

Pathogens in dry sanitation systems and risk of using sludge produced in agriculture

Blanca Jiménez*, Inés Navarro and Catalina Maya

Instituto de Ingeniería, Universidad Nacional Autónoma de México

Apdo Postal 70472; Ciudad Universitaria; 04510 COYOCAN DF, MEXICO

bjimenezc@iingen.unam.mx

Abstract: To effectively control waterborne diseases in poor urban or rural areas it is important to provide basic sanitation. The aim of this paper is to assist this process by considering the control of pathogens that are present in higher amounts in excreta produced in areas with poor health conditions. Among the pathogens of concern, helminth eggs are the most resistant due to the complex layers that protect them. According to the literature, in order to inactivate helminth eggs a temperature above 40°C, for 10-20 days or the reduction of moisture below 5% is needed, but those conditions have not always proven successful in practice, notably to achieve the standards set by US-EPA or WHO. This paper presents general information concerning the pathogen content in faecal sludge from developing countries, and the properties of helminth eggs that are important to determine their inactivation conditions. Data on the efficiency of different treatment options used to inactivate helminth eggs is discussed and information on the actual conditions that inactivate helminth eggs is presented. Due to the lack of reliable treatment methods to achieve the proposed standard at low cost, other intervention methods are proposed. Their results can be evaluated using a QRMA approach under specific conditions.

Keywords: *Ascaris*; faecal sludge, helminth eggs, inactivation, pathogens, QRMA, sanitation, treatment

Introduction: Increasing sanitation in developing countries is a key aspect in controlling waterborne diseases transmitted through faeces. The aim of this paper is to assist this process by illustrating the specific problems and requirements in low income areas to deal with pathogens. Pathogens cause diseases that are rapidly manifested, in particular affecting children and the elderly, and sometimes even resulting in fatalities (diarrheal diseases are responsible for 1.8 million deaths every year (WHO, 2004)). In contrast, safe excreta management can have positive effects when it is used as a fertilizer for agriculture. However, for this, it is necessary first to treat faecal sludge to reduce its pathogen content to levels that do not represent a risk. This requires finding low cost, efficient and robust treatment methods that, under the precarious conditions of low income regions, are capable of producing reliable results. This paper will explore the main health risks arising from the management of sludge or excreta due to the pathogens frequently present in faecal sludge arising from on site sanitation systems, intended for use in agriculture. It will describe the characteristics of pathogens, treatment options and different ways to assess and control health risks.

Biological Pollutants

There are four groups of organisms that can be found in faecal sludge: viruses, bacteria, protozoa and helminths (in the form of eggs, Jiménez 2009). The content and variety of pathogens found in low income areas will be considerably higher than that found in high income ones, as a result of the lower level of health conditions.

Viruses

Viruses are the smallest (0.01 to 0.3 μm) infectious agents. They are obligate intracellular parasites that only multiply once inside the infected host cell which they also use to reproduce. They occur in different shapes and consist of nucleic acid (DNA or RNA) surrounded by a protein layer and sometimes a lipid membrane. There are more than 150 types of enteric viruses capable of producing infections that multiply in the intestine and are expelled in faeces. Unlike bacteria, pathogenic viruses are found in faeces of infected people, independently of whether they display symptoms. The composition, type and, especially, the content of viruses contained in faecal excreta is poorly known, particularly in developing countries, as result of the complex and costly analytical techniques required to identify them. The enteric viruses most relevant to man are enteroviruses, rotaviruses, reoviruses, caliciviruses, adenoviruses and hepatitis A viruses. Rotaviruses are the main cause of infant gastroenteritis worldwide. They are responsible for between 0.5 and 1 billion cases of diarrhoea per year in children under five years of age in Africa, Asia and Latin America and up to 3.5 million deaths. It is recognized that low virus levels may cause infection or illness; some viruses contained in faeces are much more resistant to chlorine disinfection than bacteria (Jiménez, 2003).

Bacteria

Bacteria are single celled microorganisms ranging in size from 0.2-10 μm . They reproduce and grow in an appropriate environment at defined ranges of temperature, salinity, pH, etc. The environmental distribution of bacteria is ubiquitous. Many species of bacteria are not harmful to man. In fact, some even live inside humans forming intestinal colonies. Bacteria are expelled in faeces at high concentrations (more than 10^{12} g^{-1}). In faecal sludge, pathogenic bacteria are always present, but at a variable concentrations, depending on the local health conditions. Due to the high rate of endemic diseases they cause in developing countries, *Salmonella*, *Shigella* and *Helicobacter pylori* are of importance. In contrast, *Vibrio cholerae* is only present when an epidemic exists.

Salmonella spp. are almost always present in faecal sludge. A sick individual can expel up to 10^9 *Salmonella* /g of faeces. There are a wide variety of strains capable of infecting humans and animals. The incidence in humans is lower than in animals and has a seasonal variation. The most severe form of salmonellosis is typhoid fever caused by *Salmonella typhi*. Typical symptoms are chronic gastroenteritis with diarrhoea, stomach cramps, fever, nausea, vomiting and headache. In severe cases, collapse and death may occur. Transmission occurs through polluted water or food. The infective doses reported range from 10^2 to 10^5 microorganisms, 10^4 being common, but for *Salmonella typhi* doses as low as 10^2 - 10^3 have been reported (Jiménez, 2003).

Shigella spp. occurs less frequently and live for a shorter period in the environment than *Salmonella* spp. *Shigella* rarely infects animals. A common route of transmission is through swimming in polluted water, and also through the ingestion of polluted food or water. It produces bacillary dysentery or shigellosis, light watery diarrhoea that can develop into full-blown dysentery. The symptoms are fever, nausea, vomiting, abdominal pain, migraine and myalgia. The classic form of dysentery is characterized by the expulsion of faeces containing blood with or without mucus. The infective dose is less than 10^3 microorganisms.

Helicobacter pylorus is also common in faeces. The major habitat of *H. pylori* is the human gastric mucosa. *H. pylori* has a worldwide distribution, and it is transmitted by the faecal-oral or the oral-oral route and possibly also through the consumption of contaminated vegetables and water. In developing countries, the organisms are acquired early in childhood, and up to 90% of children are infected by the age of 5. This contrasts with the low infection rate during childhood observed in developed countries (0.5 to 1%, Thomas *et*

al., 1992). The higher incidence of *Helicobacter* is in poorer, lower socioeconomic groups, for which crowding and poor sanitation have been identified as risk factor. *H. pylori* is the cause of gastric ulcers and stomach cancers.

Vibrio cholerae causes a gastroenteritis referred to as cholera. The symptoms are a copious liquid diarrhoea and severe dehydration associated with vomiting. *Vibrio cholerae* is rare in developed countries but common in poor ones. Humans are the only known hosts and the most frequent vehicle for transmission is water, either through direct consumption or when used to irrigate produce that is consumed uncooked. Fish cultured in polluted water are another source of transmission. *Vibrio cholerae* has been the cause of eight pandemics, the last one between 1990 and 1995. Since 2007 there have been outbreaks of cholera in India, Iraq, Congo, Vietnam and Zimbabwe. In 2005 West Africa suffered more than 63,000 cases of cholera, leading to 1,000 deaths (Leghnton *et al.*, 2007).

Protozoa

Protozoa are the group of parasites most closely associated with diarrhoea. They are single celled organisms between 2 and 60 μm in size. They develop in two ways: as trophozoites and as cysts (or oocysts as is the case for *Cryptosporidium*). Infection occurs when mature cysts are consumed. Cysts are resistant to gastric juices and transform themselves into trophozoites in the small intestine, lodging in the wall where they feed on bacteria and dead cells. In time, trophozoites become cysts, once again, and are expelled in faeces. Infected persons may or may not exhibit the disease's symptoms. Protozoa do not reproduce in the environment, only in their host. However, they are able to survive in the environment and remain active for periods ranging from some months to up to several years, depending on the environmental conditions. Most intestinal protozoa are transmitted through polluted water and food (Jiménez, 2003). The protozoa most frequently linked to aquatic diseases are *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium spp.*

Entamoeba histolytica is one of the most significant parasites detected in faeces and is commonly known as Amoeba. It is single celled. Trophozoites are 20 to 40 μm in size, while the cysts measure between 10 and 16 μm . They usually lodge in the large intestine; occasionally they penetrate the intestinal wall, travelling and lodging in other organs. They are the cause of amoebic and hepatic dysentery. *Entamoeba histolytica* is present in 10% of the world's population resulting in approximately 500 million infected persons. There are between 40 and 50 million cases of invasive amebiasis per year resulting in up to 100 000 annual deaths (placing it second after malaria in mortality caused by protozoan parasites (WHO, 1997). 96% of these cases occur in poor countries, especially on the Indian subcontinent, West Africa, the Far East and Central America.

Giardia lamblia cause diseases which are frequently endemic. Cysts (8-14 μm long and 7-10 μm wide) can survive in water bodies for long periods, especially in winter. *Giardia* lives in the intestines of a large number of animals as trophozoites. It is the most common parasite of humans but food rather than water is the main pathway of transmission. The disease is characterized by extremely liquid, odourous and explosive diarrhoea, stomach and intestinal gases, nausea and loss of appetite. Giardiasis is endemic worldwide with infection rates of 10% in developed countries and 20% in developing ones. It mainly affects children under five suffering from malnutrition. The total number of infected people is of the order 1.1 billion, 87% of whom live in poor countries (WHO, 1997).

Cryptosporidium spp. are protozoa, widespread in nature. Oocysts are resistant to chlorine and due to their small size (4-7 μm) are difficult to remove from water. They infect a wide range of farm animals and pets and were recently recognized as human pathogens. For this reason they are considered to be emerging pathogens. *Cryptosporidium spp.* are capable of completing a life cycle within the same host and causing reinfection. Once an individual has been infected, he carries the parasite for life. The disease rate in developing countries has

been poorly studied, in particular due to the higher occurrence of other types of diseases. Cryptosporidiosis is a concern for developed countries. Outbreaks have been reported in England, USA and Japan, linked to the pollution of water sources even when disinfected with chlorine. Cryptosporidiosis in developing countries has shown a greater incidence among immune depressed people and in rural areas (Snelling *et al.*, 2007). The main symptoms of Cryptosporidiosis are stomach cramps, nausea, dehydration and headaches. Although it is known that the infectious dose varies between 1 and 10, outbreaks have always been associated with large concentrations in water.

Helminths

Some helminths (worms) are parasites to humans. Helminths are pluri-cellular animal that occur in different types and sizes (from 1 mm to several m in length), and have diverse and very complex life cycles compared to most of the known microorganisms in the sanitary field (Jiménez, 2009). The infective agents of helminthiases are the eggs, not the worms. Helminth eggs are transmitted through: (a) the ingestion of crops polluted with wastewater, excreta or sludge, (b) direct contact with polluted sludge or faecal material, and (c) the ingestion of polluted meat (WHO, 2006). Helminthiases have a high mortality rate in developing countries compared to developed ones. Globally, there are around 1-2 thousand million people infected but most of these are people from developing countries. The incidence rate may reach up to 90% where poverty and poor sanitary conditions prevail. Helminthiases have different manifestations but in general they cause intestinal wall damage, haemorrhages, deficient blood coagulation and undernourishment and can degenerate into cancer tumours. Helminthiases affect mainly children, the elderly and poor people (Jiménez, 2009). Around 94% of the more than 4 billion cases of diarrhoea in the world are caused by helminths (Murray and López, 1996). There are several kinds of helminthiases with different local names; technically, helminthiases take their name from their causative agent. For instance trichuriasis is named after *Trichuris*. Ascariasis is the most common of the helminthiases and is endemic in Africa, Latin America and the Far East. Even though the mortality rate is low, most of the people infected are children under 15 years of age with problems of faltering growth and/or decreased physical fitness. Around 1.5 million of these children will probably never bridge the growth deficit, even if treated (Silva *et al.*, 1997; Jiménez 2009). It is improper to use the terms nematodes, *Ascaris* and helminths as synonyms. *Ascaris* is only one example of a nematode that is a pathogen. Other helminths may be pathogens although they are not nematodes.

A common characteristic of helminths is that they reproduce through eggs that are also the infective agent and therefore need to be removed from faecal sludge and/or inactivated (Jiménez, 2009). Eggs contained in sludge are not always viable and infectious. To be infectious the larvae need to develop, and for that a certain temperature (around 26°C), moisture level and time are needed. The necessary conditions are frequently met in soil or crops, where eggs are deposited when polluted wastewater, sludge or excreta are used as fertilizer. Helminth eggs lie viable for 1-2 months in crops and many months (even years) in soil and water, periods much longer than those reported for microorganisms. Helminth ova are protected by a cover of 3-4 different layers that give structure and mechanical resistance to the egg and protect it from desiccation, oxidants, reducing agents, detergents and proteolytic compounds. This why eggs are very resistant to environmental conditions, and particularly those commonly found in conventional disinfection methods (Jiménez, 2009). To inactivate helminth eggs it is recommended to raise the temperature above 40°C, for 10-20 days for *Ascaris*, or to reduce moisture levels below 5% (TS > 95%).

Based on an epidemiological study, WHO (2006) suggested a value of ≤ 1 egg/gTS and 10^3 - 10^6 faecal coliforms/gTS (depending on the type of crop and the application method) in excreta intended for agricultural reuse. The high initial content of helminth eggs found in faecal sludge (Table 1) from developing countries requires the application of treatment processes with efficiencies > 90 - 99% , or to combine treatment with other intervention methods. Furthermore, there is a need to review the criteria to adapt them to local conditions and to be able to set norms that are enforceable in practice. For this QRMA evaluations should be used (Jiménez and Navarro, 2009).

Table 1. Content of selected microorganisms and helminth eggs in sludge or faecal sludge from different countries. With information from: Jiménez (2008) and Jiménez and Wang, 2006 (all concentrations are in \log_{10} units/gramme of total solids, except helminth ova which are in eggs/g TS).

Country	Faecal coliforms	<i>Salmonella</i>	<i>Pseudomonas</i>	Protozoa cysts	Bacteriophages	Helminth ova eggs/gTS
Developing countries						70-735
Brazil	5			1 - 3	<1 - 3	76
Chile		3.5			2.7	
Egypt (liquid primary)				Mean: 1. Max: 2.6		Mean: 67 Max: 735
Egypt (dewatered primary)				Mean: 0.5 Max: 1.9		Mean: 8 Max: 124
Ghana						76
Mexico	10	7 - 8	5-7	2 - 4 ^(G)	3 - 6	73 - 177 ^(v)
Developed countries						1.4 - 9.7(A)
Australia		2-3				
France						4.4-7.7
Germany						1.1
Great Britain	4-6	2-4	3-5			<6
United States	7	2	3	2	4-6.3(E)	2-13

^(G) *Giardia* spp.

^(v) viable ova. (E) Enteric viruses (A) *Ascaris*

Biological indicators

Thermotolerant coliform bacteria (commonly referred as faecal coliforms) are the group most frequently used as indicators of faecal pollution because their abundance is easy to determine (incubation in a selected media at 44°C). Thermotolerant coliforms are not specific indicators of faecal contamination since they may sometimes arise from non-faecal as well as faecal sources, especially in tropical climates (WHO, 2004). Despite this, they are universally used as they behave in a similar way to most pathogenic bacteria in the environment and during treatment. However, they are not indicators of some pathogenic protozoa of interest, such as *Giardia lamblia* or *Amoeba* neither of any type of helminth eggs. This does not mean they are useless for developing countries; it simply means that additional indicators need to be considered especially when faecal sludge is to be reused in agriculture and aquaculture, in which case the helminth egg content needs also to be determined.

Sludge characteristics

In general municipal sludge from developing countries has two common characteristics: (a) a high biological content compared to that found in developed countries' sludge, and (b) relatively low metal content (Jiménez and Wang, 2006). The high content of parasites and

pathogens in sludge limit the kind of processes that can be used to treat it. A treatment with a high disinfection capability is required (Jiménez, 2007).

Treatment requirements

One of the priorities of basic sanitation is to inactivate the pathogen content in faecal sludge. However, basic sanitation systems are not always optimized for this purpose. Therefore, it is important to find ways to properly inactivate faecal sludge extracted from basic sanitation systems prior its use. There are few options to perform this that are simple, low cost and robust enough to provide efficient results in the uncertain conditions prevailing in poor areas, notably in order to inactivate helminth eggs. According to the literature, to inactivate helminth eggs any of the following conditions can be used (Feachem et al., 1983 and US-EPA 1992): (a) a temperature above 40°C for 10-20 days; (b) a TS content above 95%; or, (c) a pH up to 12 during 2 hours in sludge, but in practice these conditions have not proven to always be effective (Jiménez et al., 2006). For instance, Mendez et al., (2002) raising the pH above 12 during 2 hours on a sludge with the helminth eggs content usually found in USA inactivated only 65% of the eggs.

According to Jiménez *et al.* (unpublished results) to fully inactivate helminth eggs it is better to combine two methods in series. The first one is used to modify the egg layer in order to increase its permeability; while the second one is applied to inactivate the egg. According to these results the following options can be used:

- (a) A temperature > 50°C during at least 2 hours and raise pH up to 12 during 3 months for sludge with a TS content of 10-15%;
- (b) A temperature > 50°C during at least 2 hours and raise pH up to 9 during 6 months for sludge with a TS content of 10-15%;
- (c) A temperature > 50°C during at least 2 hours and raise pH between 11-12 during 45 days for a sludge with a TS content of 90%.

The options recommended in common literature, yet to be evaluated under specific conditions are (Jiménez 2008):

a) Dehydration.- It is the most common method to treat sludge produced in on-site sanitation systems, but has contradictory results. Variations are due to the different storage temperatures used. Temperatures above 35°C, humidity lower than 40-50% and long retention times (1-2 years) are required for this method to be really efficient. Helminth egg inactivation remains a challenge at this level, due to a lack of basic knowledge regarding the behaviour of different genera of helminth ova. Treatment is performed empirically through storage, sometimes with the addition of dry materials and the use of solar energy to increase temperature.

b) Alkaline stabilization.-It is useful to use alkaline stabilization if lime is applied as a post-stabilization process, i.e., using predried sludge at a content of 20-40% TS. If there is not enough water the exothermic reaction will not be produced and if there is too much, the water content will absorb the heat. It is capable of inactivating 98% of helminth eggs, 6 to 8 log of coliforms, 5 to 7 log of *Salmonella*, and 4-5 log of bacteriophages provided pH is raised above 12 for at least 2 hours. This is a low cost option and is easy to operate. pH can be raised by adding lime or any other alkaline material (as ashes). When lime is added a dose of 20-40% dry weight is recommended. Notwithstanding its high efficiency, alkaline stabilization is not able to produce sludge with $\leq 1 \text{ HO g}^{-1} \text{ TS}$ from sludge with a high helminth eggs initial content. To achieve such a performance it is necessary to considerably increase the lime dose, or to use long storage times. The material produced after treatment is useful to improve soil productivity and also to amend salinity in soil (Jiménez, 2009 EOLSS).

c) Thermal drying of anaerobically digested sludge. If sludge is anaerobically digested the methane produced can be used to heat the faecal sludge in order to disinfect it. In this way 2-3 log helminth eggs can be inactivated. In addition, all other pathogens are controlled.

d) Composting.- It is a low cost and easy to operate option. It has good pathogen inactivation, as it removes 5 to 7 log of faecal coliforms, and > 95% of helminth ova. Among its major drawbacks are the high demand for bulking material, land area, and labour.

Complementary intervention methods.

As shown above, it is difficult to attain low content of helminth eggs in faecal sludge produced in low income areas, therefore it is useful to combine treatment process with other types of intervention methods, such as: (a) crop restriction; (b) timing of faecal sludge application to soil and (c) household protective measures.

a) Crop Restriction. Crop restriction is the most suitable control measure. Among the crops that tolerate the use of polluted excreta are: (a) non-food crops (e.g., cotton or flowers for trade); (b) food crops that have to be cooked (potatoes, rice) and (c) crops that are not in contact with the faecal sludge such as fruit trees.

b) Controlling the time of application.- As pathogens die in soil, partially treated excreta can be applied long before produce is harvested or even sown. To control helminth egg dissemination, this implies an application time of at least 2 months to soil prior to harvesting (Strauss and Blumethal, 1990).

c) Household Protection Measures.- There are two types of household protective measures: those that protect food consumers and those that protect people preparing food. Cooks are protected by using rigorous personal and domestic hygiene practices such as frequent hand washing with soap or detergent, carefully washing and disinfecting produce, using separate areas for food preparation and the subsequent handling of washed produce, and not tasting unwashed or uncooked food. To implement these practices proper hygiene education programs are required. The measures to protect food consumers in households are the same for cooks but also include hygiene recommendations for local food handlers (in markets, in the home, and in restaurants and food kiosks). Vigorous washing in tap water of rough-surfaced salad crops (e.g., lettuce, parsley) and vegetables eaten uncooked reduces bacteria by at least 1 log unit; for smooth-surfaced salad crops (cucumbers, tomatoes) the reduction is ~2 log units (WHO, 2006). Washing in a disinfectant solution (hypochlorite, iodine or silver solution) and rinsing in tap water can reduce pathogens by 1–2 log units. Washing in a detergent (e.g., washing-up liquid) solution and rinsing in tap water can reduce helminth egg numbers by 1–2 log units. Peeling fruits and root vegetables reduces pathogens by at least 2 log units. Cooking vegetables achieves an essentially complete reduction (5–6 log units) of pathogens.

d) Chemotherapy and Immunization. These two interventions are highly cost-effective for preventing and reducing illness. Vaccination is highly recommended to control microbial diseases but is not effective for helminthiases. Regular mass chemotherapy campaigns are a better option in high-prevalence areas and can be linked to hygiene promotion programmes for farmers and exposed communities. Additional protection may be provided by the availability of adequate medical facilities to treat diarrhoeal disease, and by regular chemotherapy. Chemotherapy must be reapplied at regular intervals to be effective and may have side effects. The frequency required to keep worm burdens at a low level (e.g., as low as in the rest of the population) depends on the intensity of the transmission, but it may be required 2–3 times a year for children living in endemic areas (WHO, 2006).

Selection of Intervention Measures

The feasibility and efficacy of any combination of these health-protection control measures will depend on several factors, which must be carefully considered before any combination of them is put into practice. These factors include (Jiménez and Navarro, 2009): (a) Availability of resources (labour, funds, land); (b) Local social and agricultural practices; (c) Type of crops to be produced; and, (d) Existing patterns of excreta-related disease. Conducting a careful analysis of local situation assisted by QRMA methods is therefore important.

Risk Assessment

The WHO (2006) guidelines are intended to be used as the basis for the development of local approaches to control health risks in order to attain local health-based targets. Health targets can be achieved by using a combination of management approaches or intervention methods, as explained previously. To verify if it is feasible to attain such goals using different combination of intervention methods a QMRA can be used. QRMA consists of four steps: 1) hazard identification, describing acute and chronic human health effects associated with any particular hazard, including pathogens or toxic chemicals; 2) hazard characterization which corresponds to dose-response assessment, to characterize the relationship between various doses administered and the incidence of the health effect; 3) exposure assessment to determine the size and nature of the population exposed and the route, amount and duration of the exposure; and 4) risk characterization to integrate the information from the exposure, dose-response and hazard identification steps in order to estimate the magnitude of the public health problem and to evaluate variability and uncertainty (WHO, 2003).

A dose response curve for helminths, developed by Navarro *et al.* (2009), can be used to evaluate different needs. This dose response curve has been applied to evaluate the risk of infection from eating raw vegetables grown on biosolid (treated faecal sludge produced in an ecosan). In this study, the effect of using faecal sludge with three different contents of helminths eggs was analyzed: (a) 0.25 HO/gTS as set by U.S. EPA, 1993; b) 1 HO/gTS as set by WHO, 2006), and (c) 4 and 37 HO/gTS. This research concluded:

(a) The risk using the faecal sludge with different helminth egg contents varied by as much as 10^{-1}

(b) A content in faecal sludge of 4 eggs/gTS was acceptable in progressively reducing helminthiases in an area where the incidence rate is less than 17 %

(c) The annual infection risk was reduced by 59% if produce is washed prior to consumption

(d) If produce washing is improved to remove 2 log instead of 1, it resulted in the same Ascariasis incidence rate in the region

(e) The infection risk depends on the type of crop produced as not all retain the same amount of eggs, for example carrots are induce less risk than spinach

(f) If a value of helminth content is set as 4 HH/gTS, instead of that proposed by WHO or U.S. EPA, the risk is acceptable as the local infection rate is not increased.

Conclusions

Helminth eggs are the major health barrier to the reuse of faecal sludge for agricultural production. As it is difficult to rely only on treatment methods to inactivate helminth eggs from faecal sludge, it is recommended to set a combination of feasible intervention methods that can be accepted locally. Prior to making such a selection of intervention measures it is

highly advisable to apply a QRMA to verify that the desired health targets can be met. And most important of all, when selecting a treatment processes to be applied in rural conditions assess first its efficiency and reliability using pilot plants.

References

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, US.

Jiménez, B. (2003). Health risks in aquifer recharge with recycle water. In R. Aertgeerts & A. Angelakis, *State of the art report health risk in aquifer recharge using reclaimed water*, Rome: WHO Regional Office for Europe.

Jiménez, B. (2007). Helminth ova control in sludge: a review. *Water Science and Technology*, 56(9), 147-155.

Jiménez, B. (2009). Helminth ova control in wastewater and sludge for agricultural reuse. Water reuse new paradigm towards integrated water resources management. In W.O.K. Grabow (Ed.), *Encyclopedia of Life Support System*, EOLSS Publishers Co Ltd.: UNESCO, Water and Health theme.

Jiménez, B. and L. Wang (2006) Sludge Treatment and Management. Chapter 10 in *Municipal Wastewater Management in Developing Countries: Principles and Engineering*, Z. Ujang and M. Henze Editors. *IWA Publishing*, London, U.K, pp: 237-292.

Jiménez, B., Austin, A., Cloete, E., & Phasha, C. (2006). Using Ecosan sludge for crop production. *Water Sciences and Technology*, 5(54), 169-177.

Jiménez, B. and I. Navarro (2009) Methodology to Set Regulations for Safe Reuse of Wastewater and Sludge for Agriculture in Developing Countries Based on A Scientific Approach and Following the New WHO Guidelines. Chapter in “Handbook of Research on IT Management and Clinical Data Administration in Healthcare”ed. ISI global

Lenghton L., Wright A. and Davis K. (2005) Health, dignity and development: what will it Take Un Millennium development Goals Task force group Earthscan UN Millennium Project 2005. *Health, Dignity, and Development: What Will it Take?* Task Force on Water and Sanitation. UNPD Earthscan, USA 228 pp.

Méndez J. M., Jiménez B. and Barrios J. A. (2002) Improved Alkaline Stabilization of Municipal Wastewater Sludge. *Water Science and Technology*, 46(10):139-146

Murray, C.J.D. and Lopez, A.D. (1996) *Global Health Statistics*. Harvard University Press. USA.

Navarro, I. Jiménez B., E. Cifuentes and S. Lucario. (2009) Application of Helminth ova infection dose curve to estimate the risks for biosolids application in soil. *Journal of Water and Health*, 7:31-43.

Silva, N., Chan, M., & Bundy, A. (1997). Morbidity and mortality due to Ascariasis: re-estimation and sensitivity analysis of global numbers at risk. *Tropical Medicine and International Health*, 2(6), 19-28.

Snelling W, Xiao L., Ortega-Pierres G., Lowery C., Moore J., Rao J., Smyth S., Millar C., Rooney P., Matsuda M., Kenny F., Xu J., Dooley J..(2007) Cryptosporidiosis in developing countries *Infect Developing Countries* 2007; 1(3):242-256

Strauss, M., & Blumenthal, U. (1990). *Use of Human wastes in agriculture and aquaculture*. IRCWD Report No 08/90, Duebenforf, Switzerland.

Thomas J., Gibson G., Darboe M., Dale A., Waever L. (1992). Isolation of *Helicobacter pylori* from human feces. *Lancet*. 340: 1194-1195.

US-EPA (1992) Control of pathogens and vector attraction in sewage sludge EPA/625/R-92-004. Washington, D.C.

WHO. (1997). Amoebiasis- an expert consultation. Weekly Epidemiological Record No. 14. Ginebra, Abril.

WHO (2004). *Guidelines for Drinking Water Quality*. 3rd ed. World Health Organization, Geneva.

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