Helminth Ova Control in Wastewater and Sludge for Advanced and Conventional Sanitation

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Abstract
Worldwide, the most important reuse of wastewater, in volume, is agricultural irrigation. Therefore, there is a need to properly treat wastewater for such purpose, considering the removal of pathogens while leaving suitable amounts of nutrients and other compounds to increase productivity. Helminth ova are one of the main targeted pathogens in the new guidelines for water reuse in agriculture and aquaculture issued in 2006 by the World Health Organization. However, relatively little research has been done recently on how to remove and inactivate helminth ova from wastewater and sludge and recommendations given several decades ago are still used, but when put in practice, particularly in developing countries, produce unsatisfactory results. One problem is that these criteria were developed using inaccurate analytical techniques and the other is the large number and variety of helminth ova species found in wastewater and sludge from the developing world. In fact, the few technological options to remove and inactivate helminth ova come from research performed using wastewater and sludge with low helminth ova content, and refers almost only to *Ascaris* (one type of helminth). This paper summarizes recent research work and results from practical experience concerning helminth ova control for advanced and conventional sanitation.

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

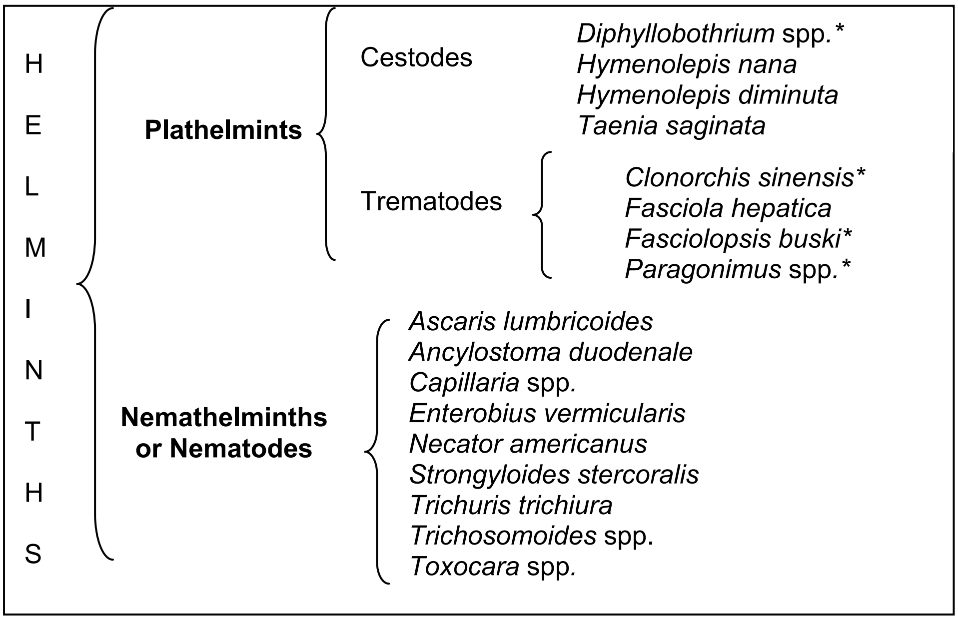
INTRODUCTION

In several regions of the world, wastewater (with or without treatment) and sludge is being used for agricultural purposes. In 1989, the World Health Organization (WHO) drew attention to diarrhoeal diseases caused mainly by helminths present in sludge and wastewater and set guidelines for safe reuse. In 1992, the USEPA published the biosolids and sludge regulation (part 503) defining the elimination of helminth ova as a key parameter for sludge reuse in agriculture. During 2006, WHO published new guidelines, this time for the safe use of wastewater, faecal material and sludge in agriculture and aquaculture, and limited helminth ova content to a value that for developing countries implies using treatment methods with 1-3 log removal or inactivation efficiencies, for which there is almost no information. In spite of the importance of helminth ova as waterborne vectors, throughout the years little attention has been given to them in terms of their control, in both wastewater and sludge. Helminth ova are poorly known and understood in our professional field because they behave different than bacteria, viruses and protozoa, problems we are more or less trained to deal with. This paper reviews from a purely sanitary engineering point of view: (a) general characteristics of the helminths; (b) common helminth ova genera found in wastewater and sludge around the world; (c) the reason why common water and sludge disinfection methods are not effective at inactivating helminth ova; (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 several research papers) should prove particularly useful for developing countries where helminths are a concern. The aim is to furnish useful information to water professionals while encouraging researchers to look for new helminth ova removal and inactivation methods.

Helminthiasis: a common disease

Globally there are 5 million people suffering helminthiasis, mainly in developing countries. Helminthiasis are particularly common in regions where poverty and poor sanitary conditions are dominant, where incidence rates may reach 90%. 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 (WHO, 2006).

Helminthiasis are named after the causative agent, being ascariasis (caused by *Ascaris* spp.), the most common and endemic in Africa, Latin-America and the Far East. Helminths are pluri-cellular worms that reproduce through ova (eggs); they are not microbes although their eggs are microscopic. They can have a flat body and are called Plathelminths, or a round body and are called Nemathelminths or Nematodes. Flat worms can have their body segmented, and are called Cestodes, or not segmented and are called Trematodes (Fig. 1). Only Cestodes and Nematodes are of sanitary importance. Helminths differ in shapes and sizes (from 1 mm to several m length), and also their eggs (Fig. 2). As shown in Fig. 1**,** it is improper to use the terms nematodes, *Ascaris* and helminths as synonyms, as frequently happens. This misunderstanding comes from the fact that *Ascaris*, a Nematode, is the most common helminth egg in wastewater and sludge (Fig. 3).



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

Figure 1. Helminth classification and common genera found in wastewater and sludge (Jiménez *et al.,* 2002).

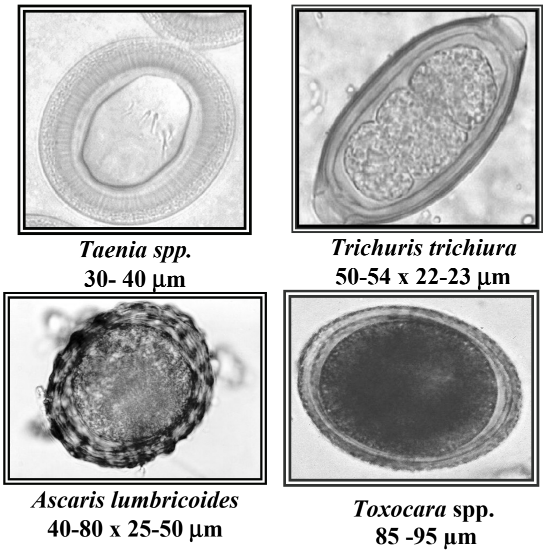


Figure 2. Helminth ova observed in wastewater and sludge (Jiménez *et al.,* 2002).

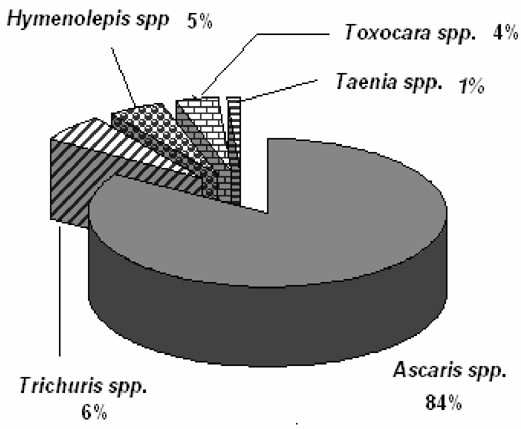


Figure 3. Genera found in wastewater (Maya *et al.,* 2006).

Helminthiasis are acquired through ingestion of polluted crops or meat, and contact with faeces, wastewater or contaminated soil. Helminths life cycles are very complex and different from that of bacteria and protozoa. The life cycle of *Ascaris lumbricoides* illustrates this complexity well. When a person ingests infectious *Ascaris* eggs, they adhere to the duodenum where the larva leaves the shell, crossing the intestinal wall into the blood stream. Through the blood, *Ascaris* travels to the heart, lungs, and bronchial tubes where it breaks the walls remaining around 10 days in the alveolus. Then travels to the trachea from where it is ingested again returning to the intestine, where it reaches its adult phase and, once mated, the female produces up to 200,000 eggs per day. During its migration, *Ascaris* may cause fever, urticaria and asthma; it may encyst in kidney, bladder, appendix, pancreas or liver, and its presence in the intestine produces abdominal pain, meteorism, nausea, vomiting, diarrhoea, and undernourishment. In general, the infective agents are the eggs, not the worms. Worms cannot live in wastewater or sludge because they need a host. Therefore, part of the control strategy for helminthiasis is to remove the eggs from wastewater and inactivate them in the sludge produced from wastewater treatment. Eggs contained in wastewater may be viable (alive) but not infective. To be infective they need to develop larvae, for which a certain temperature and moisture are required (26^{o}C and 1 month in laboratory conditions). Similar conditions are usually found in soils or crops where eggs can develop larvae in 10 days, hence the risk of using untreated wastewater or sludge in agricultural fields. Helminth ova can remain viable in water, soil, and crops for several months/years (WHO, 2006).

Helminth ova content in wastewater and sludge

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

Table 1. Helminth ova content in wastewater and sludge from different countries (Jiménez *et al*., 2002; Hays, 1977; Jiménez and Wang, in press).

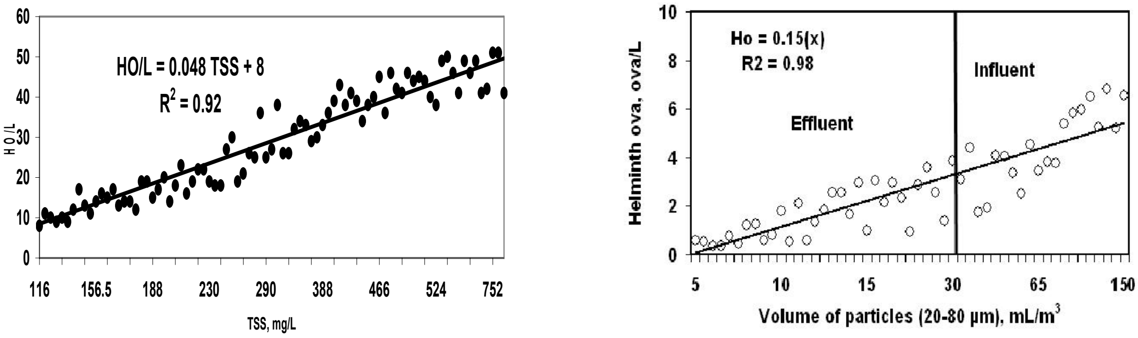
Country/region	Municipal wastewater HO/L	Sludge HO/gTS
Developing countries	70-3000	70-735
Mexico	6 – 98 in cities Up to 330 in rural and peri-urban areas	73-177
Brazil	166 – 202	75
Egypt		Mean: 67; Maximum: 735
Ghana		76
Morocco	840	
Jordan	300	
Ukraine	60	
United States	1-8	2-13
France	9	5-7
Germany		< 1
Great Britain		< 6

Helminth ova criteria. As shown in Table 1, not all wastewater and sludge contain significant amounts of helminth ova. For this reason they are not included in all countries wastewater regulations, or in all sludge revalorization options. Based on toxicological and epidemiological studies, WHO has set a recommended limit of ≤ 1 HO/L for the irrigation of crops that are eaten raw. In sludge or biosolids intended for agriculture, based on the value of ≤ 1 HO/L set for wastewater, a limit criteria of 3 to 8 HO/g TS has been calculated, depending on the sludge application rate (Hespanhol, 2002). This value is much greater than the 0.25 HO/g TS set by US EPA or 1 HO/g TS set by several countries. The US EPA value is 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 TS. This stringent limit is set only for Biosolids Class A (sludge with no restriction on use), while for Biosolids Class B there are no helminth ova limits, although sludge may be reused in agriculture with some restrictions.

Helminth ova characteristics. An important characteristic of helminth ova is that they are protected by a shell with three basic layers: a lipoid inner layer, a chitinous middle layer and an outer protein layer, making the eggs very resistant under several environmental conditions. Helminth ova of concern in the sanitary field measure 20 to 80 µm and have a density of 1.056-1.237 (David and Lindquist, 1982) and are very sticky. All these properties determine helminth ova's behaviour during treatment. First, it is very difficult to inactivate them unless the temperature is above 40°C or moisture is reduced to below 5% (TS > 95%) for a suitable contact time (Hays, 1977). However, contact time under these conditions and other related environmental factors are generally not known for high concentrations of helminth ova or for every genus. Nevertheless these inactivation conditions cannot be achieved in wastewater treatment but are common in sludge treatment.

Helminth ova removal from wastewater

Basically, the removal processes for helminth ova are those used for solid particles. In fact, helminth ova content has been related to the suspended solids content (TSS), in particular to the 20-80 µm particle content (Fig. 4). Both correlations are useful for tracking process performance when indirectly evaluating helminth ova in effluents, since they have much lower costs and response time than the analytical procedure for determining helminth ova (Chávez *et al*., 2004).



(b)

(a)

Figure 4. Correlation between helminth ova content in Mexico City's wastewater and: (a) the TSS content, (b) the 20-80µm particles content (Chávez *et al.*, 2004).

Waste stabilization ponds and reservoirs. Waste stabilization ponds are very efficient at removing helminth ova, mainly by the sedimentation process, requiring 5-20 days of retention time. In developing countries with warm climates, the use of stabilization ponds to recycle wastewater for agriculture is recommended when land is available at a reasonable price. However, care must be taken in zones with high evaporation rates, since ponds may represent a net loss of water, in some instances of up to 20-25% of the water inflow (Duqqah, 2002), and the increase in water salinity may difficult its use in agriculture. In some cases removal efficiencies are not attained in practice due to hydraulic problems, such as flow short-circuiting. Furthermore, there is little data concerning helminth ova survival at the bottom of the ponds. Nelson *et al.* (2004), in a study performed in several waste stabilization ponds in Mexico, found 14 viable HO/g TS in sludge stored for at least 9 years. Similar to stabilization ponds, reservoirs and dams may remove helminth ova from wastewater if retention time is greater than 20 days and are operated as batch systems (Juanicó and Milstein, 2004).

Wetlands. In wetlands, helminth ova are removed by filtration through the soil and adhesion to roots. There are several types of plants that may be used, 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. Pathogen removal depends on the climate, the type of wetland, and the type of plant used. Better performance is obtained when using retention times of 4 days in surface flow wetlands using duckweed. To remove 100 % of helminth ova it is necessary to couple wetlands with a horizontal flow gravel bed. Most of the removal takes place within the first 25 m (Stott *et al.*, 1999).

Coagulation-Flocculation. This process has proven to produce water fit for agricultural reuse. When this process uses low coagulant doses combined with high molecular weight/high density charge flocculants it is called chemical enhanced primary treatment (CEPT), and if it is coupled with a high rate settler, it is called advanced primary treatment (APT). APT and CEPT remove helminth ova while allowing organic matter, nitrogen and phosphorus to remain in water in the dissolved fraction or as very small particles, yielding a very good quality effluent for agriculture. The operating principle is to accelerate helminth ova's settling velocity (normally 0.39-1.53 m/h) using chemicals. An effluent with less than 20–40 mg TS/L may have a helminth ova content of 3-10 HO/L, and with less than 20 mg TS/L a content of $\leq$ 1 HO/L (Chávez *et al.*, *op.cit.*). Most common coagulants are Fe or Al. Lime has also been used but very high doses are needed and sludge production turns out to be also very high. To reduce coagulant doses, sometimes to a few mg/L, anionic polymers are used. CEPT has a total hydraulic retention time of 4-6 hours, while

APT only 0.5-1 h., thus reducing the costs to 1/3 of what a conventional activated sludge system costs, including operation and sludge treatment and disposal within 20 km (Jiménez and Chávez 1997). APT removes 90-99 % of helminth ova (Jiménez *et al*., 2001) and from a content of up to 120 HO/L, it may constantly produce an effluent with 0.5-3 HO/L (Chávez *et al.*, *op. cit.*).

Rapid sand filtration (> 2 m/h). This is a useful treatment for removing protozoa and helminth ova from physicochemical or biological effluents. Rapid filtration removes 90-99 % of helminth ova, and may be increased if coagulants are added (Jiménez *et al*., 2001). Rapid filters have a filtration media size from 0.8-1.2 mm, a minimal filter bed of 1 m and filtration rates varying from 7-10 m^3/m^2.h. Under these conditions, the effluent constantly contains < 0.1HO/L and the filtration cycles are of 20-35 h (Landa *et al*., 1997).

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

Helminth ova inactivation in sludge
Ova inactivation in sludge has been studied more than ova removal from wastewater, but unfortunately it has seldom used high initial contents of eggs. Several processes are recommended by USEPA to further reduce pathogens (PFRP) in sludge. Table 2 shows practical or theoretical application of these processes in sludge from developing countries.

Alkaline post-stabilization. Widely used to treat sludge in big and small wastewater treatment plants and even in on-site sanitation systems, since its low capital and operational costs and operational easiness. It is useful when important amount of helminth ova are involved. By adding lime (or any other alkaline material) to dewatered sludge, pH should be raised above 12 for at least 2 hours. Lime doses of 20-40% dry weight may inactivate 0.5-2 log of helminth ova (Jiménez *et al*., 2001); however, it cannot produce ≤ 1 HO/g TS from sludge with an initial high content, without adding lime doses that notably increase sludge disposal costs.

Table 2. Practical/theoretical application of USEPA-PRFP process to sludge

Process	Inactivation report	Theoretical final HO/gTS content using efficiencies reported on sludge with 75-200 eggs
Lime post stabilization	100%, initial value 8 HO/g TS, (Keller *et al*., 2004)	5-12 HO/g TS achieved in practice, (Mendez *et al*., 2002)
Thermophilic anaerobic digestion at 48°C	70-78%, initial content 2-8 HO/g TS (Gantzer *et al.,* 2001)	19-39 HO/g TS achieved in laboratory conditions (Rojas-Oropeza *et al*., 2000)
Thermal treatment at 108°C	90-93%, initial content 9.5 HO/g TS (Gantzer *et al*., 2001)	Using efficiencies reported at left, final content will be 5-16 HO/g TS
Irradiation at 1000 Gy	100%, initial content 88 HO/g TS, (Capizzi & Schwarztbrod, 2001)	Using efficiency reported at left, final content will probably be 0 HO/g TS
Pasteurization at 70°C	100%, initial value 8 HO/g TS, (Keller *et al.,* 2004)	Efficiency reported cannot be used to calculate final HO content in Mexican sludge

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

Anaerobic digestion. Thermophilic anaerobic digestion (45-65^{o}C) with retention times of 15 to 20 days inactivates low helminth ova content in sludge. Rojas-Oropeza *et al*. (2000), using sludge from Mexico in thermophilic digesters achieved 70% ova removal, although the reactor stabilization period was very long. Despite this, it seems that performance could be improved if sludge is kept longer in the acidogenic phase where toxic organic acids (acetic and propionic) are produced. An advantage of this process is that the methane produced may be used to increase the temperature in the reactor to foster helminth ova inactivation. Andreoli *et al*. (2000), used the methane to heat and dry the digested sludge to produce biosolids containing 0.99-1.1 HO/g TS. The sludge produced is easy to dewater and very stable.

Composting. No research has been done that proves that composting may produce sludge with <1 HO/g TS from sludge with an initial high content. Composting may reduce the organic matter in sludge by 20-30% and the sludge mass by 40-80% to produce stable biosolids. To produce Class B biosolids composting must be performed for 2-4 weeks at a mean temperature of 55^{o}C for 4 hours. During composting, temperature may reach values as high as 70^{o}C that are capable of inactivating helminth ova (Dougherty, 1999).

Helminth ova inactivation in faecal sludge

Dehydration has been applied in some on-site sanitation systems to inactivate helminth ova, with contradictory results (Table 3). It is performed only through storage and sometimes using solar energy. It seems that storing faeces for more than 1-2 years with a TS content >50-60%, adding bulking agents (lime, earth, leaves, etc) and at a temperature above 30^{o}C, high helminth ova content may be inactivated (Austin and Duncker, 2002). However, there is much research to be done in this matter.

Table 3. Helminth ova content in sludge of on-site sanitation systems with different operating conditions

670-2000 HO/g TS in fresh sludge coming from public systems or from latrines with a storage time of days to weeks	Strauss *et al*., 2003
580 HO/g TS in sludge from septic tanks stored for several years	Strauss *et al*., 2003
300 HO/g TS in faeces from dry sanitation systems from Guatemala, with 1 year storage at a high pH and a temperature from 17-20^{o}C	Strauss and Blumenthal, 1990
0 viable *Ascaris* eggs/g TS in sludge from dry sanitation systems from El Salvador, sun-dried at 34-44^{o}C, stored for a long period.	Strauss *et al*., 2003
0 *Ascari*s eggs/g TS in sludge from dry sanitation systems from South Africa stored for 20 months in plastic containers	Austin and Duncker, 2002

CONCLUSIONS

Though helminth ova are a major concern for the reuse of wastewater and sludge for agriculture and aquaculture, there is still little information about their behaviour during different treatment processes. Furthermore, the available information deals mainly with initial low content of *Ascaris* (especially in sludge) and does not therefore reflect real situations in the developing world, where helminth ova are really a concern, especially in the rural areas. Therefore, there is a pushing need for more research in this field. This research needs to be performed on site and with wastewater and sludge containing different helminth species, and not using only *Ascaris*, that not necessarily behaves like the rest of the helminths coming from humans.

REFERENCES

Andreoli C., Ferreira, A., Teles C., Cherubini C., Bernet P., Favarin F., Castro L. (2000). Operacionalização das alternativas de desinfecção e secagem de lodo digerido anaeróbicamente (Operation of alternatives for disinfection and drying of anaerobically digested sludge). I Seminário Nacional de Microbiologia Aplicada ao Saneamento. Anais Vitória (In Portuguese.)

Austin A. and Duncker, L. (2002). Urine-diversion Ecological Sanitation Systems in South Africa. CSIR, Pretoria,

Barrios J., Jiménez B. and Maya C. (2004). Treatment of sludge with Peracetic acid to reduce the microbial content. *Journal of Residuals Science and Technology* 1(1), 69-74.

Capizzi B. and Schwartzbrod J. (2001). Irradiation of *Ascaris* ova in sludge using an electron beam accelerator. *Water Research* **35**(9), 2256-2260.

Chávez A., Jiménez B. and Maya C. (2004). Particle size distribution as a useful tool for microbial detection. *Water Science and Technology* **50**(2), 179-186.

David E. D. and Lindquist W. D. (1982). Determination of the specific gravity of certain helminth eggs using sucrose density gradient centrifugation. *J. Parasitology* **68**(5), 916-919.

Dougherty M. (1999). *Field Guide to Farm Composting*. Ed. Natural Resources, Agricultural and Engineering Service. New York, USA

Duqqah M. (2002). Treated Sewage Water Use in Irrigated Agriculture. Theoretical design of Farming Systems in Seil Al Zarqa and the Middle Jordan Valley in Jordan. PhD thesis, Wageningen University, The Netherlands.

Gantzer C., Gaspard P., Gálvez L., Huyard A., Dumouthier N. and Schwartzbrod J. (2001). Monitoring of bacterial and parasitological contamination during various treatments of sludge. *Water Research* **35**(16), 3763-3770.

Hays B. (1977). Potential for parasitic disease transmission with land application of sewage plant effluents and sludge. *Water Research* 11, 583-595.

Hespanhol, I. (2002). Saúde Pública e Reúso Agrícola de Esgotos e Biossólidos (Public health and use of wastewater and sludge in agriculture). In: *Reúso de Água* (Water Reuse), pp 75-87 (In Portuguese).

Jiménez B. and Chávez A. (1997). Treatment of Mexico city's wastewater for irrigation purposes. *Environmental Technology* **18**, 721-730.

Jiménez B. and Wang L. (in press). Sludge treatment and management. In: *Municipal Wastewater Management in Developing Countries*, Ujang Z. and Henze M. (eds.) IWAP.

Jiménez B., Maya C. and Salgado G. (2001). The elimination of helminth ova, faecal coliforms, *Salmonella* and protozoan cysts by various physicochemical processes in wastewater and sludge. *Water Science and Technology* **43**(12), 179-182.

Jiménez B., Maya C., Sánchez E., Romero A. and Lira L. (2002). Comparison of the quantity and quality of the microbiological content of sludge in countries with low and high content of pathogens. W*ater Science and Technology* **4**(10), 17-24.

Juanicó M. and Milstein A. (2004). Semi-intensive treatment plants for wastewater reuse in irrigation. *Water Science and Technology* **50**(2), 55-60.

Keller R., Passamani F., Cassini S. and Gonçalves F. (2004). Disinfection of sludge using lime stabilization and pasteurization in a small wastewater treatment plant. *Water Science and Technology* **50**(1), 13-17.

Landa H., Capella A. and Jiménez B. (1997). Particle size distribution in an effluent from an advanced primary treatment and its removal during filtration. *Water Science and Technology* **36**(4), 159-165.

Maya C., Jiménez, B. and Schwartzbrod J. (2006). Comparison of techniques for the detection of helminth ova in drinking water and wastewater. *Water Environment Research*, **78**(2), 118-124.

Méndez J., Jiménez B. and Barrios J. (2002). Improved alkaline stabilization of municipal wastewater sludge. *Water Science and Technology* **46**(10), 139-146.

Nelson K, Jiménez-Cisneros B., Tchobanoglous G., Darby J. (2004). Sludge accumulation, characteristics, and pathogen inactivation in four primary waste stabilization ponds in central Mexico. *Water Research* 38(1), 111-127.

Paulino R., Castro E. and Thomaz-Soccol V. (2001). Helminth eggs and protozoan cysts in sludge obtained by anaerobic digestion process. *Revista de la Socciedad Brasileña de Medicina Tropical* **34**(5), 421-428.

Rojas-Oropeza M., Castro P., Ortega S. and Cabirol, N. (2000). Mesofilic and thermofilic anaerobic digestion of biological and physicochemical sludge. Proceedings of the XII National Congress of the Mexican Federation of Sanitary Engineering and Environmental Sciences. Vol 1, pp. 789-803. Morelia, México (In Spanish).

Stott R., Jenkins T., Baghat M., and Shalaby I. (1999). Capacity of constructed wetlands to remove parasite eggs from wastewater in Egypt. *Water Science and Technology*. **40**(3), 117-123.

Strauss M. and Blumenthal U. (1990). *Use of Human Wastes in Agriculture and Aquaculture.* IRCWD Report No 08/90, Duebenforf, Switzerland.

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.

US-EPA. (1992). Control of Pathogens and Vector Attraction in Sewage Sludge EPA/625/R-92-004. Washington, D.C.

Von Sperling C., Chernicharo A., Soares and Zerbini A. (2002). Coliform and helminth eggs removal in a combined UASB reactor – baffled pond system in Brazil: performance evaluation and mathematical modelling. *Water Science and Technology* **45**(10), 237–242.

WHO (2006). Guidelines for the Safe Use of Wastewater, Excreta and Greywater. World Health Organization, Geneva.