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Comparable levels of microbial contamination in soil and on tomato crops after drip irrigation with treated wastewater or potable water

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ABSTRACT

To evaluate the impact of treated wastewater (TWW) irrigation for produce safety, field experiments were conducted to compare secondary and tertiary TWW with potable water using tomatoes as a model crop. Human pathogens including a suite of obligate and opportunistic bacterial pathogens (*Campylobacter*, *Pseudomonas*, *Salmonella*, *Shigella*, *Staphylococcus*), protozoa (*Cryptosporidium* and *Giardia*), and viruses (*Adenovirus* and *Enterovirus*) were monitored in two field trials using a combination of microscopic, cultivation-based, and molecular (qPCR) techniques. Results indicate that microbial contamination on the surface of tomatoes was not associated with the source of irrigation waters; fecal indicator bacteria (FIB) contamination was not statistically different on tomatoes irrigated with TWW or potable water. In fact, indicator bacteria testing did not predict the presence of pathogens in any of the matrices tested. Indicator bacteria and the opportunistic pathogens were detected in water, soil and on tomato surfaces from all irrigation treatment schemes, and were positively correlated with duration of time in the field ($p < 0.0001$). Pathogen contamination (*Cryptosporidium*) was detected in secondary TWW (3/14 samples) and on the surface of a TWW irrigated tomato (1/41 samples). Furthermore, the *Enterobacteriaceae* species in the TWW were markedly different from those found in soil and tomato. The results indicate that (surface drip) irrigation with TWW did not result in the transfer of fecal indicator bacteria or microbial pathogens to the irrigated soil or crop. Moreover, parallel testing for pathogens with traditional culture-based and quantitative PCR indicates that specific and rapid molecular testing of pathogens appears to be a more appropriate strategy than fecal indicator testing for the determination of reclaimed water safety.

1. Introduction

In many geographic regions, demand for freshwater (FW) often exceeds availability. Globally, human populations are forecasted to increase, which will most likely compound beneficial water use issues and exacerbate regional conflicts over water resources (Sofer, 1999). Contemporary research offers potential for reduced consumption through various conservation and treatment technologies such as water desalinization, disinfection and decontamination. The use of treated wastewater (TWW) has the potential for additional conservation, specifically in the form of

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crop irrigation (Toze, 2006), considering that the amount of water used globally for agricultural purposes is increasing while the resources are limited or even diminishing (Boelee, 2013; Sofer, 1999).

Using TWW for crop irrigation represents an important opportunity for ensuring adequate sustenance in industrialized countries and food security in developing regions. An example for the implantation of this practice is found in Israel, where over the past three decades FW available to the agricultural sector was reduced while the amount of TWW supplied to farmers to irrigate fruit trees increased. At present, 96% of all municipal sewage in Israel is treated, 80% of which is reclaimed [versus 10.6% in Spain (Iglesias et al., 2010) or 2.5% in the United States (Page et al., 1996)], contributing about one-fifth of Israel's total water supply (Kfir et al., 2012). A crucial impediment to this environmentally sustainable approach is the possible contamination of produce with fecal pathogens that may be present in TWW, which carries an associated risk for foodborne illness to produce consumers.

Regardless of irrigation regimen, fresh produce eaten raw has been implicated as the major vehicle for foodborne pathogenic outbreaks in the past decade (Doyle and Erickson, 2008), mostly due to contaminated leafy greens, sprouts and low growing fruits, such as tomatoes (Warriner and Namvar, 2010). Soil might serve as a vehicle for transferring pathogens to produce due to pathogen persistence for long periods in soil following irrigation with TWW, fertilization practices (Bech et al., 2010; Gorbatsevich et al., 2013), or contaminated runoff (Ramirez et al., 2009). Some fecal pathogens may also propagate in the soil until crops are planted (Bernstein et al., 2007; Heaton and Jones, 2008), increasing the likelihood of contamination during the plant's growth cycle. Pathogens within the soil may contaminate crops directly, for instance when sprinkler irrigation cause leaf splash (Monaghan and Hutchison, 2012), or indirectly, by penetrating the plant tissues (Bernstein et al., 2007).

It has been well established that irrigation with raw WW increases the risk for bacterial, parasitic and viral infections in consumers (Campbell et al., 2001; Doyle and Erickson, 2008; Fattal et al., 1986; Nygård et al., 2008; Shuval et al., 1989). Yet, there is no conclusive evidence implicating TWW as a risk factor for crop irrigation. In fact, reports from around the world indicate that irrigation with TWW presents no greater risk than irrigation with potable water (Bichai et al., 2012; Christou et al., 2014; Cirelli et al., 2012; Forslund et al., 2012, 2010; Jang et al., 2013; Martínez et al., 2013; Shuval, 2010). However, these reports rely either on epidemiological data (reviewed in Shuval, 2010) or mainly on fecal indicator bacteria (FIB) such as coliforms or *Escherichia coli* to assess possible health risks (Bichai et al., 2012; Christou et al., 2014; Forslund et al., 2012, 2010; Jang et al., 2013), neglecting major pathogen groups such as viruses and protozoa. The lack of correlation between pathogens and FIB, currently used in microbiological monitoring standards (Bitton, 2011; Edberg et al., 2000; WHO, 2006), is well established (Harwood et al., 2005; Ottoson et al., 2006; Payment et al., 2001) and may lead to under- or over-estimation of the risks to public health.

In this study we used biochemical, molecular and microscopic methods to follow pathogens and indicators from TWW to the irrigated soil and crops. We hypothesized that the presence of FIB would not accurately predict the presence of pathogens in the crops; this lack of correlation may apply to bacterial pathogens as well as protozoa and viruses. We further postulated that soil and crops irrigated with TWW rather than potable water are more likely to be contaminated by fecal microorganisms, i.e., fecal contamination of the soil and crops would be directly correlated to the quality of the water used for irrigation.

2. Methods

2.1. Field cultivation experimental design

Tomato (*Solanum lycopersicum* L. cv Smadar) was used as a model vegetable crop to evaluate microbial contamination on crops irrigated with TWW and potable water. Lachish, the experimental station (operated by the Israeli Ministry of Agriculture), is located near the municipality of Kiryat Gat in the south of Israel. The tomato seedlings (obtained from Hishtil, Nehalim, Israel) were planted under a screen house in 11×2 m plots. Two independent field experiments were conducted with five replicates to each treatment (Fig. S1). In the first cultivation experiment (April–August, 2011), a total of 10 plots were planted and irrigated with potable water or secondary TWW effluents. In the second cultivation experiment (April–August, 2012), a total of 15 plots were planted and irrigated with potable water, secondary TWW, or tertiary TWW. Each plot experiment lasted for approximately 20 weeks from the planting of the seedling to the termination of the plats in the field.

Using a random experimental design, up to three plots were planted along a bed (10 m long and 1.9 m wide) with 1.5 m of non-irrigated soil separating plots within a bed (to ensure that roots of plants from one plot will not invade a neighboring plot). One dry bed separated adjacent beds. Each bed included one row of tomato plants, two plants per running meter, and one surface drip irrigation lateral.

2.2. TWW source and treatment

The source of the irrigation water was either potable water or secondary or tertiary treated effluent originating from the municipal WW treatment plant (WWTP) of the town of Kiryat-Gat (operated by Kal-Binyan, Caesarea, Israel). The WW was treated in an activated sludge system cycling between anoxic and aerobic conditions with a hydraulic retention time of about 28 h. The TWW was chlorinated upon leaving the WWTP. At the farm, the secondary TWW was stored in a 110 m^3 tank and for tertiary treatment was passed through a sand filtration column. In the first cultivation experiment the secondary TWW were used as is (without chlorination) mimicking a worst case-scenario. Results for this case helped to focus the detection efforts in following experiments. During the second cultivation experiment secondary and tertiary effluents were chlorinated (1 mg L^{-1} residual) at the entry point to the field. Thus, four treatments were applied in the field: in the (1) non-chlorinated secondary TWW (first cultivation study); (2) chlorinated secondary TWW; (3) chlorinated tertiary TWW by sand-filtration of the secondary TWW (second cultivation study); and (4) potable water irrigation was used as a control in both cultivation studies. We note that in accordance with the Inbar guidelines (Inbar, 2007), barriers were applied during the use of TWW for irrigation including surface drip irrigation (all treatments), chlorination (three treatments), and sand filtration (one treatment).

2.3. Method validation and limits of detection (LOD)

Limits of detection for bacteria, protozoa and viruses were estimated with preliminary spiking studies of the targets applied to the different matrices used in this study (i.e., water types, soil and tomato crops). Spiking studies were performed at high (1×10^3) and low (1–10) concentrations of biological agents of interest per test unit to estimate the recovery efficiency and LOD of each method and target combination (Table S1). Internal control surrogate microorganisms were added to the feed (*Acinetobacter baylyi* for the bacteria (Schriewer et al., 2010) and *Pseudomonas aeruginosa* phage PP7 (Rajal et al., 2007) for viruses) and their LOD monitored