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Occurrence and Fate of Human Pathogenic Parasites in Constructed Reed Beds for Wastewater Treatment

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Abstract: Pathogenic parasites are considered as the main health risk when wastewater is reused for irrigation, and one of the most targeted agents in treatment processes. The present study assessed the occurrence and fate of pathogenic helminths and protozoa in constructed reed beds under arid climate. The studied system was made of four length different beds (20-50 m) and was operated at a flow rate of 864 m^3 d^{-1} with 1-4 hours retention time. The reed beds were found to be effective in the removal of pathogenic parasites from wastewater with rates up to 98.4% for protozoan cysts and 97.7% for helminth eggs. Eggs and cysts removal was length dependent and longer beds (50 m length) were the most efficient. During the treatment process eggs and cysts concentrated in soil with mean numbers of 21.9 eggs/100 g and 7.4 $\times 10^3$ cysts/100 g dry weight, respectively, with decreasing levels from the bed's inlet towards the outlet. Furthermore, *Ascaris* and *Trichuris* eggs were found to develop in soil and 27.4% and 46% attained the infective stage, respectively; especially, during spring and summer periods. Helminth eggs persisted for longer periods in beds' soil reaching over three months versus three days for protozoan cysts.

Key words: Pathogenic helminth eggs, protozoan cysts, reed beds, removal, wastewater treatment.

Introduction

Global water resources are under increasing pressure from rapidly growing demands and climate change (Unesco, 2012). Wastewater reuse attempts to reduce the pressure on fresh water resources for irrigation use, carrying at the same time nutrients improving soil fertility and crop yields (Landa-Cansigno et al., 2013). However, the main concern of wastewater reuse is the health implications to exposed populations, primarily due to the possible presence of wide range of pathogenic organisms shed in faeces of infected humans and animals. Several researches have demonstrated that sewage reuse for irrigation purposes caused environmental contamination; and epidemiological studies revealed an increase of parasitic infections prevalence among the exposed groups (Amahmid et al., 1999; Amahmid and Bouhoum, 2005; Gumbo et al., 2010; Ensink et al., 2008). According to the World Health Organisation, soil-transmitted helminths are considered as human pathogens of primary public health concern for wastewater reuse in agriculture and are the main targeted pathogens in the new guidelines, recommending a limit value of ≤1 egg/l (WHO, 2006). The soil-transmitted helminths, *Ascaris* and *Trichuris,* often used as parasitological indicators, continue

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to be a major public health problem worldwide, affecting over 1.5 billion people (Mara, 2011; WHO, 2015). For protozoa, the main enteric agents of public health significance are *Giardia* and *Entamœba histolytica* (*E. histolytica*).

Different wastewater treatment processes with varying efficiencies have been used to limit sanitary risks related to wastewater. Investigations regarding pathogens in constructed reed beds are very scarce and not well documented. Thus, the focus of the current study was to assess the occurrence and fate of the pathogenic parasites including the helminths *Ascaris* and *Trichuris* and the protozoa *Giardia* and *E. histolytica* in a pilot of constructed reed beds wastewater treatment plant under arid climate in Marrakech (Morocco).

Materials and Methods

Pilot Details and Sampling

The studied horizontal flow reed beds system was made of four beds with 20 m of width and length ranging from 20 to 50 m corresponding to a length to width ratio ranging from 1:1 to 2.5:1. The beds were planted with *Phragmites australis* (5 shoots m^{-2}) (Figure 1). The pilot was operated at a flow of 10 l/s of raw wastewater and the retention time varied between 1 hr 30 min and 3 hrs 30 min depending on the bed's length.

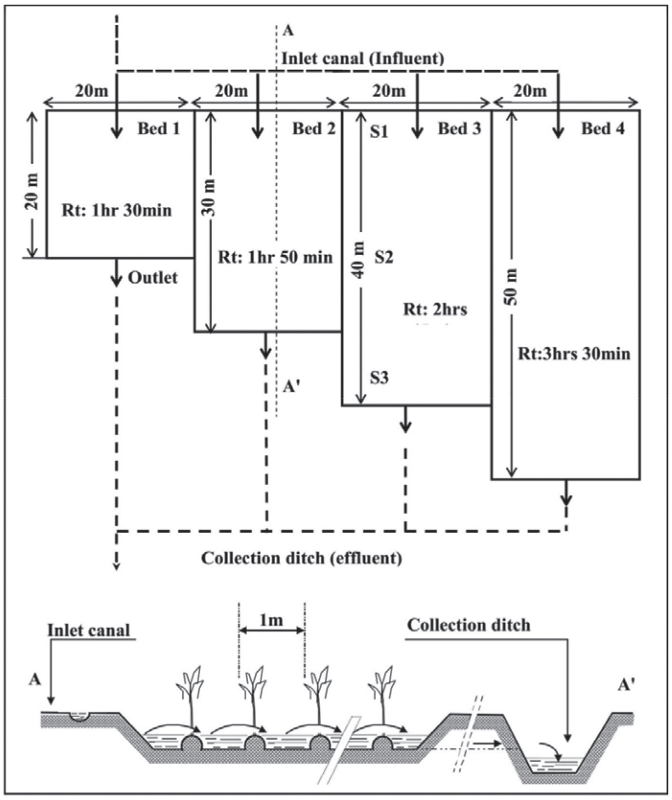

Figure 1: Diagram and profile view of the constructed reed beds system (Rt: retention time).

Wastewater samples were taken from the entrance and the exit of each bed. The sample volume was 5 litres at the entrance of the pilot and 10 litres at the exit of each bed. Water samples were collected twice monthly for 30 months. For soil, samples of 10 g weight were taken from three different stations: 50 cm from the entrance (S1), in the middle of the parcel (S2) and 50 cm before the exit (S3) (Figure 1) for 24 months. The assessment of the helminth eggs and protozoan cysts persistence was performed through the investigation of the duration of persistence after raw sewage application and by determining the development stages of eggs detected in soil samples.

Methodology

Wastewater samples were left for about 12 hours for sedimentation to take place. The top layer was discarded and the remaining residue with the suspension was centrifuged at 2164 g (3500 rpm) for 15 minutes. To ensure eggs and cysts desorption from soil particles, 40 ml of hypochlorite solution were added to soil samples for 30 min. Samples were centrifuged at 2164 g (3500 rpm) for 15 minutes. The technique of Bailenger (1962) was used to concentrate protozoan cysts in the centrifugation residues, as well as for helminth eggs in wastewater residues. In soil samples, concentration of eggs was performed with a flotation technique according to the protocol described by Bouhoum and Schwartzbrod (1989). For eggs and cysts identification and enumeration, microscopic observation was done in a Thoma counting chamber at 400× magnification for cysts and in a MacMaster counting chamber at 100× magnification for eggs.

Results and Discussion

The studied system was designed for domestic wastewater treatment. As presented in Table 1, in raw wastewater taken at the entrance of the reed beds protozoan cysts were discovered in 45.3% of samples with a mean number of 3.9×10^3 cysts/l (range 0-2.5×10^4 cysts/l). *Giardia* cysts were predominant with 34.4% of positive samples and an average concentration of 2.5×10^3 cysts/l while *E. histolytica* was discovered in 28.1% of samples with a mean number of $1.4\text{x}10^3$ cysts/l. For the helminths, 53.5% of samples were found to be positive with an average of 3 eggs/l (range 0-17.8 eggs/l). *Ascaris* eggs were detected in 36% of samples with an average of 1.6 eggs/l and *Trichuris* was observed in 25% of samples with an average concentration of 1.4 eggs/l. Several studies have reported variable levels of eggs and cysts in raw wastewater in constructed wetlands have been depending on many factors such as general health conditions of populations, season, sampling as well as techniques used for eggs and cysts purification and detection (Grimason et al., 1996; Gupta et al., 2009; Collender et al., 2015).

The analysis of treated wastewater in reed beds showed that only 15.6% of samples were positive for cysts with a range of 63 cysts/l detected at the exit of the longer bed (50 m) to 6.7×10^2 cysts/l in the effluent of 20 m bed (Table 1). For both *Giardia* and *E. histolytica*, the highest rates of positive samples and concentrations were detected in the effluent of the smaller bed with respective mean numbers of 4.3×10^2 cysts/l and 2.4×10^2 cysts/l, while the lowest levels were discovered in the effluent of the longer bed (50 m length) reaching 63 cysts/l for *E. histolytica* and *Giardia* was not detected. Of all the treated wastewater samples, 17% were positive for *Ascaris* and *Trichuris* and detected mean numbers ranged from 0.07 eggs/l in the effluent of the 50 m bed to 0.45 eggs/l for the 20 m bed (Table 1).

The wastewater treatment in reed beds has resulted in a higher reduction of cysts and eggs in the effluent. Depending on the bed length, higher removal rates of pathogens were recorded with ranges of 82.4% to 98.4% for *Giardia* and *E. histolytica* versus 85% to 97.7% for *Ascaris* and *Trichuris*. The greatest removal rates were recorded in 50 m bed while the lowest were obtained in the 20 m bed (Table 1). On the basis of the mean concentration of helminth eggs, the treated wastewater in reed beds meets the World Health Organization's recommendations for wastewater reuse for crop irrigation recommending a threshold of less than one helminth egg per litre (WHO, 2006). These findings are in accordance with those of Stott et al. (2003) who reported that helminth egg removal in reed beds was length dependent with rates of 90% (0.02 eggs/l) in 50 m beds and 100% in 100 m beds. Furthermore, the 100 m beds have demonstrated a substantial capacity (up to 100%) for helminth removal even when challenged higher parasitic loads (100-500 eggs/l). However, parasite cysts were less effectively removed and a few cysts of *Entamœba* spp. were detected in 100 m bed effluents (Stott et al., 1997; Stott et al., 1999). While,

Table 1: Occurrence and removal rates of pathogenic helminth eggs and protozoan cysts in the constructed reed beds

Pathogens	*Protozoa (cysts/l)*			*Helminths (eggs/l)*		
	Giardia	*E. histolytica*	*Total*	*Ascaris*	*Trichuris*	*Total*
Bed 1 (20 m)						
Influent	2.5×10^3	1.4×10^3	3.9×10^3	1.6	1.4	3.0
Effluent	4.3×10^2	2.4×10^2	6.7×10^2	0.36	0.09	0.45
Removal rate	82.8	82.9	82.8	77.2	93.7	85
Bed 2 (30 m)						
Influent	2.5×10^3	1.4×10^3	3.9×10^3	1.6	1.4	3.0
Effluent	3.4×10^2	2.4×10^2	5.9×10^2	0.17	0.19	0.36
Removal rate	86.4	82.9	84.9	89.2	86.6	88
Bed 3 (40 m)						
Influent	2.5×10^3	1.4×10^3	3.9×10^3	1.6	1.4	3.0
Effluent	65.6	82	1.7×10^2	0.05	0.18	0.23
Removal rate	97.4	94.1	95.6	96.8	87.3	92.3
Bed 4 (50 m)						
Influent	2.5×10^3	1.4×10^3	3.9×10^3	1.6	1.4	3.0
Effluent	0.0	63	63	0.0	0.07	0.07
Removal rate	100.0	95.5	98.4	100.0	95	97.7

the role vegetation in constructed reed beds may have in parasite removal was not well established, it has been reported an improvement in *Giardia* cysts and helminth eggs removal reaching up to 50% in planted beds compared to unplanted (Quinonez-Diaz et al., 2001; Rivera et al., 1995). The removal of pathogens contained in wastewater treated in reed beds may be achieved by entrapment and sedimentation enhanced by rhizomes and submerged stalks of plants barring the parasites outflow, as well as through adsorption on soil and organic matter particles (Mandi et al., 1996; Stott et al., 2003; Jimenez, 2007).

Analysis of soil samples taken at the reed beds has resulted in the detection of protozoan cysts and helminth eggs with respective averages of 7.4 $\times 10^3$ cysts/100 g d.w. (dry weight) and 21.9 eggs/100 g d.w. *Giardia* and *E. hsitolytica* were found in 38.9% and 50% of samples, with averages of 4.2×10^3 and 3.2×10^3 cysts/100 g d.w., respectively. For helminths, *Ascaris* and *Trichuris* were detected with a mean number of 10.9 eggs/100g d.w isolated in 31.4% and 32.6% of the analysed soil samples, respectively. The found results indicate that during the treatment process pathogenic parasite eggs and cysts were transferred from the aqueous phase (wastewater) into the solid compartment (soil). These findings are in accordance with those of studies reporting that parasite cysts and eggs tend to concentrate in sewage irrigated soil in varying levels (Blumenthal et al., 2000; Hassanain et al., 2014). Moreover, a spatial variation occurred in pathogenic parasites cysts and eggs concentrations, with a decrease from the upside to the end side of the planted beds (Figure 2). This is consistent with reports of Williams et al. (1995) and Stott et al. (1999) who found that helminth eggs removal in 100 m reed beds increases with distance along the bed with the majority of eggs removed within the first 10-25 m of the bed. The observed variation might be related to possible variation in the reed bed hydraulic conditions and parasitic loads as well as to the size and density of eggs and cysts which affects the settling velocities (Sauch, 1984; Sengupta et al., 2011).

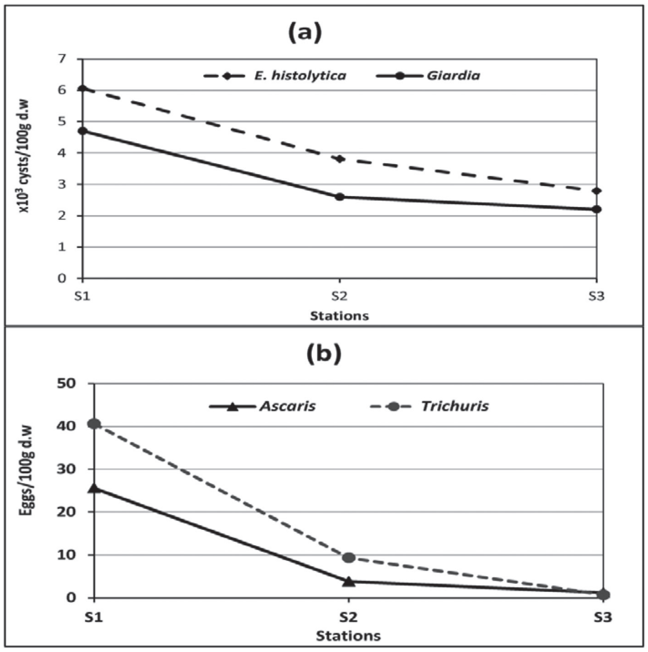

Figure 2: Horizontal spatial variation of pathogenic helminth eggs (a) and protozoan cysts (b) along the constructed reed beds.

Soil is an important environmental component for *Ascaris* and *Trichuris* transmission as they are soil-transmitted helminths (geohelminths), requiring an essential phase of their life cycle in soil during which eggs embryonate and become infective. The investigation of the development stages of *Asacris* and *Trichuris* eggs discovered in soil showed that the majority developed and 27.4% of *Ascaris* eggs and 46% of *Trichuris* attained the embryonated infective stage. By season, most of eggs developed to infective stage were detected in spring and summer with respective rates of 44.5% and 38.9% (Table 2). These findings

Table 2: *Ascaris* and *Trichuris* eggs development stages in the soil of the reed beds

Egg development stage	*Mono-cell stage (%)*	*≥2 cell-stage (%)*	*Embyonated (%)*	*Altered (%)*
Geohelminths				
Ascaris	55	17.6	27.4	-
Trichuris	30	20.5	46	3.5
Season (*Ascaris*)				
Winter	53.3	40.00	6.7	-
Spring	33.3	22.2	44.5	-
Autumn	44.4	16.7	38.9	-
Summer	95.8	0	4.2	-

indicate that conditions occurring in reed beds (i.e. humidity, temperature, oxygen …) were favourable for eggs development. Similarly, an early study by Fueki (1952) found that 36.8% of *Ascaris* eggs discovered in sewage irrigated soil were embryonated, and reached more than 70% during the period July-October, in line with the report of Ozkayhan (2006) who found *Ascaris* embryonated eggs in soil appeared in June and August.

The assessment of parasite eggs and cysts persistence in the soil of the reed beds revealed a reduction in recovered eggs and cysts counts over time (Figure 3). The protozoan cysts of *Giardia* and *E. histolytica,* initially discovered with a number of 12.6×10^3 cysts/100 g d.w. in soil, significantly decreased within three days reaching 3.5×10^3 cysts/100 g d.w. (72.2% reduction rate). Thereafter soil samples were found to be free of cysts. In contrast, helminth eggs persisted for longer periods. The initial level of helminth eggs in reed beds soil was 30.3 eggs/100 g d.w. and after 60 days, a relatively slight decrease was observed and within 90 days of exposure the number of recovered eggs declined considerably reaching 7 eggs/100 g d.w. (76.9% reduction rate); then soil samples analysed after 120-day period were free of eggs. The persistence of a given enteric pathogen in the environment is the single property most indicative of the health hazard it may pose during wastewater treatment processes and reuse (Mara, 2004). *Ascaris* eggs were reported to persist for months to years in sewage irrigated soils while protozoan may persist for days (Jimenez, 2007). A persistence period of six months was reported for *Ascaris* and *Trichuris* versus 20 days for *E. histolytica* (Duran-Alvarez and Jimenez-Cisneros, 2014). Parasite eggs and cysts gradually disappeared with time due to desiccation associated with high sunlight exposure, elevated temperatures and moisture lost mainly during dry hot period (Alum et al., 2014; Hudson et al., 2006).

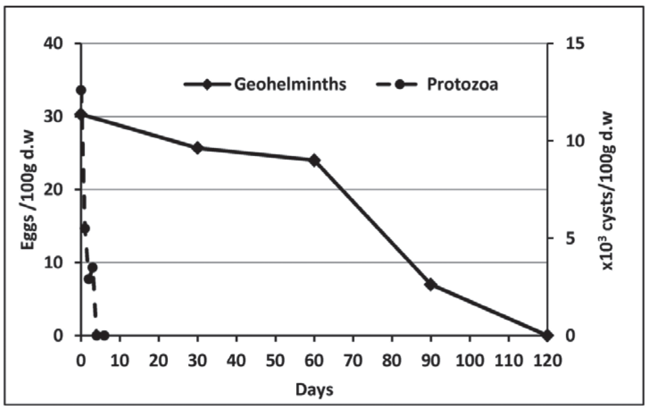

Figure 3: Persistence of pathogenic helminth eggs and protozoan cysts in the soil of the constructed reed beds.

Conclusion

The current study demonstrated that constructed reed beds, with only a 1-4 hours retention time, were very effective in the removal of pathogenic parasites from wastewater with rates up to 99%. The helminth eggs and protozoan cysts removal in reed beds was length dependent and longer beds were the most efficient. Rates of pathogens removal in constructed reed beds under arid climate compare favourably with that in other wetlands with relative short hydraulic retention times (typically hours to a few days). They may achieve standards for wastewater treatment with low cost and the process is considered convenient for developing countries. During the treatment process eggs and cysts tend to concentrate in beds' soil in considerable numbers and a persistence period up to three months for *Ascaris* and *Trichuris* eggs was recorded, versus three days for protozoan cysts. Furthermore, the majority of *Ascaris* and *Trichuris* eggs were found to develop in soil with an increase in advanced developmental stages including the infective stage. The occurrence and development of *Ascaris* and *Trichuris* eggs in the soil of the reed beds revealed the risk of transmission to the human population, especially farm workers, and raises the issue of hygiene standards and public health concerns at sites of sewage treatment, disposal and reuse.

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