Parasitological risk assessment from wastewater reuse for disposal in soil in developing countries

Silvana A. Cutolo, Roque P. Piveli, Jéferson G. Santos, Célia R. Montes, Gilberto Sundefeld, Fábio Campos, Tamara M. Gomes and Adolpho J. Melfi

ABSTRACT

The purpose of this work is to analyze the parasitological risks of treated wastewater reuse from a stabilization pond in the city of Piracicaba, in the State of São Paulo (Brazil), and the level of treatment required to protect public health. Samples were taken from raw and treated wastewater in stabilization ponds and submitted to a parasitological, microbiological and physicochemical analysis. The study revealed on treated wastewater the presence of *Ascaris* sp. and *Entamoeba coli* with an average density of 1 cysts L^{-1} and 6 eggs L^{-1}, respectively. For Ascaris, the annual risks of infection due to the accidental ingestion of wastewater irrigation were 7.5×10^{-2} in 208 days and 8.7×10^{-2} in 240 days. For Total Coliforms and *Escherichia coli* in treated wastewater, the average density was 1.0×10^5 MPN/100 ml and 2.7×10^4 MPN/100 ml respectively, representing 99% and 94% removal efficiency, respectively. For BOD, COD, TS and TSS removal efficiency was 69, 80, 50 and 71%, respectively. The removal efficiency for nitrogen; ammonia nitrogen and total phosphate was 24, 19 and 68%, respectively. The average density of helminths eggs in treated wastewater is higher compared to the density of the limit value of $\leq$1 egg L^{-1} and tolerable risk is above the level recommended by the World Health Organization. Multiple barriers are necessary for the reduction of organic matter, chemical contaminants and parasites from treated wastewater. Standards for the sanitary control of treated wastewater to be reused in agricultural irrigation areas should be compiled for developing countries in order to minimize public health risks.

Key words | developing countries, parasitological indicators, risk assessment, treated wastewater reuse

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INTRODUCTION

Agricultural irrigation represents a significant percentage of the total demand of freshwater, which represents 40% of total water demand nationwide. Over the next 50 years, over 40% of the world population will suffer water shortages (WHO 2006). In the 21st century, agriculture continues to be a key instrument for sustainable development and the reduction of poverty (World Bank 2007). In increasing food production, FAO (2006) provides some strategies for sustainable food production and preservation of natural resources, among which are the use of techniques to increase irrigation and soil fertility. At least one-tenth of the world's population is thought to consume foods produced by irrigation with wastewater (Carr 2005). It has been estimated that at least 20 million ha in 50 countries are irrigated with raw or partially treated wastewater. Wastewater and excreta are also used in urban agriculture, which often supplies a large proportion of the fresh vegetables sold in many cities in developing countries. The percentage of wastewater treated by effective treatment plants is estimated to be 35% in Asia, 14% in Latin America and the Caribbean, 90% in North America and 66% in Europe (Carr 2005).

According to Jimenez (2005) and Jimenez & Takashi (2008), the Mezquital Valley in Mexico is a semi-arid area of 76,119 ha with 73,632 farmers irrigating with wastewater for the production of main crops such as alfalfa and maize.

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Wastewater contributes some 2,400 kg of organic matter per hectare, 195 kg/ha of nitrogen and 81 kg/ha of phosphorus to the soil each year, increasing maize productivity by 150%, barley by 100% and tomatoes by 94%.Wastewater irrigation is a source of water recycling and it provides a source of *N* that can provide for plant nutrition needs and increase crop yields. Soil microorganisms have been observed to have an increasing metabolic activity when wastewater is used for irrigation (Toze 2006; Fonseca *et al.* 2007a, b; Jimenez & Takashi 2008). Also, it is a low cost and ecologically correct alternative for the wastewater destination in the environment (Toze 2006; Fonseca *et al.* 2007b). The application of treated wastewater (TW) in the soil–plant system through irrigation is a recent practice in Brazil (Fonseca *et al.* 2007a).

The great disadvantage of wastewater reuse is the effect on public health (Jimenez & Takashi 2008). There have been a number of risk factors identified for wastewater reuse for agricultural irrigation. Some risks factors are short term and vary in severity depending on the potential for human, animal or environmental contact as microbial pathogens (Haas *et al.* 1999). The most common human microbial pathogens found in wastewater are enteric in origin. A selection of pathogens was assessed for risk, represented by bacteria, viruses, protozoa and helminths (Westrel *et al.* 2004). It included pathogens that mainly cause gastroenteritis (*Salmonella*, *Giardia*, *Cryptosporidium*, rotavirus) or milder respiratory infections (adenovirus) and pathogens that can cause more severe diseases, such as haemolytic uremic syndrome, enterohaemorrhagic *Escherichia coli* O157:H7, EHEC (Carducci *et al.* 2000; Gantzer *et al.* 2001; Westrel *et al.* 2004).

The uncontrolled use of wastewater in agriculture has important health implications for product consumers, farmers and their families, producer vendors, and communities in wastewater irrigated areas (Carr 2005). Negative health impacts from the use of untreated wastewater have been documented in many studies as in work carried out in Marrakech (Amahmid *et al.* 1999); it was observed that 72% of the children presented infections caused by protozoa, mainly giardiasis and amoebic dysentery, and 73% presented infections caused by helminths. The number of infected people worldwide is about 1.05 billion for hookworm, 1.3 billion for *Trichuris trichiura*, and 1.5 billion for *Ascaris lumbricoides* (Brooker *et al.* 2007). These infections are most prevalent in tropical and sub-tropical regions of the developing world where adequate water and sanitation are lacking, with recent estimates suggesting that *A. lumbricoides* infects 1.221 million people, *T. trichiura* 795 million and hookworms 740 million (Mara 2004; Brooker *et al.* 2007). Moreover, three million children under five years of age die every year due to diarrheic complications caused mainly because of water contamination in developing countries (Cutolo *et al.* 2006; Mara 2004).

Epidemiological studies on the impacts of wastewater reuse are available such as that in Central Mexico (Jimenez 2007) which proposed that a value of $\leq$1 egg L^{-1} may not be sufficiently protective in situations where conditions are favorable for helminth eggs to survive, such as hot weather, moist soil conditions, which allow for accumulation of eggs in soil and crops, especially where children under the age of 15 consume raw vegetables irrigated with wastewater (Jimenez 2007; Navarro *et al.* 2009). In Latin America and the Caribbean (LAC), the most prevalent soil-transmitted helminth infections are Ascariasis and Trichuriasis with 84 million and 100 million of infected population respectively, (Hotez *et al.* 2008; Navarro *et al.* 2009), and the largest estimated number of cases in Brazil, Mexico and Guatemala. Of the 50 million cases of hookworm infection occurring in poor rural areas, approximately 65% occur in Brazil. In some regions of Minas Gerais (Brazil) it is estimated that 68% of the rural population is infected with hookworm (Fleming *et al.* 2006), where it is a major cause of anemia in children (Brooker *et al.* 2007). Hookworm is also a major cause of adverse pregnancy outcomes in LAC (Larocque *et al.* 2006).

Quantitative microbial risk assessment (QMRA) is a probabilistic modelling technique that can be used to estimate risks associated with different pathogens in specific wastewaters and scenarios (Seidu *et al.* 2008). It has been applied to establish the health risk associated with consuming wastewater-irrigated food crops (Tanaka *et al.* 1998) and vegetables (Shuval *et al.* 1997; Petterson *et al.* 2001; Hamilton *et al.* 2006). Mara *et al.* (2007) have also applied QMRA to assess the health risks for farmers using wastewater under different irrigation and technology regimes. The approach has also been included in the recent WHO guidelines for the safe use of wastewater, excreta and greywater (WHO 2006). QMRA consists of four steps: (i) hazard identification, (ii) exposure assessment, (iii) dose–response modelling, and (iv) risk characterization (Haas *et al.* 1999). The limited data on pathogens in developing countries require that a QMRA be based on the occurrence of indicator organisms. Despite the weaknesses of using indicator organisms, Haas *et al.* (1999) believe that many initial QMRAs will have to be performed using data on indicator organisms due to inadequate data for occurrence of pathogens. However, the use of indicator organisms does require assumptions to be made about the relationship between pathogens and indicators that introduces an additional level of uncertainty into the risk assessment (Howard *et al.* 2006). Schijven *et al.* (2011) report QMRA needs to be repeated at least every three years and three years of monitoring for pathogens may be condensed into one year to allow a higher monitoring frequency and hence provide more information about variability in pathogen concentrations in surface water for potable water.

The purpose of this study is to analyze the potential parasitological risks from wastewater in the stabilization ponds in Piracicaba city, State of São Paulo (Brazil) to determine the set of technologies, such as multi-barrier, needed before disposal of wastewater in soil for agriculture production, and to ensure protection for public health.

MATERIALS AND METHODS

Site description

The wastewater treatment plant received sewage from the town, where there are 28,600 persons connected to the town's sewage located in the city of Piracicaba (22 °42′S and 47 °38′W; and 547 m altitude) in the State of São Paulo, Brazil with subtropical humid climate, and average air temperatures of 24 °C (17–38 °C), an average of liquid temperature between 20 and 25 °C, and an annual pluviometric index of 1,253 mm.

The treatment system consists of bars, a sand box and Parshal channel followed by three parallel ponds, each with an anaerobic pond (total volume 8,500 m^3, 4 m depth, 60 m length, 40 m width) followed by a two facultative ponds (each one of total volume 14,000 m^3, 2 m depth, 100 m length, 70 m width) with a total retention time of 42 days, and flow into and out of 10.62 L/s (±1.21 L/s).

Sample collection

The project had an initial stage with characterization of hydraulic and sanitary control from the stabilization pond. The samples were collected from June 2007 to March 2008 from raw wastewater (RW) and treated wastewater (TW) to plan the best technologies to reduce the risks for public health, to reclaim the nutrients for soil and plants, to prepare the irrigation technique for disposal on soil for the experimental module with sugar cane production.

Parasitological analysis

Samples from parasites were collected bimonthly and adapted techniques from different authors were applied to concentrate helminth eggs and protozoa cysts (Ayres and Mara 1996; Cutolo *et al.* 2006; Piveli *et al.* 2008). Helminth eggs and protozoa cysts were observed with an Axioskop Zeiss microscope (Carl Zaiss, Gottingen, Germany), using Whipple reticule (Graticules LTD, Tombridge Kent, England) according to the WHO Bench Aids for Diagnosis of Intestinal Parasites (WHO 2000) and Leventhal and Cheadle (2000). The qualitative analysis was organised into representative groups of protozoa cysts (PC) and helminth eggs (HE). The quantitative results were recorded in helminth eggs and protozoa cysts per litre. Viability determination for helminths eggs (*Ascaris* spp.) was carried out using resuspended pellets with 0.1% sulfuric acid and incubation for a period of 3–4 weeks at 26 °C (Yanko 1988; Piveli *et al.* 2008). We ascertained the recovery rate of *Ascaris suum* eggs within samples of raw wastewater using a technique described by Stien & Schwartzbrod (1988), Bowman *et al.* (2003) and Maya *et al.* (2006).

Total coliforms and *Escherichia coli*

Samples for microbiological analysis were collected monthly in 250 mL samples for total coliform (TC), and *E. coli* (EC) were collected from raw and treated wastewater and analyzed with the chromogenic/fluorogenic Colilert method (APHA 1998).

Physicochemical analysis

Samples of raw and wastewater were collected bimonthly for total solids series (TS), total suspended solids series (TSS), biochemical oxygen demand (BOD) with a total of fifteen samples, chemical oxygen demand (COD) with thirteen samples, total nitrogen Kjeldahl (TNK) and ammoniacal nitrogen (N-NH_4^+) with fourteen samples, total phosphate (P-PO_4^{3-}) with eight samples and all parameters were processed in accordance with Standard Methods (APHA 1998). Climatic conditions such as air temperature (°C) and precipitation (mm) were obtained from the Integrated Center for Agrometeorological Information from São Paulo State (Brazil).

Data analysis

It was possible to determine intestinal parasites as protozoa cysts and helminth eggs with an average and standard deviation for each sample. We compared the values of helminth eggs with WHO (2006) which recommends ≤ 1 egg L^{-1} on treated wastewater. The minimum value of zero is related to no detection of helminth eggs and protozoa cysts in samples. The values of helminth eggs (eggs L^{-1}) and protozoa cysts (cysts L^{-1}) were compared with the average monthly precipitation (mm) for the period 2007 to 2008. Density of parasites, TC and EC, and physicochemical analysis were performed with SPSSw for Windows 8.0®, using a non-parametric Spearman test with positive and negative correlations ($\alpha \geq 0.50$) on raw and treated wastewater (Legendre & Legendre 1983). Data from physicochemical parameters was treated statistically with analysis with a *t*-test ($p > 0.05$) for raw and treated wastewater (Everitt 2003; Johnson 2007).

Quantitative Microbial Risk Analysis

It was possible to apply the QMRA methodology used to estimate *Ascaris* infection risks as a result of accidental ingestion of wastewater irrigation for farmers, based on the work of Haas *et al.* (1999), Mara *et al.* (2007, 2010), Mara (2011), Seidu *et al.* (2008) and Navarro *et al.* (2009). The probability of becoming infected by ingesting a number of organisms was estimated using the exponential model for *Ascaris* infection (Mara *et al.* 2007; Seidu *et al.* 2008) and is expressed as:

$$P1(\mathrm{d}) = 1 - \exp(-rd)(\text{Exponential model})$$

$P1$ (d) is the probability of becoming infected by ingesting d number of organisms, r are the dimensionless infectivity constants. The exact value of r for *Ascaris* is not yet established in dose–response studies. Therefore, a conservative value of r based on a worse case evaluation was used and the exact single-hit model with $r = 1$, which represents the maximum risk curve can be used and d is the risk of infection from a single exposure to a dose of organisms.

For the scenario of farmers ingesting wastewater, the combined annual risk of infection for multiple exposures per person PI(A) was determined by using the relation:

$$PI(\mathrm{A}) = 1 - \left[1 - P1(d)^{n}\right](\text{Annual risk of infection})$$

The annual risk of infection PI(A) is calculated from a single exposure to a dose d of organisms; n being the number of days in a year when a person is exposed to this single dose d. We used 208 day as in Seidu *et al.* (2008) and 240 days; the number being based on a region with high temperatures, intense production in peri-urban areas and constant irrigation.

RESULTS

Parasitological characterization

A total of eight samples, both raw and wastewater, were analyzed. The quantitative results showed the presence of *Entamoeba coli* represented as protozoa cysts and *Ascaris* spp. of helminths eggs as indicators in the sampling period. The frequency of *Ascaris* spp. eggs were observed in 31% of them and 11% were viable with larvae inside, were analyzed the egg in raw wastewater samples; *Ascaris* sp. was observed in 40% of them and 30% were viable in treated wastewater. The presence of *Entamoeba coli* was observed in 8%, *E. histolytica/dispar* in 5% and *Giardia* spp. in 25% in raw wastewater and was observed 11, 3 and 3% in treated wastewater, respectively.

The average density (standard deviation), minimum and maximum values of helminths eggs and protozoa cysts are presented in Table 1. In treated wastewater, we can observe the maximum value of 57 eggs L^{-1} and 11 cysts L^{-1}. Recovery efficiency of the *A. suum* eggs was between 55 and 85% according to the numbers of eggs retrieved and inoculated in eight tests with raw wastewater at the beginning of the research.

The values of the temporal series of helminth eggs and protozoa cysts were compared with the average precipitation for each month in the period of the characterization of the wastewater and an increase was observed for helminth eggs and protozoa cysts in treated wastewater from November/2007 to February/2008 as shown in Figure 1.

Four cysts of protozoa as *Entamoeba coli*, *Entamoeba histolytica/dispar*, *Giardia* spp. and *Isospora* spp. were identified in samples of raw and treated wastewater; and nine eggs of helminths as *Ascaris* spp. *Enterobius vermicularis*, hookworms, *Hymenolepis nana*, *Hymenolepis diminuta*, *Taenia* spp. *Trichuris trichiura*, *Toxocara* spp. were identified in samples of raw and treated wastewater.

Total Coliforms and *Escherichia coli*

A total of 10 samples, one each month, and the values of the concentrations of total coliforms and *E. coli* density on log scale in raw and treated wastewater from the stabilization ponds are presented in Figure 2. For TC and EC, the average

Table 1 | Results on raw wastewater (RW), treated wastewater (TW) and removal efficiency (RE) from stabilization ponds in the city of Piracicaba (State of São Paulo, Brazil)

			RW			TW			
		n	**Avg**	**SD**	**Min–max**	**Avg**	**SD**	**Min–max**	**RE (%)**
HE	eggs L^{-1}	8	1.7	1.1	1–3	6.4	1.1	0–57	−27
PC	Cysts L^{-1}	8	1.1	1.2	0–3	1.7	3.3	0–11	−19
TC	MPN100 mL^{-1}	10	1.7×10^7	2.45×10^7	10^4–10^7	1.0×10^5	1.4×10^5	10^4–10^5	99
EC	MPN100 mL^{-1}	10	1.5×10^5	1.7×10^5	10^3–10^7	2.7×10^4	4.0×10^4	10^2–10^5	94
BOD	mg L^{-1}	15	438	129	255–618	134	61	50–257	69
COD	mg L^{-1}	13	828	61	389–1570	287	143	198–428	80
TNK	mg L^{-1}	14	64.31	32.98	50.40–81.48	49	21.20	31–109	24
$N\text{-}NH_4^+$	mg L^{-1}	14	40.4	16.87	24.60–62.72	32.55	9.26	26–84	19
$P\text{-}PO_4^{3-}$	mg L^{-1}	8	4.65	1.30	3.19–5.91	1.5	1.84	2–11	68
TS	mg L^{-1}	15	1083	741	620–3010	544	115	420–875	50
TSS	mg L^{-1}	15	373	125	236–608	108	14	14–208	71

Note: RW – raw wastewater, TW – treated wastewater, *n* – number of samples, Avg – average, SD – standard deviation, HE – helminth eggs, PC – protozoa cysts, TC – total coliforms, EC – *Escherichia coli*, BOD – biochemical oxygen demand, COD – chemical oxygen demand, TNK – nitrogen Kjeldahl, $N\text{-}NH_4^+$ – ammoniacal nitrogen, $P\text{-}PO4^{3-}$ – total phosphate, TS – total solids, TSS – total suspended solids.

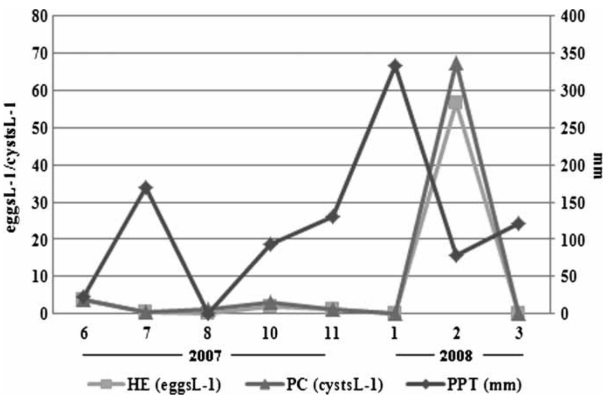

Figure 1 | Comparison of numbers of helminth eggs and protozoa cysts, and average precipitation for the period June 2007-February 2008.

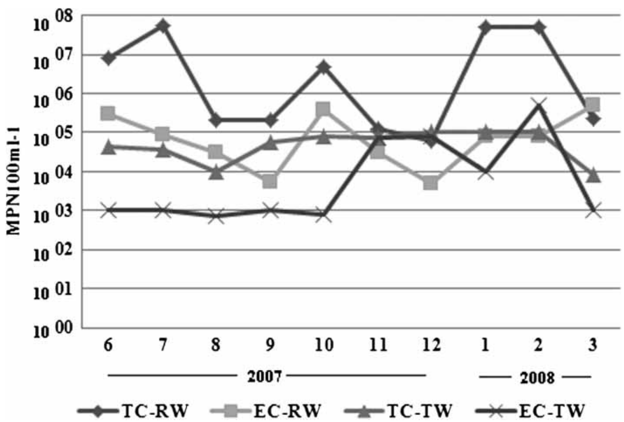

Figure 2 | Log-scale concentrations of total coliforms (TC) and *E. coli* (EC) in raw and treated wastewater from the stabilization ponds for the period June 2007-February 2008.

(standard deviation), minimum and maximum values from raw and treated wastewater are presented in Table 1. For TC, there was a reduction of 2 logs from 10^7 to 10^5/100 mL. For EC, there was a reduction of 1 log from 10^5 to 10^4/100 mL. TW samples with removal efficiency of 99% for TC and 94% for EC.

Physicochemical parameters

The average density (standard deviation) and minimum–maximum values of physicochemical analysis and removal efficiency from raw and treated wastewater are presented in Table 1.

BOD and COD

For BOD the average and *p*-value values were 438 ± 71 mg L^{-1} in raw wastewater and 134 ± 33 mg L^{-1} in treated wastewater; for COD, the average (*p*-values) were 828 ± 36 mg L^{-1} in raw wastewater and 287 ± 86 mg L^{-1} in treated wastewater.

TKN, $N\text{-}NH_4$ and Total phosphorus values

For TKN the average and *p*-values were 64 ± 19 mg L^{-1} in raw wastewater and 49 ± 12 mg L^{-1} in treated wastewater.

For N-NH_4 the average and *p*-values were 40 ± 9 mg L^{-1} in raw wastewater and 32 ± 5 mg L^{-1} in treated wastewater. For total phosphorus, average and *p*-values were 4.65 ± 1 mg L^{-1} in raw wastewater and 1.5 ± 1.5 mg L^{-1} in treated wastewater.

TS and TSS values

TS average and *p*-values were 1083 ± 410 mg L^{-1} in raw wastewater and 544 ± 63 mg L^{-1} in treated wastewater, TSS average and *p*-values were 372 ± 69 mg L^{-1} in raw wastewater and 108 ± 7.5 mg L^{-1} in treated wastewater.

Statistical analysis

There were no correlations between parasitological data, TC and EC, and physicochemical analysis in raw wastewater samples. In treated wastewater samples, there were correlations between the average density of helminths eggs and the concentration of TS (+0.60), BOD (−0.65) and TSS (−0.53).

Infection risk assessment

Using the exponential model as in Mara *et al.* (2007), Mara (2011), Seidu *et al.* (2008) and Navarro *et al.* (2009), 63% is the probability of farmers being infected after ingestion of a single dose of *Ascaris* eggs. In this case, the annual risks of infection due to the accidental ingestion of wastewater irrigation for farmers were 7.5×10^{-2} in 208 days and 8.7×10^{-2} in 240 days.

DISCUSSION

The technique applied for parasitological characterization appears adequate in laboratory conditions, being efficient and easy to apply to detect helminth eggs for developing countries. It was possible to verify the diversity of helminths eggs and protozoa cysts in samples and the presence of *Ascaris* sp. and *Entamoeba coli* as indicators as cited for Seidu *et al.* (2008), Navarro *et al.* (2009) and Mara (2011). Ascariasis is a common disease in developing countries and is endemic in Africa (Seidu *et al.* 2008), Mexico (Navarro *et al.* 2009) and Brazil (Cutolo *et al.* 2006).

Results from treated wastewater were not in compliance with the WHO recommendation (WHO 2006), which is <1 egg L^{-1}. The average density of helminths eggs was 6.4 eggs L^{-1} and for cysts was 1.7 cysts L^{-1} in treated wastewater. The high values of 57 eggs $L^{-1}$and 11 cysts L^{-1} in treated wastewater were verified in the rainy season and increased values of TC, EC, TS and TSS were observed in same period. The rainy events showed values of 168, 334 and 107 mm on December/2007, January/2008 and February/2008, respectively. There is an increase of runoff flow during the rainy events into the system of stabilization pond, indicated by increased turbidity and associated with high concentrations of bacteria and pathogens (Gaffield *et al.* 2003). Many other factors contributed to the presence of helminth eggs and protozoa cysts, among which are: the high proportion of particulate phosphorus fraction settled at a very slow sedimentation (Ulén 2004); adherence to biomass of algae such as cyanobacteria with a gelatinous mass and the colonial matrix of *Microcystis aeruginosa* (Graham *et al.* 2008); endemicity in developing countries as NTD (Hotez *et al.* 2008).

Viability technique was an important tool to verify the infectivity of *Ascaris* sp. eggs and 30% were viable in treated wastewater. According to Navarro *et al.* (2009), only one *Ascaris* sp. egg is sufficient to cause infection. For *Ascaris* infection, the tolerable risk is greater than 1×10^{-2} (Mara *et al.* 2007); in this study the annual risks of infection due to the accidental ingestion of wastewater irrigation for farmers were 7.5×10^{-2} in 208 days and 8.7×10^{-2} in 240 days. Seidu *et al.* (2008) showed the median annual risks slightly above the tolerable risk due to the accidental ingestion of drain and stream irrigation water at 8.2×10^{-2} and 8×10^{-2}, respectively. In this phase of our research, we note the need to apply methods such as QMRA to gain knowledge and reduce risks to health in developing countries for disposal in soil. Schijven *et al.* (2011) describes an interactive user-friendly computational tool, named QMRAspot, which was developed to analyze and conduct QMRA for a drinking water production chain from surface water to potable water. In order to make the process of analyzing new data more efficient and to achieve a standardized way to analyze the quality of water supplies, in 2009, Schijven & de Roda Husman (Schijven *et al.* 2011) developed a spreadsheet, to be used by companies to enter drinking water monitoring data, and a computational tool designed to automatically analyze data and perform the QMRA (Schijven *et al.* 2011).

The results show that direct contact with treated wastewater from Piracicaba can lead to increased helminth infection and the effect may be more pronounced in children than in adults. The tolerable risk is 10^{-3} per person per year, which means that an individual has a 1 in 1,000 chance to become ill from drinking fully treated drinking water over a 12-month period (WHO 2006; Mara 2004;

Mara *et al.* 2007). If the calculated risks from consuming wastewater-irrigated crops are less than 10^{-3} per person per year, the risks are acceptable (Haas *et al.* 1999; Mara 2004). The population in developing countries has diarrhea at least once a year and in industrialized countries individuals are at the high annual diarrhea disease risk of ~1 in 5 (Mara 2004). Direct contact with untreated wastewater suggests that there is an increase in diarrheal diseases, particularly in young children, in the dry seasons. Treatment may need to be below 10^4 TC/100 mL in situations where children have high amounts of contact. If this is not possible, effective measures should be introduced to reduce the contact of children with wastewater (Blumenthal *et al.* 2000; WHO 2006; Jimenez & Takashi 2008). In the case of wastewater treatment, the *Ascaris* sp. eggs found in treated wastewater are considered a neglected disease with underreporting in Brazil, so there is no systematic parasitological data at São Paulo State Department of Health, or in any of the other countries of South and Central America (Jimenez 2007; Cutolo 2009).

Based on estimates of global disease burden of life years adjusted for disability (DALYs), published by the World Health Organization (WHO 2004), estimates were obtained according to the overall percentage of disease burden determined to occur in LAC (Hotez *et al.* 2006). In the case of ascariasis, 84 million is the number of cases, 10.4% of disease burden and 124,800 to 1,092.000 in DALYs (Ault 2007; Hotez *et al.* 2008). According Mara (2011), ascariasis is one of the commonest diseases resulting from the use of wastewater in agriculture. A DALY loss due to ascariasis was 0.0026 pppy. An additional DALY loss of 10^{-4} pppy would increase to 0.0027 pppy. According to Mara (2011), this increase is not epidemiologically significant and would be very difficult to detect.

The results obtained for total coliforms showed a variation of 10^4–10^7/100 mL in raw wastewater and 10^3–10^5/100 mL in treated wastewater. For *E. coli*, the variation was 10^3–10^7/100 mL in raw wastewater and 10^2–10^5/100 mL in treated wastewater. The increase of *E. coli* was observed from November 2007 to February 2008 with values of 10^4–10^5/100 mL. Besides the rainy season, manure and septic tank sludge are released with a daily average volume of 450 m^3/month and 2,200 m^3/month, respectively; and can influence the bacterial growth. The new edition of WHO guidelines (WHO 2006) proposes a new approach based on risk evaluation allowing $\leq 10^3$ *E. coli*/100 mL for unrestricted irrigation and $\leq 10^5$ *E. coli*/100 mL for restricted irrigation with additional barriers of crop cleaning and bacteria die-off (Lazarova & Bahri 2008). The average value of *E. coli* was 2.7×10^4/100 mL with a variation of $10^3 - 10^4$/100 mL in treated wastewater and can be considered a median risk of 0.36 based on the model of Karavarsamis & Hamilton (2010) and a median risk of 0.30 based on WHO (2006) for estimating the annual rotavirus infection risks from the consumption of wastewater-irrigated lettuce by 10,000 Monte Carlo simulations (Mara *et al.* 2010).

For unrestricted irrigation, epidemiological studies in Mexico showed that when the faecal coliform level in treated wastewater was 3×10^4/100 mL, there was an excess risk of disease of 6×10^{-3} per person per year but quantitative microbial risk analysis shows that when the *E.coli* level is 1,000/100 ml, the risk of infection is ~10^{-4} per person per year (Mara 2004). For restricted irrigation, epidemiological studies in Israel, Mexico and US showed that faecal coliform levels of 10^6/100 mL of treated wastewater were associated with excess viral infection but there is no association with levels of 10^4/100 mL, thus suggesting that *E. coli* $\leq 10^5$/100 mL would be an appropriate guideline value (Mara 2004).

As observed, there was a correlation between the average density of helminths eggs and TS (+0.60), BOD (−0.65) and TSS (−0.53). Cutolo *et al.* (2006) obtained a correlation between outflow variation and density of helminth eggs (+0.60) in research on wastewater reuse in urban areas. As for the coliform group, it is known that a correlation does not necessarily occur in the presence of pathogens (Ferguson *et al.* 1996).

According to the Conselho Nacional de Meio Ambiente (Territory Environment Ministry 2005) in CONAMA Resolution No 357, the results of the physicochemical parameters from treated wastewater are above Brazilian regulations for discharge into a water body and showed a BOD of 134(±33) mg L^{-1}, COD of 287 (±86) mg L^{-1}, ammoniacal nitrogen of 32.55 (±5.35) mg L^{-1}, and total phosphate of 1.5 (±1.5) mg L^{-1}. The typical range in wastewater for BOD was 110–400 mg L^{-1} and for COD was 250–1,000 mg L^{-1}, the impacts on the water body system were aesthetic and nuisance problems, providing food for microorganisms and contributing to chlorine demand (Lazarova & Bahri 2008); the BOD can enhance soil fertility by increasing humic content, aiding moisture retention and enhancing microbial biomass (Godfree & Godfrey 2008). The typical average concentration in wastewater from anaerobic followed aerobic ponds for ammoniacal nitrogen was >15 mg L^{-1} and for total phosphate was >4 mg L^{-1}. A positive environmental benefit from nitrogen and phosphorus is that they are absorbed by the crop and therefore are removed from the water cycle, minimizing the eutrophication of water body nutrients (Godfree & Godfrey 2008). Fonseca *et al.* (2007a, b) observed in an experiment under controlled conditions on soil nitrogen concentrations (mineral and total), total carbon and pH with surface irrigation of treated wastewater from a secondary system of stabilization pond in Lins, São Paulo State (Brazil) that the application increased the nitrogen content and soil pH.

The implementation of a tertiary treatment is restricted in Brazil by (i) absence of national policies for sewage treatment over the long term (von Sperling & Oliveira 2009); (ii) absence of at least one of the sanitation units (general network, sanitary facilities and garbage collection) in 43.5% of the permanent households in Brazil (Fonseca *et al.* 2007a, b). Due to the lack of space to introduce a maturation pond, and the stringent water quality requirements imposed upon water reuse applications, it is interesting to consider simplified and low-cost technologies such as those being applied in developing countries. The most economical method to date is chemical treatment, including coagulation and precipitation with lime and salts of aluminum or iron, and ion exchange (Cornwell 1987; Ayoub *et al* 2001).

Filtration is considered an alternative to remove turbidity and flocculated particles. The process depends on the filter medium, concentration and type of solids to be filtered out and the operation of the filter (Bitton 2005). Slow sand filtration is a simple technology and may be adapted for wastewater disinfection but only a few studies have been conducted on tertiary treatment of wastewater (Langenbach *et al.* 2010). Rock filters were investigated and show that the ammonia and nitrate concentrations in the effluent from the aerated filter were <3 and ~5 mg N/L, respectively, whereas the ammonia concentration in the effluent from the control filter was ~7 mg N/L. Typical FC numbers in the facultative pond effluent were 10^4 per 100 mL in winter and 10^3 per 100 mL in summer. Faecal coliform removal in the aerated filter was much higher than expected. Aerated rock filters are thus a useful land-saving alternative to aerobic maturation ponds (Mara & Johnson 2006).

Disposal in soil has been considered an alternative to solve the adverse effects of treated wastewater and can be used as a natural filter for the soil–plants system (Pollice *et al.* 2004). To use the nutrients present in wastewater, drip irrigation has a more advantages in terms of the environment and public health but has serious limitation in that the emitters tend to clog (Feigin *et al.* 1991; Ravina *et al.* 1997), which affects water application in the soil–plant system. Organic material sedimentation, bacterial growth, iron oxidation, high sulfur content, and high pH values (>7), promote biofilm growth and obstruction of drip emitters and the irrigation line (Cararo *et al.* 2006). Filtration is a key aspect in drip irrigation with wastewater, but this does not prevent emitters from clogging (Tajrishy *et al.* 1994). Chlorination has been found to be an effective way to reduce clogging caused by bacteria and algae when wastewater is used (Tajrishy *et al.* 1994; Ravina *et al.* 1997; Puig-Bargués *et al.* 2005); applying other oxidant or flocculant substances is also effective, as is a combination of both treatments (Chica *et al.* 2000a).

The lack of national guidelines points to the emerging need to establish national quality standards by which to dispose of and recycle wastewater, to protect and preserve the health of the exposed population, such as farmers, rural workers, consumers, and susceptible people. Hespanhol (2003) emphasizes that normalizations and regulations adopted in foreign countries should not be allowed and the precautionary principle should be applied to establish water quality criteria to protect and maintain individual uses of water. Moreover, the risk assessment should be considered as an important tool to characterize and estimate the adverse potential effects on health when people are exposed to risk factors, such as pathogenic microorganisms (Piveli *et al.* 2008).

CONCLUSION

The helminth eggs found in wastewater treatment, especially eggs of *Ascaris* sp. and the associated disease are considered a neglected disease which is underreported in Brazil, so there is no systematic parasitological data for São Paulo State Department of Health. The characterization of treated wastewater proved to be an important tool for disposal in soil and is considered a first step towards applying QMRA with hazard identification.

The results of helminths eggs, *E. coli* and physicochemical parameters of treated wastewater and the low removal efficiency of the stabilization pond demonstrated the risk to health. For wastewater disposal it is necessary to use technologies such as multiple barriers to reduce and, if possible, eliminate parasites from treated wastewater. In Brazil, there are no standards; guidelines for wastewater reuse must be developed for the protection of public health in order to contribute to agricultural production, without endangering workers, children and consumers.

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