Chapter 3

Helminths and their Role in Environmental Engineering

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Additional information is available at the end of the chapter

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Abstract

Helminth eggs represent an important challenge to environmental engineers as they are among the most difficult biological parasites to inactivate in wastewater and sludge. Even though no official data on helminthiasis exist, it is estimated that more than 2.6 billion people are affected. These parasites are of concern in developing countries, particularly in those areas where the reuse of wastewater and sludge for agriculture is common. With regard to this, the unrestricted use of wastewater for irrigation presents a serious health risk due to the dissemination of pathogens, particularly helminth eggs. Helminth eggs survive in water, soil, and crops for several months and over much longer periods than other microorganisms. Therefore, and in order to minimize risk, several guidelines and regulations exist which limit their content in wastewater and sludge. Risk assessment estimates that such regulations may be less strict in developing countries where higher concentrations of helminth eggs occur in wastewater and sludge. These eggs need to be removed from wastewater and inactivated in sludge using certain treatment processes, some of which are not feasible in developing countries. Adequate methods are needed to precisely identify and quantify helminth eggs in environmental samples. Therefore, a multidisciplinary approach is needed to address helminthiasis in environmental engineering issues.

Keywords: Biosolids, guidelines and regulations, helminth eggs, helminthiasis, inactivation processes, microbial risk assessment, wastewater

1. Introduction

For centuries, wastewater has been used for agricultural irrigation throughout the world, and this is still a common practice in several countries. There are many examples that show wastewater reuse is key to increasing food production and improving local economies. This

is simply due to the high water demand for irrigation. In developing countries, this amounts to 81% of the total water extracted and to around 45% in developed countries [1]. As an example, in Mexico, where 75% of the territory is semiarid, wastewater reuse for agriculture is often practiced. Mexican farmers have long appreciated wastewater use because it contains, in addition to water, organic matter, nitrogen, and phosphorus, which act as fertilizers, increasing crop yield. Jiménez [2] summarized the reasons why wastewater is reused for irrigation: (a) it significantly saves fresh water; (b) it provides nutrients to soil; (c) it reduces or eliminates the need for chemical fertilizers; (d) it contributes to the expansion of agricultural land in arid and semi-arid areas; (e) it increases farmers' income; and (f) it is a relatively cheap disposal method for wastewater, reducing surface water pollution. It is estimated that there are 20 million hectares in at least 50 developing countries where wastewater is used to irrigate [3], representing around 10% of the total irrigated land. Even though the reuse of wastewater for agricultural irrigation has several benefits, it poses a public health risk due its pathogenic content. Among these pathogens, helminth eggs are of particular concern.

Helminths are parasitic worms transmitted to humans via their eggs (infective life stage, **Figure 1**). They are considered to be the biological structures most resistant to inactivation in the environmental engineering field [4]. Most helminths are transmitted by direct contact with contaminated soil, crops, or wastewater (e.g., *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms), but some require the presence of intermediate hosts (e.g., freshwater snails in the case of schistosomiasis) [5].

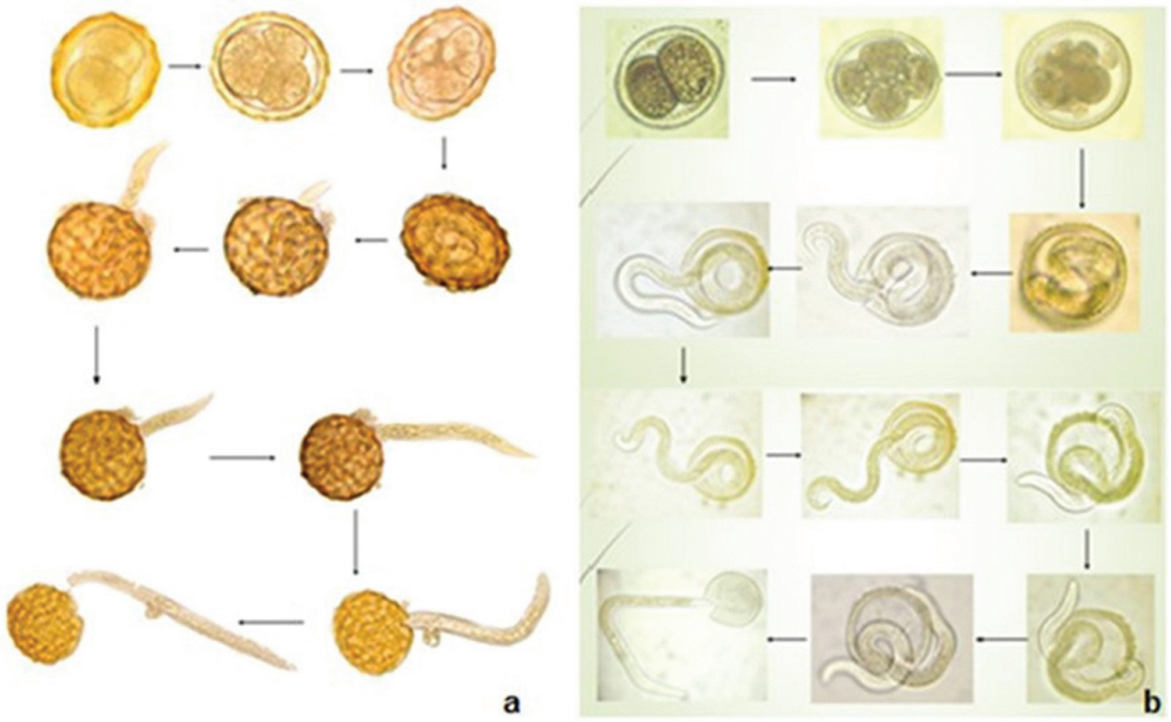

Figure 1. Fertilized eggs with larval growth and development of the worm for (a) *Ascaris lumbricoides* and (b) *Toxocara canis* (Photographic archives, Treatment and Reuse Group, Instituto de Ingeniería, UNAM).

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Concerns about their presence are related to their very low infectious dose, their high rate of survival in the environment for extended periods of time (up to several years, compared to weeks for other pathogens), and their high resistance to conventional disinfection processes [6]. Helminth eggs are a particular threat to health where sanitation conditions are poor, polluted water is used for irrigation, or where excreta or non-treated sludge is disposed of in an uncontrolled manner. They cause a set of diseases call "helminthiasis" that are given specific names based on the genera involved (for instance, ascariasis for genus *Ascaris*). Symptoms include deterioration of the intestines, toxic effects, blood loss, diarrhea, undernourishment, and anemia. Some helminths gnaw away at the intestinal wall causing hemorrhages while simultaneously secreting an anti-coagulant. The damage caused by inflammation, and the wounds they open generate tumors and excrescences. In addition, helminths can block conduits (for example, biliary) or cause intestinal obstruction and perforation of the digestive tract causing peritonitis [6, 7].

Helminthiases may lead to serious collateral effects limiting the physical and mental development of children, notably when repeated infection occurs between 5 and 15 years of age. It is estimated that 182 million children of preschool age are infected, that is, around 33% of those living in developing countries [8–10]. These children display lower height and weight due to undernourishment compared to well-nourished children living in healthy environments. At the same time, malnutrition results in a low school performance and a decrease in intelligence quotient (IQ) [11, 12]. Other effects, such as epileptic seizures, violent headaches, vertigo, local paralysis, vomiting, and optical and physical perturbations have been reported [7].

In spite of that, the control of helminth eggs was neglected until the late 1980s when the World Health Organization (WHO) developed guidelines for water and sludge reuse in agriculture. In 2006 the World Health Organization [6] confirmed the need to control the associated health hazards through an updated version of the guidelines for safe use of wastewater, excreta, and sludge in agriculture and aquaculture. These guidelines recommend limiting the helminth egg content in wastewater, excreta, and sludge; however, to achieve this, it is necessary to remove and inactivate the eggs. The following sections discuss the data collected for helminth eggs in environmental matrices, public health risks, regulations in different countries, treatment methods for wastewater and sludge, and analytical methods to determine their concentrations in environmental matrices.

2. Helminth egg content in wastewater and sludge

Helminths are multi-cellular organisms (worms) with a wide variety of shapes and sizes. Some of them are free-living (like earthworms), while others are parasites. Helminths can be classified in different ways, but the classification most frequently used considers their body shape. Roundworms are called Nematodes while flatworms are called Plathelminthes; if the latter have segments then are referred to as Cestodes while non-segmented ones are termed Trematodes [5]. As mentioned previously, helminths reproduce via the production of eggs, which are microscopic structures whose shape, size (20–180 m), and density (1.06–1.23) vary.

A fertilized *Ascaris lumbricoides* female roundworm can lay around 200,000 eggs per day and up to 27,000,000 eggs over a lifespan of 10–24 months, while the *Taenia saginata*, a beef tapeworm, can produce as many as 800,000 eggs per segment per day (a typically sized worm may have around 1000 segments) [13–15]. Helminth eggs contained in wastewater, sludge, or excreta may be viable (alive) but not infective. To be infective, the eggs need to develop into larvae, which require a temperature of around 25°C combined with a moisture content of at least 5% [16]. These conditions are found in soils or crops where eggs develop into larvae within a few days, remaining viable for several months or years [6]. In addition, infective doses are very low, within the range of 1–10 eggs. Most helminthiases are, therefore, transmitted via the eggs through a human-water-soil-crop-human pathway.

The content of helminth eggs in developing countries' wastewater and sludge differs considerably from that of developed ones (**Table 1**). Helminth egg concentrations may be 7–80 times greater in developing countries. For instance, the helminth egg content in developing countries ranges from 70 to 3000 helminth eggs per liter (HE/L) in wastewater and 70–735 helminth eggs per gram of total dry solids (HE/g TS) in sludge. In comparison, for developed countries, the content in wastewater is only 1–9 HE/L and that for sludge is 2–13 HE/g TS [17]. Not all eggs are viable, but, in general, mean viability generally reaches 88%, with an even higher percentage for *Ascaris* spp. (90%) as a result of their high environmental resistance [4, 18, 19].

Country/region	Helminth eggs in wastewater (HE/L)	Helminth eggs in sludge (HE/g TS)	References
Developing countries	70–3000	67–735	[13, 18, 20]
Tropical countries		300–3000	[18]
Brazil	166–202	1–76	[21]
Cayman islands	100–1230		[14]
China	840	2300	[22]
France	9	5–7	[20]
Egypt (liquid primary)	6–42	Mean: 67, Max: 735	[23–24]
Egypt (dewatered primary)		Mean: 8, Max: 124	[24]
Ghana		76	[20, 24]
Germany		<1	
Great Britain		<6	
Japan	80	1–51	[25]
Jordan	300		[14]
Mexico	6–98 (up to 330 in poor areas)	73–177 (viable eggs)	[17]
Morocco	840		[26]
South Africa	772	2–40	[27, 28]
Syria	800		[29]
Tunisia	30		[15]
Ukraine	60		[13]
United States of America	1–8	2–13	[20]

Table 1. Helminth ova content in wastewater and sludge for selected countries.

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3. Prevalence of helminthiasis

Stoll N. [30] calculated that there were 2200 million nematode infections worldwide and predicted that by 1991, there would be 5000 million infected people. Similarly, he estimated that the Chinese population produced a total of 18,000 tons of *Ascaris* eggs per year. Currently, it is estimated that more than 2.6 billion people are infected with helminths (**Table 2**), and although helminthiases are globally distributed, the prevalence of disease is mostly limited to developing countries [5, 7, 31]. They are endemic in regions of Africa, Central America, South America, and the Far East, with incidence rates as high as 90% for specific regions and sectors of the population [6, 7]. The dominant species depends on local conditions. However, most of the information related to procedures to control helminth eggs in sludge was gathered in developed countries where the low concentrations found render it difficult to utilize the data collected [20].

Around 96% of the helminthiases reported are induced by infection by Nematoda and Trematoda classes, with 4% due to Cestoda [5, 32]. The most common genus is *Ascaris lumbricoides* [33] with more than 819 million people infected. The eggs are highly infective (commonly, one egg suffices), very persistent in the environment, and very resistant to conventional disinfection/inactivation processes [10–12, 34]. For example, embryonated eggs of *Ascaris* spp. can survive for 20 days at temperatures of -20.9 to -27°C and survive in frozen Siberian soils for 10 years [35]. This resistance comes from the fact that eggs are surrounded by a series of layers functioning as a barrier that protects the larva against harsh environmental conditions. Indeed, the different helminth egg species possess 3–4 layers with different physical and chemical characteristics: (a) The 1–2 outer layers are formed of mucopolysaccharides and proteins; (b) the middle layers have chitin which provides structure and mechanical resistance; and (c) the inner layer, composed of lipids and proteins, protect the egg from desiccation, strong acid and bases, oxidants, reductive agents, detergents and proteolytic compounds [36–39].

The interaction of the layers' components with external agents may cause a change in their chemical composition, as well as in the specific function of each layer, which in turn modifies their permeability, their mechanical and chemical resistance and, finally, the egg viability. These mechanisms may be similar to those that take place during the hatching process, during which the larva secretes certain enzymes that induce the loss of permeability of the ascaroside layer and attack the outer eggshell layers. However, in this case, the layer remains intact until the hatching of the larva with no detectable chemical change [40–41].

The impact on the quality of life of infected populations may be illustrated using an estimation made in 2013 for years lived with disabiliy (YLDs) [42]. This amounted worldwide to 1,004,000 years for anemia caused by hookworm disease, and 671,000 years for schistosomiasis. Based on the analysis of YLDs estimates, ascariasis was among the eight most prevalent causes of disease, affecting more than 10% of world's population in 2013, such that if the mean prevalence of chronic sequelae is selected, for periods longer than 3 months, the impact of ascariasis was estimated in 819 million cases.

Helminth species	Common name	Prevalence (million inhabitants)	Regional presence
Nematoda			
Ascaris lumbricoides	Roundworm	819	Many regions of South-East Asia, Africa, and Central and South America
Ancylostoma duodenale	Hookworm	439	Tropical and subtropical countries (Sub-Saharan Africa), Central and South America, the Caribbean, Asia and in the Pacific islands
Necator americanus			
Strongyloides stercoralis		370	
Trichuris trichiura	Whipworm	465	Moist, warm, tropical regions of Asia, Africa, Central and South America, and the Caribbean islands
Trichostrongylus orientalis	Roundworm	Several	Mainly rural communities in Asia
Cestoda			
Hymenolepis diminuta	Rat tapeworm	50	Most occurrences in areas which lack adequate sanitation but may be found around the world in South America, Southeast Asia, Western Africa and East Africa; and in areas of the tropics and subtropics and some areas of Southern and Eastern Europe, the United States of America and Mexico.
Hymenolepis nana	Dwarf tapeworm		
Taenia saginata	Beef tapeworm	50	
Taenia solium	Pork tapeworm		
Trematoda			
Schistosoma mansoni	Blood fluke	207	Tropical and subtropical regions. This species occurs widely throughout Africa and South America, especially in Brazil, Venezuela, Surinam, Guyana and several Caribbean islands, including Puerto Rico, St. Lucia, Martinique, and Guadalupe
Clonorchis sinensis *Echinostoma* spp. *Fasciola gigantica* *Fasciola hepatica* *Fasciolopsis buski* *Heterophyes* spp. *Metagonimus* spp. *Opisthorchis felineus* *Opisthorchis viverrini* *Paragonimus* spp.	Food-borne Trematodes	56	Largely in Southern and Eastern Asia but also in Central and Eastern Europe.
Fasciolopsis buski	Intestinal fluke		
Other groups		100	Worldwide
Total		Over 2.6 billion infections worldwide	

Source: Ref. [5].

Table 2. Common helminth species and helminthiasis by region.

4. Guidelines and regulations

As mentioned previously, the use of domestic wastewater for irrigation, even without treatment, is an established practice in many countries. However, it leads to the proliferation of helminthiasis in the exposed population [6, 43]. Following a series of epidemiological studies, since 1989, the World Health Organization has recommended a limit for the content of nematode eggs in wastewater reused for agricultural irrigation [44]. Similarly, sludge produced during wastewater treatment may be reused in agriculture or soil remediation after adequate treatment to reduce its microbial content. Once treated, wastewater and sludge need

to meet national regulations or international criteria in order to be safely reused. For instance, in 2006, the World Health Organization suggested that a limit of less than 1 viable helminth egg per liter (HE/L) in wastewater makes it acceptable for agricultural reuse, while less than 1 viable helminth egg per gram of total dry solids (1 HE/g TS) is required in sludge [6]. However, these limits are difficult to meet in countries or regions with endemic diseases, requiring effective inactivation processes to control the high concentrations of helminth eggs.

Following the WHO guidelines [6], many countries have set standards for helminth eggs in treated water, including Brazil, Colombia, Costa Rica, Chile, Israel, Jordan, Mexico, Saudi Arabia, and Tunisia. These standards also set recommendations for the types of crops to be irrigated, irrigation methods, and other intervention measures to manage risks. In addition, sludge and biosolids (treated sludge) regulations have been developed by several countries, led principally by the United States (**Tables 3** and **4**).

Country	Regulation-standard-guidelines	Type	Helminth eggs limit, eggs/L
Australia	Australian regulations, 1995	U	–
		N/A	Absent
Argentina	Resolución 778/96	U	–
		R	<1 Nematode
Chile	Norma 1333 (1978)	U	-
		R	<1 Nematode
Mexico	NOM-001-SEMARNAT-1996	U	<1
		R	<5
United States of America	Department of Health Services (DHS), California, 1918.	N/A	Absent
WHO	WHO, 2006	U	≤1 (arithmetic mean)[b]
		R	
		Localized (drip) irrigation)	Low-growing crops: ≤1 (arithmetic mean) High-growing crops: no recommendation

U: Unrestricted irrigation: For agricultural irrigation of all kinds of crops, including those that are eaten uncooked (lettuce, onion); R: Restricted irrigation: For agricultural irrigation of all kinds of crops, except that are eaten uncooked (highly mechanized, labor intensive).

[a]When children under 15 years of age are exposed, additional health protection measures should be used (e.g., treatment to ≤0.1 egg per liter, protective equipment such as gloves or shoes /boots or chemical treatment);

[b]An arithmetic mean should be determined throughout the irrigation season. The mean value of ≤1 egg per liter should be obtained for at least 90% of samples in order to allow for the occasional high-value sample (i.e., with >10 eggs per liter). In some wastewater treatment processes (e.g., waste stabilization ponds), the hydraulic retention time can be used as a surrogate to assure compliance with a limit of ≤ 1 egg per liter;

[c]Including fruit trees and olives, not crops harvested directly from the soil.
Source: Refs. [6, 45–47].

Table 3. Regulations for helminth egg content in treated wastewater use in agriculture.

Country	Regulation standard guidelines	Class/type	Helminth egg limit, eggs/g TS
Brazil	CONAMA 375/2006	A	0.25
		B	10
Chile	No. 123 (30/08/2006)	A	0.25
		B	-
France	Directive 86/278	-	0.3
Mexico	NOM-004-SEMARNAT-2002	A	1
		B	10
		C	35
New Zealand	Guidelines for the safe application of biosolids to land in New Zealand	A	0.25
Norway	Regulations for the treatment, use and disposal of sewage sludge	–	Absent
South Africa	Guidelines for the utilisation and disposal of wastewater sludge (Vol. 2)	A	0.25
		B	1
The United States of America	CFR 40 Part 503 /1993	A	0.25
The WHO	WHO, 2006		1

[V]Viable eggs.

Table 4. Regulations for helminth egg content in sludge.

In general, the standard values have been set to protect human health, allowing sludge application at sites where human contact may occur [48]. Under those conditions, a value of <1 HE/g is to be achieved (Class A). Only Brazil and Mexico allow higher concentrations of helminth eggs. However, in the case of Brazil, the regulation states that Class B sludge may be land applied only after risk assessment determines that this is a secure practice. In contrast, the limits for Class B and C sludge in the Mexican regulations refer to total eggs, assuming not all of them are viable, and is intended for land application without human contact (e.g., agriculture, soil restoration, forestry). However, to enforce these limits, it is necessary to correctly measure the helminth egg content in wastewater and sludge (treated or untreated).

5. Quantitative microbial risk assessment (QMRA)

WHO guidelines and US EPA limits for helminth eggs were based on limited epidemiological evidences, and on the performance of different sludge treatment methods, rather than the results of a risk assessment. Neither of the organizations based their considerations on results of a dose response-curve "because methodologies had not been developed sufficiently enough to make such calculations" [6, 49]. As a result, no human risk assessment was conducted, due to the lack of a dose-response model appropriate to describe helminth eggs infection. At the time, of the three different methods used to evaluate microbial risks: microbiology laboratory analysis, epidemiological studies, and quantitative microbial risk assessment (QMRA), only

the first two have been applied to helminth data [50–52]. In contrast, a number of human dose-response relationships have been developed for bacteria, viruses, and protozoa [53–59]; consequently, a dose-response function for helminth infections was required for applying a QMRA.

QMRA is a modeling approach used to predict the human health risks from exposure to pathogens and has been shown to be effective in assessing the transmission of water and food-borne infections. It estimates the risk of infection or illness based on the concentration of infectious pathogens in wastewater, on surfaces or in drinking water, or from other environmental sources (e.g., crops, biosolids, air), the estimated ingestion rate, and the established dose-response models for a given population.

Since 2009 a dose-response curve is only available for *Ascaris* [60], although other helminths genus are highly relevant in wastewater and sludge reuse, especially in developing countries [61]. This curve was derived from *A. lumbricoides* prevalence data obtained from stools of a large sample of children in the Mezquital Valley in Mexico, rather than conducting human *Ascaris* dose-challenge studies. Navarro et al. [60] found that their *Ascaris* infection data best fitted the β-Poisson dose-response equation [Eq. (1)].

$$P(d) = 1 - \left[1 + \left(d / N_{50}\right)\left(2^{1/\alpha} - 1\right)\right]^{-\alpha} \tag{1}$$

where P(d) is the risk of infection in an individual who has ingested *d Ascaris* eggs on one occasion; N_{50} is the mean *Ascaris* infective dose; and α is an *Ascaris* 'infectivity constant'. They found the values of N_{50} and α to be 859 and 0.104, respectively.

Use of the QMRA tool allowed an initial target estimation of *Ascaris* concentration in sludge and wastewater. These values were greater than WHO and US EPA limits [62], but with an estimated probability of infection smaller than the prevalence rate observed on site. Such scenarios may allow a gradual improvement in population health conditions, as well as working towards an *Ascaris* eggs content in sludge and/or wastewater which gradually should approach regulation limits. The authors concluded from these risk analyses that regulations targeting biosolids reuse in developing countries should address the challenge of firstly deciding an acceptable infection risk and, secondly, putting in place an integrated framework for risk management, involving additional health protection measures. They suggested factors to be considered, which include, amongst others: (a) an affordable treatment process, (b) crop restriction policy, (c) different sludge application rates, and (d) efficient produce washing methods.

Further research should focus on QMRAs with the β-Poisson dose-response model for *Ascaris lumbricoides*, which have demonstrated through case studies its application to analyze the tolerable risk and to evaluate additional control measures, considered as potential interventions for health risk reduction. They illustrates the importance of two recommendations from WHO guidelines [3]: The first is that in some circumstances, "it is recommended that, initially, a national standard is established for a locally appropriate level of tolerable additional burden of disease based on the local incidence of diarrheal disease," and the second that post-treatment

health-protection control measures can achieve significant pathogen reductions, so that wastewater treatment does not have to achieve the total pathogen reduction required to protect consumer health.

In the first case, it was shown that a maximum tolerable additional disability-adjusted life year (DALY) loss per person per year (pppy) of 10^{-4} is an appropriate value, especially in low-income countries [63]. It is more applicable than the WHO guidelines [3] default value of $\leq 10^{-6}$ DALY loss for the tolerable additional burden of disease due to wastewater pathogens. A tolerable *Ascaris* infection risk of 1.2×10^{-2} [64] corresponds to a tolerable DALY loss of 10^{-4} pppy. Therefore, the QMRA results, applying the β-Poisson dose-response model, indicate that a ova reduction to 10 eggs/L results in a risk of $\sim 3 \times 10^{-3}$, which could be used as a guideline value. This is in agreement with previous recommendations of ≤15 eggs/L [65, 66] who also suggested that pathogen reduction may be achieved by simple wastewater treatment.

QMRA applications using the β-Poisson dose-response model have illustrated the health protection levels that may be achieved with some control measures. Experimental work by [67] showed that a waiting period of 120 days at 25°C or 90 days at 37°C following land application of biosolids to lettuce fields would result in acceptable yearly risks of less than 10^{-4} for *Ascaris* for the planting of the crop and acceptable yearly risks of less than 10^{-4} for *Ascaris* from the consumption of lettuce. They were able to evaluate the effect of different incubation temperatures of biosolids on the inactivation of *Ascaris* eggs and estimated an appropriate time between the application of biosolids to land and the harvesting of lettuce to achieve an acceptable risk for consumers at the end of the exposure pathway.

An important topic with regard to the safe use of wastewater in agriculture is the estimation of the number of days of irrigation cessation required to achieve tolerable annual infection risk. The on-site die-off of pathogens through irrigation cessation is considered a potential intervention for health risk reduction. Seidu et al. [68] undertook the challenge of comparing the best fit die-off model for *Ascaris* derived in their study with existing die-off models in a QMRA. The β-Poisson dose-response model was used in order to assess the effect of different die-off models on the days required to achieve the tolerable annual infection risk associated with the consumption of wastewater-irrigated lettuce. The study showed that none of the survival curves of *Ascaris suum* fitted the log-linear model, indicating that the classical first-order kinetic approach is inadequate in many cases. The implication of using die-off models for health risk assessment was an underestimation of the number of days of irrigation cessation necessary to reduce *Ascaris* infection risk for the log-linear die-off rate compared to the biphasic die-off rate of their study. Therefore, cessation of irrigation as a health risk reduction measure appeared to be impractical, given the prevailing conditions, in their study area. This also indicated that assessing the health risk reduction efficacy of intervention using QMRA models is dependent on accurate characterization of the die-off of pathogenic organisms.

Studies of Barker *et al* [69] and Kundu *et al* [70] assessed the risk of gastrointestinal infections caused by *Ascaris lumbricoides*, among other pathogens, associated with the consumption of raw vegetables. They used a QMRA in two developing regions (Kumasi, Ghana, and the Arias-Arenales River, Argentina). Both studies are examples of risk estimations that consider the *Ascaris* infection risk as a result of the quality of the water resource used for irrigation, the

results after harvesting, and due to crops being eaten raw. They also included the *Ascaris* infection risk from accidental ingestion of contaminated water and produce washing behaviors of the population. They developed different approaches for following the steps of a QMRA, showing that a wide range of assumptions may be made for specific exposure scenarios to identify the reduction measures needed to protect human health. Their results and discussion also made it evident that the variability observed is a consequence of the inherent characteristics of each case study and that the uncertainties analyzed in the different scenarios illustrated the variables that played a major role in the risk estimates. Barker et al. [69] concluded that "particularly in the context of developing countries, it is important to balance the risks with the benefits of access to fresh vegetables. It is important that the consideration of the benefits is not lost in the discussion of the risks, which remain real and significant".

Finally, it was shown that these case studies illustrate that QMRA is a useful tool for developing standards for human exposure to pathogens. Clearly, additional interventions and changes in behavior need to be investigated and implemented to reduce the risks to acceptable levels; however, in the meantime, a more efficient and rapid analytical technique of helminth egg detection in environmental samples is needed, as well as high-quality control of local data to improve the exposure scenarios.

6. Wastewater treatment processes

In order to remove helminth eggs from wastewater, processes that remove particles, such as sedimentation, filtration, or coagulation-flocculation, are employed [71–73]. In practice, and in contrast to other microorganisms, helminth ova cannot be inactivated with chlorine, UV light, or ozone (in the latter case at least not with economical doses since a concentration of >36 mg/L ozone is required with 1 h of contact time). As discussed previously, their morphology, in terms of their external structure, protects them from inactivation.

The main removal mechanisms employed during wastewater treatment are associated with the size and density of helminth eggs. Since they are denser than water, they may be correlated with solid particles in wastewater [measured as total suspended solids (TSS)] and this, therefore, aids their separation. This correlation is used to indirectly measure their content and evaluate the performance of treatment processes in terms of their removal [74]. Due to their size and adhesiveness, helminth eggs can also be removed from wastewater through filtration [33, 71, 75, 76]. In terms of wastewater treatment, the following processes may achieve good removal of helminth eggs [33, 73].

- *Stabilization ponds (SP)*. Stabilization ponds are large shallow basins enclosed by earth embankments, in which raw wastewater is treated by entirely natural processes. Several factors contribute to removing helminths, namely sedimentation, temperature, sunlight, pH, microorganism predation, adsorption, and absorption, although sedimentation is the most effective. There are three types of SP: anaerobic, facultative, and maturation ponds. Anaerobic (1-day retention time) and facultative ponds (5–15 days) are best for helminth ova removal.

- *Reservoirs*. Similarly to stabilization ponds, reservoirs and dams can remove helminth ova from wastewater if retention times longer than 20 days are used.
- *Coagulation-flocculation*. This process promotes aggregation of solid particles with the use of metallic salts (mainly alum or iron salts) and produces water suitable for agricultural reuse. When this process uses low coagulant doses (50–65 mg/L) combined with synthetic polymers as flocculants (0.8–1.2 mg/L), it is called chemical enhanced primary treatment (CEPT) or advanced primary treatment (APT). CEPT or APT promote particle aggregation, including helminth eggs, into flocs that increase their settling velocity and allow separation via sedimentation.
- *Filtration*. This consists of passing primary or secondary effluent through a porous material that retains the solids and produces a good quality effluent. Rapid filtration (rate >2 m/h) is one of the most useful treatments to remove helminth eggs from effluents, either physicochemical or biological, with values consistently below 1 HE/L.
- *Constructed wetlands*. These are designed to operate by gravity, and they are generally shallow to allow a better removal of pollutants. The plants typically used are as follows: (a) large plants with floating or aerial leaves; (b) plants with well-developed and submerged roots, such as rushes, water hyacinth, reeds, and water lilies; and (c) very small floating plants with few roots or no roots at all, such as those of the *Lamenacea* family, *Lemna* or duckweed, *Spirodela*, *Wolffia*, *Wolffiela*, and *Salvinia* [77, 78]. Most of the removal of helminth eggs occurs within the first 25 m in a horizontal flow gravel bed wetland (100 m long), reaching 100% after the entire process [79, 80].

7. Sludge stabilization

Once separated from wastewater, most helminth eggs are concentrated in sludge, which needs to be adequately treated before its land application or disposal. Since helminth eggs are the most resistant form of the parasites [4, 18–19], sludge treatment processes must consider their initial concentrations to achieve their inactivation and comply with regulations. In this respect, helminth eggs have the ability to survive for long periods of time in raw sludge and soil (up to 6 years from their initial application [81]). As a result, different sludge treatment processes may be used to inactivate helminth eggs, often including thermal treatment.

As an example, some of the best results for helminth egg inactivation in sludge have been obtained using thermal treatment at 108°C, irradiation at 1000 Gy or greater, pasteurization at 70°C, and thermophilic (55°C) anaerobic digestion [82–85]. In addition, the US EPA [48] allows the use of different alternatives to produce a sludge that meets class A limits (<0.25 HE/g TS) which include processes such as composting, irradiation, pasteurization, and heat treatment. However, all these advanced treatments require investments that are not always feasible in developing countries, where basic sanitation strategies are of greater importance.

With respect to more conventional treatment processes, mesophilic aerobic or anaerobic digestion do not reduce viable eggs of *Ascaris, Trichuris, Toxocara,* or *Capillaria* [84]. When

mesophilic anaerobic digestion has been evaluated, at least 50% of *Ascaris suum* eggs were found to survive for up to 5 weeks [86]. In contrast, studies of alkaline pre- and post-stabilization with 40% (w/w) lime [71, 87] have reported inactivation efficiencies between 92 and 95%.

Several studies have evaluated the combination of different adverse conditions to inactivate helminth eggs, with some of them including the use of a range of chemicals. For example, Ghiglietti *et al* [88] found that a combination of sludge alkalinization with ammonium hydroxide (NH_4OH) at 30°C causes the inactivation of *A. lumbricoides*, *A. suum*, and *Trichuris muris*, and that a temperature of 40°C, with or without ammonia, was unfavorable for the development of the eggs; however, at 22°C, there was no effect, even with the addition of ammonia. Another study [87] also obtained a 69% reduction of viable helminth eggs after 2 h contact time with 10% (w/w) ammonia. When 50% ammonia was added, inactivation reached 94%. In addition, optimal conditions for egg inactivation have been found to include a combination of temperature, pH, and dryness of the sludge (45°C, pH of 5.3 and 90% dryness within 6 days or 45°C, pH of 12.7 and 90% dryness within 19 days) [4]. These results also confirm that the acidification process with peracetic acid was more effective than lime treatment. In this respect, Barrios *et al* [89] also evaluated the use of peracetic acid for microbial inactivation in sludge; results showed that the application of 550 ppm for 30 min reduced viable helminth eggs by 94% from an initial concentration of 93 viable HE/g TS. Aguilar et al. [90] evaluated the use of silver for inactivating high concentrations of helminth eggs in sludge (168–215 viable HE/g TS); the use of silver alone was not sufficient for complete inactivation. However, when silver was applied with copper and a synergistic agent (5:50:13.3 mg Ag–Cu–SA/g TS), total inactivation was achieved after 60 min.

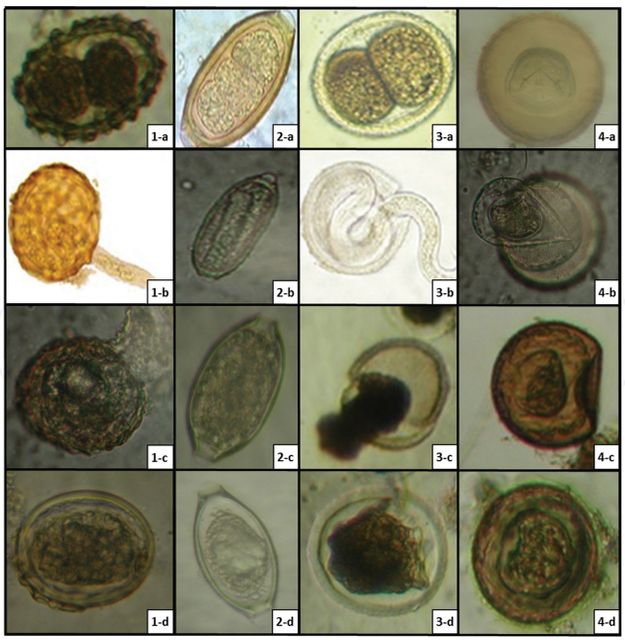

Figure 2. Viable eggs: 1-a *Ascaris*; 2-a *Trichuris*; 3-a, *Toxocara*; and 4-a *Hymenolepis diminuta*. Larval eggs: 1-b *Ascaris*; 2-b *Trichuris*; 3-b *Toxocara*; 4-b *Hymenolepis*. Morphological damage observed when eggs were subjected to different chemical reagents: 1-c and 1-d *Ascaris*; 2-c and 2-d *Trichuris*; 3-c and 3-d *Toxocara*; and 4-c and 4-d *Hymenolepis*. (Photographic archives, Treatment and Reuse Group, Instituto de Ingeniería, UNAM.).

Following some of those studies, the effect of different chemicals on the morphology of helminth eggs has been evaluated. After the addition of peracetic acid, lime, increased temperature, and desiccation, serious malformations of the embryos and larva, and even the destruction of the embryo, have been documented (**Figure 2**).

8. Analytical techniques

In order to quantify helminth eggs in wastewater, sludge, and excreta, an analytical procedure based on their visual identification and enumeration is used. However, current methodologies are not always effective for identification since experienced technicians are required, and therefore, results are often neither accurate nor reliable. Moreover, there is no universally accepted method to quantify them in sludge and biosolids [91]. The available analytical procedures commonly have two steps: (a) the separation of as many eggs as possible from other particles in the sample and (b) their visual identification under the microscope, where the concentrated sediment (pellet) contains many other types of particles. Only a properly trained technician is able to discriminate such particles from the eggs, and this technician has to visually identify the different species of helminth eggs. This is critical and constitutes the main source of error and uncertainty in the methodology. In particular, it is problematic when processing samples with a high content of helminth eggs because the discrimination process is tedious and time-consuming. In short, the challenge for the analysis is to separate, correctly identify, and enumerate helminth eggs in samples that, even after processing, contain many impurities, which render this difficult. Several alternative methods have been proposed to quantify helminths, considering the complexity of dealing with environmental samples. However, the efficacy of their application is yet to be proven.

As part of newly proposed analytical techniques, molecular methods have reported variable results. Some of these have been tested with synthetic samples and as a result, they still need to demonstrate their feasibility where actual environmental samples are analyzed. A recent study [92] compared different methods to detect hookworm ova over a range of concentrations and concluded that direct DNA extraction exhibited some limitations due to polymerase chain reaction (PCR) inhibitors present in environmental samples. In contrast, another study reported a sensitivity of one to four hookworm ova in wastewater samples [93]. Using quantitative PCR methods, *Ascaris* eggs may be detected [94, 95]; however, this technique still has some limitations, such as the low recovery rates obtained in samples with low numbers of eggs or the production of false positives.

A recent approach has proposed the use of image processing algorithms to identify and quantify helminth eggs from photographs taken using a microscope. This method is based on selected characteristics of helminth eggs that allow their correct identification without the need for a highly trained technician; it is capable of identifying up to eight species of helminths with a specificity (capacity to discriminate between species of helminth eggs and other objects) and sensitivity (capacity to correctly identify and classify the different species of helminth eggs) of 0.99 and 0.90, respectively [5].

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9. Final remarks

Much knowledge has been gained since the early 80s but there are still major challenges in the field of environmental engineering. A large number of species of helminth eggs are excreted by humans and are discharged into wastewater, representing a significant source of pollution to the environment. In particular, when untreated or partially treated wastewater is discharged or used for agriculture, the risk of transmitting these types of diseases increases. The concentrations of helminth eggs in environmental matrices (wastewater and sludge) are directly related to public health. Therefore, until the millennium goals of 100% sanitation worldwide are met, about 2.6 billion cases of helminthiasis will prevail. Meanwhile the integrated management of wastewater and sludge remains as an engineering challenge.

Wastewater and sludge regulations focus on public health protection, assuming agricultural reuse and land application is practiced. Very low concentrations are permitted in treated effluents and biosolids (<1 HE/L or gram of dry solids, respectively). In some cases, these levels are not easy to achieve, considering the high concentrations occurring in developing countries. Helminth eggs are associated with solid particles in wastewater, either because they behave in the same way or they are part of aggregated solids. As a result, processes related to solids removal (e.g., sedimentation, filtration, or coagulation-flocculation) will also remove most of the helminth eggs contained in wastewater. Once separated, they will concentrate in sludge where they need to be inactivated before the sludge is reused or disposed of. For this purpose, processes that increase temperature and/or pH, reduce sludge humidity, as well as the use of certain chemicals (e.g., lime, organic acids, or ammonia) achieve high inactivation (>90%). Within conventional sludge treatment methods, the use of quicklime has proven effective where high concentrations occur. However, in developing countries where high concentrations of helminth eggs are found, there is still a need for economic yet efficient inactivation methods.

In order to demonstrate that these processes meet the strict limits established by regulations and guidelines, adequate methods for identification and quantification are required. Current analytical techniques to quantify helminth eggs in environmental samples are usually time-consuming and require a high level of expertise to differentiate between species. Promising alternative methods are being developed, including the use of molecular tools as well as image processing technologies that could improve sensitivity and reduce processing time. Further validation will prove their applicability under different laboratory and/or field conditions.

Quantitative microbial risk assessment has been used to predict the human health risks from exposure to *Ascaris* eggs. Some QMRA applications suggest that helminth egg limits estimated may be initial targets and may be included in national regulations (wastewater and sludge for agricultural reuse), even though they may be less strict than those proposed by WHO and US EPA, but still they will gradually reduce infection risks from these parasites. Moreover, several QMRA results have demonstrated that additional interventions and behavior changes may be implemented to reduce the risks to acceptable levels. Nonetheless, more high-quality data is

needed to improve the evaluation of different exposure scenarios and to reduce uncertainties in risk estimates.

From the point of view of environmental engineering, helminth eggs are relevant since they need to be removed from wastewater and inactivated once in sludge to break their life cycle. However, to further improve public health, other strategic measures, such as preventive chemical treatment, and specific agricultural practices, must be implemented, and thus, a multidisciplinary approach is needed to address this global problem.

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