Urban water reuse: microbial pathogens control by direct filtration and ultraviolet disinfection

Ricardo de Lima Isaac, Luciana Urbano dos Santos, Mariana S. Tosetto, Regina Maura Bueno Franco and José Roberto Guimarães

ABSTRACT

Physicochemical treatment efficiency for unrestricted urban water reuse was evaluated at a conventional activated-sludge wastewater treatment plant (WWTP). Pilot plant set-up consisted of an alum coagulation step, granular media upflow flocculation and direct downflow dual-media filtration followed by ultraviolet disinfection (dose of 95 mJ cm^{-2}). Optimum aluminum sulfate dosage of 10 mg L^{-1} and coagulation pH 7.0 were preset based on bench scale tests. Under WWTP stable operation, water quality met United States Environmental Protection Agency (USEPA) suggested guidelines for unrestricted urban reuse regarding turbidity (mean value 1.3 NTU) and suspended solids (mean value 2.1 mg L^{-1}). When WWTP overall plant performance dropped from 90 to 80% (although BOD value stayed below 6 mg O_2 L^{-1}, suggesting unrestricted reuse), solids breakthrough in filtrate was observed. Microorganism removal rates were: total coliforms 60.0%, *Escherichia coli* 63.0%, *Giardia* spp. 81.0%, and helminth eggs 62.5%; thus organisms still remained in filtrate. Ultraviolet (UV) disinfection efficiency was 4.1- and 3.8-log for total coliforms and *E. coli*, respectively. Considering low UV efficiency obtained for helminths and the survival of protozoa and helminths in the environment, effluent quality presents risk to public health if destined for unrestricted urban reuse.

Key words | bacteria, eggs, granular filtration, helminths protozoa, wastewater reclamation

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INTRODUCTION

Water reuse plays an increasingly important role in sustainable water resources management. High demographic and economic growth rates and fast urbanization observed in many parts of the world impose high pressure on natural resources leading to qualitative and quantitative shortage scenarios. In order to mitigate water scarcity issues, wastewater reclamation and reuse is one among several interesting alternatives to reduce pressure on the aquatic environment. Reclaimed wastewater has been applied predominantly in agricultural irrigation, landscape irrigation, industrial recycling and reuse, and groundwater recharge (Huertas *et al.* 2008). Reclaimed water availability from engineered systems for non-potable use can help to preserve natural sources and relieve urban water supply system. Pathogen removal efficiency of wastewater treatment plants must be optimized in order to assure safe reuse practices, as for irrigation. In addition, to protect receiving waters, eventually the major source of water to supply systems downstream (unplanned indirect potable reuse taking place). Unexploited natural resources sometimes are far away from large consumption centers, reinforcing a need for microbiologically safe reclaimed water (Graczyk & Lucy 2007).

The World Health Organization (WHO 2013) reported that 768 million people relied on unimproved drinking-water sources for drinking water (89% of world population coverage) and 2.5 billion people still did not use an improved sanitation facility (64% sanitation coverage only)

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by the end of 2011, leading to a serious global health problem. According to a national assessment on water supply and sanitation, water consumption *per capita* grew 3.8% and 4.2% in 2011 over the 2007–2009 period, reaching 162.6 and 189.7 liters/person/day in Brazil and Southeastern Brazil, respectively (SNIS 2013). This scenario of greater demand for potable water creates the need to increase production. Nonetheless, effluent discharges (from domestic and non-domestic sources) in waterways pushes water treatment plants to deal increasingly with public health risks associated with biological agents present in raw water.

The most common human microbial pathogens found in recycled water include viruses, bacteria, protozoa, and helminths. The most prevalent infections of the small intestine are caused by diarrheagenic *Escherichia coli*, particularly enterotoxigenic and enteropathogenic, rotavirus, *Giardia* spp., and *Cryptosporidium parvum* (Putignani & Menichella 2010).

Reclaimed water quality criteria which aims to protect public health are related to degree of human exposure considering each proposed reuse. They are usually based on pathogen control assessed through indicator organisms. Monitoring the concentration of helminth eggs is also recommended by the World Health Organization (WHO 2006). This recommendation is based on the conclusion that the main health risks for most developing countries are associated with diseases caused by exposure to helminths. One billion people in the world are infected with *Ascaris lumbricoides*, 800 million with *Trichuris trichiura*, and 700 million with hookworms, while an estimated 200 million people suffer from schistosomiasis. Due to high prevalence and close association with poor infrastructure, helminths are used as a social indicator of a country (Bethony *et al*. 2006). These organisms are considered a problem in wastewater reuse due to their high resistance in the environment and low infectious doses. These pathogens are the primary health hazards associated with the use of wastewater (Kamizoulis 2008).

The protozoa *Cryptosporidium* spp. and *Giardia* spp. are among the major causal agents of diarrhea disease in humans and present a potential public health hazard, especially to those with compromised immune systems, such as the elderly and infants. Both protozoa should be monitored in cattle, in sources of water used for recreational purposes, and in artificial waterways used by farmers as in water channels, animal drinking water, and drainage systems (Castro-Hermida *et al*. 2009). Due to their great resistance to environmental stress, these pathogens have a high potential for water transportation, recently becoming one of the main problems in water supplies around the world (Mons *et al*. 2009). Cysts/oocysts show certain characteristics that increase the probability of waterborne dissemination: the abundance and the resistance in the environment, as well as the small size of cysts and oocysts allow them to pass through physical barriers within water treatment plants. These characteristics and the resistance to chemical disinfectant agents employed in the plants, and the low infecting dose are three of the factors that contribute to the occurrence of cryptosporidiosis and giardiasis outbreaks (Karanis *et al*. 2007; Baldursson & Karanis 2011).

In Brazil, waterborne outbreaks caused by protozoa contamination in public owned water supply systems have been reported (Zini *et al*. 2004; Moura *et al*. 2006), and although no waterborne outbreaks of *Cryptosporidium* spp. or *Giardia* spp. were documented, concentrations of *Cryptosporidium* spp. oocysts (15 to 60 oocysts per L) and *Giardia* spp. (2.5 to 120 cysts per L) have been observed, respectively, in surface water and wastewater in the country (Cantusio *et al*. 2010). Brazilian Drinking Water Legislation recommends the monitoring of these pathogenic protozoa in surface water supply. The need for controlling resistant forms (cysts and oocysts) of protozoa in reclaimed water is reinforced since no correlation was found between the concentration of cysts/oocysts and physicochemical and microbiological parameters, such as turbidity, used to evaluate the water quality regarding total and thermotolerant coliforms (Cantusio 2004).

Physicochemical removal of cysts/oocysts by filtration under optimized coagulation and filtration conditions rather than disinfection by chlorine is among the best available technologies to control these organisms at water treatment plants as indicated in the literature (e.g., Edzwald & Kelley 1998; Emelko 2003; Emelko *et al*. 2005; Tufenkji *et al*. 2006). Granular-medium filtration has for decades also been recommended for removal of residual biological flocs in settled effluents from secondary treatment. This physical unit operation is most commonly used before

discharge to the receiving waters or to activated carbon adsorption units. In water reuse, filtration of treated wastewater is required for application to food crops, park and playground irrigation, and body-contact recreational impoundments (Tchobanoglous 1979). Considering the relative low costs for construction, operations and maintenance of filters, in addition to the ease of operation, the authors intended to look at filters feasibility based on performance to controlling protozoa, helminths, and other pathogenic microorganisms. Because the performance of wastewater filters is affected by many factors, pilot studies have been recommended to meet strict effluent quality as for urban water reuse.

The objective of the present research was to evaluate in pilot scale: (1) the efficiency of coagulation, gravel-medium upflow flocculation and direct downflow filtration of wastewater treatment plant (WWTP) effluent to remove total coliforms, *E. coli*, cysts, oocysts of pathogenic protozoa (*Giardia* spp. and *Cryptosporidium* spp.), and helminth eggs; and (2) the efficiency of disinfection by ultraviolet (UV) light of filtered effluent to inactivate total coliforms, *E. coli*, and helminth eggs. Viruses are one of the most common pathogenic organisms found in recycled water. Bacteriophages are often suggested indicators/model organisms used to measure the removal efficacy of treatment systems. However, monitoring viruses was not considered for the present study due to the authors' expertise and lab skills on target organisms.

MATERIALS AND METHODS

Samambaia WWTP

The experimental investigation was carried out in a filtration pilot plant installed at Samambaia WWTP, Campinas city, Southeast Brazil (22°56′00″S/47°00′00″W). The activated-sludge plant has a nominal flow rate of 150 L s^{-1}. WWTP effluent major quality parameters are shown in Table 1.

Pilot plant

The tertiary advanced wastewater treatment pilot plant operated at a flow rate of 2 L min^{-1}. The treatment consisted of chemical coagulation, gravel-medium upflow flocculation and direct downflow filtration, followed by UV disinfection.

Table 1 | Samambaia WWTP effluent quality during research period

Parameter	Minimum	Maximum
BOD – biochemical oxygen demand (mg O_2/L)	10	16
COD – chemical oxygen demand (mg O_2/L)	40	53
SS – suspended solids (mg/L)	3	17
VSS – volatile suspended solids (mg/L)	2	14
pH	6.6	7.5
Alkalinity (mg $CaCO_3$/L)	36	120
Turbidity (NTU)	2.1	12.6
Apparent color (mg Pt-Co/L)	53	136
True color (mg Pt-Co/L)	25	47

Optimum coagulant dosage and pH were preset based on previous bench scale (jar-test) tests (unpublished data). Coagulant dosage ranged from 2.5 to 15.0 mg L^{-1} (every 2.5) and pH values ranged from 4.0 to 7.5 (every 0.25) giving 90 experimental conditions for aluminum sulfate and the same figure to ferric sulfate. Flocculation time and velocity gradient were preset at, respectively, 10 min and 52 s^{-1}, to simulate pilot plant operating conditions. Jar-test samples were filtered and tested for turbidity and apparent color. Experimental conditions that led to higher removals were then repeated and samples analyzed for suspended solids, nutrients, BOD, COD, and coliforms. Best results were obtained for chemical coagulation at an aluminum sulfate dose of 10 mg L^{-1} and coagulation pH 7.0 and then applied in pilot scale tests.

Flocculation was achieved at a granular media unit, as follows: column internal diameter (I.D.) = 175 mm, flow rate = 120 $m^3.m^{-2}.d^{-1}$; gravel grain size range from 25.0 to 12.5 mm, E.S = 14.0 mm, U.C. = 1.5, layer depth = 190 cm. Dual media filter setup was as follows: column I.D. = 110 mm; filtration constant rate = 300 $m^3.m^{-2}.d^{-1}$; *anthracite* grain size range from 2.4 to 0.8 mm, effective size (E.S.) from 0.9 to 1.0 mm, uniformity coefficient (U.C.) < 1.6, layer depth = 50 cm; *sand:* grain size range from 1.2 to 0.45 mm, E.S. = 0.5 mm, U.C. = 1.65, layer depth = 25 cm (Figure 1). Filter backwashing was completed with chlorinated filtrate hence recirculated to the WWTP inlet.

The UV reactor consisted of a 39 cm length and 4.0 cm-I.D. borosilicate glass cylinder. A germicide lamp (15 W,

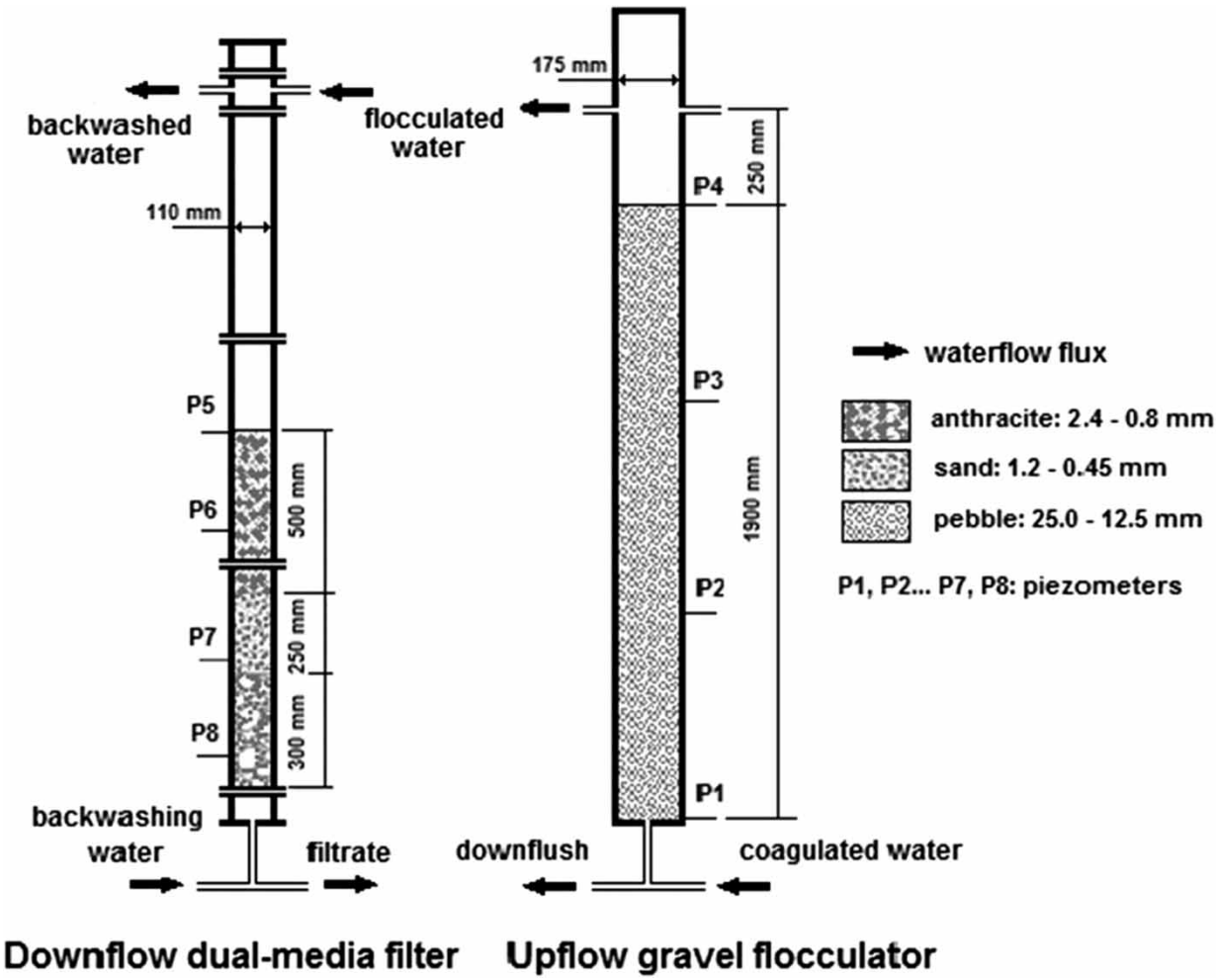

Figure 1 | Pilot plant scheme of gravel flocculator and dual media filter installed at Samambaia WWTP.

$\lambda_{max} = 254$ nm and 2.5 cm external diameter) was positioned in the center of the reactor giving a 299 mL hollow-working volume, a hydraulic detection time of 9 s and an average low-pressure UV dose of 95 mJ cm^{-2}. The disinfection system was completed by a 15 L volume reservoir containing filtered effluent, which was continuously pumped into the reactor. Monitoring of the microorganism concentration occurred at the reactor inlet (sample C) and outlet (sample D).

Sampling points

In order to evaluate pilot plant performance, 15 full experiments (filtration runs) were carried out at intervals of 15 days, five of them under challenged operation, i.e., when WWTP overall efficiency dropped from 90 to 80% of BOD reduction. Four sampling points were considered: (1) secondary effluent of WWTP, designated here as influent (A); (2) flocculated effluent (B); (3) filtered effluent (C); and (4) filtered effluent disinfected by UV light (D). Filtrate samples were grabbed at filtration run times of 30 and 150 min, aiming to evaluate the difference between the effluent microbial quality produced at considered filter ripening (B) and filter stable operating condition (C). Water quality parameters analyses were done in triplicate.

Detection and quantification of total coliforms and *E. coli*

Samples from the four points (A, B, C, and D) were collected in sterilized glass bottles. The Quanty-Tray/2000 method

(IDEXX Laboratories, Inc.) was applied, using IDEXX Enzymatic Defined Substrate Technology®, according to the manufacturer's instructions.

Detection and quantification of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts

Samples were grabbed from point A (2,000 mL) and points B and C (4,000 mL) and were examined for protozoa after cellulose esters membrane filtration (porosity of 3 μm; diameter of 47 mm; Millipore®).

Each membrane underwent sample elution by alternately scraping the membrane with a smooth-edged plastic loop and rinsing it with the elution solution. The resultant liquid was centrifuged at 1,050 × g for 10 min and the concentrated pellet was washed and centrifuged again (Franco *et al*. 2001). The density of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts was determined by fluorescent monoclonal antibody tests (IFA) (Merifluor® kit; Meridian Bioscience, Cincinnati, Ohio) according to the manufacturer's instructions.

The criteria considered in this study to identify cysts/oocysts were based in the Method 1623.1 (USEPA 2012a).

The estimate of the number of cysts/oocysts per liter was computed using the formula

$$X = \frac{n}{v} \cdot \frac{s}{V_f}$$

where X = cyst and oocyst concentration (number of cysts and oocysts per liter); n = number of cysts and oocysts; v = volume of the aliquot (mL); s = volume of the sediment (mL); and V_f = filtered volume of sample (L).

Detection and quantification of helminth eggs

For the quantitative analysis of helminth eggs in treated wastewater (samples A and D), the Mexican Official Norm methodology *NOM-001-ECOL* (México 1996) was used. Briefly, a 1 liter volume sample (A and D) was twice left for sedimentation for 24 h. The sediment was then transferred to 15 mL centrifuge tubes and centrifuged at 400*g* for 3 min. The pellet was re-suspended in 9 mL of $ZnSO_4$ ($d = 1.3$) and again centrifuged at 400*g* for 3 min. The supernatant was pooled and diluted with distilled water for additional sedimentation (24 h). The sediment was homogenized and transferred to centrifuge tubes (15 mL). The pellet was re-suspended in 2.3 mL 0.1 N H_2SO_4 in 33–35% ethanol and 1.5 mL of ethyl ether (centrifugation at 600 × g for 3 min), resulting in two tubes for each sample, both with 1 mL of sediment stored in a microcentrifugation tube.

The helminth eggs/larvae concentrations were estimated as follows:

$$C = \frac{N}{v} \cdot \frac{s}{V_i}$$

where C = helminth eggs/larvae concentration (number of eggs or larvae per liter); N = number of eggs; v = volume of the aliquot (mL); s = volume of the final sediment (mL); and V_i = initial volume of sample (L).

UV efficiency for total coliforms, *E. coli* and helminth eggs inactivation

The same protocol used for total coliforms and *E. coli* detection was used for evaluation of UV light efficiency in the inactivation of these parameters. To accomplish this, C samples aliquots were evaluated after being submitted to disinfection by UV light (D).

The viability of helminth eggs was evaluated using the Trypan Blue staining method (Victorica & Galván 2003). Briefly, 6 μL aliquots of samples were examined in triplicate after staining with 6 μL of 0.1% Trypan Blue on slides and examined by optical microscope. Unstained eggs are considered viable while blue stained eggs are considered non-viable. The modified method of incubation plate was also performed (USEPA 1992). Briefly, the pellet (1 mL) remaining in the tube is placed in a Petri dish with filtered water (2 mL) and incubated at 27 °C for 15 days. Small portions of each sample were daily microscopically examined for larvae observation.

RESULTS AND DISCUSSION

Sample C turbidity ranged from 0.4 to 3.6 NTU (mean value 1.3 NTU, consistently <2 NTU, USEPA (2012b) suggested

guideline criteria for unrestricted urban reuse). Turbidity removal efficiency ranged from 60.9 to 83.7% (sample A and sample C). No statistically significant difference ($p < 0.05$) was observed between organisms and mean turbidity concentrations after filter ripening (B) and stable operating condition (C). Sample C maximum SST value was 15 mg L^{-1} (consistently <30 mg L^{-1}, USEPA criteria for urban reuse-restricted). BOD maximum observed value was 8 mg L^{-1} (consistently <10 mg L^{-1}).

Removal of total coliform and *E. coli*

Comparing samples A and C, the concentration difference for total coliforms was 3×10^4 MPN/100 mL with a mean removal rate of 0.40 log. The average removal rate of *E. coli* was 0.43 log (1.3×10^4 MPN/100 mL of concentration difference between samples A and C) (Figure 2).

Removal of *Giardia* spp. and *Cryptosporidium* spp.

An average concentration of 620 cysts per liter of *Giardia* spp. (ranging from 500 to 800 cysts per liter) was observed in the influent samples and in the filtrated effluent samples the average concentration was 108 cysts per liter (ranging from 37 to 260 cysts per liter). These results indicate that the average removal of cysts by the direct filtration process was about 82.5%. Although the removal rate attained in this study was high, the remaining concentration in filtered effluent can be considered high ranging from 25 to 430 cysts per liter (Table 2).

Cryptosporidium spp. oocysts were observed in just one sample of influent (concentration of 50 oocysts per liter), and were not detected in the filtered effluent; for this reason, the oocysts removal rate could not be determined.

Removal of helminth eggs

The average filtrated effluent concentration observed was 50 helminth eggs per liter. When the average concentration of helminth eggs for the four independent experiments is considered, the removal efficiency was 62.5% using the Trypan Blue staining methodology. The removal rate of helminth eggs is shown in Table 2.

The removal rates obtained in this study differed from those obtained by Jiménez-Cisneros *et al.* (2001), who obtained 99.0% of removal of *E. coli*, 67.0% of *G. duodenalis* cysts, and 96.0% of helminth eggs.

Inactivation of total coliform, *E. coli*, and helminth eggs by UV light

The average inactivation verified for total coliforms and *E. coli* was approximately 4-log (Figure 2), at an average UV dose of 95 mJ cm^{-2}, that resulted in densities, respectively,

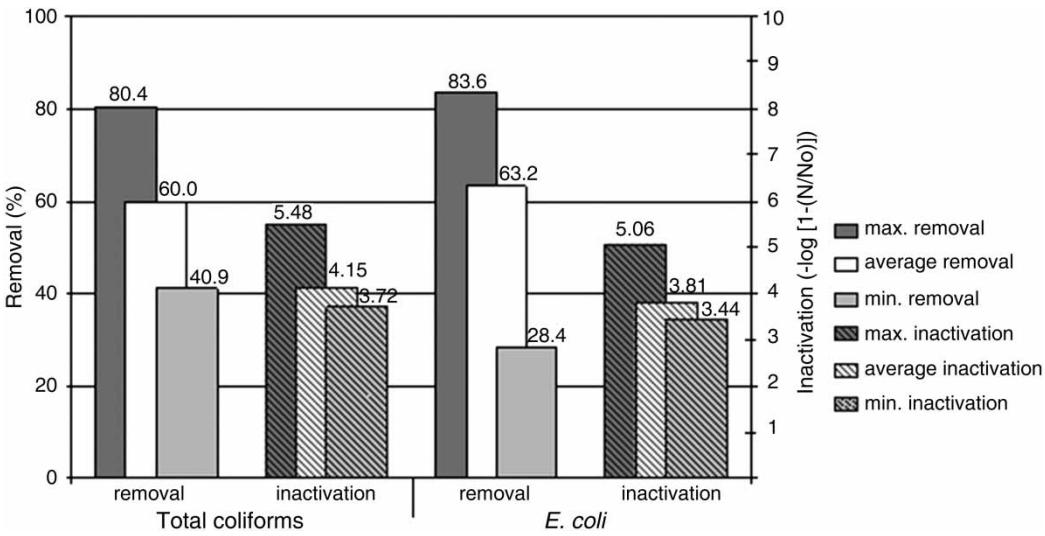

Figure 2 | Filtration removal (A to C sampling points) and UV inactivation (C to D sampling points) of total coliforms and *E. coli*.

Table 2 | Number of protozoa and helminth ova, as well as removal of *Giardia* spp. cysts, *Cryptosporidium* spp. oocysts and helminth eggs for experiments performed at Samambaia WWTP

Parameters	Experiment samples	1	2	3	4	5	Average
Giardia spp. (cysts/L)	A	8.0×10^2	7.1×10^2	5.0×10^2	5.5×10^2	5.5×10^2	6.2×10^2
	C	1.0×10^2	8.0×10^1	2.6×10^2	6.2×10^1	3.75×10^1	1.3×10^2
	% removal	87.4	87.9	47.5	88.6	93.2	80.9
Cryptosporidium spp. (oocysts/L)	A	ND	ND	5.0×10^1	ND	ND	–
	C	ND	ND	ND	ND	ND	–
	% removal	–	–	100	–	–	100
Helminth (larvae/L)	A	5.6×10^1	5.6×10^1	1.7×10^2	8.3×10^1	ND	7.2×10^1
	D	ND	ND	8.3×10^1	8.3×10^1	8.3×10^1	5.0×10^1
	% removal	100.0	100.0	50.0	0.0	–	62.5

Sample A, secondary effluent; sample C, filtered effluent (average of filtered effluent after 30 min and after 2 h and 30 min); sample D, effluent disinfected by UV.
ND, not detectable.

from 1 to 18 MPN/100 mL and from 1 to 2 MPN/100 mL in final effluent (sample D).

Ultraviolet doses ranging from 40 to 80 mJ cm^{-2} led to about 4-log reduction of *E. coli* (Guo *et al.* 2012). Amin *et al.* (2010) found granular filtration followed by medium pressure lamp exposure at a dose of 230 mJ cm^{-2} effective to reduce coliform counts in the secondary effluent sufficiently to meet local standards for effluent discharge used for unrestricted agricultural irrigation. Amoah *et al.* (2005) found that the addition of particulate matter was correlated with a statistically significant reduction in *C. parvum* and *G. muris* inactivation of 0.8 log and 0.4 log, respectively, even after the fluence was adjusted for increased absorbance due to the presence of particles. These results indicate that the particulate matter present in natural surface waters may interact with oocysts and cysts and thereby result in a reduction in UV inactivation over and above that attributable to simple absorbance.

Inactivation of helminth eggs could not be estimated for the Trypan Blue method, since no eggs were observed in sample D (post-UV). Regarding Petri dish incubation method, larvae were observed thus suggesting that UV inactivation of helminth eggs was not 100% efficient.

Studies have reported that UV is effective against all waterborne pathogens including viruses, bacteria, and protozoa (Hijnen *et al.* 2005). However, conflicting studies showed that helminth eggs were not completely inactivated at UV doses as high as 15,300 and 45,700 mJ cm^{-2}. However, Mun *et al.* (2009) obtained 2-log *Ascaris* eggs inactivation by UV doses below 400 mJ cm^{-2}.

Cantusio *et al.* (2006) evaluated effluent disinfection by UV light regarding animal infectivity assay for *Giardia* spp. cysts at the same WWTP. One animal of the UV-treated group revealed trophozoites in intestinal scrapings. Thus, the efficiency of inactivation by UV treatment, under field conditions, was not complete.

The high values of total coliform, *E. coli*, *Giardia* spp. Cysts, and helminth eggs in the filtrated effluent samples (sample C) reinforce the importance of a proper disinfection step to provide tertiary effluent for reuse applications without representing a public health threat.

According to USEPA (2012b) the final effluent (1 to 2 fecal coliform/100 mL) is not suitable for unrestricted urban reuse (non-detectable).

When the helminth eggs parameter is considered, the WHO guidelines (1989) recommends densities lower than 1 egg per liter. As the average concentration found in this study for the tertiary treated effluent (after UV disinfection) was much higher than this recommendation (50 eggs per liter), the tertiary effluent produced could not be used for irrigation of crops that are likely to be eaten uncooked, or used at sports fields, public parks, and other areas where there is public access.

Due to occurrence of cysts of *Giardia* spp. in some treated wastewaters intended to be used as reclaimed water, Hachich *et al.* (2013) concluded that studies should be

conducted to establish pathogen quantitative criteria for a future Brazilian water reuse regulation.

CONCLUSIONS

The rates of organism removal obtained in this study were quite limited (total coliforms, 60.0%; *E. coli*, 63.0%; *Giardia* spp., 81.0%; and helminth eggs, 62.5%), corresponding to less than 1-log efficiency, and a remarkable number of organisms still remained in the treated wastewater. UV disinfection efficiency was 4.1- and 3.8-log for total coliforms and *E. coli*, respectively. Larvae presence denoted that UV inactivation of helminth eggs was not completely efficient. It is important to emphasize that the present quality requirements for wastewater reuse do not limit the presence and/or the concentration of protozoa. Considering the survival of these pathogens in the environment, the tertiary effluent obtained in this study presents a risk to public health if destined for reuse in localities with public access.

Findings reinforce direct filtration high dependence on adequate coagulation. Laboratory-scale alum optimum dosage (10 mg L^{-1}) was not effective in pilot plant processes under challenged operating conditions and filtration could be improved by changing the chemistry of the system. Organic content is known to increase colloidal particles and microorganisms' stabilization, which would demand higher amounts of coagulant that reduce filtration run duration making the proposed system not feasible.

The authors conclude that the low quality of treated wastewater generated at the activated-sludge WWTP during the current study resulted in not very efficient physicochemical tertiary treatment.

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