Number of residual thermotolerant coliforms on plants and in soil when using reclaimed domestic wastewater for irrigation

Sasirot Khamkure, Edmundo Peña Cervantes, Alejandro Zermeño González, Rubén López Cervantes, Prócoro Gamero Melo and Homero Ramírez

ABSTRACT

The reclamation of domestic wastewater for irrigation is one alternative approach to solve the water scarcity crisis, but it is essential to control the microbiological quality of wastewater used for irrigation. The removal of thermotolerant coliforms, also known as faecal coliforms (FC), from treated domestic wastewater by intermittent media infiltration (IMI) in column was studied. The columns were filled with natural filter media (soil, soil/charcoal and zinc-modified zeolite, Zeo-Zn), and wastewater, IMI-treated wastewater and disinfected wastewater were compared. The numbers of residual FC on Swiss chard (Beta vulgaris) and in agricultural soil were determined over a 4-month period. The column using Zeo-Zn had a higher FC removal efficiency (2.98 log) than columns with other filter media and disinfection (1.87–2.57 log) due to the bactericidal properties of Zn$^{2+}$. The treatment of wastewater using Zeo-Zn and disinfection both decreased the accumulation of FC on plants and in soil to approximately 1–20 MPN/g dry matter. IMI-treated wastewater using the column with Zeo-Zn was suitable for unrestricted agricultural use, complied with Mexican regulations (as did disinfected wastewater) and had a low risk of FC contamination of plants and soil.

Key words | crop, intermittent media infiltration, irrigation, reclaimed wastewater, residual bacteria, soil

INTRODUCTION

Although domestic wastewater is harmful due to its pathogenic load, wastewater reuse is one important method to sustain water resources, especially for agricultural irrigation. Wastewater reuse can reduce treatment costs and increase agricultural productivity (Pedrero et al. 2010). Improvements in wastewater treatment methods to remove pathogens and chemicals can minimise negative impacts on health and the environment.

Mexico is one of the ten largest water users in the world. This country’s freshwater resources are surface water (65%) and underground water (37%). The distribution of freshwater use in Mexico for irrigation, public supply, industrial and thermoelectric use is 77, 14, 4 and 5%, respectively (CONAGUA 2010). In some areas of Mexico, reclaimed wastewater is used to irrigate crops and recharge aquifers to maintain surface resources and to reduce the overexploitation of groundwater aquifers (Espino et al. 2004; Jimenez & Chávez 2004). The Mezquital Valley, a district with a large area that is irrigated in the state of Hidalgo, Mexico, has been using municipal wastewater from Mexico City to irrigate vegetable and forage crops. The use of this water has resulted in a significant increase in crop yields because of the high levels of organic matter (OM) and nutrients in the wastewater, but the frequency of waterborne diseases has also increased (Lucho-Constantino et al. 2005).

Thermotolerant coliforms, also known as faecal coliforms (FC), are non-disease causing organisms that are always found in the intestinal tracts of warm-blooded animals (Asano et al. 2007). Faecal contamination is a risk...
factor for different types of diseases; thus, FC bacteria are used as a biological indicator for wastewater (Salgot et al. 2006). According to the Mexican regulations (SEMARNAP 1996), the FC limit based on the most probable number (MPN) is 1,000 MPN/100 mL in wastewater reused for unrestricted agricultural irrigation, corresponding with the WHO guidelines (WHO 1989).

Not only should regulatory mechanisms governing the characteristics of treated wastewater be applied, but also suitable cost-effective wastewater treatments must be developed and implemented (Salgot 2008). The challenge is to find alternative disinfection processes or to improve the chlorination procedure to control the levels of common disinfection by-products (DBPs) (Hrudey 2009). Residual chlorine causes severe damage to plants when in excess of 5 mg/L (Pedrero et al. 2010).

An appropriate filtration technique could improve disinfection and the quality of treated wastewater (Ausland et al. 2002; Zhang et al. 2007; Rajeb et al. 2009). The survival of bacteria in porous media is reduced by the attachment of the bacteria to the media, by transport of bacteria into the pores and by die-off due to the high surface area (Smith & Badawy 2010; Travis et al. 2010). Many studies have been published on the effects of irrigation with treated municipal wastewater on the levels of nutrients, heavy metals and on crop biomass (Rusan et al. 2007; Kalavrouziotis et al. 2008; Pedrero et al. 2010). However, only a few studies have investigated the pathway of residual bacteria (Akponikpè et al. 2011).

A previous study of wastewater treated by intermittent media infiltration (IMI) showed that the levels of FC were effectively decreased over a 4-week period (Khamkure et al. 2012a, 2012b). IMI is a low-cost treatment method and is easy to perform, allowing unrestricted water reuse for irrigation. The aim of this study was to determine the number of residual FC on plants and in soil after irrigating plants with reclaimed domestic wastewater treated by IMI.

**MATERIALS AND METHODS**

The experiments were carried out in the greenhouse of the Department of Soil Science at the Universidad Autónoma Agraria Antonio Narro (UAAAN), located in a semi-arid region to the south of Saltillo, Coahuila, Mexico. The average annual precipitation is 369 mm, and the average temperature is 18°C. The greenhouse’s ambient temperature was maintained on average at 25 and 18°C during the day and night, respectively. The columns were prepared using 100% soil (IMI-S), 75% soil and 25% charcoal (Mesquite wood) (IMI-C) and 100% zinc-modified natural zeolite (clinoptilolite) (IMI-MZ) (Khamkure et al. 2022b). The zeolite was modified using zinc acetate dihydrate (Zn(CH₃COO)₂ · 2H₂O) following the method described by Khamkure et al. (2022a). Treated domestic wastewater (unchlorinated wastewater) was obtained from a wastewater treatment plant located to the south of Saltillo. The studied wastewater types were unchlorinated wastewater (WWI), chlorinated wastewater treated with sodium hypochlorite (NaOCl) (WWC), and effluents from the IMI-S (WES), IMI-C (WEC) and IMI-MZ (WEZ) columns (Table 1). NaOCl (household bleach, 5.25%, w/w) was applied at 25 ppm with a contact time of 15 min. A water depth of 270 mm during the crop season (60 days) was applied for 4 months. No fertiliser was applied during this study.

Swiss chard (Beta vulgaris) was planted as a control plant to study the effects of wastewater reuse. The chard was planted in pots containing 5 kg of agricultural soil (UAAAN source). The agricultural soil was classified

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### Table 1 | Characteristics of raw wastewater and treated wastewater used for irrigation and the Mexican water quality guidelines for various water uses

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WWI</th>
<th>WWC</th>
<th>WES</th>
<th>WEC</th>
<th>WEZ</th>
<th>Guidelines for discharge*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Temperature (°C)</strong></td>
<td>23.8 ± 1.0</td>
<td>23.6 ± 3.6</td>
<td>24.4 ± 4.1</td>
<td>24.5 ± 3.8</td>
<td>24.6 ± 3.8</td>
<td>N.A. b</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.6 ± 0.4</td>
<td>7.7 ± 0.2</td>
<td>8.2 ± 0.8</td>
<td>8.1 ± 0.8</td>
<td>7.5 ± 0.3</td>
<td>5–10</td>
</tr>
<tr>
<td><strong>Conductivity (μS/cm)</strong></td>
<td>893 ± 113</td>
<td>1037 ± 120</td>
<td>912 ± 77</td>
<td>979 ± 126</td>
<td>817 ± 150</td>
<td>5–10</td>
</tr>
<tr>
<td><strong>TS (mg/L)</strong></td>
<td>709 ± 164</td>
<td>841 ± 186</td>
<td>767 ± 147</td>
<td>751 ± 139</td>
<td>622 ± 38</td>
<td>N.A.</td>
</tr>
<tr>
<td><strong>TSS (mg/L)</strong></td>
<td>12.5 ± 2.9</td>
<td>8.8 ± 6.3</td>
<td>10.0 ± 7.0</td>
<td>9.2 ± 3.0</td>
<td>7.5 ± 2.9</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

*SEMARNAP (1996); bnot applicable; the data are the mean and standard deviation for four samples taken at different times during the irrigation period.*

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as clay loam (34% sand, 36% clay and 30% silt), and the pH, OM, N and carbonate levels were 7.5, 4.2, 0.210 and 67.5%, respectively. Swiss chard is a salt-tolerant vegetable (Shannon et al. 2000). A randomised complete block design was used, with five treatments and five replications. The applied treatments were the five different types of wastewater, with one plant representing an experimental unit.

The soil was screened with a 5-mm sieve to remove stones and then dried using sunlight for 7 days before it was placed into the plastic pots. Freshwater was added to each pot to saturate the soil 2 days before seeding (three seeds per pot). Two weeks after seeding, the seedling with best development in each pot was selected, and the others were discarded.

The effluents of each column were collected manually in 100 mL sterile bags (Whirl-Pak). These effluents were analysed monthly according to the standard method (APHA 1998) over a period of 4 months. The FC bacteria were counted using the MPN technique and analysed using A-1 medium (Becton, Dickinson and Company, USA) accepted by the USEPA, which can be used in a single-step procedure.

Plant samples were collected 2 and 4 months after planting, and leaf samples were used for analysis. Soil samples were analysed at the end of the experiment. Undisturbed plants and soil samples were steriley collected for bacterial analysis. Soil samples were obtained from the surface to a depth of 5 cm from each pot, corresponding to the depth profile over which most FC bacteria are found (Entry et al. 2000; Jamieson et al. 2002). 10-g samples (wet weight) of Swiss chard leaves and experimental soil were placed in sterile flasks, to which was added 90 mL of sterile peptone water (5 g/L of peptone, MCD LAB). The leaves were gently shaken for 2–3 min, and the soil samples were shaken at low speed for 5 min to rinse off most of the bacteria present (Estrada et al. 2004). Three 1 mL aliquots of each of three dilutions prepared using 0.1, 1.0, and 10.0 mL sample volumes were used to estimate the number of FC using the MPN test as described above. Plant and soil samples were dried in the oven at 70 °C for 48 h or until a stable weight was observed, and then the number of FC bacteria was reported per gram of dry weight.

Statistical analyses were carried out using R version 2.14.1. One-way analysis of variance (ANOVA) was performed to determine the effects of irrigation with different types of wastewater on the number of FC on plants and in soil at a significance level of $p = 0.05$. Then, Tukey’s HSD (honestly significant difference) test was used for multiple comparisons.

RESULTS AND DISCUSSION

FC removal performance

The physical and chemical properties of the irrigation water are shown in Table 1. The values were lower than those established by the Mexican regulations. The fewest FC bacteria were found in WEZ, followed by WWC, WES and WEC (Table 2). WEZ had the highest reduction in the number of FC, virtually 100% (99.9%) or 2.98 log removal. The other filter media reduced the number of FC bacteria by 98.6–99.7% or 1.87–2.57 log removal. The number of FC in the irrigation water is presented in Figure 1. The numbers of FC in the treated wastewaters were lower (1–3 log MPN 100/mL) than that in WWI by approximately 2–4 orders of magnitude. The primary bacterial transport mechanisms in filter media are straining and adsorption, which are related to the grain surface roughness and the difference in the surface charges between the media and the bacteria (Poppen & Schijven 2006). Similar trends for FC removal were observed between WWC and WEZ and between WES and WEC. WWC and WEZ had lower FC values than the Mexican limits. The IMI-MZ, prepared by substituting Zn$^{2+}$ for alkaline and alkaline earth cations on the surface of clinoptilolite, had an FC antibacterial capacity as good as that of chemical disinfection (Nibou et al. 2009). The bacteria or some parts of them interact with the empty orbitals of the zinc atoms that are chemically anchored to the clinoptilolite surface. The interaction between the zinc atoms and the bacteria can damage the bacteria’s cell walls, inhibit the functions of some enzymes and lead to the death of the bacteria (Malachová et al. 2011).

<table>
<thead>
<tr>
<th>Type of Irrigated water</th>
<th>Mean</th>
<th>S.E.M.</th>
<th>% Reduction</th>
<th>Log removal</th>
<th>Range of log removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWI</td>
<td>4.82</td>
<td>0.24</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>WWC</td>
<td>1.74</td>
<td>0.64</td>
<td>99.7</td>
<td>2.57</td>
<td>1.36–4.43</td>
</tr>
<tr>
<td>WES</td>
<td>2.74</td>
<td>0.37</td>
<td>98.9</td>
<td>1.94</td>
<td>1.35–3.18</td>
</tr>
<tr>
<td>WEC</td>
<td>2.77</td>
<td>0.37</td>
<td>98.6</td>
<td>1.87</td>
<td>1.19–3.06</td>
</tr>
<tr>
<td>WEZ</td>
<td>1.43</td>
<td>0.52</td>
<td>99.9</td>
<td>2.98</td>
<td>1.67–4.43</td>
</tr>
</tbody>
</table>

Note: The data are four samples taken at different times during the irrigation period. ND – Not detectable.
Thus, IMI-MZ may have disinfecting activity against FC bacteria due to the availability of Zn$^{2+}$ (Bonferoni et al. 2007). The column using the zinc-modified natural zeolite (Zeo-Zn) is recommended over the chlorination process for wastewater treatment to prevent chlorine toxicity, which can result in chlorosis and significant suppression of plant growth (Carrillo et al. 1996).

WES and WEC had FC values lower than the limit (3 FC log MPN/100 mL) at 2 months but increased thereafter (Figure 1). However, the FC contents of WES and WEC were in the lower range, according to the study of Akponikpè et al. (2011). Due to the long study period under greenhouse conditions, both the column of IMI-S and IMI-C contained green slime in the filter media, and the IMI-S also had a low permeability. Although the adsorption capacity of charcoal is high, the biofilm concentration is also high. The biological clogging of filter media was caused by the accumulation of microorganisms in the form of biofilms in the upper layer, which was the main reason for the pore size reduction. This clogging increased the water retention and reduced the effective area for filtration (Jung-Woo et al. 2010). Thus, the number of FC in the effluents of these columns increased with increasing operation time (Table 2) due to the reduction in the hydraulic conductivity of the columns.

**Effects of watering with wastewater and treated wastewater on plants and in soil**

The FC contents of the samples of plants and soil treated with the five different types of irrigation water (WWI, WWC, WES, WEC and WEZ) are shown in Figure 2. At the first sampling, plants irrigated with WWI had significantly higher FC contents than plants irrigated with treated wastewater. When compared with soil, plants had higher FC contents by approximately 1 order of magnitude for both WWI samplings. All types of water were directly applied to the plant leaves to achieve the worst possible results for the microbial contamination of vegetables that are eaten raw. The behaviour of FC bacteria survival in soil has a direct influence on soil moisture and temperature (Entry et al. 2000). The average die-off rate ($k$) of the FC is in the range of 0.043–0.152 day$^{-1}$, which has a significant effect on the moisture content of soil (Kouznetsov et al. 2004). The die-off may be the result of a lack of sufficient nutrients or biodegradable OM (Candela et al. 2007) or to an increase in the interactions between various microorganisms in the soil matrix (Rajeb et al. 2009). The factors influencing FC die-off may include soil type, pH, the manure application rate, nutrient availability and competition (Jamieson et al. 2002).

At the first plant sampling, no difference was observed in the FC contents among the plants irrigated with WWC, WES and WEC. However, the FC contents were higher on the plants watered with WWI than on the plants watered with WEZ ($p < 0.05$) (Figure 2). At the second plant sampling, plants watered with WEC had lower FC contents than the plants watered with WWI. At the two plant samplings, the plants watered with WWI had the highest FC contents, and the plants watered with the treated wastewater (WWC, WEC, WES and WEZ) had lower FC contents. The trend for the residual FC levels on plants irrigated with

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**Figure 1** | Time variations in the log levels of thermotolerant coliforms in the irrigation water over the 4-month study period. WWI: unchlorinated wastewater; WWC: chlorinated wastewater; WES, WEC and WEZ: effluents from the IMI-S, IMI-C and IMI-MZ columns, respectively.
WWC, WEC, WES and WEZ exhibited an increase with increasing time. This result is similar to that obtained by Al-Sa`ed (2011) for a short period (4–6 months). However, Cirelli et al. (2015) have recently reported low faecal contamination of agricultural products over the long term (2 years).

Furthermore, plants and soil watered with WWI had higher levels of residual FC than the plants and soil watered with other treated wastewaters except for the soils watered with WES and WEC, which will be explained later. The concentration of FC bacteria in WWI (Table 2) was significantly higher than those in the treated wastewaters (WWC, WEC, WES and WEZ), containing approximately 2–3 orders of magnitude more bacteria.

The soil microbiology results show that the FC contents were significantly lower for soil irrigated with WWC and WEZ, respectively (Figure 2). The results presented in Figure 1 show that the FC contents of the effluents of WWC and WEZ during the study period were acceptable according to the Mexican and WHO guidelines. The numbers of FC in soil irrigated with WWI and treated wastewater (WWC and WEZ) were in good agreement with the levels reported by Travis et al. (2010). A similar trend was found for FC survival after WWC and WEZ irrigation in both plant sampling and soil. These results demonstrated that the IMI-C and IMI-MZ treatments were able to reduce the FC content of the wastewater and the levels on plants and in soil. The wastewater treated with these methods meets the levels for unrestricted agricultural utilisation. Figure 2 also shows that soil containers irrigated with WEC had FC levels significantly higher than those of soils irrigated with other types of wastewater, with a difference of approximately 1–2 orders of magnitude. Although the levels of residual FC for WEC irrigation were high, these levels were in the range of coliform contamination (10^2–10^3 CFU/g soil) resulting from the use of treated wastewater in soil at the surface (5–10 cm), corresponding to the values reported by Candela et al. (2007). Regarding soil containers irrigated with WEC, the numbers of FC could increase again due to the detachment of biomass, as with WES (Rajeb et al. 2009), because the average FC levels in the WEC and WES effluents (Table 2) were higher than those of other effluents and the microbial biomass growth, as previously discussed.

**CONCLUSIONS**

The treatment of domestic wastewater using IMI-MZ reduces the FC content by up to 99.9%. This FC removal efficiency (2.98 logs) is higher than that of other filter media (soil and soil/charcoal) and disinfection. The FC contents of different types of irrigated wastewater have a significant effect on the levels of FCs on plants and in soils. Irrigation with WEZ resulted in a lower residual FC level in the soil (1.66 ± 1.07 MPN/g) and on Swiss chard after 2 and 4 months (5.45 ± 2.13 and 16.38 ± 8.00 MPN/g dry matter, respectively); these results are similar to those for chemical disinfection. The use of IMI-MZ is recommended over chlorination when the treated wastewater will be used for...
agricultural irrigation because of the ability to regenerate the filter media, the lack of residual chlorine and the lack of toxic effects on vegetable crops.

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