Helminth ova removal from wastewater for agriculture and aquaculture reuse

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Abstract In the new version of the World Health Organization (WHO), water reuse guidelines helminth ova are considered one of the main target pollutants to be removed from wastewater reuse for agriculture and aquaculture purposes. In spite of this, along with the fact that helminth ova have been considered the main health risk to wastewater reuse for agriculture for at least 20 years, relatively little research has been done to control helminth ova in the wastewater treatment field. This paper addresses (1) characteristics of helminth ova and differences with microorganisms; (2) the most frequent helminth ova genus found in wastewater; (3) helminth ova content in developed and developing countries wastewater; (4) reasons why conventional disinfection methods cannot be applied; (5) main removal mechanisms; and (6) processes that in practice have effectively removed or inactivated helminth ova.

Keywords Agriculture; aquaculture; guidelines; helminth ova; reuse; wastewater treatment

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Introduction

The 1989 WHO agricultural water reuse guidelines draw attention to risks caused by helminths when wastewater was used for irrigation. In the 2006 guidelines, helminth ova are pointed out as one of the major concerns to reuse not only in wastewater in agriculture but also in aquaculture, particularly in developing countries. For these reasons, WHO establishes efficiencies of several log removal for parasites to safely reuse wastewater. Helminth ova limits are set not only to regulate conventional wastewater treatment plants effluents, but also on-site sanitation systems. In spite of the importance of helminth eggs as waterborne vectors, little attention has been given to them in the literature in terms of their removal during wastewater treatment processes. Helminth eggs are very poorly known and understood; most professionals believe they behave similarly to microbes in wastewater. However, the reality is quite another: not only do they behave very differently to bacteria, viruses and protozoan, they also cannot be monitored using the commonly accepted microbial indicator, faecal coliforms, for several reasons. This paper reviews, from a purely sanitary engineering point of view: (1) general characteristics of helminths; (2) common helminth ova genus found in wastewater and their content in developed and developing countries; (3) reasons why the conventional disinfection methods frequently used in wastewater treatment are not effectively inactivating helminth ova presence; (4) the main removal mechanisms; and (5) the processes that have proven efficient in removing helminth ova from wastewater. This paper presents compiled information that should be useful for developing countries practitioners and researchers where helminths are a concern. The aim is to provide engineers with basic information about feasible processes to control helminth ova in specific situations, but also with a view to stemming the commercialisation of ineffective methods that have begun to proliferate. The paper also aims to spark some much-needed curiosity in researchers to look for new helminth removal/inactivation methods.

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Helminthiasis: its importance

Helminthiasis are common diseases with an uneven distribution around the world. In developing countries, the affected population is 25–33% (Bratton and Nesse, 1993) whereas in developed ones it is less than 1.5% (WHO, 1997). Thus it is a problem that mostly concerns developing countries, particularly in regions where poverty and poor sanitary conditions are dominant; under these conditions helminthiasis incidence rates reach 90% (Bratton and Nesse, 1993). There are several kinds of helminthiasis; ascariasis is the most common and is endemic in Africa, Latin America and the Far East. There are 1.3 billion infections globally. Even though it is a disease with a low mortality rate, most of the people affected are children under 15 years with problems of faltering growth and/or decreased physical fitness. Approximately 1.5 million of these children will probably never catch up the growth deficit, even if treated (Silva *et al.*, 1997). Helminthiases are transmitted through: (1) consumption of polluted crops; (2) direct contact with polluted faeces or polluted wastewater; and (3) ingestion of polluted meat. Environmental and sanitary engineers intervene in the control of the pathways that spread helminths eggs, which are the infective agent (helminths or worms cannot live in wastewater).

Characteristics of helminths

Helminths are pluricellular worms; they are not microbes although their eggs are microscopic. Helminths come in different types and sizes (from around 1 mm to several metres in length) with various life cycles and ideal living environments. Their life cycle is very complex and different from that of bacteria and protozoan, which are well known microbes in the wastewater treatment field. The life cycle of *Ascaris lumbricoides* illustrates this complexity well. When a person ingests the eggs (1 to 10 is the infective dose according to US EPA, 1992), they stick to the duodenum where the larvae leaves the egg, crossing the wall into the blood stream. Through the blood, *Ascaris* travels to the heart, lungs and the bronchus tubes. There, it breaks the walls, remaining for around 10 days in the alveolus. It then travels to the trachea from where it is ingested again returning to the intestine. Back in the intestine, *Ascaris* reaches its adult phase, and, if female, produces up to 27×10^6 eggs during its 10–24-month life. It is estimated that around 200,000 eggs per day can be excreted with the faeces of an infected person (Ellis *et al.*, 1993). During its migration, *Ascaris* provokes allergic reactions (fever, urticaria and asthma). But also, sometimes *Ascaris* lodges in the kidney, bladder, appendix, pancreas, heart or liver forming cysts when they die that need to be removed through surgery. In the intestine, *Ascaris* produces abdominal pain, meteoroism, nausea, vomiting, diarrhoea and undernourishment. Helminthiasis diseases have different manifestations, but in general they cause intestinal wall damage, haemorrhages, deficient blood coagulation and undernourishment. Helminthiasis can degenerate into cancerous tumours.

There are three different types of helminth (Figure 1): (1) platyhelminths or flat worms; (2) nemathelminths or round worms; and (3) aschelminths. In municipal wastewaters only the first two are of importance. A common characteristic of helminths is that they reproduce through eggs. Eggs of different helminths differ in shape and size (Figure 2), but in general, those of importance in wastewater vary from 20–80 μm, have a density of 1.06–1.15 (Ayres *et al.*, 1992) and are very sticky. As can be seen in Figure 1, it is incorrect to use the terms nematodes, *Ascaris* and helminths as synonyms. Nevertheless, the nematode *Ascaris* is the most common helminth egg observed in wastewater and sludge (Figure 3). Eggs contained in wastewater are not infective. To be infective they need to develop larva, for which a certain temperature and moisture are required. These

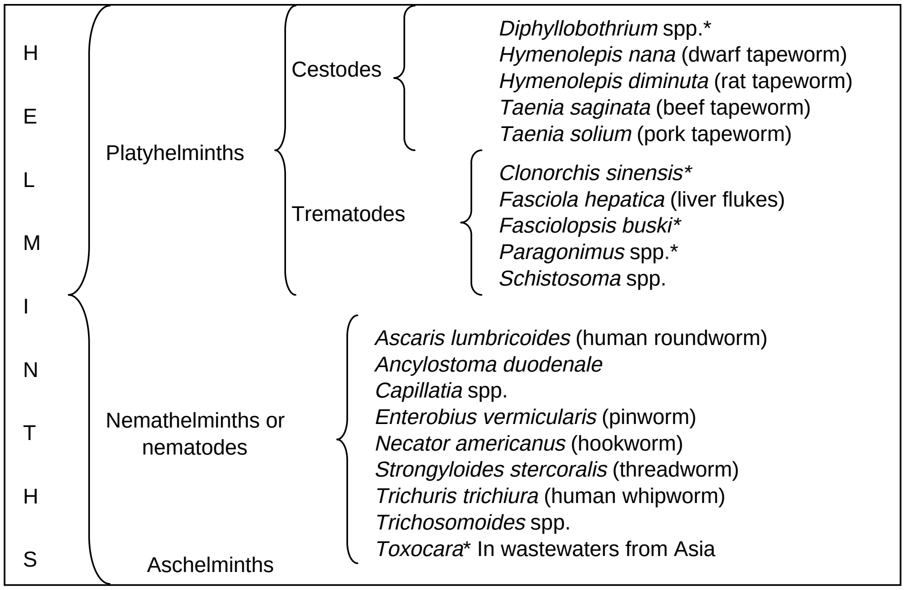

Figure 1 Classification of helminths and common genera found in wastewater

conditions are usually found in soils or crops irrigated with wastewater where eggs can develop larva in 10 days. Helminth eggs can live in water, soil and crops for several months/years (Feachem *et al.*, 1983).

Helminth ova in wastewater

Because of the difference in the general health conditions of people living in developed and developing countries, helminth ova content in wastewater and sludge is very different (Table 1), according to the little literature available on this subject, and so it follows that the technology for treating the wastewater and a monitoring strategy should also be different.

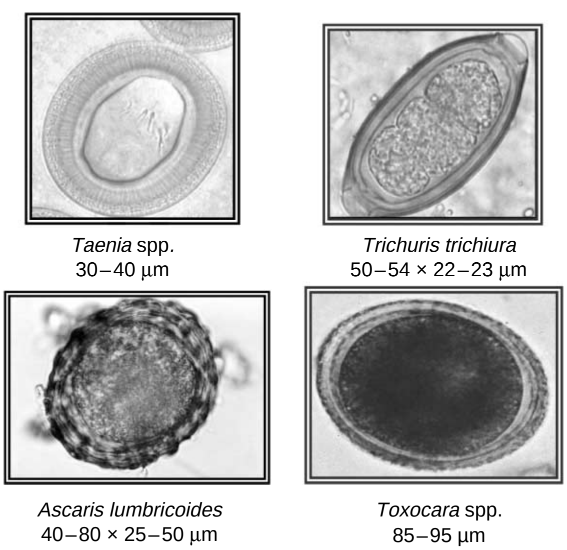

Figure 2 Some helminth eggs observed in wastewater

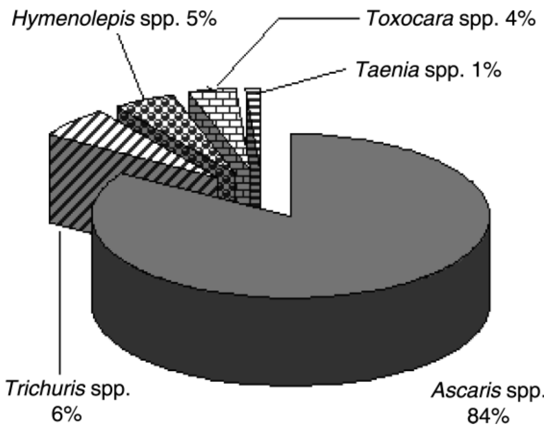

Figure 3 Distribution of helminth ova genera in wastewaters (Hays, 1997)

Faecal coliforms as indicators

Faecal coliforms are the bacterial pollution indicators most extensively used, and it is frequently, and wrongly, assumed that they are indicators of biological pollution in general. Furthermore, even though faecal coliforms might be a useful indicator of faecal pollution in developed countries, this is not the case in developing ones due to the presence of a wide variety and greater quantities of microorganisms. That is not to say that that faecal coliforms are not useful pollution indicators in developing countries, but rather that care must be taken to select an additional indicator for specific purposes. For example, agriculture and aquaculture wastewater reuse, which is where helminth ova fit in, given that helminth ova are more resistant to environment conditions. Helminth ova cannot be inactivated with chlorine, UV light or ozone (at least with economical doses because $>36\,mgO_3/L$ are needed during 1 hour, Rojas *et al.*, 2004) and behave differently to Faecal coliforms during treatment processes.

Helminths ova criteria

As shown in Table 1, not all wastewaters contain significant amounts of helminths ova; that is why they are not considered in all countries' norms as is the case of BOD or faecal coliforms. The WHO has performed research to establish recommended limits. For agricultural irrigation of crops that are eaten uncooked, it recommends a value of ≤ 1 HO/L (WHO, 1989), and recent epidemiological research work shows that a limit <0.1 HO/L is needed if children under 15 years are exposed (Blumenthal *et al.*, 2000).

Table 1 Helminth ova content in wastewater and sludge from different countries, with information from Jimenez (2003), Jimenez and Wang (in press) and Jimenez *et al.* (2005)

Country/regions	Helminth ova in wastewaters, HO/L	Helminth ova in sludge HO/gTS
Developing countries		70–735
Mexico	6–98 up to 330 in poor areas	73–177
Brazil	166–202	75
Egypt		Mean, 67; Max, 735
Ghana		76
Morocco	840	
Jordan	300	
Ukraine	60	
United States	1–8	2–13
France	9	5–7
Germany		<1
Great Britain		<6

For fish culture, trematode eggs (*Schistosoma* spp., *Clonorchis sinensis* and *Fasciolopsis buski*) must be zero HO/L as these worms multiply by the tens of thousands in their first intermediate aquatic host (an aquatic snail) according to Mara (2003).

Helminth ova removal

Helminth ova possess a shell that consists of three basic layers secreted by the egg itself: a lipoidal inner layer, a chitinous middle layer and outer proteinic layer. All these layers give high resistance to eggs under several environmental conditions. Helminth eggs of concern in wastewater used for irrigation have a size between 20 and 80 μm and a relative density of 1.06–1.15. These three properties determine the helminth ova's behaviour during treatment. First, it is very difficult to inactivate them, unless temperature is increased above 40°C or moisture is reduced to less than 5% (Feachem *et al.*, 1983; Hays, 1997). The exposure time has only been studied for temperature, setting it over several days (US EPA, 1994). These conditions are not often achieved in wastewater treatment but are common in sludge treatment. Thus, in wastewater it is not common to inactivate helminth ova but to remove them. This is done by processes that remove particles through sedimentation or filtration. Actually, there are correlations between the helminth ova content and the TSS or with particles with sizes between 20 and 80 μm content (Figure 4). Both correlations are useful in indirectly evaluating helminth ova content and tracking how the process performs. However, this correlation is not universal and needs to be established for each type of wastewater and process. It is certainly worth it, because helminth ova detection costs around 70 US$, while solid detection is only 7–12 US$ and particle counts is 3 US$ (Chavez *et al.*, 2004).

Wastewater treatment processes

Waste stabilisation ponds

Stabilization ponds constitute a very efficient process for removing all kinds of pathogens. According to Feachem *et al.* (1983), it removes up to 6 log units of bacteria, up to 5 log units of viruses and 100% of protozoa and helminths ova compared to 1–2 log units removal of bacteria and viruses and 90–99% removal of protozoan cysts and helminths ova in conventional treatment processes. Several factors contribute to this removal (sedimentation, temperature, sunlight, pH, microorganism predation, adsorption and absorption), but concerning helminth ova, sedimentation is the most effective. To remove helminth ova, a minimum retention time of 5–20 days depending on the initial content is required, with at least twice as much time to reduce thermotolerant coliforms to less than 1,000/100 mL. To control cryptosporidia, almost 38 days are needed (Shuval *et al.*, 1986; Grimason *et al.*, 1993; Mara, 2003). Most ova are retained normally in the

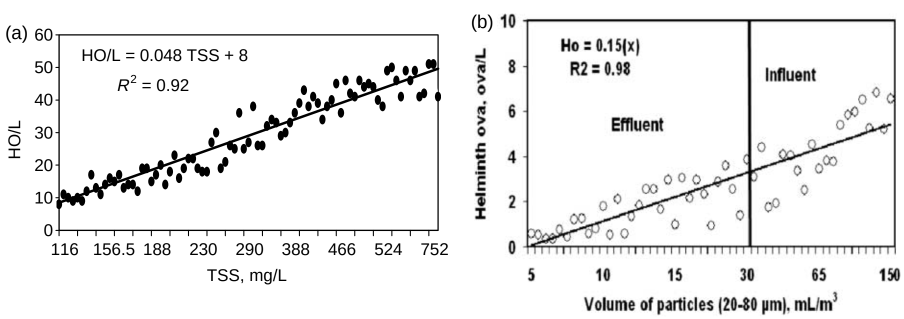

Figure 4 Correlation between (a) TSS and (b) 20–80 μm particles size with the helminth ova content in wastewater from Mexico City. From Jimenez and Chavez (2002) and Chavez *et al.* (2004)

first anaerobic pond. Using data from Brazil, India and Kenya, the following equation to calculate the percentage of egg removal in ponds (Ayres *et al.*, 1992) was established:

$$R = 100(1 - 0.41\,e^{-0.49\Theta+0.0085\Theta(\exp2)}) \quad (1)$$

where Θ is the retention time in days in the pond. Equation (1) is to be applied sequentially to each pond in the series.

In developing countries with warm climates, the use of stabilisation ponds to recycle wastewater for agriculture is recommended when land is available at a reasonable price (WHO, 1989). Yet, care must be taken in arid zones where evaporation/transpiration rates are high, since ponds may contribute to the net loss of water. For instance, in the system of Khirbet As Samra near Amman, Jordan, with a surface of 181 ha, around 13–18,000 m^3 per day of water evaporates in the summer, when the need for the resource is highest. This volume accounts for 20–25% of the water flow (Duqqah, 2002). It has been reported, in some cases that design removal efficiencies are not attained in practice due to hydraulic problems, such as flow bypasses (Huntington and Crook, 1993; Yates and Gerba, 1998).

Reservoirs

Like stabilisation ponds, reservoirs and dams can remove helminth ova from wastewaters if retention times of >20 days are used. This infrastructure is useful both in removing helminth ova and reconciling constant wastewater production with variable water demand by crops. According to Juanicó and Milstein (2004), all helminth ova are removed from reservoirs when they are operated as batch systems.

Constructed wetlands

Wetlands generally consist of reservoirs or ponds where plants are grown. They are built on a slant surface so that water may flow by gravity, and they are generally shallow to allow for better removal of contaminants. There are several types of plants that can be used, such as very small floating plants with few roots or no roots at all like *Lemna* or duckweed, or long plants like *Phragmites*, a common reed. Wetlands are also efficient at removing nitrogen, phosphorus and heavy metals (Brix, 1993). Several wetlands have been installed in different countries, but few microbiological studies have been carried out because of the high cost involved. Pathogen removal depends on the climate and the type of wetland and plant used. This process removes 90–98% of the thermotolerant coliforms, 67–84% of MS2 coliphage and 60–100% of protozoan (Jimenez, 2003). Better performances are obtained when using retention times of 4 days in surface flow wetland and with duckweed. To remove 100% of helminth ova it is necessary to couple the wetlands with a horizontal flow gravel bed, and most of the removal is achieved in a 25 m length (Rivera *et al.*, 1995; Stott *et al.*, 1999). In general, wetlands have very variable efficiency and it is considered difficult to control the processes, thus more research is needed in this field since it is a process considered convenient for developing countries.

Coagulation-flocculation

Jimenez *et al.* (1997) and Harleman and Murcott (1999) recommend the use of coagulation–flocculation processes to produce water for agricultural reuse. When this process is used with low coagulant doses combined with high molecular weight and high density charge flocculants, it is called chemical enhanced primary treatment (CEPT), and if besides this it is coupled with a high rate settler instead of a conventional one, it is then called advanced primary treatment (APT). APT and CEPT are both efficient at removing helminth ova allowing organic matter, nitrogen and phosphorus to remain in water in the dissolved fraction or as very small particles, which improves soil productivity. The resulting effluent has a low content of suspended solids and helminth ova, but still needs disinfection to inactivate bacteria. This can be done with chlorine or UV light. The low TSS effluent can be used in sprinklers to irrigate with no problem at all. The operating principle is very simple; it consists of accelerating the settling velocity of helminth eggs (0.39–1.53 m/h; Mara, 2003) with chemicals. It is considered that effluents with <20–40 mg TSS/L have a helminth ova content of around 3–10 HO/L and with <20 mg TSS/L the content is ≤ 1 HO/L (Chavez *et al.*, 2004). Different coagulants can be used (Jimenez, 2003). Lime applied at a dosage of 1000 mg/L to raise pH to 11 with retention times of 9–12 removes 4 log units of helminth ova and 4.5 log units of faecal coliforms, directly producing a safe effluent for unrestricted irrigation, but produces large amounts of sludge (around 0.14 m^3/m^3), which is certainly one of the inconveniences of the process (Gambrill, 1990 in Mara, 2003). When combined with proper polymers (anionic most often) coagulant doses can be considerably reduced to 40–50 mg/L of $FeCl_3$, 50–70 mg/L of Al_2SO_4 and 200 to 300 mg/L of $Ca(OH)_2$. Doses with PACS as the main coagulant are only some mg/L. The CEPT version has a total hydraulic retention time of 4–6 hours, while for the APT it is only 0.5 to 1 h. The cost of this latter process is only one third of the cost of a conventional activated sludge system, including sludge treatment and disposal within 20 km (Jimenez and Chavez, 1997). APT removes 1 log of faecal coliforms, 1 log *Salmonella* spp., 50–80% of protozoa cysts (*Giardia* and *Entamoeba coli*, *E. histolytica*) and 90–99% of helminth ova (Jimenez *et al.*, 2001). When optimised and well operated with an initial content of helminth ova from 20–100 HO/L, it is possible to produce an effluent with 0.5–3 HO/L constantly (Chavez *et al.*, 2004).

Rapid filtration (>2 m/h)

It is a useful treatment to remove protozoa and helminth ova from effluents, either physicochemically (Landa *et al.*, 1997) or biologically. Rapid filtration removes 90% of faecal coliforms, pathogenic bacteria (*Salmonella* and *Pseudomonas aeruginosa*) and enteroviruses, 50–80% of protozoan cysts (*Giardia* and *Entamoeba coli*, *E. histolytica*) and 90–99% of helminths ova (Jiménez *et al.*, 2001). This removal can be increased by 2–4 log if coagulants are added (US EPA, 1992). Rapid sand filtration is performed in sand filters (helminth ova stick very easy to silica, and is actually why silica glass material is not used for sampling or during the analytical technique). Specific size of media is from 0.8–1.2 mm, the minimal filter depth is 1 m and filtration rates are 7–10 $m^3/m^2/h$. Under these conditions, the effluent constantly has a HO content of <0.1/L and the filtration cycles are 20–35 h (Landa *et al.*, 1997).

UASB

The upflow anaerobic sludge blanket is an anaerobic biological reactor that can remove helminth ova through sedimentation and filtration in the sludge bed. Von Sperling *et al.* (2002) in a UASB with 5.5 h retention time, with waste water containing between 64 and 320 HO/L produced an effluent with 1.3–45 HO/L with a mean value of 16 HO/L and a mean removal efficiency of 96%. It has been recommended to couple the UASB system with stabilisation ponds in order to completely remove the fluctuations observed in the effluent.

Conclusions

Even though helminth ova are a major concern for the reuse of wastewater for agriculture and aquaculture, there is still little information about their behaviour during different wastewater treatment processes. Therefore, there is a pressing need for more research in this field. This research needs to be performed on site and with real wastewater, as studies performed in places where helminths are scarce use helminth eggs that come from animals, which do not necessarily behave like those from humans. Another issue to address is the need for the mass training of laboratory personnel from the developing world to measure helminths in wastewater and sludge and obtain more information about their content and their removal in wastewater treatment plants that already exist. Alongside this the same problem for sludge treatment in which helminth ova inactivation takes place also needs to be resolved.

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