Parasite removal by natural wastewater treatment systems: performance of waste stabilisation ponds and constructed wetlands

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Abstract Parasite removal and low cost systems for wastewater treatment have become increasingly important requirements in developed and developing countries to safeguard public health from wastewater-associated intestinal diseases. Pilot and field-scale ponds and wetlands in Brazil and Egypt have been investigated for the fate and removal of eggs of human intestinal parasites from domestic wastewater. In northeast Brazil, parasite removal was investigated for a series of five waste stabilisation ponds treating raw wastewater. In Egypt, parasite removal was studied for Gravel Bed Hydroponic constructed wetlands treating partially treated wastewater. Influenus to ponds and wetlands contained a variety of parasite helminth eggs (e.g. *Ascaris*, hookworm, *Trichuris*, and *Hymenolepis* spp.). The ponds consistently removed parasite eggs though rate of removal by individual ponds may have been related to influent egg numbers and extent of short-circuiting. Parasite eggs were reduced on average by 94% and 99.9% in the anaerobic and facultative ponds respectively. No eggs were found in effluent from the second maturation pond. In the wetland system, parasite removal varied with reedbed length. The majority of parasite eggs were retained within the first 25 m. Parasite eggs were reduced on average by 98% after treatment in 50 m beds and completely removed after treatment in 100 m beds.

Keywords Constructed wetlands; parasite removal; waste stabilisation ponds; wastewater treatment

Introduction Infectious diseases caused by faecal pathogens are a principal cause of human morbidity worldwide. In many countries, the transmission of these enteric diseases is often associated with exposure to faecally contaminated water due to inadequate sanitation facilities, or wastewater management practices of disposing wastewater into receiving waters later abstracted for potable supplies or from the reuse of inadequately treated wastewaters for agricultural irrigation (Cairncross and Feachem, 1993).

The removal of excreted pathogens from wastewaters is an important yet often neglected public health consideration for treatment systems, particularly in developing countries. However, the increasing emergence of waterborne parasitic diseases e.g. *Cryptosporidium* in industrialised countries associated with wastewater contamination of sourced water supplies (Patel *et al*., 1998), and the development of bacterial and parasite guidelines for the agricultural reuse of wastewaters (WHO, 1989; Blumenthal *et al*., 2000) has created interest in the removal of parasites from wastewaters in developed and developing countries alike.

Treatment of wastewaters in waste stabilisation ponds (WSP) and constructed wetlands (CW) utilises physical and ecological processes to remove nutrients and pathogens. These natural wastewater treatment systems have been used as primary, secondary or tertiary systems to treat a variety of wastewaters worldwide. Their low operational and maintenance requirements have promoted their use for rural communities in developed countries and for
developing countries as appropriate technology (Mara et al., 1992; Green and Upton, 1994; von Sperling, 1996; Kivaisi, 2001). A variety of studies conducted worldwide have shown that WSP can effectively remove faecal pathogens (Ayres, 1991; Dixo et al., 1995) and subsequently WSP have often been the preferred method of wastewater treatment. However, there is little information available on the fate and removal of parasitic pathogens such as the eggs of intestinal helminths in Constructed Wetlands (CW), especially in gravel-based systems which have been shown to be more effective in pathogen removal than soil-based wetland systems (Rivera et al., 1995). The assessment of CW systems as a viable alternative to WSP for wastewater reclamation and reuse or disposal is therefore limited.

The removal of eggs of human intestinal parasites from domestic wastewater has been investigated for pilot and field-scale ponds and wetlands in Brazil and Egypt and is compared here.

**Methods**

**Site description**

*Waste stabilisation ponds.* Parasite removal was investigated for a series of pilot-scale waste stabilisation ponds treating raw wastewater in north east Brazil. The multistage system comprised of 5 ponds in series: an anaerobic pond fed with raw sewage, followed by a secondary facultative pond and 3 maturation ponds. Each of the ponds received an average inflow of 14.7 m$^3$/d and had the following dimensions: 10 m $\times$ 3.35 m $\times$ 2.20 m deep. The mean hydraulic retention time for each pond was 5 days. A full description of the ponds is given by de Oliveira (1990).

*Constructed wetlands.* The constructed wetland system in Egypt was a field-scale Gravel Bed Hydroponic (GBH) horizontal subsurface flow (HSF) system consisting of 6 inclined gravel channels planted with reeds (predominantly *Phragmites australis*). The beds were either 50 m or 100 m long and were 2 m wide and 0.3 m deep. Bed gradients ranging from 1:20–1:50 were introduced into the first 23 m to reduce clogging with a final gradient of 1:100 from 25 m onwards. Reedbeds received partially treated domestic wastewater from a conventional trickling filter plant at a rate of 20 litres/min and were operated on an intermittent 12 h on: off regime starting at 08:00 h equivalent to a hydraulic loading of 72 mm/d and average inflow of 14.4 m$^3$/d. Further details of design and construction are reported elsewhere (Butler et al., 1990).

**Wastewater collection and analysis**

*Wastewater sampling.* A variety of sampling regimes and detection methods were used to determine parasite removal efficiencies. Diurnal studies were carried out on anaerobic pond influent i.e. raw wastewater (Brazil) and CW influent wastewater (Egypt) in which samples were taken every 2–3 hours commencing at 08:00–10:00 h. Daily, weekly and monthly sampling programmes were also carried out on raw, influent and effluent wastewaters over 2–12 months. On each sampling occasion, one litre grab samples of raw wastewater and pond effluents were collected usually at 08:00 h. One litre grab samples were collected for raw and CW influent wastewaters between 10:00–12:00 h. One and 10 litre samples were also collected from CW effluents at 15:00 h to allow for wastewater infiltration through the system. Wastewater samples were enumerated for parasite eggs and analysed for BOD, TSS, temperature and pH to investigate the effect of organic loading rates and physicochemical parameters on parasite removal performance.

*Parasite analysis.* Eggs of intestinal parasites (helminths) were identified and enumerated using the Leeds I and II methods for raw wastewater and pond effluents, respectively, in
Brazil and the modified Bailenger and Leeds II method for raw and CW influent and effluent wastewaters in Egypt (Ayres et al., 1991 and 1992). Samples of treated effluent were also analysed using the Leeds II method. The stage of egg development was recorded for Ascaris lumbricoides eggs detected in wastewater samples. The viability of these eggs was also determined, where possible, after 4 weeks incubation in 0.1N H₂SO₄ at 30°C (Hinck and Ivey, 1976).

Results
Parasite eggs in raw and influent wastewaters
A comparable range of parasite eggs were found in raw and influent wastewaters in Brazil and Egypt. The majority of eggs present in wastewaters were those of Ascaris lumbricoides. However, eggs of Trichuris trichiura, hookworm and Hymenolepis species (H. nana and H. diminuta) were also found. On a few occasions, eggs of Toxocara spp. were also detected in wastewaters in Egypt suggesting faecal contamination from animal sources. The variety of parasite eggs found in wastewaters in Brazil and Egypt are typical of that reported worldwide where intestinal parasite diseases are prevalent. A similar diversity of parasites has also been reported in UK and European wastewaters (Watson et al., 1983; Schwartzbrod et al., 1986).

Temporal variation was observed in parasite egg loading rates for both treatment systems. Numbers of parasite eggs varied diurnally in wastewaters entering the WSP and CW systems (Figure 1).

Significant diurnal fluctuations in parasite eggs were found in raw wastewater in Brazil (ANOVA F = 3.55, p = 0.013) with egg numbers recovered ranging from 40–700 eggs/l (mean 356 eggs/l). All species of parasites (excluding hookworm) were found to fluctuate significantly and showed similar diurnal trends. Egg numbers also varied diurnally, though not significantly, in Egyptian wastewaters (ANOVA, p = 0.669) with a range of 2–35 eggs/l (mean 14 eggs/l) in raw wastewater and a range of 0–9 eggs/l (mean 3.7 eggs/l) for influent. It is likely that short circuiting or contamination of influent is occurring in the conventional treatment plant before the partially treated wastewater is delivered to the reedbeds.

Similar diurnal trends in egg distribution were observed in both studies with the smallest numbers of eggs generally recorded at 05:00 h and between 04:00 and 06:00 h in Brazil and Egypt respectively. Diurnal variation in the numbers of parasite eggs entering the WSP and CW systems indicate temporal variation in egg loading rates probably correlated with human activity patterns of the catchment population. Parasite egg numbers also varied, though not significantly, with diurnal sampling occasion in the Brazil studies indicating longer term trends in egg loading in wastewaters.

Parasite egg removal in waste stabilization ponds: Brazil
The removal performance for the pond series is shown in Table 1. Eggs per litre in raw wastewater entering pond and wetland systems in Brazil and Egypt, respectively.
wastewater and pond effluents are shown as adjusted values for method recovery success (Leeds I: 23.7% recovery success – Ayres et al., 1989).

Parasite eggs were found in several pond effluents during the study. Eggs of *Ascaris*, *Trichuris* and hookworm were reported in anaerobic pond effluent; facultative and maturation pond effluents contained eggs of *Ascaris* only. All eggs of *Ascaris* recovered were undeveloped. No eggs were found in effluent from the second maturation pond.

The highest number of parasite eggs was removed in the anaerobic pond followed by the facultative pond. On average, parasite eggs were reduced by 94.6% in the anaerobic pond but effluent still contained around 50 eggs/l. Egg removal increased to 99.6% in the secondary facultative pond but eggs were not completely removed despite their relatively low numbers in the influent. Eggs were also found in tertiary treated pond effluent from the first maturation pond (0.1 eggs/l) which demonstrated a parasite removal rate of 50%. Complete removal of parasite eggs was not observed until after the second maturation pond with a total retention time of 20 days. A negative but non significant association was found between suspended solids and egg counts in facultative and maturation pond effluents.

**Parasite egg removal in constructed wetlands: Egypt**

The parasite removal performance of the Gravel Bed Hydroponic wetland system is shown in Table 2. *Ascaris lumbricoides* was the predominant species of parasite helminth found in raw and influent wastewaters to the GBH reed-beds during the sampling period. Of the *Ascaris* eggs recovered, 81% were unembryonated and 19% were partially developed (2–8 cell stage) in raw wastewaters in comparison to 73% unembryonated and 27% partially developed in influent wastewaters; egg viability was 75% and 100% in raw and influent wastewaters respectively. The presence of developing and viable eggs in GBH influent indicates that the conventional treatment plant had no deleterious effect on egg survival and suggests that non viable eggs may have been selectively removed during treatment.

All eggs of *Ascaris* were removed from wastewaters during treatment in 50–100 m reedbeds in contrast to the pond system. However, there was slight variability in parasite egg removal efficiency between beds. The rate of removal of helminth eggs was 100% in 100 m beds with a retention time of about 6 hours. However, cestode eggs of *Hymenolepis diminuta* were detected on one occasion in effluent from a 50 m bed. The concentration of parasite eggs in the influent was reduced on average by 91% after treatment in the 50 m bed

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Raw wastewater</th>
<th>Primary Anaerobic pond</th>
<th>Secondary Facultative pond</th>
<th>Tertiary Maturation ponds 1–3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasite eggs/l:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean:</td>
<td>992.6</td>
<td>54.0</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>95% CI:</td>
<td>787.9–1197.3</td>
<td>32.0–76.0</td>
<td>0.06–0.34</td>
<td>0.01–0.19</td>
</tr>
<tr>
<td>n:</td>
<td>13</td>
<td>13</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Parasite eggs present</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascaris</td>
<td>Ascaris</td>
<td>Ascaris</td>
<td>Ascaris</td>
<td></td>
</tr>
<tr>
<td>Trichuris</td>
<td>Trichuris</td>
<td>Hookworm</td>
<td>Hookworm</td>
<td></td>
</tr>
<tr>
<td>Hymenolepis spp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% removal of eggs for each pond</td>
<td>94.56</td>
<td>99.63</td>
<td>50.00</td>
<td>100</td>
</tr>
<tr>
<td>% removal of eggs from raw w/w</td>
<td>94.56</td>
<td>99.98</td>
<td>99.99</td>
<td>100</td>
</tr>
<tr>
<td>Mean SS mg/l</td>
<td>48.5</td>
<td>37.6</td>
<td>28.3</td>
<td>22.7</td>
</tr>
</tbody>
</table>

(a): *Necator americanus*
indicating that shorter length beds were less effective in helminth parasite removal. No significant association was found between eggs/l and physicochemical parameters in raw, influent or effluent wastewaters suggesting that eggs were present in wastewaters independently of organic loading.

### Discussion

Parasite removal in Waste Stabilisation Ponds and Constructed Wetlands appeared to be a stable process. Consistent removal of parasite eggs was demonstrated in both systems despite temporal variation in egg loading rates entering the treatment systems.

The primary removal mechanism for parasite eggs in anaerobic, facultative and maturation ponds is thought to be sedimentation facilitated by the long hydraulic retention times (HRT) in ponds (Ayres et al., 1992). However, the dynamics of removal may be different between anaerobic and aerobic ponds. Lower rates of egg removal are generally seen in anaerobic ponds (77–98%) probably due to shorter designed HRT in comparison to facultative/maturation ponds (83–99.99%). In Brazil, egg removal rates varied between anaerobic, facultative and maturation ponds despite the same HRT of 5 days in each pond of this particular system. Most of the eggs were removed in the anaerobic pond followed by the facultative pond. Differences in removal in individual ponds may be related to influent loading rates and conditions within the ponds preventing sedimentation and removal. Anaerobic ponds removed around 95% of eggs when fed with high parasite loading rates of $1.5 \times 10^7$ eggs/d (surface loading $4.4 \times 10^3$ eggs/m²/day). Similar removal success (99%) was observed in facultative ponds with mean daily loading rates of $8 \times 10^5$ eggs/d ($2.4 \times 10^4$ eggs/m²/d). In contrast, tertiary maturation ponds demonstrated poorer mass removal even with low mean daily loading rates of $3 \times 10^3$ eggs/d ($1 \times 10^2$ eggs/m²/d). Discharge rates from the first maturation pond were equivalent to $1.5 \times 10^3$ eggs/d.

Tertiary maturation ponds are often used to remove pathogens from wastewaters and should demonstrate good removal as HRT are usually longer. However, removal rates vary considerably between pond systems and species with removal rates for parasites ranging between 36–100% (Maynard et al., 1999). Increasing the HRT may not improve performance. Parasite eggs have been reported in effluent from 3 maturation ponds in series with a...
cumulative retention of 15 days (Ayres et al., 1989) and from a single facultative pond with a total retention time of 15 days (Ayres, 1991). Results from the Brazil study indicate that whilst high numbers of eggs are relatively easy to remove in ponds, low numbers of eggs entering maturation ponds may be more difficult perhaps due to short circuiting reducing the HRT within the ponds. All Ascaris eggs recovered from pond effluents were undeveloped suggesting that eggs were fresh or had been resuspended from sludge in ponds by gas production. Nevertheless, the pond series in Brazil achieved <1 nematode eggs/l in the secondary facultative pond satisfying WHO guidelines for restricted agricultural irrigation use of wastewaters, whilst effluent from the first maturation pond (0.1 egg/l) satisfied recent recommended revisions to the WHO guidelines (Blumenthal et al., 2000).

Constructed wetlands can demonstrate comparable rates of parasite egg removal to WSP. In this study, secondary treatment 50 m and 100 m reedbeds received mean daily parasite loading rates of $4.4 \times 10^4$ eggs/day. In 50 m beds loaded at $4.4 \times 10^2$ eggs/m²/d and 100 m beds at $2.2 \times 10^2$ eggs/m²/d, removal rates of 90% and 100% reported respectively were greater than that for tertiary maturation ponds with comparable parasite daily and surface loading rates in Brazil. Results suggest removal is improved with increasing bed length confirming the work of others (Mandi et al., 1996) as no parasite eggs were detected in effluents from the longer 100 m beds with retention times of less than 0.5 days despite the high number of eggs applied. In contrast, a 75% rate of removal has been predicted from design equations for egg removal in a WSP with a retention time of 1 d (Ayres et al., 1992). Furthermore, nematode eggs have been found in maturation pond effluent despite a retention time of 11 days and an influent of <5 eggs/l (Ayres et al., 1992) comparable to that of wastewater entering the wetland system. Wetlands may even have the potential to demonstrate greater removal than secondary facultative ponds loaded under similar conditions. Stott et al. (1999) reported that 100 m beds challenged with a mean daily load rate of $1–7 \times 10^6$ eggs/d ($0.5–3.6 \times 10^4$ eggs/m²/d) completely removed all eggs. However, the removal performance of wetlands needs to be evaluated for systems operated under continual high parasite loading rates.

Sedimentation of eggs may not be the principal removal process in subsurface flow wetlands unlike ponds, due to the relatively shorter retention times and horizontal flow of wastewater through the beds. No correlation was found between the levels of suspended solids and parasite eggs counts in raw and wetland influent and effluents. Results suggest that adsorption of eggs onto solids followed by sedimentation is not an important removal process unlike that reported for viral pathogens (Gersberg et al., 1989). Bed length may be important for egg removal. Helminth eggs might be removed in wetland systems as a result of entrapment and localised settling within the reed bed root matrix as wastewater percolates through the bed substrate. Profile analysis along 100 m reedbeds in Egypt has shown that parasite egg removal increases with distance along the bed with the majority of eggs removed within the first 10 to 25 m of the bed (Stott et al., 1999). Particulate matter has been found to accumulate within the first 10 to 20 m of the 100 m reed beds in earlier studies (Williams et al., 1995) suggesting that parasite eggs might be removed by mechanical filtration in subsurface flow systems as wastewater is treated along the bed length.

Wetlands such as GBH reed beds are capable of consistently removing eggs of intestinal helminths to produce effluents compliant with current WHO helminthological guideline levels of 1 nematode egg or less per litre for wastewater irrigation. Assuming that eggs, if present in GBH effluent, is a rare event that occurs randomly and independently, then the eggs in GBH effluent would follow a Poisson distribution similar to that observed for eggs in WSP final effluents (Ayres et al., 1989). For a count of zero eggs based on the examination of a total of 272 litres, 95% confidence limits for egg numbers in GBH effluent from
100 m planted beds is 0 to 0.014 eggs per litre. Such low limits indicate that GBH effluent quality is estimated to be well within WHO guideline levels.

**Conclusions**

Parasite eggs in wastewater can be significantly removed during treatment in CW and WSP. In these studies, wetlands and ponds demonstrated a consistent rate of egg removal despite temporal variation in egg numbers and egg loading rates. Nematode eggs were effectively removed in 50 m reedbeds and after secondary facultative pond treatment. All helminth parasite eggs were removed in 100 m wetland beds and in second maturation ponds.

Parasite removal performance of treatment systems may be related to loading rates. Very low numbers of eggs may be harder to remove particularly where opportunities exist for short circuiting such as in WSP. At high parasite loading rates, WSP and CW can remove intestinal parasite eggs very efficiently and consistently. Sedimentation is considered to be one of the principal egg removal mechanisms in pond systems with long retention times of days, yet the role of this or other physical processes such as filtration in the removal of parasite eggs in CW with a short retention time of <1 day and horizontal water flow is unclear. Some studies indicate that microbial predation may be an important factor in the removal of parasite cysts in CW (Stott *et al.*, 2001).

Where financial constraints exist, WSP or CW are low-cost treatment alternatives with a proven ability to maximise effluent reuse for agricultural production. WSP systems are effective in removing pathogens such as parasites and producing effluents satisfying microbial guidelines for crop irrigation. CW can also remove a variety of intestinal parasites effectively and reliably from wastewaters and may have practical applications and advantages as a component part of hybrid systems in combination with ponds or other treatment technologies.

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**References**


