HELMINTHS (WORMS) EGGS CONTROL IN WASTEWATER AND SLUDGE

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ABSTRACT

Worldwide, the most important reuse of wastewater, in volume, is agricultural irrigation. Therefore, there is a need to properly treat wastewater for such purpose, considering the removal of pathogens. According to the WHO agriculture and aquaculture water reuse guidelines, helminth ova are one of the main targeted pathogens. However, in spite of this, little research has been done recently on how to remove and inactivate helminth ova from wastewater and sludge. Thus, available recommendations developed several decades ago are still in use, and when put in practice, particularly in developing countries, produce unsatisfactory results. This is due to three reasons: (a) design criteria was developed long time ago using inaccurate helminth ova analytical techniques; (b) design criteria has been developed using sludge from developed countries having a low helminth ova content; and, (c) in developing countries there are a larger number and variety of helminth ova species found in wastewater and sludge. This paper summarizes recent research work and results from practical experience concerning: (a) the general characteristics of the helminth ova; (b) the common helminth ova genus found in wastewater and sludge around the world; (c) the reason why common wastewater and sludge treatment methods are not effective at removing or inactivating helminth ova; (d) the main removal and inactivation mechanisms, and, (e) the processes that in practice have proven to remove or inactivate helminth ova.

Key words: Agricultural reuse, helminth ova, pathogens, sludge, treatment, wastewater.

1. INTRODUCTION

In several regions of the world, wastewater (with or without treatment) and sludge is being used for agricultural purposes. In 1989, the World Health Organization (WHO) drew attention to diarrheic diseases caused mainly by helminths contained in sludge and wastewater reused for agriculture, and in agreement, set guidelines for safe reuse. In 1992, the US-EPA published the biosolids and sludge regulation (part 503) defining the elimination of helminth ova as a key parameter for sludge revalorization in agriculture. At the end of 2006, once again WHO set up a helminth ova limit for the reuse of wastewater and sludge in agriculture or aquaculture. These limits imply for developing countries using treatment methods with 1-3 log removal or inactivation efficiencies, for which there are very few options and available information. In spite of this, little attention has been given to helminth ova in terms of their control, in both wastewater and sludge. Helminth ova are poorly known and understood in the environmental engineering field because they behave different than bacteria, viruses and protozoan that cause problems worldwide. This paper reviews from a purely sanitary engineering point of view general characteristics of the helminths, main removal and inactivation mechanisms in wastewater and sludge and processes that in practice have effectively removed or inactivated helminth ova. This consolidated information (until now spread across several research papers) should prove particularly useful for developing countries where helminths are a concern.

2. HELMINTHIASES: A COMMON DISEASE

Globally there are 5 million people suffering helminthiases (UN, 2003), mainly in developing countries. Helminthiases are particularly common in regions where poverty and poor sanitary conditions are dominant, reaching incident rates of up to 90% (Bratton and Nesse, 1993). There are several kinds of helminthiases, ascariasis is the most common and is endemic in Africa, Latin-America and the Far East. Even though the mortality rate is low, most of the people infected are children under 15 years with problems of faltering growth and/or decreased physical fitness. Around 1.5 million of these children will probably never bridge the growth deficit, even if treated (Silva *et al.,* 1997). Helminthiases are transmitted through: (a) the ingestion of polluted crops, (b) contact with polluted faeces or polluted wastewater, and (c) the ingestion of polluted meat. Helminthiases infective agents are the eggs, not the worms. Worms cannot live either in wastewater or in sludge because they need a host. Therefore, part of the helminthiases control strategy is to remove the eggs from wastewater to later inactive them in the sludge. Helminths are pluri-cellular worms (they are not microbes) although their eggs are microscopic. Helminths come in different types and sizes, from 1 mm to several m length, have different life cycles and ideal living environments.

There are three different types of helminths: (a) Plathelminths, or flat worms, (b) Nemathelminths, or round worms, and (c) Annelides. In the sanitary engineering field 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 and therefore can only be identified by properly trained people. As shown in figure 1**,** it is improper to use the terms nematodes, *Ascaris*, and helminths as synonyms, as frequently happens in the sanitary engineering literature. This misunderstanding comes from the fact that *Ascaris* (a nematode) is the most common helminth egg in wastewater and sludge. Eggs contained in wastewater are not always infective. To be infective they need to develop larva, for which a certain temperature and moisture are required (26^{o}C and 1 month in laboratory conditions). These conditions are usually found in soil or crops where eggs, deposited through irrigation with wastewater or sludge, can develop larva in 10 days. Helminth ova (or Helminth eggs) can live in water, soil, and crops for several months/years (Feachem *et al*., 1983).

3. HELMINTH OVA IN WASTEWATER AND SLUDGE

3.1. Content

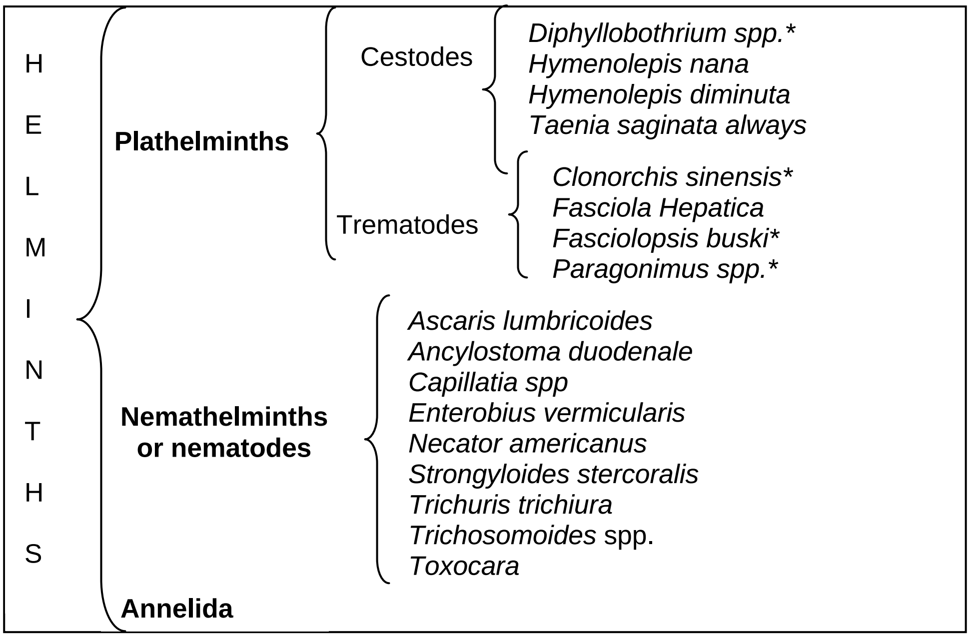
Due to the difference in health conditions of people living in developed and developing countries, according to the scarce literature available on the subject, helminth ova content in wastewater and sludge is very different (Table 1) and therefore the treatment methodology for wastewater and sludge significantly differs.

3.2. 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 any kind of biological pollution. Even though faecal coliforms are useful indicators of faecal pollution in developed countries, this is not so in developing ones owing to the presence of a wide variety and greater quantities of microorganisms. This does not mean that faecal coliforms are not useful, it simply means that care must be taken to select additional indicators for specific purposes, such as agriculture and aquaculture wastewater and sludge reuse, which is where helminth ova fit in, given that they are more resistant to environmental conditions than bacteria. Actually, in contrast to faecal coliforms, helminth ova cannot be inactivated with chlorine, UV light or ozone, at least in economical doses.

3.3. Helminth ova criteria

As shown in table 1, not all wastewater and sludge contain significant amounts of helminth ova. For this reason they are not considered in all wastewater and sludge countries' regulations as is the case of BOD or faecal coliforms. Based on epidemiological studies, WHO set limit of ≤ 1 HO/L for the irrigation of crops that are eaten uncooked and < 1 HO/gTS in faecal sludge. In the case of aquaculture, the limit is of 0 HO/L or g TS because the trematode egg (*Schistosoma* spp., *Clonorchis sinensis*, and *Fasciolopsis buski*) develop worms in an snail that is an intermediate aquatic host, that produce millions of eggs infecting fish and humans.



*Reported only in wastewater and sludge from some regions of Asia.

Figure 1 Helminth classification and genera found in wastewater and sludge (Jimenez, 2007).

Table 1 Helminth ova content in wastewater and sludge from different countries. Modified from Jiménez, 2007; Hays, 1977; Schwartzbrod *et al*., 1989; Bennani *et al*., 1992; Strauss, 1997* and Ensink *et al*., in press.

Country/region	Municipal wastewater, HO/L	Sludge, HO/g TS
Developing countries	70-3000	70-735
Mexico	6 – 98 in cities, Up to 330 in rural and peri-urban areas	73-177
Brazil	166 – 202	75
Egypt		67 (mean); 735 (max)
Ghana		76
Morocco	214-840	
Jordan	300	
Ukraine	60	
United States	1-8	2-13
France	9-10	5-7
Germany	≤ 40	< 1
Great Britain		< 6
Pakistan (Faisalabad)	142 (*Ascaris*)	
	558 hookworms (*Ancyllostoma, Necator* and *Ascaris*)	
Russia	≤ 2000	
Irkutsk, USSR	19	

*cited as: from an oral communication with Schwartzbrod.

3.4. Helminth ova characteristics

An important characteristic of helminth ova is that they have a shell that consists of 3-4 basic layers with a specific chemical composition: a lipoid inner layer, a chitinous middle layer and outer protein layer. All these layers render the eggs very resistant to several environmental conditions. Helminth ova of concern in the sanitary field have a size between 20 to 80 μm and a density of 1.06-1.15 (Ayres *et* *al*., 1992) and are very sticky. All these properties determine helminth ova's behaviour during treatment. First, it is very difficult to inactivate them unless the temperature is above 40°C or moisture is reduced to below 5% (TS > 95%), according to Feachem *et al*. (1983) and Hays (1977). But details about the contact time under these conditions and other related environmental factors are generally not known. Only contact time at temperatures of around 40°C has been established for one genus of helminth, *Ascaris*, and, according to US-EPA (1992), it is around 10-20 days. These inactivation conditions cannot be achieved in wastewater treatment but are common in sludge treatment. Thus, helminth ova are removed from wastewater and inactivated in sludge.

4. HELMINTH OVA REMOVAL FROM WASTEWATER

Helminth ova are particles forming a fraction of the suspended solids. Actually, the helminth ova content is related to the suspended solids one (TSS) and, in particular to the 20-80 μm particle content. Both correlations are useful for tracking process performance when indirectly evaluating helminth ova in wastewater. However, it seems that this correlation is not universal and needs to be established for each type of wastewater and treatment process. Nevertheless, it is worth it because 70 USD are needed to determine helminth ova content using the optical microscope procedure, while the TSS or the particle evaluation procedures have a cost of 7-12 USD or 3 USD per sample, respectively (Chavez *et al*., 2004).

4.1. Wastewater treatment processes

Waste stabilization ponds.- Waste stabilization ponds are very efficient at removing all kinds of pathogens. They remove up to 6 logs of bacteria, up to 5 logs of viruses, and practically all the protozoan and helminth ova (Feachem *et al*., 1983). These performances are higher than those observed in conventional processes (1-2 logs of bacteria, and 70-99 % of protozoa and helminth ova) using specific disinfection steps. Several factors contribute to this removal (sedimentation, temperature, sunlight, pH, microorganisms predation, adsorption and absorption), but concerning helminth ova, sedimentation is the most effective. To efficiently remove helminth ova, a minimum of 5-20 days depending on the initial content is required, with a least twice as much to reduce thermo-tolerant coliforms to less than 1000 MPN/100 mL. To control *Cryptosporidia,* almost 38 days are needed (Mara, 2003). Care must be taken, because in practices removal efficiencies are not attained in practice due to hydraulic problems, such as flow short circuiting (Huntington and Crook, 1993).

In developing countries with warm climates, the use of stabilization ponds to recycle wastewater for agriculture is recommended when land is available at a reasonable price. However, care must be taken in arid zones with high evaporation rates because ponds may represent a net loss of water. Water losses trough evaporation can account for 20-25% of the water inflow (Duqqah, 2002). Water evaporation also increases salinity content in the effluent making difficult its use for agricultural irrigation. There is little data concerning helminth ova survival at the bottom of the ponds. Nelson *et al.* (2004), in a study performed in several waste stabilization ponds in Mexico, found 14 viable HO/g TS in sludge stored for at least 9 years.

Reservoirs.- Similar to stabilization ponds, reservoirs and dams remove helminth ova from wastewater if retention time is greater than 20 days (Juanicó and Milstein, 2004). This way, reservoirs are useful for both removing helminth ova and supplying variable water flows to irrigate crops from wastewater that is constantly produced.

Wetlands.- In wetlands, helminth ova are removed by filtration through the soil and adhesion to roots. Besides removing pathogens, wetlands are also efficient at removing nitrogen, phosphorus and heavy metals. Several wetlands have been installed in different countries, but few data concerning pathogen removal is available out due to the high cost involved to monitor the system. Wetlands remove 90-98% of thermo-tolerant coliforms, 67–84% of MS2 coliphages, and 60-100% of protozoa. To remove 100% of helminth ova it is necessary to couple wetlands with a horizontal flow gravel bed where removal takes place within the first 25 m (Stott *et al*., 1999 and Rivera *et al*. 1995). Practical data show that pathogen removal is very variable depending on the climate, the type of wetland, and the type of plant used.

Coagulation-Flocculation.- Jiménez (2002) recommends coagulation-flocculation to produce water fit for agricultural reuse. When this process uses low coagulant doses combined with a high molecular weight and high density charge flocculants it is called chemical enhanced primary treatment (CEPT), and if it is coupled with a high rate settler instead of a conventional one, it is called advanced primary treatment (APT). APT and CEPT are both efficient at removing helminth ova while allowing organic matter nitrogen and phosphorus to remain in water in the dissolved fraction or as very small particles. This produces an effluent of an adequate quality for agriculture, with low TSS and helminth ova content, but that still requires disinfection to inactivate bacteria. This can be done with chlorine, UV-light or a combination of both. The operating principle is very simple: it consists of accelerating the helminth ova's settling velocity (normally of around 0.39-1.53 m/h; Mara, 2003) using chemicals. An effluent with less than 20–40 mg TS/L may have a helminth ova content of 3-10 HO/L, and with less than 20 mg TS/L a content of ≤ 1 HO/L (Chavez *et al*., 2004). Different coagulants may be used, Fe or Al being the most common. When combined with proper polymers (regularly anionic ones), coagulant doses may be considerably reduced (40-50 mg/L of $FeCl_3$ or 50-70 mg/L of Al_2SO_4). If poly-aluminium chlorides (PACS) are used as main coagulants, doses are reduced to only some mg/L. The CEPT version has a total hydraulic retention time of 4-6 hours, while for APT it is only 0.5-1 h. Consequently, this latter process costs 1/3 of what a conventional activated sludge system, including operation and sludge treatment and disposal within 20 km. APT removes 1 log of faecal coliforms, 1 log of *Salmonella spp.*, 50-80% protozoan cysts (*Giardia* and *Entamoeba coli*, *E. histolytica*), and 90-99% of helminth ova. From a content of up to 120 HO/L, APT may constantly produce an effluent with 0.5-3 HO/L (Jiménez *et al*., 2001 and Chavez *et al*., 2004).

Rapid Filtration (> 2 m/h).- This is a useful treatment for removing protozoa and helminth ova from effluents, either physicochemical or biological ones. Rapid filtration removes 90% of faecal coliforms, pathogenic bacteria (*Salmonella and Pseudomonas aeruginosa*), enteroviruses, 50-80% of protozoan cysts (*Giardia, Entamoeba coli*, *E. histolytica*), and 90-99% of helminth ova (Jiménez *et al*., 2001). These removals may be increased by 2-4 log if coagulants are added (US EPA, 1992). Rapid filtration is performed in sand filters (helminth ova sticks easily to silica, a reason why silica glass material should not be used for sampling or during helminth ova analysis). Specific filtration media size is from 0.8-1.2 mm, with a minimal filter bed of 1 m for filtration rates varying from 7-10 m^3/m^2.h. Under these conditions, the effluent constantly has a helminth ova content of < 0.1HO/L with filtration cycles of 20-35 h (Jimenez, 2007).

UASB.- Upflow Anaerobic Sludge Blanket reactors remove helminth ova through filtration in the sludge bed and sedimentation. Von Sperling *et al*. (2002) in a 5.5 h UASB produced an effluent with 1.3-45 HO/L from wastewater containing 64-320 HO/L. The mean removal efficiency obtained was 96%, so he recommended coupling UASB reactors with stabilization ponds in order to completely and constantly remove them from wastewater. Paulino *et al*. (2001) in an anaerobic fluidized bed also observed a variable removal efficiency of 60 to 93%.

5. HELMINTH OVA INACTIVATION IN SLUDGE

Helminth ova inactivation from sludge has been studied more than helminth ova removal from wastewater, but unfortunately, it has seldom use high initial contents of helminth ova.

Alkaline post-stabilization.- This process is widely used to treat sludge because it is easy to operate and has low capital and operational costs. For these reasons, it is applied in big and small wastewater treatment plants and even in on-site sanitation systems. To inactivate helminth ova, only alkaline post-stabilization is useful when important amount of helminth ova are involved. By adding lime (or any other alkaline material) to dewatered sludge, pH is raised above 12 for at least 2 hours. Lime doses of 20-40% dry weight may inactivate 6-8 logs of faecal coliforms, 5-7 logs of *Salmonella*, 0.5 - 2 logs of helminth ova, and 4-5 logs of bacteriophages (Méndez *et al*., 2002). Notwithstanding these high efficiencies, post-stabilization cannot produce sludge with ≤ 1 HO/g TS from sludge with an initial high content, with out adding lime doses that notably increase sludge disposal costs. To achieve such numbers, it is necessary to considerably increase the lime dose or alternatively to use closed vessels with recirculation to use the ammonia produced as an additional disinfecting agent (Mendez *et al*., 2002).

Acid treatment.- Since 1984, patents for stabilizing sludge using acids have been developed with very good results. Besides the pH effect, acids might have a toxic effect that will depend on their chemical composition. In general, organic acids are more toxic than mineral ones because they interfere with cellular reactions. Common acids used to inactivate pathogens are sulphuric, hydrochloric, propionic, acetic, and peracetic, the latter two being the best ones. Using 550 ppm of peracetic acid in physicochemical sludge with a high pathogen content (for instance, helminth ova of 74-142 HO/g TS), 5-6 logs of fecal coliform, 4-5 logs of *Salmonella*, and 2-3 logs of helminth ova are inactivated in only 10 minutes (Barrios *et al.,* 2004). Acids damage helminth ova membrane entering to the egg and affecting nuclei. Besides disinfecting, acid treatment is very useful for controlling bad odours and for oxidizing organic matter without significantly increasing the sludge mass.

Anaerobic digestion.- Thermophilic (45-65^{o}C) anaerobic digestion with retention times of 15 to 20 days inactivates low helminth ova content in sludge. It is a costly process, complex to operate, and produces a very low quality supernatant (WPCF, 1988). Oropeza *et al*. (2000), using sludge from Mexico in thermophilic digesters found that helminth ova removal was 70% and that the reactor stabilization period was very long. Despite this, it seems that performance could be improved if sludge is kept longer in the acidogenic phase where toxic organic acids (acetic and propionic) are produced. One important advantage of this process is that the methane produced may be used to increase the temperature in the reactor to foster helminth ova inactivation. The sludge produced is easy to dewater and very stable.

Thermal drying of anaerobically digested sludge.- An interesting option developed in Brazil is to use the methane produced during anaerobic sludge digestion to heat and dry the digested sludge to destroy helminth ova. Biosolids containing 0.99 -1.1 HO/g TS have been produced this way (Andreoli *et al*., 2000).

Composting.- No research has been done that proves that composting may produce sludge with < 1 HO/g TS from a sludge with an initial high content, even though it is considered a process with good pathogen inactivation potential. Composting may reduce the organic matter in sludge by 20-30% and the sludge mass by 40-80% to produce stable biosolids. To produce Class B biosolids according to the US EPA (with no helminth limits) composting must be performed for 2-4 weeks at a mean temperature of 55^{o}C for 4 hours. During composting, temperature may reach values as high as 70^{o}C that are capable of inactivating helminth ova.

Dehydration.- Dehydration have been applied in some on-site sanitation systems to inactivate helminth ova, with contradictory results (Table 2). Dehydration is performed only through storage and sometimes using solar energy. It seems that storing faeces for more than 1-2 years with a TS content > 50-60% adding bulking agents (lime, earth, leaves, etc.) and at a temperature above 30^{o}C, high helminth ova content may be inactivated (Austin and Duncker, 2002). Controlling helminth ova content in rural areas of poor countries is a particular challenge because: (a) very high helminth ova content is found; (b) it is practically impossible to perform periodic surveys; (c) installed processes need to be low cost and very easy and simple to operate; and (d) there is little research and information on the subject.

Table 2 Helminth ova content in sludge of on-site sanitation systems with different operating conditions, from Strauss *et al*., 2003 and Austin and Duncker 2002

670-2000 HO/g TS in fresh sludge coming from public systems or from latrine with a storage time of days to weeks
580 HO/g TS in sludge from septic tanks stored for several years
300 HO/g TS in faeces from dry sanitation systems from Guatemala, with 1 year storage at a high pH and a temperature of between 17-20^{o}C
0 viable *Ascaris* eggs/g TS in sludge from dry sanitation systems from El Salvador, sun-dried at 34-44^{o}C and stored for a long period.
0 *Ascari*s eggs/g TS in sludge from dry sanitation systems from South Africa stored for 20 months in plastic containers

6. CONCLUSIONS

Even though helminth ova are a major concern for the reuse of wastewater and sludge for agriculture and aquaculture, there is still little information about their behaviour during different treatment processes. Furthermore, the available information deals mainly with initial low content of *Ascaris* (mainly in sludge) and does not therefore reflect real situations in the developing world, where helminth ova are really a concern. Therefore, there is a pushing need for more research in this field. This research needs to be performed on site and with wastewater and sludge containing different helminth species, and not using only *Ascaris*, that not necessarily behaves like those coming from humans. Another issue to address is the need for training laboratory personnel from the developing world to measure helminth ova in wastewater and sludge and obtain more information about their content and their removal in treatment processes that already exist.

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