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HELMINTH OVA CONTROL IN WASTEWATER AND SLUDGE FOR AGRICULTURAL REUSE

B. E. Jimenez-Cisneros
Universidad Nacional Autónoma de México *(UNAM)*

Keywords: Agricultural reuse, helminth ova, pathogens, sludge, treatment, wastewater.

Contents

Summary

A new version of the WHO Guidelines for the Safe Use of Waste Water, Excreta and Greywater in Agriculture and Aquaculture has been released in 2006. These guidelines, among other things, establish criteria for the helminth ova content, considering them as one of the main targeted pollutants for developing countries. However, in spite of this breakthrough and the fact that helminth ova have been considered the main health risk when wastewater is reused for irrigation or aquaculture, relatively little information exists on how to remove and inactivate helminth ova from wastewater and sludge and, consequently, there are few technological options for controlling them. Moreover, it is still common nowadays to find recommendations on how technology can be applied to solving this problem based on data related to the inactivation of thermo-tolerant coliforms, even though it is well known that these are not indicators of helminth ova behavior. Furthermore, treatment methods are unable to produce treated wastewater and sludge with the low helminth ova content required by such criteria due to the high initial content found in the developing world. Due to the great need to apply adequate control methods in developing

countries to address helminthiasis problems, this paper presents useful information for environmental and sanitary engineers 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 water and sludge disinfection methods are not effective at inactivating helminth eggs; (d) the main removal and inactivation mechanisms, (e) the processes that in practice have effectively removed or inactivated helminth ova and (f) how its content is measured in wastewater and sludge.

1. Introduction

In several regions of the world, wastewater (treated or untreated) and sludge, are being used for agricultural works. In 1989, the World Health Organization (WHO) drew attention to diarrheic diseases caused by these practices and the presence of helminth ova. In agreement, WHO set guidelines for its safe reuse. In 1992, the US-EPA published biosolids and sludge criteria (part 503) defining the elimination of helminth ova as a key parameter for sludge revalorization in agriculture. In 2006, WHO once again released guidelines, this time for the safe use of wastewater, fecal material and sludge in agriculture and aquaculture with a recommendation on the helminth ova content to a value that for developing countries will imply using treatment methods with 1-3 log removal or inactivation efficiencies, for which there is almost no information available. In spite of the importance of helminth ova as waterborne vectors, throughout the years, little attention has been paid to them in terms of their characterization and control, in both wastewater and sludge. Helminth ova are still poorly known and understood in the water profession and are often thought to be similar to microorganisms (bacteria, viruses and protozoa) even though they behave very differently. This chapter reviews from a practical point of view: (a) general characteristics of helminth ova; (b) common helminth ova genus found in wastewater and sludge around the world; (c) the reason why common water and sludge disinfection methods are not effective at inactivating helminth eggs; (d) main removal and inactivation mechanisms, and, (e) processes that in practice have effectively removed or inactivated helminth ova. This consolidated information (until now spread across research and internal reports papers) addresses to water professionals dealing with wastewater and sludge problems in developing countries. Information should also encourage researchers to look for more useful information on helminth ova characteristics but as well on removal and inactivation methods.

2. General Information

2.1. Helminthiasis: A Common Disease

Globally there are 5 million people suffering helminthiasis, mainly in developing countries. Helminthiasis is particularly common in regions where poverty and poor sanitary conditions are dominant. Under these circumstances helminthiasis incident rates reach 90%. There are several kinds of helminthiasis, Ascariasis being the most common and 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. Helminthiasis is transmitted through: (a) the ingestion of polluted crops, (b) contact with polluted sludge, faeces or wastewater, and (c) the ingestion of polluted meat.

2.2. Helminths' Life Cycle

Helminthiasis 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 control strategy for helminthiasis is to remove the eggs from wastewater and later inactive them in the sludge produced from wastewater treatment. 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 in length), with several life cycles and ideal living environments. Besides humans some of them have intermediary hosts (such as *Schistosoma* spp. that live temporarily in a snail). Helminths' life cycle is very complex and different from that of bacteria and protozoan, which are well-known microbes in the wastewater treatment field. The *Ascaris lumbricoide's* life cycle illustrates this complexity well. When a person ingests *Ascaris* eggs (1-10), they adhere to the duodenum where the larva leaves the shell, crossing the wall into the blood stream. Through the blood *Ascaris* travels to the heart, lungs and bronchus tubes where it breaks the walls remaining in the alveolus around 10 days. The larva 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 million eggs. During, its migration, *Ascaris* provokes allergic reactions (fever, urticaria and asthma); it may also sometimes lodge in the kidney, bladder, appendix, pancreas or liver forming cysts that can only be removed through surgery. In the intestine, *Ascaris* produces abdominal pain, meteorism, nausea, vomiting, diarrhea and undernourishment. Helminthiasis diseases have different manifestations but in general they cause intestinal wall damage, hemorrhages, deficient blood coagulation and undernourishment. Helminthiasis can degenerate into cancer tumors.

2.3. Classification

There are three different types of helminths: (a) Plathelminths, or flat worms, (b) Nemathelminths, Nematodes or round worms, and (c) Annelids (see Figure 1). In the sanitary engineering field only the first two are of importance. A common characteristic of helminths is that they reproduce through eggs. The eggs of different helminths differ in shape and size (see Figure 2). As can be seen 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 (see Figure 3).

Normally, eggs contained in wastewater are not 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. Conditions usually found in soil or crops are suitable for the development of larvae in 10 days, hence the risk of using polluted wastewater or sludge in agricultural fields. According to information that is now several years old and obtained, using a much less sensitive helminth ova analytical technique than the one available nowadays, it was found that they live in water, soil and crops for several months, a period of time that it is much longer than the one for microorganisms (1-2 months in crops and many months in soil, see Table 1).

Table 1: Survival time of different pathogens in soil and crops Table 1: Survival time of different pathogens in soil and crops

(From: Feachem R., Bradley D., Garelick H. and Mara D. (1983) Sanitation and disease: Health aspects of excreta and wastewater management. John Wiley and Sons, New York, NY.)

Organisms	Soil		Crops	
	Absolute Maximum	Common Maximum	Absolute Maximum	Common Maximum
Bacteria	70 days	20 days	30 days	15 days
Viruses	100 days	20 days	2 months	15 days
Protozoan Oocyts	20 days	10 days	10 days	2 days
Helminth ova	Many months	Many months	2 months	1 month

Note: Periods of time may vary according to weather conditions.

3. Helminth Ova in Wastewater and Sludge

3.1. Type and Content

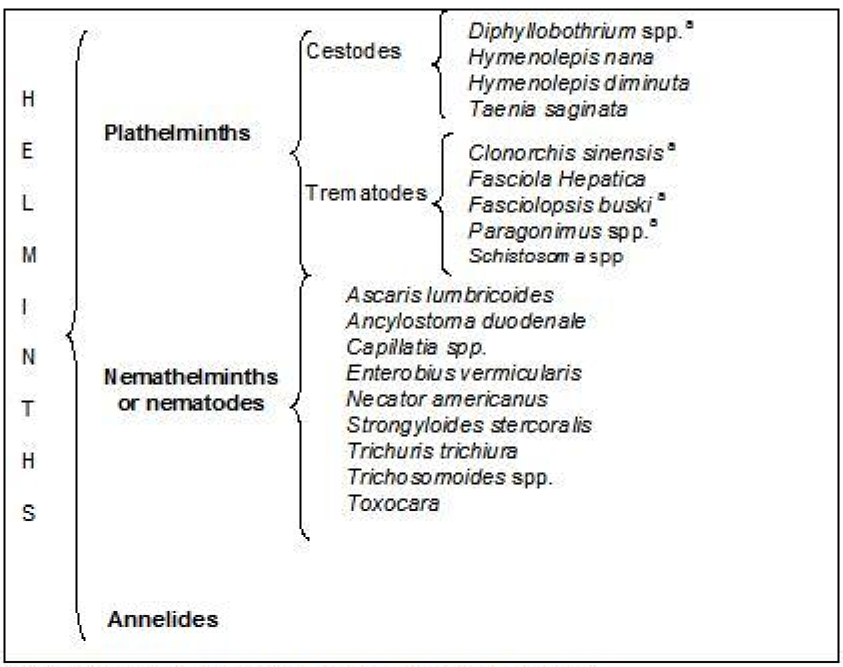
Due to differences in health and conditions according to the little literature available on the subject, helminth ova content in wastewater and sludge is very different (Table 2) in developed and developing countries. Moreover, the distribution genera presented vary from country to country, reflecting local health conditions. Different helminth ova genus reported as detected either in wastewater or sludge are mentioned in Figure 1, while a general distribution of genus, but not representative for all countries is presented in Figure 3.

Table 2: Helminth ova content in wastewater and sludge from different countries T With information from: Jimenez B. (2003) Health Risks in Aquifer Recharge with Recycle Water in State-of-the-Art Report Health Risk in Aquifer Recharge Using Reclaimed Water. Aertgeerts R: and Angelakis A. Editors. WHO Regional Office for Europe. , Jiménez B. and Wang L. (2006) Sludge Treatment and Management, Chapter 10, pp 237-292 in Municipal Wastewater Management in Developing Countries: Principles and Engineering, Ujang Z. and Henze M. Editors. International Water Association Publishing. London, U.K., Using Mara D. (2003) Domestic Wastewater Treatment in Developing Countries Ed. Earth Scan, London

Country/region	Municipal wastewater HO L^{-1}	Sludge HO g^{-1} TS
Developing countries	70-3000	70-735
Brazil	166–202	75
Egypt	No data	Mean: 67; Maximum: 735

Ghana	No data	76
Jordan	300	No data
Mexico	6–98 in cities Up to 330 in rural and peri-urban areas	73-177
Morocco	840	No data
Ukraine	60	No data
France	9	5-7
Germany	No data	< 1
Great Britain	No data	< 6
United States	1-8	2-13

Figure 1: Helminth classification and common genus found in wastewater and sludge.



[a] Found only in wastewater and sludge from some regions of Asia.

Figure 1 Helminth classification and common genera found in wastewater and sludge.

With information from: *A Practical Guide for the Enumeration of Intestinal Helminths in Raw Wastewater and Effluent from Stabilization Ponds*; *Low Cost Technology for Reliable Use of Mexico City's Wastewater for Agricultural Irrigation*; *Environmental Health Engineering in the Tropics*; *Helminth egg concentration in wastewater helminth egg concentration in wastewater: Influence of rainwater*; *Destruction of fecal bacteria, enteroviruses and ova of parasites in wastewater sludge by aerobic thermophilic and anaerobic mesophilic digestion*; *Parasite ova and cysts in waste stabilization ponds*; *Potential for parasitic disease transmission with land application of sewage plant effluents and sludge* and *sludge from twelve U.S. urban areas*

3.2. Fecal Coliforms as Indicators

Fecal 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 fecal coliforms are useful indicators of fecal pollution in developed countries, this is not the case in developing ones owing to the presence of a wide variety and larger quantities of microorganisms. This does not mean that fecal coliforms are not useful in developing countries, simply 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 one of the main associated health risks displaying a much higher resistance to environmental conditions than viruses, bacteria and protozoa. Actually, in contrast to fecal coliforms, helminth ova cannot be inactivated with chlorine, UV light or ozone (in the latter case at least not with economical doses because >36 mg O_3 L^{-1} are needed with 1 hour contact time.

Figure 2: Helminth eggs observed in wastewater and sludge

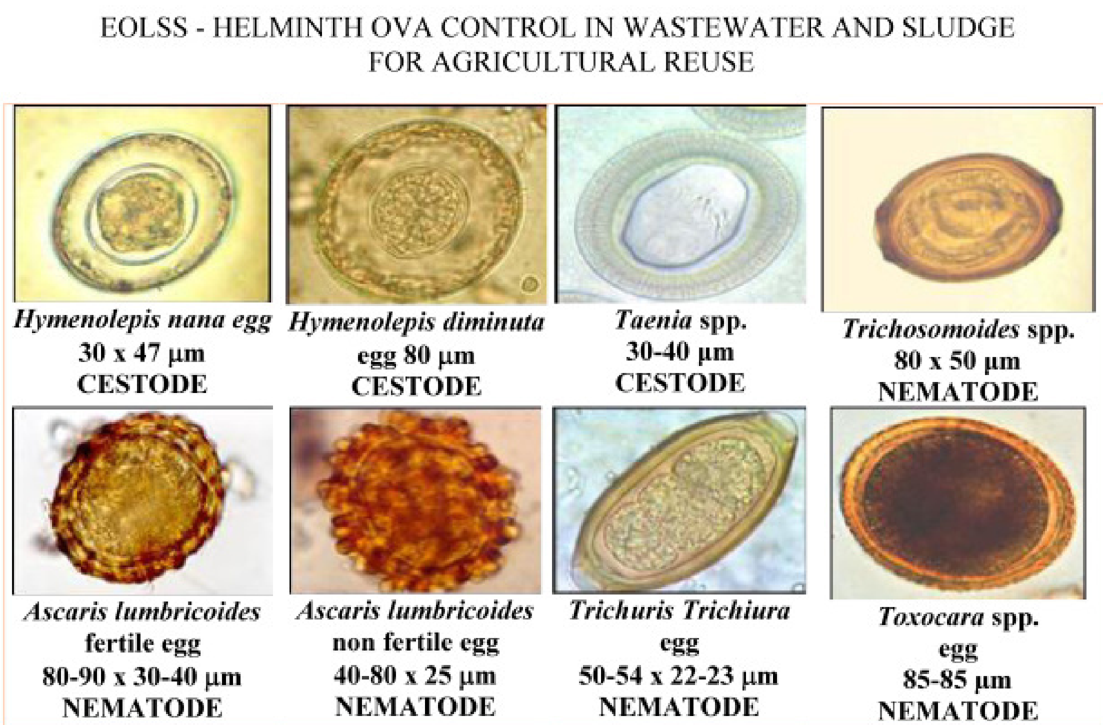


Figure 2. Helminth eggs observed in wastewater and sludge
(from *Atlas of Medical Parasitology: Intestinal Parasites helminthes;* for *Toxocara* and *Trichosomoides* courtesy of the Treatment and Reuse Group, UNAM)

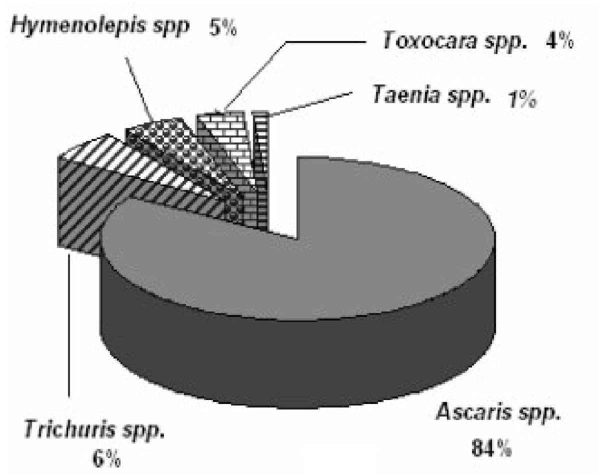


Figure 3. Genus found in wastewater
(From: *Comparison of Techniques for the Detection of Helminth Ova in drinking water and Wastewater*)

Figure 3: Genus found in wastewater

3.3. Helminth Ova Criteria

As shown in Table 2, not all wastewater and sludge contain significant amounts of helminth ova. For this reason they are not considered in all wastewater and sludge countries' norms as is the case of BOD or fecal coliforms, which are universal parameters. Based on epidemiological studies WHO has set a recommended limit of ≤ 1 HO L^{-1} for the irrigation of crops that are eaten uncooked for both restricted and unrestricted irrigations, but for drip irrigation of high growing crops (crops not growing down or on the soil), there is no recommendation. For fish culture with wastewater, the trematode eggs (*Schistosoma* spp., *Clonorchis sinensis* and *Fasciolopsis buski)* maximum content has been set as zero, as these worms multiply by tens of thousand producing millions of eggs in their first intermediate aquatic host (a snail) before infecting fish and humans.

In sludge or biosolids intended for agriculture, based on the value of ≤ 1 HO L^{-1} set for wastewater, some authors have calculated a limit criterion of 3 to 8 HO g^{-1} TS for sludge depending on its application rate. This value is much higher than the 0.25 HO g^{-1} TS set in the United States as standard or the 1 HO g^{-1} TS set as criteria by WHO (for fecal sludge). The US EPA value has been set based on the inactivation removal achieved by most of the available treatment technologies (with efficiencies of around 90%) to treat sludge with a maximum helminth ova content of 10 HO g^{-1} TS. Anyway, in practice, both, standard and criteria mean very high inactivation efficiencies (< 99%) for sludge and fecal sludge (due to high initial content normally found in developing countries) not affordable in practice.

The US-EPA standard value was defined for biosolids Class A (sludge with no restriction on use), while for biosolids Class B there are no helminth ova limits, although sludge can be reused in agriculture with some restrictions. Unfortunately, for sludge with greater content than those reported for the USA, very few economical feasible options are available for inactivating helminth ova in sludge or fecal sludge with low content values. Not applying any limit as is done for class B in US-EPA would be dangerous for the revalorization of sludge in developing countries.

4. Helminth Ova Characteristics

An important characteristic of helminth ova is that they are covered by 3-4 layers. The 1-2 outer layers are formed with mucopolysacharides and proteins. The middle layers consist of chitinous and serve to give structure and mechanical resistance to the eggs. Finally, the inner layer is composed of lipids and proteins and is useful to protect eggs from desiccation, strong acid and bases, oxidants and reductive agents as well as detergent and proteolytic compounds. Thus the combination of all these layers is responsible for making eggs very resistant to several environmental conditions.

Helminth ova of concern in the sanitary field measure between 20 and 80 μm with a density of 1.06-1.15 and are gelatinous which makes them very sticky. All these properties determine helminth ova's behavior during wastewater and sludge treatment. First, it is very difficult to inactivate them unless the temperature is raised above 40°C or moisture is reduced to below 5% (TS > 95%. But details about the contact time under these conditions and other related environmental factors are generally not well-defined for every type of helminth ova genus or for high helminth ova content. Only for *Ascaris* has a contact time of 10-20 days at temperatures above 40°C been reported. In wastewater treatment, the inactivation conditions mentioned can hardly be achieved while in sludge treatment they are feasible. Thus, helminth ova are normally removed from wastewater and inactivated in sludge.

5. Helminth Ova Removal from Wastewater

Basically, to remove helminth ova from wastewater it suffices to realize that they are in fact particles forming a fraction of the suspended solids. This is why the helminth ova content is related to the total suspended solids content (TSS) in wastewater-specifically, to the amount of particles measuring 20-80 μm (Figure 4). As helminth ova are particles, mechanisms used to remove suspended solids are also useful removing helminth ova from wastewater. These mechanisms are sedimentation, filtration and coagulation-flocculation.

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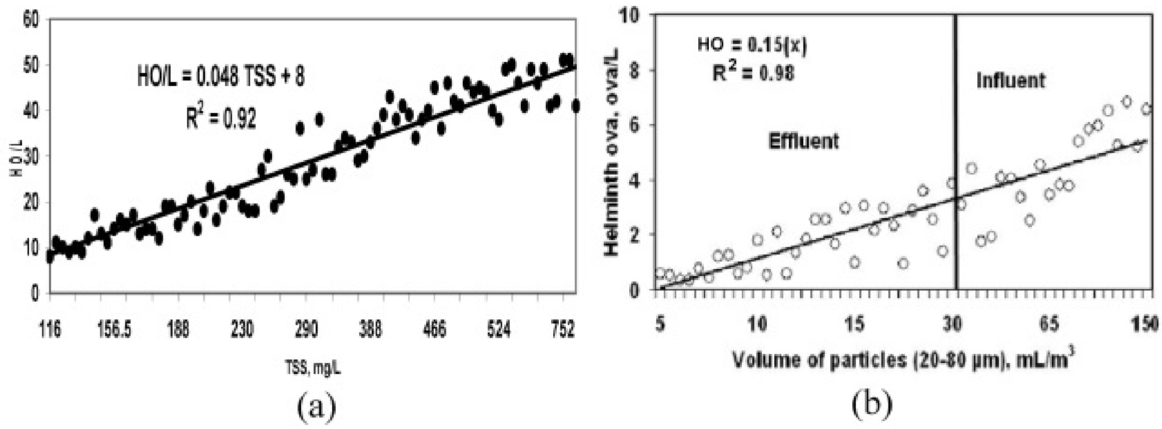

Figure 4. Correlation between helminth ova (HO) content in Mexico City's wastewater and (a) the TSS content and (b) the 20-80µm particles content

(From: *Low Cost Technology for Reliable Use of Mexico City's Wastewater for Agricultural Irrigation and Particle size distribution as a useful tool for microbial detection*)

Figure 4: Correlation between helminth ova content in Mexico City's wastewater and (a) the TSS content and (b) the 20-80µm particles content

5.1. Waste Stabilization Ponds

Waste stabilization ponds are very efficient at removing all kinds of pathogens. They remove up to 6 bacteria log, up to 5 viruses log and almost all the protozoa and helminth ova. These performances are higher than those observed in conventional processes (1-2 bacteria log and 70-99% of protozoa and helminth ova) without disinfection, as is the case of the activated sludge process. Several factors contribute to producing this efficiency (sedimentation, temperature, sunlight, pH, microorganisms predation, adsorption and absorption), but concerning helminth ova, sedimentation is the main one.

To efficiently remove helminth ova in waste stabilization ponds a minimum of 5-20 days, depending on the initial content, is required, with at least twice as much to reduce thermo-tolerant coliforms to less than 1000 MPN/100 mL. Furthermore, in order to control *Cryptosporidia,* around 38 days hydraulic retention time is needed. Most helminth eggs are retained in the first anaerobic pond. Using data from Brazil, India and Kenya, Eq. (1) was established to calculate the percentage of ova removal in ponds. This equation is to be applied sequentially to each pond in the series.

$$R = 100(1 - 0.41e^{(-0.49\theta + 0.0085\theta^2)}) \qquad (1)$$

Where θ is the hydraulic retention time, in days, in the pond.

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The use of stabilization ponds to recycle wastewater for agriculture is recommended in developing countries with warm climates if land is available at a reasonable price. However, in arid zones with high evaporation/transpiration rates ponds may represent a net loss of water. For instance, in the 181 ha system of Khirbet As Samra near Amman, Jordan, around 13000 -18000 $m^3 d^{-1}$ of water evaporates during summer when water demand is highest. This volume accounts for 20-25% of the incoming flow. Similar data has been reported for other stabilization ponds in same weather conditions. Besides losing water, evaporation increases water salinity in ponds, thus the waste stabilization pond effluent needs to be handled properly during irrigation to avoid soil salinization problems. Furthermore, in some waste stabilization ponds its low bacterial removal has been reported due to hydraulic problems, such as flow bypasses.

Concerning the quality of sludge produced in waste stabilization ponds, there is little data on the helminth ova content and their survival. However, it seems that they can survive in the pond for more than 9 years.

5.2. Reservoirs

Using a similar procedure (sedimentation) to waste stabilization ponds, reservoirs and dams remove helminth ova from wastewater if retention times are greater than 20 days. In this way, they are useful for both removing helminth ova and reconciling constant wastewater generation with the variable water demand by crops. Experience in Israel has proven that helminth ova can be totally removed from wastewater if reservoirs are operated as batch systems.

5.3. Wetlands

In artificial wetlands, helminth ova are removed by filtration through the soil and adhesion to roots. There are several kinds of plants that can be used for this purpose, such as very small floating plants with few or no roots, like *Lemna* or duckweed, or long plants like *Phragmites*, a common reed. Besides removing pathogens, wetlands are also efficient at removing nitrogen, phosphorus and heavy metals. Several wetlands have been installed in different countries, but few pathogen removal studies have been carried out due to the high cost and complexity of the analytical techniques involved. Pathogen removal depends on climate, type of wetland and the kind of plant used. Wetlands remove 90-98% of thermo-tolerant coliforms, 67 -84% of MS2 coliphages and 60-100% of protozoa. Better performances are obtained with hydraulic retention times of over 4 days in surface flow wetlands. To remove 100% of helminth ova it is necessary to combine wetlands with filtration. Horizontal flow gravel bed has been used as a filter for this purpose. The removal takes place mostly within the first 25 m, but the length can be considerably reduced if sand is used as filtration media instead. As yet there is not enough knowledge on how to better control wetlands' efficiency, and concerning helminth ova more research is still needed to define specific conditions for removing them in different circumstances. Additionally, there is no available information on the survival of helminth ova retained in wetlands.

5.4. Coagulation-flocculation

Coagulation-flocculation has been highly recommended to produce water fit for agricultural reuse because it removes helminth ova while preserving nutrients. When this process is applied using 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 also coupled with a high-rate settler instead of a conventional one, it is then called advanced primary treatment (APT). CEPT has a total hydraulic retention time of 4-6 hours while for APT it is only 0.5-1 h. The low hydraulic retention time in an APT system reduces the treatment cost to 1/3 of that of an activated sludge system, even considering sludge treatment and disposal within 20 km cost. APT removes 50-80% of protozoan cysts (*Giardia, Entamoeba coli* and *E. histolytica)* and 90-99% of helminth ova. And, from a content of up to 120 HO L^{-1}, an APT can constantly produce an effluent with 0.5-2 HO L^{-1}.

The operating principle of coagulation-flocculation processes is very simple: it consists of accelerating the helminth ova's settling velocity (normally of around 0.39-1.53 m h^{-1}) using chemicals. Normally, effluents with < 20 -40 mg TSS L^{-1} have helminth ova content of 3-10 HO L^{-1} and when it is possible to reduce it to < 20 mg TSS L^{-1} the helminth ova content will be ≤ 1 HO L^{-1}. Concerning chemicals, different coagulants have been used, iron and alum compounds being the most common ones. Lime has also been applied at very high doses (more than 1000 mg L^{-1}) to coagulate but also to raise pH (9-11). Under these circumstances 4 helminth ova log and 4.5 fecal coliform are reduced in 9-12 h contact time. But the sludge production turns out to be very high (around 0.14 m^3 m^{-3}) limiting the application of the process. Low coagulant doses can be used [40-50 mg L^{-1} of $FeCl_3$, 50-70 mg L^{-1} of $Al_2(SO_4)_3$, and 200-300 mg L^{-1} of $Ca(OH)_2$] in combination with proper polymers (regularly, cationic ones). Also, PACS can be used alone as coagulants at doses of some mg L^{-1}.

Both APT and CEPT have proven efficient at removing helminth ova while preserving this way the organic matter, nitrogen and phosphorus dissolved content in wastewater, producing an effluent particularly suitable for agricultural irrigation. But although the effluent has nutrients and a low or nil helminth ova content, it still requires disinfection to inactivate bacteria. This is normally done using chlorine, UV-light or a combination of both.

5.5. Rapid Filtration

Filtration is very efficient at removing protozoa and helminth ova from wastewater, primary and secondary biological or physicochemical effluents. Rapid filtration (rates over 2 m^3 m^{-2} h^{-1}) removes 90% of fecal coliforms, pathogenic bacteria (*Salmonella* and *Pseudomonas aeruginosa*) and enteroviruses, 50-80% of protozoan cysts (*Giardia, Entamoeba coli* and *Entamoeba histolytica*) and 90-99% of helminth ova. Protozoan and helminth ova removal can be increased by 2-4 log if coagulants are added. Rapid filtration is performed in sand filters (helminth ova sticks easily to silica, which is the reason why silica glass material should not be used for sampling or during helminth ova analysis). Filtration media size is 0.8-1.2 mm, filter beds are 1 m in height and filtration rates are of 7-

10 m^3 m^{-2} h^{-1}. Sand filtration is able to produce an effluent with a constant helminth ova content of < 0.1 HO L^{-1} in filtration cycles of 20-35 h from as primary effluent.

5.6. UASB

Upflow Anaerobic Sludge Blanket (UASB) reactors remove helminth ova through filtration in the sludge bed and sedimentation in the reactor. From wastewater containing 64-320 HO L^{-1} UASB it produces effluents with 1.3-45 HO L^{-1}, that is to say, with highly variable efficiencies, from 60-96%. Therefore to treat wastewater it is recommended that UASB reactors be combined with stabilization ponds to completely and constantly remove helminth ova.

6. Helminth Ova Inactivation in Sludge

Helminth ova inactivation in sludge has been studied more than helminth ova removal from wastewater, but unfortunately seldom using initial high helminth ova content, as is commonly the case in developing countries. Table 3 shows the application of the PFRP (*Process to further reduce pathogens*) to sludge with contents normally present in sludge from developing countries.

Table 3: Practical or theoretical application of US EPA 's PRFP process to high helminth ova content sludge Table 3: Practical or theoretical application of US EPA's PRFP process to high helminth ova content sludge

(Aguilar P., Jiménez B., Maya C., Orta T. and Luna V. (2006) Disinfection of sludge with high pathogenic content using silver and other compounds. *Water Science and Technology*. 5(54): 179-187., Keller R., Passamani F., Cassini S. and Goncalves F. (2004) Disinfection of sludge using lime stabilization and pasteurization in a small wastewater treatment plant. *Water Science and Technology* 50(1): 13-17., Gantzer C., Gaspard P., Gálvez L., Huyard A., Dumouthier N. and Schwartzbrod J. (2001) Monitoring of bacterial and parasitological contamination during various treatment of sludge *Water Research* 35(16): 3763-3770., Méndez J., Jiménez B. and Barrios J. (2002) Improved Alkaline Stabilization of Municipal Wastewater Sludge. *Water Science and Technology* 46(10): 139-146., Capizzi B. and Schwartzbrod J. (2001) Irradiation of Ascaris ova in sludge using an electron beam accelerator. *Water Research* 35(9):2256-2260., Oropeza M., Castro P., Ortega S. and Chabirol N. (2000) Mesofilic and thermofilic anerobic digestion of biological and physicochemical sludge. XII National Congress of the Mexican Federation of Sanitary Engineering and Environmental Science 1:789-803. Morelia, México (In Spanish).)

Process	Inactivation reported in bibliography	HO g^{-1} TS content in treated sludge (data from full scale reactor or calculated using reported efficiencies on sludge with 75-200 eggs g^{-1} TS)
Lime post stabilization	100% of HO from an initial value of 8 HO L^{-1}	5-12 HO g^{-1} TS
Thermophilic anaerobic	70 to 78% with an initial HO content of 2-8 g TS	19 to 39 HO g^{-1} TS

digestion at 48°C		
Thermal treatment at 108°C	90 to 93% with an initial content of 9.5 HO g^{-1} TS	Estimated value of 5- 16 HO g^{-1} TS
Irradiation at 1000 Gy	100% with an initial content of 88 HO g^{-1} TS	Estimated value 0 HO g^{-1} TS
Pasteurization at 70°C	100% with an initial value of 8 HO g^{-1} TS	Estimated value of 0 HO g^{-1} TS

6.1. Lime Post-stabilization

This process is widely used to treat sludge due to its low investment and operational costs as well as because it is very easy to operate. It is applied at big and small wastewater treatment plants and even to in-site sanitation systems. To inactivate helminth ova only alkaline post-stabilization is useful. 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 basis inactivate 6-8 fecal coliforms log, 5-7 *Salmonella* log, 0.5-2 helminth ova log and 4-5 bacteriophages log. Notwithstanding these high efficiencies, lime post-stabilization is not able to produce sludge with ≤ 1 HO g^{-1} TS from sludge with a high initial content. To achieve such a performance it is necessary to considerably increase the lime dose or alternatively to perform it in closed vessels with recirculation to use the ammonia produced during the alkaline reactions as an additional disinfecting agent. Furthermore, lime post-stabilization does not destroy organic compounds that allow microbial re-growth in sludge after treatment and produce bad odors. Also, it increases sludge mass by 20-40%, and, as a consequence, increases the final disposal cost. However, the sludge produced is useful to improving productivity or amending salinity in soil when applied.

6.2. Acid Treatment

Since 1984 patents for stabilizing sludge using acids have been in existence with good results. Besides the pH effect, acids have a toxic effect that depends 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 sulfuric, 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^{-1} TS), 5-6 fecal coliform log, 4-5 *Salmonella* log and 2-3 helminth ova log are inactivated in only 10 minutes. Acids destroy helminth ova layers entering to the egg and damaging nuclei. Sludge acid treatment can also be used to remove metals such as arsenic, cadmium, copper, manganese, nickel and zinc, and even to recover some of them for its reuse (like alum*)*. Besides disinfecting, acid treatment is very useful for controlling bad odors and for oxidizing organic matter without significantly increasing the sludge mass.

6.3. Anaerobic Digestion

Thermophilic anaerobic digestion (at 45-65°C) with retention times of 15 to 20 days has proven to inactivate helminth ova in sludge with a concentration commonly found in developed countries. It is a costly process, complex to operate and produces a very low quality supernatant. In literature, very few works present results concerning its efficiency with microorganisms other than fecal coliforms, and there are even less reporting on helminth ova inactivation. From a single experience in an anaerobic reactor at 55°C with 21 days retention time using Canadian sludge, a treated sludge containing no *Salmonella* and a fecal coliform content of 10^2 g^{-1} TS was produced. Using sludge from Mexico in thermophilic digesters helminth ova removal was found to be 70 % with a very long reactor stabilization period. Despite this, it seems that performance could be increased if sludge is kept for longer in the acidogenic phase where toxic organic acids (acetic and propionic) are produced. An important modification to this process is that the methane produced can be used to increase reactor temperature to foster helminth ova inactivation. The sludge produced is easy to dewater and very stable.

6.4. Thermal Dry of Sludge Anaerobic Digested

An interesting and low cost option developed in Brazil is the use of methane produced during anaerobic sludge digestion to heat and dry digested sludge to destroy helminth ova. Biosolids containing 0.99-1.1 HO g^{-1} TS have been produced this way.

6.5. Composting

No data is available concerning the production of treated sludge with < 1 HO g^{-1} TS from sludge with high pathogenic content. Nevertheless, it is a process with good microbial inactivation and apparently with the potential to inactivate helminth ova content commonly present in developing countries' sludge, as composting temperature can reach values as high as 70°C. Composting has the additional advantage of reducing the organic matter content by 20-30% and the sludge mass by 40-80% in sludge. To produce US-EPA class B biosolids (with no helminth limits) composting must be performed for 2-4 weeks at a mean temperature of 55°C for 4 hours.

7. Helminth Ova Inactivation in Fecal Sludge

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 very little research and information on the subject. Dehydration is the most common method used in some on-site sanitation systems to inactivate helminth ova but displays contradictory results (Table 4). Dehydration is performed only through storage, with the addition of dry material and sometimes using solar energy. It seems that only by storing feces for more than 1-2 years with a TS content > 50-60% achieved by adding bulking agents (lime, earth, leaves, etc.) and at a temperature above 30°C, can high helminth ova content be inactivated.

Table 4: Helminth ova content in sludge of on site sanitation systems with different operating conditions Table 4: Helminth ova content in sludge of on site sanitation systems with different operating conditions

(Strauss M., Drescher S., Zurbrügg CH. and Montangero A. (2003) Co-composting of Faecal Sludge and Municipal Organic Waste. A Literature and State-of-Knowledge Review, Swiss Federal Institute of Environmental Science and Technology (EAWAG) and IMWI. Strauss M. and Blumenthal U. (1990) Use of Human wastes in agriculture and aquaculture. IRCWD Report No 08/90, Duebenforf, Switzerland. and Austin A. and Duncker L. (2002) Urine-diversion ecological sanitation systems in South Africa. CSIR, Pretoria.)

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

8. Analytical Techniques

Besides being hard to remove and inactivate, helminth ova are difficult to detect and even more so to enumerate. This will certainly be a limitation to applying WHO guidelines in any country if not properly addressed. Problems enumerating helminth ova and comparing data among laboratories arise because there is no standardized technique, and it seems that most of the few laboratories trained to detect the helminth ova content are using different techniques or similar ones with self modifications. Moreover, most of the laboratories only report *Ascaris* content, and not the total helminth ova content.

Analytical techniques for enumerating helminth ova can be classified in two: direct and indirect methods. Some examples of direct techniques are the US-EPA one, the membrane filter, the Leeds I and Leeds II, and the Faust technique. All four techniques have two general steps. The first step is to separate, recover and concentrate the helminth ova from the sample sediment. In the second step, the helminth ova are identified and counted visually with the aid of a microscope. The techniques can be categorized in two ways: (a) based on the means used to separate, recover and concentrate the helminth ova, and; (b) whether the microscopic analysis is of all, or only part, of the concentrated volume containing the ova. Another important difference among the techniques is the initial volume of sample employed. The EPA technique uses a 5-L sample, combining flotation, biphasic and sedimentation steps to separate the ova from the water. The microscope readings or ova counts are performed on the entire mass recovered. The membrane filter technique, originally developed for detecting protozoa, uses flotation and an indirect concentration method of a 1-L sample. The ova are retained on a cellulose acetate membrane (10 µm pore, 25 mm diameter) that is later dehydrated and treated with ethanol, and is rendered transparent by treatment with glycerol. The ova count is made on the residual obtained from the whole sample. The Leeds I technique is based on several successive centrifugation and flotation steps. The sample size is 1 L for wastewater with high solids content and 40 L for wastewater with low solids content (filtered using a micro-wynd device to recover the ova). The final reading is made on only part of the flotation, which is carried out in a $ZnSO_4$ saturated solution. The ova are recovered by taking the upper part of the solution from six 15-mL tubes with a slide cover glass (4 per tube), which is then placed over a slide and quantified under the microscope. The individual results are added to give the final concentration. The Faust technique is similar to the Leeds I, and also uses samples sizes of 1 and 40 L, but involves more flotation, centrifugation and sedimentation steps. Readings are taken on five aliquots of 50 µL each of the final sediment, and then the results are averaged and extrapolated to give the final concentration. Some laboratories have modified the Leeds I and Faust techniques by filtering the 40-L samples through a Micro-wynd system to concentrate such samples, only when dealing with treated wastewater. Helminth ova recovery from samples varies widely from one technique to another (ranging from 20-80%), and they also have a very different sensitivity to accurately measuring low helminth ova content such as 1 HO L^{-1} or 1 HO g^{-1} TS, as requested by the new WHO criteria. The best technique to date seems to be the US-EPA one developed by Yanko, but unfortunately it is being applied with different modifications by several laboratories that affect differently the recovery percentage.

Indirect techniques are based on measuring an alternative property in samples other than helminth ova. Most of the time it is the particle content that is measured. An interesting indirect method that is useful from a practical point of view is based on the correlation between the particle content (Total suspended solids content or particle count) and the helminth ova content in wastewater. However, it seems that correlations are not universal and need to be established for each type of wastewater and treatment process. Nevertheless, it is a worthy method because the determination of the helminth ova content using microscopic methods costs around 70 USD, while its evaluation through the TSS and particle count procedures has a cost of 7-12 USD and 3 USD, respectively.

Furthermore, because only the viable helminth ova content can be infective, strictly speaking, it would be important to distinguish between fertile viable and non fertile eggs. This cannot be done visually, as it requires special procedures. One procedure is to incubate, once enumerated, the recovered helminth ova at 26ºC for 3-4 weeks. Alternatively different stains (such as trypan blue and safranin) can be used to determine within minutes whether eggs are viable or not.

9. Conclusion

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 characteristics, content in wastewater and sludge and behavior during different treatment processes. This is due to several reasons one being capability restrictions to analyzing helminth ova content in regions where they are a concern. Furthermore, available literature deals mainly with initial low content helminth ova which refers mainly to *Ascaris* and comes mostly from the sludge treatment field. Unfortunately this is not enough to address problems observed in the developing world, where helminth ova are a real concern. There is therefore a pressing need for more research in this field.

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Glossary

Activated sludge process	: A biological wastewater treatment process in which a mixture of wastewater and microorganisms is mixed and aerated to remove organic pollution from wastewater.
Alkaline post stabilization	: A method of disinfecting sludge produced during wastewater treatment using a high pH value due to the addition of an alkaline material.
Biosolids, Class A and Class B and in general	: Solid organic matter recovered from municipal wastewater treatment that can be used beneficially, especially as a fertilizer. Class A biosolids are those which have no detectable pathogens and can be used without restriction. Class B biosolids have a reduced level of pathogens and can therefore be used with some restrictions.
Fecal sludge	: The sludge formed from the excreta disposed of in latrines or individual sanitation systems.
Genus	: A category of biological classification concerning the family and the species, comprising structurally or phylogenetically-related species or an isolated species exhibiting unusual differentiation designated by a Latin or Latinized capitalized singular noun.
Helminth ova	: The female reproductive cells or gametes of parasitic worms.
Inactivation	: The action and effect of making a life form inactive or biologically inert, especially because of the loss of some quality (such as infectivity or antigenicity).
MS-2 coliphages	: Viruses, primarily of human origin, which infect only male strains of *Escherichia coli* and which, given their size and behavioral characteristics, are used as indicators of enteric viruses.
Ova/ovum	: A mature egg ready to undergo fertilization, the first term is plural while the second is singular.
Primary effluent	: Treated wastewater leaving a primary treatment unit.
Secondary effluent	: Treated wastewater leaving a secondary treatment facility, usually having 5-day biochemical oxygen demand and suspended solid

	concentrations of less than 30 mg L^{-1}.
Sludge	**:** Accumulated and concentrated solids generated as part of the wastewater treatment process.
Specie	**:** A category of biological classification ranking immediately below the genus or subgenus, comprising related organisms or populations potentially capable of interbreeding, and being designated by a binomial consisting of the name of a genus followed by a Latin or Latinized uncapitalized noun or adjective agreeing grammatically with the genus name.
Thermotolerant coliforms	**:** Bacteria that can grow at a temperature of 44.2ºC. They are fecal index organisms which are non-pathogenic and used to indicate the degree of fecal contamination.
TSS	**:** Total suspended solids are the measure of particulate matter suspended in a sample of water or wastewater. After filtering a sample of a known volume, the filter is dried and weighed to determine the residue retained.
UASB	**:** The Upflow Anaerobic Sludge Blanket is an anaerobic wastewater treatment technology. Treatment is performed by passing wastewater through a sludge blanket formed by microorganisms.
Water borne vectors	**:** Organisms supported, carried, or transmitted by water containing a pathogen.
Waste stabilization ponds	**:** A wastewater treatment system that refers to a pond or lagoon used to treat organic waste by biological and physical processes.
Wetlands (artificial)	**:** A man-made wetland is used to treat wastewater using plants and organisms.

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Biographical Sketch

Blanca Jimenez was born in Mexico City, where she obtained a *Bachelor's degree in Environmental Engineering.* She has Masters and a PhD degree in Wastewater Treatment from the Institut National de Sciences Appliquées, Toulouse, France. She works since 1985 at the National Autonomous University (UNAM) where she is Senior Researcher. In 1992 she founded the graduate program in Environmental Engineering in the state of Morelos and in 1994 launched the prestigious UNAM Group of Wastewater Treatment and Reuse.

Dr Jiménez has published more than 180 international papers and has 4 patents. She has published the book: "Environmental Pollution in Mexico. Causes, Effects and Technology": She has been responsible for more than 117 research projects for several public and private institutions. Due to her professional reputation Dr. Jiménez has been invited to lecture more than 100 conferences in several countries. She has been awarded several prizes like the National Ecology Award (as best academic in the environmental) 2006, the Environment and Ecology Award "Miguel Alemán Valdés" (2001), the Award for Scientific Research in the area of Technology Research, granted by the Mexican Academy of Sciences, (1997) and the Ciba Award for Technological Innovation in Ecology (1993). She is the chairperson of the Water Reuse Specialty Group in the International Water Association. She was President of the Mexican Association of Environmental Engineers, the Mexican Federation of Sanitary Engineering and Environmental Sciences (the oldest environmental professionals association in the country), and belongs to the Executive Committee of the International Water Association.

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