathogens (WHO 2006). Moreover, the Tanzania Bureau of Standards (TBS) has set a wastewater discharge limit of 10,000 (4 log unit) colony-forming units (cfu)/100 mL for coliform bacteria (TBS 2005).

Many studies have documented the performance of CW systems in removing physicochemical pollutants. However, information is lacking on how efficiently they remove pathogenic organisms such as parasites and bacteria, as few parasitological and microbiological studies of that question have been conducted (Redder 2010). Apart from the limited availability of information on the efficiency of removal of parasites and bacteria by CW systems, there is also a lack of data on full-scale CW systems monitoring, as most of the available data are based on experimental systems, an understandable limitation given the nascent state of CW technology (Mbuligwe 2011). Insufficient information on the parasitological and microbiological qualities of full-scale CW effluents potentially endangers the health of people and animals exposed to effluents and effluent-produced products.

The main objective of the present study was to determine how efficiently three full-scale CW systems treating domestic and municipal wastewater effluents removed pathogenic parasites and fecal coliform (FC) indicator bacteria from secondary schools' septic tanks and municipal WSP systems in the Dar es Salaam and Iringa Regions of Tanzania.

Materials and Methods

Study design

This was a cross-sectional study conducted from February to August 2018.

Study sites

St. Anthony Secondary School constructed wetland: The St. Anthony Secondary School CW system is located in Mbagala, a ward on the south side of Dar es Salaam region (6°53'49.82" S, 39°16'17.46" E). The system has two HSS CW cells operating in parallel and is used to treat wastewater generated from student hostels and pre-treated by the septic tank system. Effluents from this system are drained and collected to an underground storage tank, then used for gardening. At the time of the present study, the system was planted with *Phragmites mauritianus* (a perennial reed grass; Figure 1) and used gravel media as substrates.

Figure 1: Part of the St. Anthony Secondary School constructed wetland (photo by first author).

Ruaha Secondary School constructed wetland: Ruaha Secondary School CW system is located in Ipogolo, Iringa Region (7°48'09.15"S 35°40'45.09"E). The system has two HSS CW cells operating in parallel and is used to treat wastewater generated by the school community. The two cells receive wastewater from the septic tank system. Effluents from this system are collected by local residents for irrigation farming. The system was planted with *Phragmites mauritianus* and gravels were used as substrates: however, the plants became dominated by intruder plants.

Iringa Municipal constructed wetland: Iringa Municipal CW is located at the Iringa municipal wastewater treatment plant (7°45'31.47"S 35°40'12.34"E), at Don Bosco area in Iringa Region. The system is a FWS CW type; it is used to polish wastewater effluents from the last maturation pond of the Iringa municipal WSP system. The system consists of two parallel wetland cells with several combinations of wetland plant species, including *Phragmites* spp., *Typha* spp and *Cyperus* spp. The effluent is discharged to a nearby stream and used by local residents for irrigation and brickmaking.

Wastewater samples collection and transportation

Wastewater samples were collected in order to test for parasites and FC bacteria. Briefly, for parasitological examination, 10 L of wastewater were collected at each sampling point and allowed to settle for 2 hours before collecting 1 L of sediment. At each sampling point, 250 mL of wastewater were collected in a sterile glass container for FC bacteria analysis. Samples were collected at monthly intervals for a total of 4–5 months at each site (4–5 visits per site).

During each site visit, four samples (two for parasitological analysis and two for FC bacteria analysis) were collected: two at the inlet of the system and two at an outlet. Over the course of the study, 56 wastewater samples were collected from all three CW systems. Of these, 28 samples were collected for parasitological analysis and 28 samples were collected for FC bacteria analysis. A total of 28 samples were collected from the inlet of all the three CW systems (14 for parasitological and 14 for FC bacteria). In addition, the same numbers of wastewater samples were collected from the outlets of each of the three CW systems. Samples for bacterial tests were transported to the University of Dar es Salaam Water Resources Laboratory; those for parasitological tests were transported to the Muhimbili University of Health and Allied Sciences Parasitology Laboratory in an ice box 6–36 hours after collection. All samples were analysed immediately upon arrival at the laboratory.

Wastewater analysis

Two methods were used to analyse the parasites: the modified Bailenger method and the modified Ziehl-Neelsen stain (Ayres and Mara 1996, Cheesbrough 2009). FC bacteria were processed by using the membrane filtration method (Ayres and Mara 1996).

Modified Bailenger Method: Briefly, each 1 L sample was centrifuged at 1,000×g for 15 min. The pellet was suspended in an equal volume of acetoacetic buffer, pH 4.5. Then, two volumes of ether were added and the sample was mixed for 10 min. The sample was then centrifuged at 1,000×g for 15 min. After the volume of the pellet was recorded, it was resuspended in five volumes of zinc sulfate solution (gravity 1.3) and mixed thoroughly. Immediately, 0.15 mL was transferred to a slide for microscopic counting with a x40 objective.

Modified Ziehl-Neelsen Stain: A smear was prepared using 0.05 mL of each modified Bailenger–processed sample, air-dried and fixed in methanol for 3 min. The methanol-fixed smear was stained with cold strong carbol-fuchsin for 15 min, then rinsed with water and decolourised with a 1% acid-alcohol for 10–15 s. The slide was rinsed with water and counterstained with 0.3% malachite green for 30 s, after which it was rinsed in distilled water. The slide was allowed to air-dry, then was examined under the microscope with a x100 oil immersion objective. For both procedures, the number of eggs or (oo)cysts/L of wastewater was calculated by using the following equation:

$$N = \frac{ax}{pv} \qquad (1)$$

where: *N* is the number of parasites' eggs or (oo)cysts per litre of wastewater, *a* is the number of eggs or (oo)cysts counted, *x* is the volume of the final product in mL, *p* is the volume examined in 0.15 mL or 0.05 mL, and *v* is the original sample volume in litres.

Membrane filtration method: Briefly, 1 mL of wastewater sample was diluted several times by adding sterile distilled water. The sample was vacuum filtered through a membrane filter. The membrane filter was placed on a petri dish containing 45 mm absorbent pads with m-fecal broth; then, the lead was put on the petri dish and the petri dish was inverted. The petri dish was incubated at 44.5 °C for 24 hours. After 24 hours, FC colonies were counted and the results were recorded in terms of cfu/100 mL.

Data analysis

Statistical analysis was performed with statistical software IBM SPSS version 20. Data for FC concentrations were transformed from their reported form of cfu/100 mL to log concentration (log units/100 mL). Log reduction of FC was calculated by substituting an average inlet log concentration for the average outlet log concentration. Percentage reductions of parasites by a system were computed by using the following equation:

$$p = \frac{(\mu i - \mu o) \times 100}{\mu i} \qquad (2)$$

where: *p* is the percentage reduction, μi is the mean concentration of the inlet samples, and μo is the mean concentration of outlet samples.

The Shapiro-Wilk test was used to test for normality of the data, which were considered normally distributed at $p > 0.05$. One-way ANOVA, Kruskal-Wallis, and Mann-Whitney U tests were conducted to make comparisons of the means and medians of the concentrations of parasites (protozoa and helminth) and FC concentrations between the influents and effluents of the three treatment systems. When the one-way ANOVA result was significant, the Tukey post hoc test was used to determine the differences between specific means of compared groups.

Results

Out of the 28 wastewater samples analysed for the detection of parasites, eight (28.6%) showed positive results. Of the eight positive samples, three had been collected at the inlet of the St. Anthony Secondary School CW, three at an inlet of the Iringa municipal CW, and one at an inlet and one at an outlet of the Ruaha Secondary School CW. Seven parasite species were recovered. Three were helminths, *Ascaris lumbricoides*, hookworm, and *Taenia* spp., and four were protozoa, *Entamoeba coli*, *Entamoeba histolytica/dispar*, *Giardia lamblia*, and

Cryptosporidium spp. Among the positive samples, helminths were detected in only two, while protozoa were found in all eight samples. *Entamoeba coli* was the most frequently identified parasite, detected in seven of the eight positive samples, followed by *Giardia lamblia* and *Cryptosporidium* spp., which were identified in three of the eight positive samples. Each of the remaining parasites was found in only one sample. No helminth eggs were detected in either influent or effluent samples from the Ruaha CW system.

When all the samples were combined, a Mann-Whitney U test indicated that the parasite concentrations were significantly greater for protozoa than for helminths, $U = 308$, $p = 0.039$. The mean concentrations of protozoa (oo)cysts and helminth eggs in the influents of all the treatment systems are shown in Table 1. Protozoa mean concentration was highest in the influents of the St. Anthony Secondary School CW, followed by the Ruaha Secondary School CW. The highest mean concentrations of helminth eggs were detected in the Iringa municipal CW. A Kruskal-Wallis test showed that there was no statistically significant difference in influent protozoa concentrations between the different CW systems, $H(2) = 0.987$, $p = 0.61$: The mean ranks were 8.8 for the St. Anthony CW, 6.25 for the Iringa CW, and 7.2 for the Ruaha CW. The Kruskal-Wallis test also indicated that there were no statistically significant differences in influent helminth concentrations between the different CW systems, $H(2) = 1.31$, $p = 0.52$: The mean ranks were 7.8 for the St. Anthony CW, 8.38 for the Iringa CW, and 6.5 for the Ruaha CW system.

Table 1: Protozoa and helminth concentrations in influents (inlets) of the three constructed wetland systems

Constructed wetland	*n*	Protozoa, (oo)cysts/L				Helminths, eggs/L			
		Min	Max	Mean	Median	Min	Max	Mean	Median
St. Anthony Secondary School	5	0.0	120.0	52.0	40.0	0.0	20.0	4.0	0.0
Iringa treatment plant	4	0.0	3.0	1.0	0.5	0.0	31.0	7.8	0.0
Ruaha Secondary School	5	0.0	214.0	45.8	0.0	0.0	0.0	0.0	0.0

Notes: n = number of samples, Min = minimum, Max = maximum.

The St. Anthony Secondary School and Iringa municipal CWs removed 100% of both protozoa and helminth parasites from their influents. As there were no helminths detected in influents and effluents of the Ruaha Secondary School CW, determination of removal efficiency was not possible. Effluents of the Ruaha CW had a mean protozoa (*Cryptosporidium* spp.) concentration of 0.1 oocysts/L, equivalent to a 99.8% reduction of all protozoa (oo)cysts detected in the influent samples.

Summarized statistics for the FC log concentrations in the influent samples of the three CW systems are provided in Table 2. There were statistically significant differences in FC log concentrations between the influent samples of the three CW systems as determined by one-way ANOVA, $F(2, 11) = 4.158$, $p = 0.045$. A Tukey post hoc test revealed that the FC log concentrations were significantly higher in influent samples of the St. Anthony Secondary School CW (8.1 log units/100 mL, $p = 0.037$) than in the Iringa municipal CW (6.1 log units/100 mL). There was no significant difference between FC log concentrations in the influents of the St. Anthony and Ruaha CW systems ($p = 0.429$), or between the influents of the Ruaha and Iringa system ($p = 0.261$).

Table 2: Log concentrations of fecal coliforms in influents (inlets) of the three constructed wetland systems

Constructed wetland	*n*	Fecal coliforms (log units/100mL)				Tukey post hoc test		
		Min	Max	Mean	Median	St. Anthony	Iringa	Ruaha
St. Anthony Secondary School	5	6.7	9.9	8.1	7.9		*s*	*ns*
Iringa municipal system	4	5.0	7.2	6.1	6.0	*s*		*ns*
Ruaha Secondary School	5	5.8	8.1	7.3	7.5	*ns*	*ns*	

Notes: n = number of samples, Min = minimum, Max = maximum.

Figure 2 displays the mean FC log concentrations of effluent samples from each CW system. Effluent FC log concentrations ranged from 5.1 log units/100 mL in the Iringa municipal CW to 6 log units/100 mL in the St. Anthony Secondary School CW. The one-way ANOVA indicated that the observed difference in effluent FC log concentrations between the three CW systems was not statistically significant, $F(2, 11) = 1.947$, $p = 0.189$.

The FC removal efficiency of each treatment system is presented in Table 3. The one-way ANOVA showed a statistically significant difference in FC log reduction, $F(2, 11) = 5.274$, $p = 0.025$. A Tukey post hoc test showed that there was strong evidence that FC log reduction was higher in the St. Anthony Secondary School CW (2.13 log units/100 mL, $p = 0.033$) and Ruaha Secondary School CW (2.07 log units/100 mL, $p = 0.043$) than in the Iringa municipal CW (0.98 log units/100 mL). There was no significant difference in FC log reduction between the St. Anthony and Ruaha CWs ($p = 0.986$).

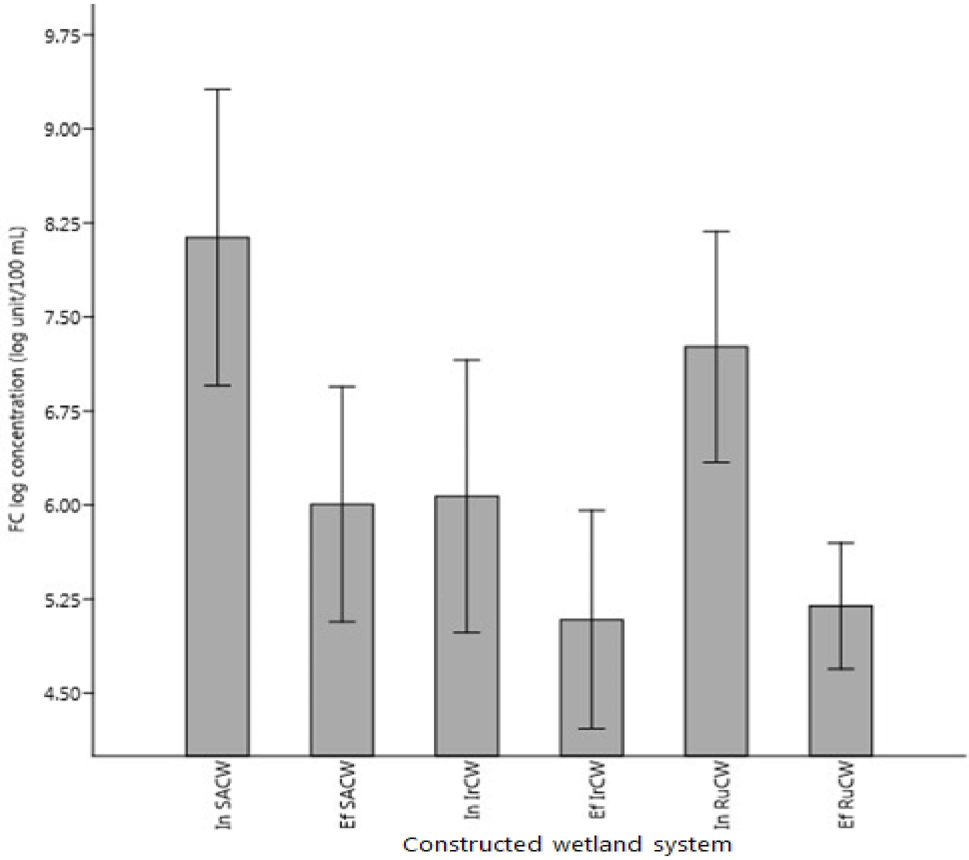

Figure 2: Log concentrations of fecal coliform in each constructed wetland system. *Note:* In SACW and Ef SACW indicate, respectively, influent and effluent of St. Anthony Secondary School constructed wetland. In IrCW and Ef IrCW indicate, respectively, influent and effluent of Iringa Municipal constructed wetland. In RuCW and Ef RuCW indicate, respectively, influent and effluent of Ruaha Secondary School constructed wetland.

Table 3: Fecal coliforms removal efficiency of the three constructed wetland systems

Constructed wetland		Fecal coliform reduction (log units/100 mL)				Tukey post hoc test		
	n	Min	Max	Mean	Median	St. Anthony	Iringa	Ruaha
St. Anthony Secondary School	10	1.55	2.61	2.13	2.24		*s*	*ns*
Iringa municipal system	8	0.10	1.39	0.98	1.23	*s*		*s*
Ruaha Secondary School	10	1.08	2.80	2.07	1.97	*ns*	*s*	

Notes: n = number of samples, Min = minimum, Max = maximum.

Discussion

The primary health hazards associated with the use of wastewater in agriculture and aquaculture include excreta-related pathogenic organisms (WHO 2006). Pathogenic parasites are considered wastewater-associated pathogens of great public health importance due principally to their environmentally persistent transmissive stages, a low infective dose, and limited or transient acquired immunity and morbidity, particularly in immunocompromised hosts (Stott 2003). In the present study, protozoa were identified more frequently and at higher concentrations than helminths. Protozoa (oo)cysts predominated compared to helminth eggs, perhaps due to their ubiquitous presence and higher prevalence in wastewater (Zacharia et al. 2018). Other possible reasons are the higher amount of sedimentation of helminths eggs than of protozoa (oo)cysts in both types of pre-treatment systems (septic tank and WSP), lower prevalence of helminths in the sewered communities possibly resulting from an ongoing anthelminthic campaign (national mass drug administration) conducted by the national neglected tropical diseases control program, and easy accessibility of anthelminthic drugs. In addition, epidemiological studies conducted in Tanzania have revealed more than 50% prevalence of *Entamoeba coli* cyst in school-age children (Speich et al. 2013, Venkatajothi 2017). The high prevalence of *Entamoeba coli* may also have contributed to the high concentrations of protozoa in wastewater.

Entamoeba coli was the most frequent identified among the seven detected parasite species. This protozoan parasite is a non-pathogenic species of *Entamoeba* that frequently exists as a commensal parasite in the human gastrointestinal tract. We have reported on this parasite because in medicine, *Entamoeba coli* can be confused during microscopic diagnosis with the pathogenic *Entamoeba histolytica*. Moreover, when this protozoan is detected in domestic wastewater, it is an indication of unhygienic conditions in the sewered community whereby people consume fecally contaminated products that present the possibility that pathogenic organisms will be consumed at the same time (Issa 2014).

Influent of the Iringa municipal CW (WSP effluent) was observed to have lower concentrations of protozoa parasites than the influents of the St. Anthony and Ruaha Secondary School CWs (septic tank effluents). The low protozoa concentrations in the Iringa CW influent possibly are attributable to good performance of the discharging WSP system in comparison to septic tanks, or might also be attributed to lower protozoa concentrations in WSP influents than in septic tank influents. Helminth concentrations were higher in the effluents of discharging WSP system than in that of discharging septic tanks. However, there was a possibility of onsite contamination in the WSP system, as no helminth presence was detected in the influents of maturation ponds discharging to CW system during the study period (Zacharia et al. 2019).

The three CW systems removed both protozoa (99.8%–100%) and helminths (100%) to the level of minimum or no risk of parasitic diseases to the wastewater users and the community. Similar performance was observed in a study in Iran, where it was found that 100% of protozoa and 99.7% of helminths were removed by CW systems (Sharafi et al. 2015). However, other researchers have reported less effective performance of CWs in removing parasites. Studies conducted in Brazil and Spain found that effluents of HSS-CWs, FWS-CWs, and a combination of an HSS-CW and vertical subsurface flow CW contained more than one helminth egg/L (Bastos et al. 2010, Garcia et al. 2013). In a study conducted in the United States, it was found that both HSS-CW and FWS-CW systems removed less than 90% of protozoa (*Giardia* spp. and *Cryptosporidium* spp.), with effluent concentrations being found of up to 7.4 oocysts/L (Gerba et al. 1999).

In the present study, filtration and sedimentation were hypothesised to be the main parasite (helminths and protozoa) removal mechanisms in HSS-CW and FWS-

CW systems, respectively. The effectiveness of both mechanisms depends on the size and density of the removed organisms (parasites), with larger ones being removed more efficiently than smaller ones (Maiga et al. 2017). In this study, among the seven parasites species detected in influent samples of all the systems, *Cryptosporidium* spp. was detected only in one effluent sample, from the Ruaha Secondary School CW, which has an HSS design. Judging from data presented in other studies, *Cryptosporidium* spp. appears to be the smallest parasite, with lower settling velocity, among those identified in the present study (Feachem et al. 1981, Mara 2003). However, the concentration detected in the effluents of the Ruaha CW was very low (0.1 oocysts/L).

FC bacteria indicate the presence of fecal material from warm-blooded animals, and these bacteria display a survival pattern similar to that of the pathogenic bacteria (Bitton 2005). As was the case for protozoa parasites, FC concentrations were higher in the influents of the St. Anthony and Ruaha Secondary School CWs (septic tank effluents) than in the influents of the Iringa municipal CW (WSP effluent). The studied septic tank effluents had FC concentrations within the range of the summarized results for other septic tank effluents (7.0–7.3 log units/100 mL) in Tanzania (Mbuligwe et al. 2011). The effluent of WSP in the present study had higher FC concentrations than the 3 log units/100 mL reported in a WSP in the city of Moshi, Tanzania, and slightly lower concentrations than the 6.9 log units/100 mL detected in a WSP in Lugalo, Tanzania (Kaseva et al. 2008, Kihila et al. 2014). All in all, the FC contents of effluents we studied in septic tank and WSP systems required further treatment before reuse or discharge into the environment.

HSS-CW systems performed better in removing FC bacteria than FWS-CW systems. Both the St. Anthony and Ruaha Secondary Schools CWs (both HSS-CWs) removed FC bacteria by approximately 2 log units/100 mL, while the Iringa municipal CW (FWS-CW) removed FC bacteria at a rate of approximately 1 log unit/100 mL. FC log reductions in these systems achieved the expected levels for both HSS-CW and FWS-CW systems (Maiga et al. 2017). However, in research conducted in Brazil, a reduction of up to 4 log units of FC by the two types of CW was obtained (Bastos et al. 2010). In CW systems, physical contact of FC with wetland substrates and/or plant roots may be the primary mechanism of removal (Gerba et al. 1999). The significantly higher FC reduction achieved by the St. Anthony and Ruaha CWs may be attributable to increased physical contact and filtration as result of the use of filled gravel media and rooted plants, a practice unlike that of the Iringa CW, which only had rooted plants.

Despite greater reductions in FC in the St. Anthony and Ruaha Secondary School CWs relative to the Iringa municipal CW, all the three systems produced effluents with higher FC concentrations than the required TBS discharge standard limit (TBS 2005). Moreover, FC concentrations detected in the effluents of the three CW systems were higher than the 3.7 log units/100 mL reported in a fully operating HSS-CW in the city of Moshi (Kihila et al. 2014). However, according to WHO, wastewater with FC concentrations between 4 and 5 log units may, with regular monitoring, be used in some unrestricted and restricted agricultural irrigation activities. The WHO guidance implies that effluents from the three CW systems could be applied in restricted circumstances in the cultivation by drip irrigation of high-growing-plants. This restricted irrigation should be integrated with other health protection measures such as hygiene promotion—for example, washing and cooking produce before consumption (WHO 2006). Wetland plant coverage is one of the most important factors in the removal of pathogens and indicator bacteria in CW systems (Hogan et al. 2013, Wu et al. 2016). Performance of assessed CW systems may be improved by adding more plants in areas without them and removing intruder plants.

Conclusion

In general, the three assessed CW systems produced effluents that met parasitological quality standards as described in the WHO 2006 guideline. Therefore, these three CWs proved to be important facilities for the control of wastewater-associated pathogenic parasites transmission. However, FC concentrations in the effluents of the three assessed systems did not meet the local discharge standards as called for by the TBS. Therefore, further improvements to the CW systems' design, operation, and maintenance are required for more efficient removal of bacteria.

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Conflict of interest
The authors confirm that this work has no conflicts of interest.

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