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Removal capacity of faecal pathogens from wastewater by four wetland vegetation: *Typha latifolia, Cyperus papyrus, Cyperus alternifolius* and *Phragmites australis*

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The ability of four wetland vegetation: *Typha latifolia, Cyperus papyrus, Cyperus alternifolius* and *Phragmites mauritianus* in removing pathogenic and indicator microorganisms in the wetlands were studied in bucket experiments. The findings suggested that vegetated systems can effectively reduce faecal pathogens in wastewater. Both *Salmonella* species and *Escherichia coli* removal efficiencies were above 98%. This proved the positive use of plants in bacteria removal from wastewater. Nevertheless, removal of faecal bacteria differed significantly between macrophytes where *C. alternifolius* and *T. latifolia* were the most effective followed by *C. papyrus* and the least was *P. mauritianus*. The study also observed no significant difference between planted and unplanted buckets. The effect of physicochemical parameters such as dissolved oxygen, pH, temperature and salinity were thought to influence the bacterial removal.

Key words: Constructed wetland, *Typha latifolia*, *Cyperus papyrus*, *Cyperus alternifolius*, *Phragmites mauritianus*, *Salmonella* species, *Escherichia coli*.

INTRODUCTION

Macrophytes play an important role in maintaining the wetland ecosystem. They have the capacity to improve water quality by removing faecal pathogens present-in with wastewater. The influence is principally explained by supply of oxygen to the roots, which plays a crucial role in the activity and type of metabolism performed by microorganisms in the root zone (Stottmeister et al., 2003), especially the grazing predators like protozoan, nematodes and zooplankton and lytic bacteria and viruses (Vymazal, 2005). Another potential source of removal is the adsorption by bio-films on the rock media and plant roots (Stevik et al., 2004; Stott and Tanner, 2005). Macrophytes also affect faecal pathogens by excreting toxic antimicrobial substances from their roots (Sundaravadivel and Vigneswaran, 2001; Stottmeister et al., 2003). The roots which grow vertically and horizontally favour the removal by enhancing hydraulic pathways and increase the contact time (Vymazal et al., 1998; Stottmeister et al., 2003). Other mechanisms reducing microbial contaminants in vegetated systems are natural

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Table 1. Characteristic of the selected macrophytes and their use in CWs of Sub-Saharan Africa.

Macrophytes	Common name	General characteristic	Maximum water depth	pH tolerance	% reduction of faecal indicator bacteria	Use in CWs of sub-Saharan Africa
Typha latifolia	Cattail	Grow aggressively with large biomass	30 cm (Reed, 1993)	3.0-8.5 (Davis, 1995)	91% (Mashauri et al., 2000)	Tanzania (Mashauri et al., 2000; Njau et al., 2011).
Cyperus papyrus		Unique due to its C_4 photosynthetic pathway (Mnaya et al., 2007)	-	-	>98% (Kansiime and Mwesigye, 2012; Abou-Elela et al., 2014)	Kenya (Vymazal, 2013); Ethiopia (Tadesse, 2010); Uganda (Okurut et al., 1999; Kansiime and Mwesigye, 2012).
Cyperus alternifolius	Umbrella sedge	-Strong-growing rhizomes which quickly establish a large clump -Positive visual impact	-	-	90% (Leto et al., 2013)	-
Phragmites mauritianus	Reed	-Highly invasive with poor wildlife value -Not recommended for storm-water wetlands (Davis, 1995)	60 cm (Reed, 1993)	3.7- 8.0 (Davis, 1995)	>96% (Reinoso et al., 2008; Abou-Elela et al., 2014)	Sudan (Vymazal, 2013); Tanzania (Njau et al., 2011; Mairi et al., 2013)

temperature, unfavourable pH, and presence of toxic chemicals (Vymazal et al., 1998; Stevik et al., 2004) and sedimentation (Stott, 2003) as well as pathogen-sediments interaction (Searcy et al., 2006). Removal may also depend on water type and salinity which significantly affect pathogens settling (Hogan et al., 2013).

The contribution of UV light may not be effective in some wetlands due to shadowing effects of full growing macrophytes which protect pathogens from effective exposure (Naja and Volesky, 2011) or presence of substrate in case of sub-surface wetland systems.

Nevertheless, various studies have demonstrated that the effect of macrophytes on pathogen reduction may be irrelevant when compared with unplanted beds (Sleytr et al., 2007; Mburu et al., 2008; Torrens et al., 2009). The comparative effect brought by unplanted system was described by a tracer test study from Torrens et al. (2009), who discovered there was enough oxygen transfer in unplanted system facilitated by batch loading and diffusion process from the air. Although, the discovery provides clues on observed differences, which earlier sought to be unclear, yet the information is insufficient, because other factors such as macrophyte type and system design might also contribute. Macrophytes differ and their efficiency on pollutant removal is not similar; varying across plant species and with plant phenology (Fu et al., 2002). As reviewed by Faulwetter et al. (2009), root oxygen release and the diversity of the rhizosphere microbial community differs according to macrophyte species and on environmental conditions. Hogan et al. (2013) cited that different aquatic plants may vary in the ability to remove parasites due to distinct surface properties, unique biofilms, and differential effects on water flow and drag. Therefore, the choice of the macrophytes is of particularly important aspect in the design of a constructed wetland. The choice should include several factors, such as geographical distribution, climate and habitat conditions, wastewater composition, availability of the plants, long term maintenance, agronomic management costs, and the project aims (Leto et al., 2013).

In this study, four types of macrophytes: *Typha latifolia, Cyperus papyrus, Cyperus alternifolius* and *Phragmites mauritianus* commonly occurring in natural wetlands of Tanzania were selected to evaluate how vegetation type affects the removal of faecal indicator bacteria and pathogens under controlled laboratory conditions.

The evaluated plants are capable of surviving and proliferating in extreme tropical climates (Katsenovich et al., 2009). Characteristics and the use of the selected macrophytes in constructed wetlands (CWs) of sub-Saharan Africa are described in Table 1.

METHODOLOGY

Experimental site and design

The set-up was conducted at the Nelson Mandela African

Figure 1. Buckets experimental set-up with various macrophytes at NM-AIST, Tanzania.

Institution of Science and Technology (NM-AIST) in Arusha, Tanzania. The area is at an altitude of 1,400 m above sea level on the slopes of Mount Meru (Latitude 03° 24' S, Longitude 036° 47' E). The region is characterized with distinct wet and dry season and cool, dry air for much of the year. The temperature ranges between 13 and 30°C with an average of around 25°. The experiment was performed in a greenhouse with an area of 120 m^2. Inside the greenhouse, the temperature was averaged 27°C during the study period. Fifteen circular buckets made of plastic (PVC), with dimension 80 cm long and 54 diameter were filled with graded gravel, granite type, with size 12 to 20 mm to cover a depth of 60 cm. All the gravel was thoroughly washed with tap water to remove silt and debris before use. The porosity of the media was 0.35. Twelve buckets were planted with four wetland-macrophytes, each in triplicates, and three buckets were left unplanted to stand as controls.

Planting and macrophytes growing

Four types of wetland vegetation were studied: *T. latifolia, C. papyrus, C. alternifolius* and *P. mauritianus* (Figure 1). These plants were preferred based on the criteria of locally and widely distributed, easily propagated and able to tolerate waterlogged-anoxic and hyper-eutrophic conditions (Thomas et al., 1995). The plants were transferred from the surrounding natural marshes and planted on the same day in the buckets. Each plant composed of a piece of rhizome cut into lengths of 8 inches (20 cm) or two to three nodes. The cuts were evenly spaced in the buckets at densities of 4, 4, 4 and 8 cuts per bucket for *T. latifolia, C. papyrus*, *C. alternifolius* and *P. mauritianus*, respectively. After planting, the buckets were filled and kept in irrigation with hydroponic nutrient solution (Hoagland) to almost 10 cm beneath the gravel layer. The systems were emptied and refilled with the new nutrient solution once a week. Six months later, before sampling, tape water replaced the hydroponic nutrient solution as the medium solution.

Organisms

Escherichia coli (strain K-12) and *Salmonella enteric Serovar Typhimurium* labelled with green fluorescent protein (GFP) were used in this study. Both *E. coli* and *GFP Salmonella* were prepared by incubating broth with isolated colony on rotating shaker at 37°C, 120 rpm for 12 to 16 h. After a late log-phase, a measure of 10^8 cells per millilitre was obtained from Optical Density (OD) measurement using a Spectrophotometer (UNICO 2800 UV/VIS) set at OD_{600} and dilution reads 0.1. The broth for culturing *E. coli* and *GFP Salmonella* comprised of tryptic soy broth (TSB; Difco Laboratories, France) and Luria-Bertani broth (LB; Difco Laboratories, France) mixed with antibiotic Carbenicillin (Fisher

Table 2. Average value of physical and chemical parameters.

Vegetation type	No. of samples	Temperature (°C)		pH		DO (mg/L)		Salinity (ppt.)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Typha latifolia	18	24.6	0.30	6.95	0.03	1.43	0.19	0.47	0.01
Cyperus papyrus	18	24.8	0.16	6.74	0.02	1.30	0.10	0.50	0.02
Cyperus alternifolius	18	24.4	0.18	7.09	0.02	1.26	0.14	0.53	0.02
Phragmites mauritianus	18	25.0	0.26	7.18	0.07	1.19	0.08	0.50	0.05
Unplanted (control)	18	24.7	0.20	8.08	0.09	2.07	0.66	0.40	0.00

BioReagent), respectively.

Sampling and analysis

The amount of 5 × 10^8 cells per millilitre for both *Salmonella* spp. and *E. coli* were mixed together with 500 L of water in a plastic tank and then introduced to the buckets by using a hosepipe. Samples were collected in each bucket at the bottom tap in the interval of 6, 12, 24, 48, and 72 h. They were collected in sterile glass bottles and kept in an ice-packed cooler and transported to the laboratory at NM–AIST. They were processed within 4 h of collection. Both samples of *E. coli* and GFP *Salmonella* were analysed using membrane filtration protocol in accordance with conventional methods (Standard Methods for Examination of Water and Wastewater - (APHA, 1998)). The membranes for *E. coli* were placed in plates with Hach's m-ColiBlue24 Broth and incubated at 35°C for 24 h for complete enumeration. *E. coli* colonies appeared blue. The membranes of *GFP Salmonella* were plated in LB Carb+ and incubated at 37°C for 24 h followed by verifying their green autofluorescence colonies under UV illumination (Cole-Palmer UV-Transilluminator). Physical/Chemical parameters such as DO, pH, and temperature were measured directly by using a Multi-Parameter Digital Meter (Thermo Scientific Orion 4 Star) and recorded onsite.

Statistical analysis

Data were processed by using the Origin Version 8 software to obtain the trends in the concentration and the IBM-SPSS Version 20 for comparison and testing significance under one-way analysis of Variance (ANOVA). Comparison was considered significantly different at $p < 0.05$.

Evaluation of the results

It is assumed that the bucket experiments can be modelled as batch reactors; however, mixing was very poor due to lack of stirring or flowing of water. In this regard, faecal bacterial removal is modelled based on a first order kinetics model where removal depends on influent concentrations, retention or travel time, and a first order rate constant (Vymazal, 2005; Kadlec and Wallace, 2008). The first-order reaction equation is described as:

$$\ln\frac{C_t}{C_0} = -k \times \mathrm{HRT}$$

where C_t is the microbial concentration at a given time (cfu/100 ml), C_0 is the initial microbial concentration (cfu/100 ml), k is the first-order rate constant (h^{-1}) and HRT is the hydraulic retention time (h). Since all parameters are known, the observed values of k as the slope of the regression line were obtained by plotting $\ln\frac{C_t}{C_0}$ against the values of HRT. Comparing k- values for various macrophyte species helps to determine the different removal rate constants for each species.

RESULTS AND DISCUSSION

Effect of physicochemical parameters on reduction of *Salmonell*a spp. and *E. coli*

Table 2 summarises the average value of physical and chemical parameters in the planted and unplanted buckets. Temperatures in all buckets were almost identical, ranging between 24 and 25°C. The highest temperature was observed in *P. mauritianus* (25.0± 0.2°C) and the lowest was in *C. alternifolius* (24.4± 0.18°C). The pH in planted buckets was almost neutral while the unplanted buckets were alkaline. Very little variation in DO was observed in the planted systems (1.2 to 1.4 mg/L). The highest oxygen concentration was observed in unplanted system (2.07 ± 0.66). All experimental buckets had low salinity (<1 ppt.).

Usually, the removal of bacteria and other pollutants depend on biological and chemical reactions occurring at specific physicochemical environmental parameters such as temperature, pH and dissolved oxygen (Naja and Volesky, 2011). Temperature in all systems was almost the same with average values ranging from 24 to 25°C. This range normally favours the elimination of micro-organisms in porous media. The review done by Kristian et al. (2004) in a survival experiment using *Pseudomonas* spp. in soils revealed no difference between 5 and 15°C, but a significant reduction of the bacterial numbers at 25°C. Temperature appeared to be a factor with a positive influence upon the behaviour and removal of bacteria in surface and subsurface flow CW in Leon, Spain (Molleda et al., 2008).

The survival of most bacteria decreases at both low

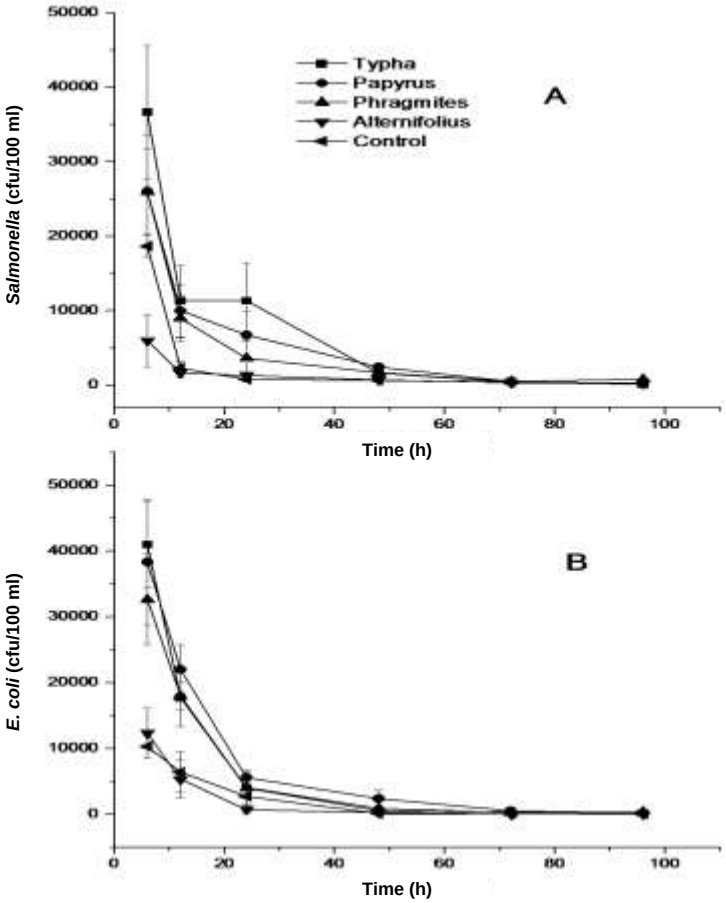

Figure 2. Concentrations of *Salmonella* spp. (A) and *E. coli* (B) per 100 ml volume of water plotted over time from buckets experiments planted with *T. latifolia, C. papyrus, C. alternifolius* and *P. mauritianus* and in unplanted buckets that stands as a control. Three replicates under each wetland condition were tested to evaluate the effect of vegetation on the removal of faecal pathogen and indicator bacteria from the system.

and high pH (Mawdsley et al., 1995; Stevik et al., 2004). The survival of *Salmonella* spp. and *E. coli* was found to be optimal when pH falls between 5 and 6.4 (Stevik et al., 2004). Therefore, as the average pH was around neutral and alkaline in planted and unplanted systems respectively, a considerable reduction of both *Salmonella* spp. and *E. coli* can be expected.

Most of enteric bacteria such as *Salmonella* spp. and *E. coli* are facultative or obligate anaerobes and thus the presence of oxygen creates unfavourable growth conditions (Vymazal, 2005). The presence of oxygen also facilitates the survival of predators for bacteria such as protozoans and lytic bacteria and viruses (Vymazal, 2005). However, this experiment observed less DO in planted than unplanted systems (Table 2). This was in contrast to other findings where the values of DO were greater in the planted systems due to the release of oxygen through roots into the rhizosphere (Stottmeister et al., 2003). It may be that more oxygen in planted systems was utilized for decomposition of organic matter released from root decay and droppings from macrophytes (Hench et al., 2003; Kyambadde et al., 2004). Similarly, the absence of macrophytes, in unplanted systems, encouraged greater atmospheric aeration in the substrate which facilitates, in some cases, the growth of algae with significant release of oxygen during algal photosynthesis (Leto et al., 2013). This increases pH as well as the carbonate-bicarbonate equilibrium is destabilised (Mashauri et al., 2000).

Effect of macrophytes on reduction of *Salmonella* spp. and *E. coli*

Figure 2 shows the trends on the removal of *Salmonella* spp. and *E. coli*. The overall removal for both *Salmonella*

Table 3. Effect of macrophytes on reduction of *Salmonella* spp. and *E. coli*.

Vegetation type	No. of samples	*Salmonella*				*E. coli*			
		k	SE	R^2	% Removal	k	SE	R^2	% Removal
Typha latifolia	36	0.055	0.006	0.946	99.04	0.057	0.009	0.878	99.59
Cyperus papyrus	36	0.045	0.006	0.927	98.75	0.055	0.005	0.964	99.39
Cyperus alternifolius	36	0.051	0.006	0.933	99.31	0.080	0.007	0.967	99.89
Phragmites mauritianus	36	0.046	0.007	0.889	98.55	0.053	0.010	0.841	98.88
Unplanted (control)	36	0.067	0.012	0.865	99.97	0.071	0.012	0.878	99.61

and *E. coli* was above 98% (Table 3). *C. alternifolius* achieved the greatest removal for both bacteria followed by *T. latifolia, C. papyrus* and the least was *P. mauritianus*. The same trend was also observed along the values of kinetic rate constant (k). When statistically tested, the vegetation types differed significantly in the removal of faecal bacteria ($p < 0.05$). A plot of fitted regression line for all treatments showed a very good linear correlation with R^2 above 0.84 (Table 3).

Both *Salmonella* spp. and *E. coli* were reduced in all treatment systems by almost two orders of magnitude (99%). These reductions are in line with other reports on removal of faecal coliforms in wetlands (Okurut et al., 1999; Molleda et al., 2008). The reductions were encouraging since the average concentrations for both *Salmonella* spp. and *E. coli* after 4 days retention time were below 400 cfu/100 ml. *C. alternifolius* has the least concentration (*Salmonella* 55 cfu/100 ml; *E. coli* 10 cfu/100 ml) and the *P. mauritianus* observed the highest (*Salmonella* 400 cfu/100 ml; *E. coli* 350 cfu/100 ml). Findings from various operating systems suggested that removal efficiency of faecal pathogens in planted systems is primarily influenced by hydraulic characteristics and presence of vegetation (Vymazal et al., 1998; Stottmeister et al., 2003; Vymazal, 2005). The effect of retention time can simply be explained; the longer the hydraulic retention time, the longer the bacteria are exposed to unfavourable conditions (Vymazal, 2005).

Despite the high removal of faecal bacteria during the 4 days of operation, substantial differences in performance were noted between the vegetation. When the comparison was established between kinetic rate constant (k), *C. alternifolius* outperformed other vegetation. The effect is probably caused by relative high pH, DO and salinity (Table 2) as compared to other vegetated systems. *T. latifolia* and *C. papyrus* appeared the second and third, respectively, and the least was *P. mauritianus*. Abou-Elela et al. (2014) found that *C. papyrus* which grows and distributes more widely in the bed was more effective in the removal of bacterial indicators as compared to *P. mauritianus.* Higher removal of faecal indicator bacteria was observed in *T. latifolia* as compared to *P. mauritianus* (Kaseva, 2004). In contrast to this study, Leto et al. (2013) and Katsenovich et al. (2009) observed better removal of faecal indicator bacteria in *T. latifolia* as compared to *C. alternifolius.* It was explained that significantly higher yields of biomass above and below the ground contributed to the highest performance in *T. latifolia* (Leto et al., 2013).

Planted vs. unplanted treatments system

Comparing the rate of removal between planted and unplanted systems, there were no significant differences ($p<0.05$) on removing both *Salmonella* and *E. coli*. This can be explained by relative high DO and pH, which facilitates the reduction of bacteria in unplanted as compared to planted systems. Similar to these findings, several studies had also experienced no significant difference between planted and unplanted systems for removal of indicators organisms and pathogens (Sleytr et al., 2007; Mburu et al., 2008; Torrens et al., 2009). The comparable effect might also be attributed to diffusion of oxygen from the air (Torrens et al., 2009) and exposure to UV light (Sleytr et al., 2007) in unplanted systems.

Conclusion

Findings of the present study suggested that vegetated systems can effectively reduce faecal pathogens in wastewater. Both *Salmonella* spp. and *E. coli* removal rates were above 98%. This indicates the positive use of plants in bacteria removal from wastewater. Removal of faecal bacteria differed significantly between macrophytes where the comparison of the rate constants showed that *C. alternifolius* and *T. latifolia* were the most effective followed by *C. papyrus* and the least was *P. mauritianus.* On the other hand, no significant difference was observed between planted units compared with the unplanted ones. The observed physicochemical parameters such as DO, pH, temperature and salinity were thought to influence the differences. However, the results of pot/ bucket experiments might not reflect the actual field conditions due to several factors such as growing conditions, choice of the type of pot, application of

nutrients and general adaptation of bacteria. Therefore, the present study recommends further researches to be conducted in the field on evaluating the removal efficiency of faecal pathogens using different macrophytes.

Conflicts of Interests

The authors have not declared any conflict of interest.

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