Identification of pathogen bacteria and protozoa in treated urban wastewaters discharged in the Ebro River (Spain): water reuse possibilities

R. Mosteo, M. P. Ormad, P. Goñi, J. Rodríguez-Chueca, A. García and A. Clavel

ABSTRACT

The aim of this research work is to identify the presence of pathogens, bacteria and protozoa, in different treated urban wastewaters and to relate biological pollution with the processes used in wastewater treatment plants. A study of the possibilities for water reuse is carried out taking into account bacterial and parasite composition. The analysed bacteria and protozoa are: Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Clostridium perfringens (spore), Salmonella spp., Legionella spp., helminths eggs, Giardia, Cryptosporidium spp. and free-living amoebae (FLA). The selected municipal wastewater treatment plants (MWTPs) are located in Navarra (Spain) and the main difference between them is the use of natural lagoons as tertiary treatment in some plants. The results concerning bacteriological identification showed contamination of mainly faecal origin, and the use of natural lagoons as tertiary treatment in some MWTPs produced an important disinfection effect. Moreover, pathogen parasites such as Giardia and Cryptosporidium were not detected in the samples studied although FLA were identified in all cases.

Key words | faecal indicators, Legionella spp., pathogen protozoa, Salmonella spp., water reuse

INTRODUCTION

The quality of treated urban wastewater depends to a great extent on the quality of the municipal water supply, the nature of the wastes added during use and the degree of treatment the wastewater has received (Pedrero et al. 2010). Wastewater treatment plants are usually designed to efficiently remove solids and dissolved pollutants (organic matter and nutrients) but are seldom planned specifically to remove pathogenic microorganisms from wastewaters. The removal efficiency of pathogenic microorganisms during wastewater treatment is characterized by high variations and depends on factors such as the type of treatment process, hydraulic retention time, retention of solids, dissolved oxygen concentration, pH, temperature and the efficiency in removing suspended solids (Tyagi et al. 2011).

Due to the increase in demand for water, the reuse of treated urban wastewater has been extended for agricultural, industrial, environmental and municipal uses over the last few decades. One of the main concerns for the reuse of treated urban wastewater is human exposure to biological pollution, not only pathogen bacteria but also helminth eggs, protozoa and enteric viruses, and the possibility of diseases being spread since biological pollution is not commonly eliminated by conventional secondary wastewater treatment (Castro-Hermida et al. 2008). Microbiological control is therefore necessary for water that is to be reused, in order to avoid risk. The most important microorganisms able to be detected in treated urban wastewaters are the following: putrefaction bacteria (Pseudomonas aeruginosa, Pseudomonas fluorescens, Proteus vulgaris, Bacillus cereus, Bacillus subtilis, Enterobacter cloacae); faecal indicators (Escherichia coli, Enterococcus spp., sulphite-reducing Clostridia) and other human pathogens (Salmonella spp., Shigella spp., Mycobacterium spp., Brucella spp., Legionella spp., Vibrio spp., Clostridium spp., sulpho-reducing bacteria etc.). Fungus and yeast are also detected in treated urban effluents (Moreno et al. 1998).

Various guidelines on water reuse have appeared in recent years which establish the criteria for reusing
wastewater (USEPA 2004; EPA (Queensland State) 2005; WHO 2006). In Spain, the reuse of treated urban wastewater is regulated by legislation, under Royal Decree 1620/2007 (published in the Spanish Official Bulletin), in which maximum contaminant levels are established taking into account the type of water reuse: urban, agricultural, industrial, recreational or environmental. In the case of microbiological contamination, the pathogens controlled by this law are Escherichia coli, and intestinal nematodes in all cases, and Legionella spp. and Salmonella spp. for several uses including industrial use.

Consequently, there is a need to find treatment technologies that reduce the sanitary risk related to water reuse, producing high quality effluents suitable for water reuse. Waste stabilization ponds have been considered as a well-established method of biological wastewater treatment being particularly efficient in the removal of pathogens. However, there is still debate regarding the importance of the mechanisms by which pathogens are removed: sedimentation, sunlight, natural die-off and environmental variables (Reinoso et al. 2011).

To control the microbiological pollution of wastewaters, faecal contamination indicators are usually employed. The choice of these microorganisms is based on two main factors: first, their detection and quantification is easy and cheap, and second, this determination provides information related to the presence and behaviour of the principal human pathogens present in wastewaters (Abreu-Acosta & Vera 2011). Nowadays, the main indicator microorganisms of faecal pollution are faecal coliforms, Escherichia coli, Enterococcus spp. and Clostridium perfringens.

Escherichia coli is the most widely accepted faecal indicator since this bacterium is present in large quantities in the human digestive tract and it is not usually found in other environments. The presence of this bacterium in water indicates recent faecal contamination and the possible existence of other pathogens (Molleda et al. 2008). The use of Enterococcus as indicator bacteria is frequently suggested as an alternative to Escherichia coli. Their main advantage lies in their greater resistance and their inability to grow in any environment, such as soil and water (Ryu et al. 2007). In the last few years, Clostridium perfringens has been used as a faecal indicator since it is an anaerobic spore-forming sulphite-reducing bacterium found in high concentrations in human and animal faeces. Moreover, its spores are very resistant to environmental conditions and have greater resistance to disinfection treatment than conventional faecal contamination indicators, while its vegetative forms do not seem to multiply in aquatic environments (Dunlop et al. 2008; Lanoa et al. 2010). The spores, therefore, can indicate remote or discontinuous pollution, and possible contamination with other resistant pathogens such as Cryptosporidium or Giardia.

However, faecal contamination indicators have a limited predictive value for various pathogens, especially human viruses and protozoa. These microorganisms are now recognized as being more resistant to natural inactivation and to water treatment processes than the current bacterial indicators of water quality (Abreu-Acosta & Vera 2011).

Human pathogenic protozoa, which are not well represented by faecal contamination indicators, are present in water in resistant forms (cysts and oocysts) that protect them from environmental stress. Cryptosporidium and Giardia are genera of protozoan parasites potentially found in water and other media. Cryptosporidium spp. and Giardia duodenalis are major causes of diarrhoeal disease in humans and animals worldwide and of protozoan waterborne diseases (Moulin et al. 2010). Moreover, the oocysts are resistant to conventional wastewater treatment processes and disinfectants, at least at the concentrations commonly used to reduce bacterial contamination. Cryptosporidium and Giardia have been detected in irrigation water, and in effluents and biosolids emanating from wastewater treatment plants (Castro-Hermida et al. 2008).

Free-living amoebae (FLA) are protozoa prevalent in the environment. The most extensively studied of the FLA is Acanthamoeba spp. because of its abundance and its medical significance as an agent of human infections such as amoebic keratitis and granulomatous amoebic encephalitis. Another area of concern regarding FLA in water is their relationship with waterborne pathogenic bacteria, including Legionellaceae, Mycobacteriaceae, Enterobacteriaceae, Vibrionaceae and many others (Greub & Raoult 2004; Thomas et al. 2010). They improve bacterial survival and resistance to disinfectants, making possible the recolonization of artificial water systems after disinfection programmes (Storey et al. 2004; Thomas et al. 2004). Previous research has shown the presence of potentially pathogenic FLA in treated urban wastewaters (García et al. 2011).

Helminths may also be found in wastewaters, the most important group being nematodes. Their main epidemiological characteristics are long persistence in the environment, a minimal infective dose, limited immune response and the ability to remain viable in solids over long periods of time (Molleda et al. 2008).

On the other hand, reported data on Salmonellae and Legionellae in treated urban wastewaters are scarce. Despite
the importance of *Salmonella* spp. as one of the major causes of food-borne infections worldwide, data regarding the presence of these organisms in the environment are limited (Shannon et al. 2007; Levantesi et al. 2010). Similarly, few reports may be found about the presence of *Legionella* spp. in urban wastewater (Shannon et al. 2007; Brissaud et al. 2008).

One of the major limitations in this field of investigation is the lack of studies comparing removal rates of indicator bacteria and pathogenic parasites in natural wastewater treatment systems such as natural lagoons (Reinoso & Bécares 2008).

The aim of this research is: (1) to identify the presence of pathogens, bacteria and protozoa in different treated urban wastewaters and to relate biological pollution with the processes used in certain urban wastewater treatment plants; and (2) to study the efficiency in the removal of bacteria and protozoa in natural lagoons. The analysed bacteria and protozoa are: *Enterococcus, Pseudomonas aeruginosa, Staphylococcus aureus, Clostridium perfringens* (spore), total anaerobic bacteria, *Salmonella* spp., *Legionella* spp., helminth eggs, *Giardia duodenalis* and *Cryptosporidium* spp.

**MATERIALS AND METHODS**

**Sample collection**

Samples were collected at five municipal wastewater treatment plants (MWTPs). All of the MWTPs are located in the region of Navarra (Spain) and the final effluents from the MWTPs are discharged into the Ebro River.

One isolated sample was collected from secondary and tertiary effluents during the spring of 2009. The samples were taken in sterilized flasks (10 L for parasitic pathogens and physico-chemical parameters and 1 L for bacteriological analysis) and were preserved in a refrigerator prior to analysis and processed within six hours of sample collection. The sampling protocol, handling and storage were carried out following ISO 5667-3:2003.

The wastewaters treated in the selected MWTPs are composed of domestic sewage and biodegradable industrial wastewaters as considered by Directive 91/271 regarding urban wastewater treatment.

The specific steps in the treatment process used in each of the five MWTPs and the basic influent characteristics are described in Table 1. The treatment carried out at MWTP1 and MWTP3 differs from the other three plants in that they use natural lagoons as tertiary treatment. The tertiary treatment in MWTP1 consists of three lagoons being identical in size (width × length × height = 87 × 166 × 2.5 m). The total hydraulic residence time (HRT) in the lagoons is 50 d, prior to discharge. The hydraulic load is 0.05 m³/(m² · d). MWTP3 has four natural lagoons in series with the same dimensions (width × length × height = 23 × 46 × 2 m), the total HRT is 24 d and the hydraulic load is 0.09 m³/(m² · d).

**Physico-chemical analysis**

Physico-chemical parameters such as pH, conductivity, turbidity, dissolved organic carbon, chemical oxygen demand (COD), biological oxygen demand (BOD), dissolved oxygen and suspended solids (SS) were measured in duplicate in the water samples according to *Standard Methods* (Clereschi et al. 2005).

**Bacteriological analysis**

The analysis of *Escherichia coli, Enterococcus* spp., *Pseudomonas* spp., *Staphylococcus aureus* and *Salmonella* spp.

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**Table 1 | Main features of treatment plants**

<table>
<thead>
<tr>
<th></th>
<th>Influent m³/d</th>
<th>Population equivalent</th>
<th>Primary treatment</th>
<th>Secondary treatment</th>
<th>Tertiary treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWTP1</td>
<td>2,129</td>
<td>15,869</td>
<td>Grit and grease separation, sedimentation</td>
<td>Trickling filter</td>
<td>Natural lagoons</td>
</tr>
<tr>
<td>MWTP2</td>
<td>16,734</td>
<td>46,237</td>
<td>Screen, grit and grease separation, sedimentation</td>
<td>Trickling filter</td>
<td>–</td>
</tr>
<tr>
<td>MWTP3</td>
<td>361</td>
<td>3,453</td>
<td>Screen, sedimentation</td>
<td>Trickling filter</td>
<td>Natural lagoons</td>
</tr>
<tr>
<td>MWTP4</td>
<td>983</td>
<td>4,376</td>
<td>Screen, anoxic reactor</td>
<td>Activated sludge</td>
<td>–</td>
</tr>
<tr>
<td>MWTP5</td>
<td>3,400</td>
<td>27,154</td>
<td>Screen, grit and grease separation, anoxic reactor, sedimentation</td>
<td>Aerated trickling filter</td>
<td>–</td>
</tr>
</tbody>
</table>

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was performed using the membrane filtration method (using a cellulose nitrate filter of 0.45-μm pore size, Millipore®) in triplicate. The culture and enumeration of *Escherichia coli* were carried out according to procedure ISO 9308-1, using MacConkey agar (Scharlau®). The culture and enumeration of *Enterococcus* spp. were carried out according to procedure ISO 11291 using Cetrimide agar (Scharlau®), respectively. The culture and enumeration of *Staphylococcus aureus* and *Salmonella* spp. were performed using Manitol agar (Scharlau®) and XLD (xylose lysine deoxycholate) agar (Scharlau®), respectively. *Legionella* spp. analysis was carried out under ISO 11731: part 1. The analysis of spores of *Clostridium perfringens* was performed through heat shock on SPS (sulfite polysulphide) agar medium (Scharlau®) using the membrane filtration method (with a cellulose nitrate filter of 0.22-μm pore size, Millipore®), according to the procedure UNE-EN 264612.

The enumeration of colonies was carried out in terms of colony-forming units (CFUs) per 100 mL of sample in each contact time. These concentrations were transformed to log_{10}. The removal efficiency was determined by log (Nt/N0) and removal percentage [(Nt – N0)/100] × 100, where N0 and Nt are the concentration before and after lagoons. All zero counts were replaced by the lowest possible count.

### Parasite analysis

To detect parasites, helminth or cestode eggs, and *Cryptosporidium* or *Giardia* (oo)cysts, the Bailinger method as modified by Bouhoum & Schwartzbrod was performed (Ayres & Mara 1996). Ten litres of water samples were filtered with a cellulose nitrate filter of 0.45-μm pore size (Millipore®). The filters were then washed twice with Tween 80 0.1% solution and centrifuged at 1,000 g for 15 min. A small fraction of the pellet was microscopically observed and the formalin ethyl acetate concentration procedure was performed. Microorganisms were identified by their morphology. Direct microscopic examination of a few microlitres of each concentrated sample was performed, along with microscopic visualization of trichrome and modified Ziehl–Neelsen staining of each sample, in order to detect cysts or trophozoites of *Giardia* and *Entamoeba*, and *Cryptosporidium* oocysts, respectively, or other protozoa cysts. At least four preparations of each sample were checked in a Nikon Eclipse 80i microscope. An initial observation was performed with the ×10 objective, examining all the possible fields in a smear of 22 × 22, later moving to ×40 and ×100. Also, an observation for each sample was sequentially performed by two skilled and experienced microscopists. A sample was concluded as positive for a parasite or protozoa when it was detected in at least one preparation.

Culturing and genotyping for FLA were performed in non-nutrient agar as previously described (García et al. 2011).

### RESULTS

#### Results of physico-chemical parameters analysis in final effluents

Table 2 shows the results obtained in the physico-chemical characterization of the MWTP final effluents, these being secondary effluents for MWTP2, MWTP4 and MWTP5 and tertiary effluents for MWTP1 and MWTP5. As can be observed, the measurements show slight variations in the different parameters such as pH, conductivity and SS, due mainly to their original concentration in the water supply, the uses to which the water has been put and the technologies used in each MWTP. MWTP1 final effluent shows a high conductivity associated with an industrial biodegradable effluent generated from the cutting industry, in which high quantities of salts (sodium chloride) are employed during industrial processes.

### Table 2 | Results of physico-chemical parameters

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Conductivity (μS/cm)</th>
<th>Turbidity (NTU)</th>
<th>SS (mg/L)</th>
<th>COD (mg/L)</th>
<th>BOD (mg/L)</th>
<th>O₂ diss. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWTP1</td>
<td>14.7 ± 0.3</td>
<td>8.61 ± 0.02</td>
<td>3,220 ± 0.5</td>
<td>34 ± 0.2</td>
<td>56 ± 5.2</td>
<td>111 ± 1.0</td>
<td>16.4 ± 1.8</td>
</tr>
<tr>
<td>MWTP2</td>
<td>15.8 ± 0.3</td>
<td>7.93 ± 0.02</td>
<td>1,867 ± 0.5</td>
<td>123 ± 0.2</td>
<td>52 ± 5.2</td>
<td>60 ± 1.0</td>
<td>17.5 ± 1.8</td>
</tr>
<tr>
<td>MWTP3</td>
<td>11.1 ± 0.3</td>
<td>7.39 ± 0.02</td>
<td>1,086 ± 0.5</td>
<td>1.74 ± 0.2</td>
<td>4 ± 5.2</td>
<td>77 ± 1.0</td>
<td>15.3 ± 1.8</td>
</tr>
<tr>
<td>MWTP4</td>
<td>18.6 ± 0.3</td>
<td>7.22 ± 0.02</td>
<td>2,950 ± 0.5</td>
<td>34.7 ± 0.2</td>
<td>32 ± 5.2</td>
<td>62 ± 1.0</td>
<td>45.4 ± 1.8</td>
</tr>
<tr>
<td>MWTP5</td>
<td>13.5 ± 0.3</td>
<td>7.27 ± 0.02</td>
<td>832 ± 0.5</td>
<td>5.43 ± 0.2</td>
<td>20 ± 5.2</td>
<td>50 ± 1.0</td>
<td>29.5 ± 1.8</td>
</tr>
</tbody>
</table>

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Results of bacteriological characterization in secondary and tertiary effluents

Table 3 shows the concentrations of bacteria identified in secondary effluents for all the facilities. In the case of MWTP1 and MWTP3, bacteriological characterization of tertiary effluents is also shown in Table 3. It can be seen that all the microorganisms studied, with the exception of *Salmonella* spp. and *Legionella* spp., were detected in the secondary effluents in all the MWTPs. The concentrations of each microorganism were similar in all the facilities. The presence in treated wastewater of *Escherichia coli*, *Enterococcus* spp., *Pseudomonas* spp., *Staphylococcus aureus* and *Clostridium perfringens* reflects a high faecal pollution load in waters due mainly to domestic sewage discharged into the MWTPs. These results are in keeping with values described by other authors (Levantesi et al. 2010; Moulin et al. 2010). It is important to mention that neither *Salmonella* spp. nor *Legionella* spp. were detected in secondary effluents. This absence of *Salmonella* in treated urban wastewaters is consistent with findings in other research works (Shannon et al. 2007). These bacteria can be present in urban wastewater but they are usually removed or reduced to undetectable levels in MWTPs (Levantesi et al. 2010).

Taking into account the secondary treatment used in each MWTP (Table 1), it can be said that there were no significant differences in bacteriological pollution present in secondary effluents compared with treatments using the activated sludge process or trickling filters.

As mentioned previously, MWTP1 and MWTP3 have an additional step consisting of four lagoons in series. The treated wastewaters thus remain in the plant for a longer period of time before being returned to the Ebro River. Comparing results between the secondary and tertiary effluents of MWTP1 and MWTP3 (Table 3), it can be concluded that tertiary effluents have lower concentrations of microorganisms than secondary effluents. Previous studies have indicated that sunlight may be the main factor for the inactivation of microorganisms in these systems (Curtis et al. 1992; Davies-Colley et al. 1997; Reinoso & Bécares 2008; Maiga et al. 2009). The disinfection effect is observed in all the microorganisms studied, the best disinfection results being obtained for *Enterococcus* spp. (reduction of 5.51 log in MWTP1 and 3.61 log in MWTP3). *Escherichia coli* also reaches significant removal rates, higher in MWTP3 (reduction of 2.14 log) than MWTP1 (1.38 log). In the case of *Staphylococcus aureus*, a relatively high disinfection efficiency was obtained in MWTP1 (reduction of 1.78 log)

Table 3 | Results of bacteriological analysis from secondary and tertiary effluents

<table>
<thead>
<tr>
<th>Microorganism (CFU/100 mL)</th>
<th>Secondary treatment</th>
<th>Tertiary treatment</th>
<th>Secondary treatment</th>
<th>Secondary treatment</th>
<th>Tertiary treatment</th>
<th>Secondary treatment</th>
<th>Secondary treatment</th>
<th>Tertiary treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>7.25 ± 0.5 × 10^5</td>
<td>&lt;1</td>
<td>3.20 ± 0.1 × 10^5</td>
<td>&lt;1</td>
<td>3.85 ± 1.0 × 10^5</td>
<td>3.20 ± 0.2 × 10^5</td>
<td>&lt;1</td>
<td>0</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp.</td>
<td>3.05 ± 0.5 × 10^5</td>
<td>1.16 ± 0.9 × 10^5</td>
<td>3.85 ± 1.0 × 10^5</td>
<td>3.20 ± 0.2 × 10^5</td>
<td>1.40 ± 0.4 × 10^5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas</em> <em>aeruginosa</em></td>
<td>3.5 ± 0.3 × 10^5</td>
<td>5.80 ± 0.8 × 10^4</td>
<td>1.16 ± 0.9 × 10^5</td>
<td>3.85 ± 1.0 × 10^5</td>
<td>1.64 ± 0.4 × 10^5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>1.05 ± 0.5 × 10^5</td>
<td>5.90 ± 0.7 × 10^4</td>
<td>1.20 ± 0.1 × 10^4</td>
<td>3.85 ± 1.0 × 10^5</td>
<td>1.64 ± 0.4 × 10^5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>2.00 ± 0.5 × 10^3</td>
<td>1.60 ± 0.9 × 10^5</td>
<td>1.20 ± 0.1 × 10^4</td>
<td>3.85 ± 1.0 × 10^5</td>
<td>1.64 ± 0.4 × 10^5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> <em>spp.</em></td>
<td>0</td>
<td>1.40 ± 0.4 × 10^5</td>
<td>1.20 ± 0.1 × 10^4</td>
<td>3.85 ± 1.0 × 10^5</td>
<td>1.64 ± 0.4 × 10^5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Legionella</em> <em>spp.</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
while in MWTP3 inactivation was not high enough for this microorganism. On the other hand, the removal rate of *Pseudomonas* spp. and *Clostridium perfringens* is lower than a reduction of 1 log, reflecting the greater resistance of these bacteria (Hassen et al. 2000; Liberti et al. 2001).

In Figure 1, it can be observed that the majority of the microorganisms achieve removal rates close to 90% and the total removal rates for bacteriological pollution are 95 and 73%, in MWTP1 and MWTP3, respectively. The difference in removal rates is probably due to lagoon design. If design parameters such as hydraulic load and HRT are considered, it is noticed that disinfection efficiency increases with higher HRT and lower hydraulic load according to design criteria (Ortega et al. 2010). Hydraulic loads are 0.05 m³/(m²·d) in MWTP1 lagoons and 0.09 m³/(m²·d) in MWTP3 lagoons and the total HRTs are 50 d (MWTP1) and 24 d (MWTP3). On the other hand, there are some physical factors such as temperature and sedimentation which are involved in the inactivation of pathogens. Microorganisms tend to conglomerate forming flocs that settle. The sedimentation process improves with the presence of SS. In Table 2, it can be seen that secondary effluent from MWTP1 presents the highest concentration of SS, the difference with MWTP3 effluent being around 90%.

### Results of pathogen characterization in secondary and tertiary effluents

Identification and characterization of protozoa and parasites present in sampled treated urban wastewaters were carried out in this research work. The results are summarized in Table 4. The pathogen protozoa analysed are *Cryptosporidium* and *Giardia*, while the parasites selected were helminth eggs including *Ascaris*, *Trichuris* and *Ancylostoma*,

![Figure 1](https://iwaponline.com/wst/article-pdf/68/3/575/440130/575.pdf)

### Table 4 | Results of protozoa and parasite analysis

<table>
<thead>
<tr>
<th>Microorganism (500 mL)</th>
<th>Secondary treatment</th>
<th>Tertiary treatment</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>Rodents, nematode</td>
<td>Rodents, nematode</td>
<td>Acanthamoeba spp.</td>
</tr>
<tr>
<td><em>Enteroxoccus</em> sp.</td>
<td>larvae, algae</td>
<td>larvae</td>
<td>sp.</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp.</td>
<td>Rodents</td>
<td>Acanthamoeba spp.</td>
<td>Hartmannella vermiformis</td>
</tr>
<tr>
<td><em>Sphaerobacter</em> sp.</td>
<td>larvae, rosettes</td>
<td>Rodents, nematode</td>
<td>sp.</td>
</tr>
<tr>
<td><em>Clostridium</em> sp.</td>
<td>larvae, rosettes</td>
<td>Rodents, nematode</td>
<td>sp.</td>
</tr>
</tbody>
</table>

**Figure 1** | Removal rates in lagoons.
and potentially pathogenic FLA. All of them are subject to legislative control. These analyses were carried out in secondary and tertiary treatments in sampled MWTPs.

As can be observed in Table 4, neither Giardia, Cryptosporidium nor cestode eggs were found in any effluents. Free-living helminth eggs were detected in MWTP effluents, except in secondary and tertiary effluents of MWTP1. Note that these effluents have a higher pH and conductivity that could select the specimens which inhabit this ecological niche (WHO 2004). On the other hand, the presence of free-living nematodes is not unusual because they can originate from soil which many species inhabit. The most important result obtained in this characterization is the detection of potentially pathogenic FLA in the treated urban wastewaters, as was reported by García et al. (2011). Also, the analysis showed presence of non-pathogenic protozoa (ciliates and rotifers), which are indicative of good water quality because they participate in the processes of natural purification of water. All these results showed the excellence of the purification processes used without differences between them.

CONCLUSIONS

Bacteriological identification in treated urban wastewaters reveals bacteria of mainly faecal origin since Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa and Clostridium perfringens are detected in high concentrations. Moreover, Staphylococcus aureus, a species of bacteria normally found in human skin and mucus, was also detected. On the other hand, Salmonella spp. and Legionella spp. were not detected in the treated urban wastewaters.

Tertiary treatments by natural lagoons used in MWTP1 and MWTP3 has a significant disinfection efficiency, reducing biological pollution in the final effluent. For the majority of investigated microorganisms, removal rates achieved are close to 90% and the total removal rates for bacteriological pollution are 95 and 73%, in MWTP1 and MWTP3, respectively.

In the study of pathogen parasites, neither Giardia, Cryptosporidium nor cestode eggs were detected in the secondary and tertiary effluents. Non-pathogenic protozoa, ciliates, rotifers and free-living nematodes were detected in the analysed samples. This indicates good water quality since these protozoa are involved in natural depuration processes.

The most important result obtained in this characterization is the detection of potentially pathogenic FLA in the treated urban wastewaters (García et al. 2011). The identification of FLA in the sampled treated urban wastewaters is important due to their potential pathogenicity since they allow bacterial survival in adverse conditions. Their presence improves bacterial survival and resistance to disinfection processes and recolonization takes place in water systems (Thomas et al. 2010), but living amoebae are very difficult to remove. In fact, studies carried out where many disinfection methods were tested conclude their ineffective action against Acanthamoeba cysts (Coulon et al. 2010). Thus it is important to decrease the protozoa concentration in order to control the potential pathogen population.

Although further researches are necessary with a great number of samples and at different sampling periods in order to establish the possibilities for water reuse, the results of the study suggest that the final effluent from MWTP3 could be reused taking into account WHO faecal bacteria limits for use of treated wastewater in agricultural unrestricted irrigation. Furthermore, MWTP3 final effluent could be reused in Spain for the majority of water uses established in current law (RD 162/2007), taking into account both physico-chemical and microbiological parameters.

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