Risk assessment of treated municipal wastewater reuse in Sicily
Rosa Aiello, Giuseppe L. Cirelli, Simona Consoli, Feliciana Licciardello and Attilio Toscano

ABSTRACT
In Italy, the restrictive approach for treated wastewater reuse in agriculture has led to some difficulties in promoting this practice. In order to assess the health risk associated with the use of wastewater in agriculture, an experiment was conducted in an open field near the constructed wetland (CW) system of San Michele di Ganzaria (Eastern Sicily), during the irrigation seasons 2004–2009. In particular the impact on tomato crops of drip and sub-drip irrigation with treated municipal wastewater, as well as effects of wastewater reuse on the irrigation system, main production features, hydrological soil behaviour, and microbial soil and products contamination were investigated. Notwithstanding the fact that globally CW effluents did not match microbiological standards for wastewater reuse of Italian legislation, the median infection risk (function of the recommended tolerable additional disease burden of 10^-6 DALY (disability-adjusted life year) loss per person per year) suggested by the 2006 World Health Organization Guidelines for rotavirus, Campylobacter and Cryptosporidium for lettuce irrigation under unrestricted irrigation scenario was achieved.

Key words | constructed wetland, herbaceous crops, irrigation, microbiological risk, treated wastewater reuse

INTRODUCTION
Irrigation with treated wastewater (TWW) is a common practice in many parts of the world, although its diffusion is still a debatable point (Scott et al. 2004). A realistic estimation confirms that 20 million ha in the world are irrigated by raw, treated and/or partially diluted wastewater (Hamilton et al. 2006). TWVs are produced in large volumes, which if not reused would be discharged into the environment and do not contribute to increased water availability for the agricultural context. Furthermore, it is well known that discharge of raw effluents into the environment, particularly natural water bodies such as lakes, rivers and the coastal marine environments, may cause severe degradation of these water bodies. The degradation is often related to the presence of organic and inorganic nutrients, which cause problems such as eutrophication and algal blooms. Reusing these discharged effluents can significantly reduce or completely remove the impact of these effluents on receiving bodies. In addition, the TWW reuse for agricultural irrigation reduces the amount of water that needs to be extracted from environmental water resources. TWW can often contain significant concentrations of organic and inorganic nutrients for example nitrogen and phosphate that may be used as a fertilizer source when the water is recycled for irrigation. Moreover, soil microorganisms have been observed to increase their metabolic activity when sewage effluent is used for irrigation (Toze 2006).

One of the most economically feasible agricultural uses of reclaimed TWW is the irrigation of high-value horticultural crops, which typically has high returns per volume of water invested in (Hamilton et al. 2006). But this practice has been approached with trepidation, owing primarily to concerns about risks to human health via contamination of food with pathogenic microorganisms (Hamilton et al. 2005; Lazarova 2005; Toze 2006). There have been a number of risk factors identified for using TWVs for agricultural irrigation purposes. Some risk factors are short term and vary in severity depending on the potential for human, animal or environmental contact (e.g. microbial pathogens), while others have longer term impacts which increase with continued use of recycled water (e.g. saline effects on soil).

A pragmatic approach to allow and justify such concerns, which has been gaining favour in recent years, is the application of WHO 2006 Guidelines (updated in 2008) based on a risk assessment and management procedure. In particular, the approach for microbial risks is: (i) to define a tolerable maximum additional burden of disease; from which it is possible (ii) to derive tolerable risks of disease and infection; (iii) to set health-based targets for pathogens reductions; (iv) to determine how the required pathogen reductions can be achieved; and (v) to set up a system for verification monitoring.

Finally, the main purposes of this study were to assess the health risk associated with the use of WW in irrigation and to monitor the impact on tomato crops of drip and sub-drip irrigation with treated municipal WW. Faecal coliform (FC), *Escherichia coli* (*E. coli*), Enterococcus faecalis (*EF*) were selected as indicators of faecal contamination, whereas *Salmonella* was selected as pathogenic bacteria. In addition, effects of TWW reuse on an irrigation system, main production features, hydrological soil behaviour and microbial soil contamination were investigated.

**MATERIALS AND METHODS**

**Irrigation systems description**

The experiment was conducted near the constructed wetland (CW) system of San Michele di Ganzaria (eastern Sicily), during the irrigation seasons 2004–2009. The CW unit treats secondary urban effluents from the conventional WW treatment plant of the municipality (Cirelli et al. 2007). The area under study is characterized by a Mediterranean semi-arid climate, with a mean annual rainfall of 600 mm and a mean daily temperature of 18°C in the observation period.

The experimental field was moved during the years. In particular, the soil was sandy-loam during the first four years of the experiment (2004–2007) and clay (USDA textural soil classification) during the following period (2008–2009). The areas of trial were pretty flat with slopes in the order of a few per mille (‰).

**Monitoring period 2004–2007**

In 2004, the site was equipped with two parallel testing systems, each one of four plots (plots S1...S4 were replicated two times); in Figure 1 just one testing system is reported. The performance of two different filtration technologies (120 mesh screen filter and disk filter) and four different drip laterals types were evaluated: (i) surface light pipe P1, which permits notable branch lengths with small loss in pressure; (ii) sub-surface light pipe P1 FLAP, which includes a flap in the emitter orifice to prevent soil clogging; (iii) sub-surface rigid pipe MONO, with co-extruded dripper; and (iv) sub-surface light pipe P1 Rootguard (RTG). The polyethylene laterals (four in each plot) had length of 45 m, external diameter of 16 mm and an in-line labyrinth dripper (nominal water flow of 2.1 L h⁻¹ at a pressure of 100 kPa) spaced by 0.33 m (Figure 1). A control system (S5), supplied with fresh water (FW) and equipped with surface light pipe P1 was also installed; in this experiment we preferred to compare the performance of TWW plots with FW plots equipped just with the surface lateral P1, showing the best laboratory performance as confirmed by the Irritec & Siplast company. The Irritec & Siplast company manufactured the whole laterals pattern used in the study.

![Figure 1](https://iwaponline.com/wst/article-pdf/67/1/89/441375/89.pdf)
By taking into account the technological results of the first year of experiment, the irrigation system lay-out was simplified during the 2005–2007 period. In particular, the drip lateral types P1 and MONO, which achieved the highest performance in 2004 (Aiello et al. 2007), were adopted in the following trials. In 2004, each plot was divided into two sub-sections differentiated by the presence of a plastic mulching soil coverage or bare soil; during the following years’ trial the whole irrigation area was covered by black/white plastic mulching.

Prior to planting, N (26 kg ha$^{-1}$), P$_2$O$_5$ (35 kg ha$^{-1}$) and K$_2$O (35 kg ha$^{-1}$) were applied uniformly to the experimental field.

Cultivars ‘Incas’ and ‘Missouri’ of tomato crop were transplanted into the experimental field (about 1,550 m$^2$) at a density of 3.6 plant m$^{-2}$. TWW was applied in a randomized block design. Standard cultivation practices were adopted during crop growing seasons. Tomatoes were hand harvested at full red maturity.

Irrigation scheduling was based on a simplified soil water balance method, by recording climatic data and evaporation rates through a Class A pan evaporimeter. The total irrigation volume measured by volumetric meters was about 6,000 m$^3$ ha$^{-1}$ during each year of the trial.

Monitoring period 2008–2009

Figure 2 reports the experimental system design during 2008–2009 monitoring periods. In the scheme, two plots (S1 and S4) were supplied by TWW and two (S2 and S3) by FW. FW plots were sized to have half of the TWW plots length; this was due to the limited FW availability in the experimental area.

Three repetitions of crops were considered for each plot. Tomato crops were transplanted into the field at a density of 2.5 plant m$^{-2}$. All plots were equipped with surface (SP) or subsurface (SSP) (buried at 0.10 m from the soil surface) polyethylene laterals with 16 mm external diameter. The chosen drip laterals were the same (P1 and MONO) showing the highest performance during the 2004 monitoring program.

The system was supplied with two different filtering technologies, 120 mesh screen (for S2 and S3 plots) and disk filter (for S1 and S4 plots), that showed quite similar performance in previous works (Aiello et al. 2007; Cirelli et al. 2012). The whole irrigation area (less than 200 m$^2$) was covered by black/white plastic mulching.

Standard cultivation practices were adopted during crop growing seasons. N (89 kg ha$^{-1}$), P$_2$O$_5$ (35 kg ha$^{-1}$) and K$_2$O (35 kg ha$^{-1}$) were applied uniformly to the experimental field, during the irrigation periods. Tomatoes were hand harvested at full red maturity. The different rates of N during the two monitoring programs (2004–2007 and 2008–2009) were related to the soil composition and soil availability of N and to a slight tomato transplanting delay during 2008–2009 period.

Irrigation scheduling was based on the advection-aridity model as function of evapotranspiration rates (Parlange & Katul 1992), rainfall, and soil water content (evaluated by time domain reflectometry method). The total irrigation volume measured by volumetric meters was about 5,500 m$^3$ during each year of trial.

Determination of irrigation uniformity

The uniformity of drip irrigation units was determined by means of the method described by Keller & Karmeli (1975). From 16 emitters for each surface laterals, the discharge was fortnightly collected by means of graduated cylinders. These data were used to calculate the average flow for each lateral. The uniformity of subsurface laterals

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**Figure 2** | Lay-out of the experimental irrigation system at S. Michele di Ganzaria during 2008–2009.
was evaluated before they were buried and at the end of the irrigation season. The data obtained were used to calculate emission uniformity (EU), the flow rate reduction factor ($R_d$) and the coefficient of variation of the emitter flow ($C_v$). The formulas used for calculating these parameters are shown in Table 1.

In addition, filtering system performance was determined by evaluating: (i) the rate of pressure loss across the tested manual filters; (ii) the time and effort required to clean the manual filters; and (iii) the need for operation and maintenance.

### Irrigation water contamination analysis

The main physical–chemical and microbial characteristics of both kinds of water (TWW and FW) were monitored during the irrigation period with intervals of about 10 days. TWW samples were collected at the beginning of the irrigation system and FW samples were collected at the tank where FW was stored. Sterile 1,000 mL glass bottles used for microbiological analysis were stored at 4 °C.

The following chemical–physical parameters were measured in the laboratory according to standard methods (APHA 1998): total suspended solids (TSS) (at 105 °C), 5-day biochemical oxygen demand (BOD$_5$), chemical oxygen demand (COD), total phosphorus (TP), total nitrogen (TN), electrical conductivity (EC), pH, dissolved oxygen (DO) and temperature. BOD$_5$ and COD were evaluated on samples filtered by GF/C Whatmann glass fibre, as required by the current Italian legislation (Law Decree 152/2006, Italian Government 2006). EC, pH, DO and temperature were further controlled in situ through piezometers. Microbiological analysis included: FC, *E. coli*, EF and *Salmonella*. FC, *E. coli* and EF were analysed according to standard methods (APHA 1998); *Salmonella* was examined according to the methodology reported in Giammanco et al. (2002).

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emission uniformity (EU)</td>
<td>$EU = \frac{q_{25}}{q} \times 100$</td>
</tr>
<tr>
<td>Reduction factor</td>
<td>$R_d = \left(1 - \frac{q_d}{q_i}\right) \times 100$</td>
</tr>
<tr>
<td>Coefficient of variation of the emitter flow ($C_v$)</td>
<td>$C_v = \frac{SD(q)}{q}$</td>
</tr>
</tbody>
</table>

$q_{25}$: average discharge of 25% of emitters with the lowest flow (L h$^{-1}$); $q$: average discharge of all emitters (L h$^{-1}$); $SD(q)$: average discharge of each plot (L); $C_v$: discharge of new unclugged emitters.

### Soil contamination analysis and soil hydraulic behaviour

During 2004–2007 monitoring period, soil contamination analyses were carried out to assess *E. coli*, EF and *Salmonella* concentrations within soil columns (from 0.1 to 0.4 m of depth) collected close to emitters. Laboratory processing for soil microbial and constituent analyses were performed as outlined in APHA (1998). Soil sample (about 20 g) microbial levels (MPN 100 mL$^{-1}$) were enumerated using the phosphate (about 180 mL) buffer solution (PBS) technique.

To determine changes induced in soil hydraulic and transport properties at the end of the TWW application cycle, soil characterization analyses were performed by laboratory test. At the beginning and end of each irrigation season about 60 undisturbed soil samples were collected along a diagonal transect (north–south oriented) in the field at a depth between 0.15 and 0.3 m. Soil hydraulic properties, such as volumetric water content ($\theta_v$) (%) and hydraulic conductivity at saturation ($k_S$ cm h$^{-1}$) were analysed with the falling-head method (Klute & Dirksen 1986).

### Crop yield features and microbiological contamination

The effect on crop production features due to water qualities (TWW and FW), drip lines (SP and SSP) and soil coverage (just during 2004) and their interactions were evaluated during the trials. Marketable yield (MY), marketable fruits number (MN), fruit mean weight (MW) and unmarketable fruits number (UMN) were determined and processed. Analyses of variance (two-way ANOVA) identified the effects of main factors and their interactions. Treatment means were compared by the least significance test, considering * for $P < 0.05$, ** for $0.01 < P \leq 0.05$ and *** for $0.001 < P \leq 0.01$.

Samples of 0.5 kg of harvested tomatoes for each plot (and eventually sub-sections) were used to investigate the fruits’ microbial contamination. Samples were fortnightly collected at the experimental field before irrigation rates application. In the laboratory, 100 g of tomatoes, including fruit skin and flesh, were homogenized with 900 mL of sterile water by a stomacher. Then 10-fold dilutions were carried out within the same medium. *E. coli* and EF were enumerated using membrane filtration techniques (APHA 1998).

The *Salmonella* detection protocol consisted of a ‘pre-enrichment’ stage using a solution of buffered peptone water, a non-selective culture medium to revitalize microorganisms. Then, an inoculum culture was prepared in selenite and cystim medium and incubated at $36 \pm 1$ °C.
for 48 h. After the incubation period, several cultures were inoculated and incubated in parallel on ss-agar gel to identify and enumerate any \textit{Salmonella} colonies (Giammanco \textit{et al.} 2002).

**The disability-adjusted life years (DALYs)**

The health risk associated with the use of TWW for tomato crop irrigation was evaluated following the disability-adjusted life years (DALYs) tool (WHO 2006). The DALYs index calculates the time lost because of disability from a disease compared with a long life free of disability in the absence of disease. Three index pathogens were selected: rotavirus (a virus), \textit{Campylobacter} (a bacterium) and \textit{Cryptosporidium} (a protozoan). Results of the QMRA (quantitative microbial risk analysis, WHO 2006) were used to determine the total pathogen reductions targets to be achieved by a combination of wastewater treatment and a selection of post-treatment health protection control measures, based on a tolerable maximum additional burden of disease of one million of a DALY loss per person per year (1 x 10^-6 DALY loss pppy).

In the adopted approach, the model exposure scenario of ‘unrestricted irrigation’, based on the consumption of TWW-irrigated lettuce (Shuval \textit{et al.} 1997), was considered within the ‘planned TWW use’ definition. The selected scenario corresponds with the controlled use of TWW to grow crops that are normally eaten raw (WHO 2006). The QMRA procedure is illustrated in Table 2 for the consumption of TWW-irrigated lettuce.

As can be seen, the risk of rotavirus infection is higher than those of \textit{Campylobacter} and \textit{Cryptosporidium} and thus rotavirus infection risk must be used to assess the safety of TWW irrigation practice. In particular, the calculation shows that, for the parameter value selected, the required rotavirus reduction from TWW to lettuce ingestion is 6 log units. This total reduction is achieved partially by wastewater treatment and partially by a selection of post-treatment health-protection control measures (i.e. low-cost drip irrigation techniques, pathogen die-off, produce washing and peeling, etc.).

In the study, the risk associated with the use of TWW to grow tomato crops, evaluated by the described DALY tool, was compared with the very restrictive approach adopted by the Italian legislation for TWW reuse (Ministry Decree, n. 185/03, Ministry for Environment 2003).

**RESULTS AND DISCUSSION**

**Treated wastewater quality and technological monitoring results**

The evolution of the physical–chemical and microbial composition of the effluent during the irrigation periods (May–September of each monitoring year) is shown in Table 3, where it can be observed that the TSS were lower than 50 mg L^{-1} in 2004–2007 period and 2008. Only in 2009, according to the classification proposed by Bucks \textit{et al.} (1979), the analysed effluent would constitute a medium-high physically clogging hazard.

The higher mean TSS concentrations during 2008–2009 irrigation periods are strictly related to the worsening of CW inlet characteristics, showing a mean TSS of about 107 mg L^{-1}.

In the whole pattern of analysed samples BOD$_5$ and COD concentrations were compatible with Italian law limits for disposal in water bodies and with the more restrictive limits for reuse (Cirelli \textit{et al.} 2007). The removal efficiency of nutrients (TN and TP), within the CW system, was fairly well related to the vegetation management practices adopted (i.e. only two harvests have been carried out in the whole period 2004–2009). Globally CW effluents did not match the microbiological standards for TWW reuse of Italian legislation. In particular, \textit{E. coli} concentration (up to 100% of samples in 2007 and 2009) were

<table>
<thead>
<tr>
<th>TWW quality (E. coli per 100 mL)</th>
<th>Rotavirus</th>
<th>Campylobacter</th>
<th>Cryptosporidium</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^-7–10^8</td>
<td>1</td>
<td>1</td>
<td>0.91</td>
</tr>
<tr>
<td>10^-3–10^4</td>
<td>0.3</td>
<td>1.1 x 10^-2</td>
<td>2.4 x 10^-4</td>
</tr>
<tr>
<td>100–1,000</td>
<td>3.4 x 10^-2</td>
<td>1.1 x 10^-3</td>
<td>2.3 x 10^-5</td>
</tr>
<tr>
<td>10–100</td>
<td>3.5 x 10^-3</td>
<td>1.1 x 10^-4</td>
<td>2.3 x 10^-6</td>
</tr>
<tr>
<td>1–10</td>
<td>3.5 x 10^-4</td>
<td>1.1 x 10^-5</td>
<td>2.5 x 10^-7</td>
</tr>
</tbody>
</table>
above the limits of 50 and 200 CFU 100 mL\(^{-1}\), required for 80 and 100% of samples, respectively, by Italian law (Ministry Decree, M.D. 185/03) for TWW reuse. A detailed description and analysis of the removal rates in the CW system, covering all the months of each year, is reported in Barbagallo et al. (2011).

Figure 3 shows the mean variation observed in emission uniformity (EU, %) according to the Rodríguez (1990) classification. In particular, EU is described as excellent for 96–100%, good for 86–96%, acceptable for 86–80% and unacceptable for less than 80%.

In our study, the values of EU were excellent or good for all the plots throughout the tests. During 2008, SP showed EU reductions and increments of \(R_d\) despite the working pressure being slightly higher than that required. No cases of complete clogging were observed in the emitters. Taking into account Bralts & Kesner's (1985) classification, the coefficient of variation (\(C_v\)) of the emitter flow was excellent or very good (results not shown) during the entire experiment.

Data on EU for sub-surface light pipe P1 FLAP and sub-surface light pipe P1 RTG were analysed just for the monitoring year 2004 and thus they were not included in the graph of Figure 3; however, mean EU data were in the order of 0.85 and 0.91 for P1 FLAP and P1 RTG, respectively.

### Risk assessment analysis

TWW contamination by *E. coli* varied considerably from null values to \(4 \times 10^5\) CFU 100 mL\(^{-1}\) during the trials (Figure 4). In most cases (70%) the *E. coli* count was over the current Italian threshold of 50 CFU 100 mL\(^{-1}\) (M.D. 185/2003). In 16% of samples *E. coli* contamination was above the value of \(10^4\) CFU 100 mL\(^{-1}\) fixed by the World Health Organization in 2006 for the ‘unrestricted irrigation’

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**Table 3** | WW average quality during the monitoring periods (May–September of each year)

<table>
<thead>
<tr>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS (mg L(^{-1}))</td>
<td>62.0</td>
<td>3.3</td>
<td>4.5</td>
<td>7.8</td>
<td>12.0</td>
<td>5.6</td>
<td>25.7</td>
<td>25.7</td>
</tr>
<tr>
<td>BOD(_5) (mg L(^{-1}))</td>
<td>25.7</td>
<td>6.0</td>
<td>15.8</td>
<td>10.6</td>
<td>10.4</td>
<td>19.5</td>
<td>31.4</td>
<td>26.1</td>
</tr>
<tr>
<td>COD (mg L(^{-1}))</td>
<td>48.3</td>
<td>15.7</td>
<td>33.6</td>
<td>17.7</td>
<td>5.6</td>
<td>15.0</td>
<td>19.3</td>
<td>26.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.6</td>
<td>8.2</td>
<td>7.5</td>
<td>7.3</td>
<td>6.5</td>
<td>7.7</td>
<td>6.0</td>
<td>6.0–9.5</td>
</tr>
<tr>
<td>EC (µS m(^{-1}))</td>
<td>1.4</td>
<td>2.4</td>
<td>1.1</td>
<td>1.2</td>
<td>1.1</td>
<td>1.5</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>TN (mg L(^{-1}))</td>
<td>7.7</td>
<td>26.2</td>
<td>1.1</td>
<td>2.4</td>
<td>1.0</td>
<td>1.5</td>
<td>1.5</td>
<td>3.4</td>
</tr>
<tr>
<td>TP (mg L(^{-1}))</td>
<td>2.3</td>
<td>7.7</td>
<td>9.8</td>
<td>4.5</td>
<td>8.6</td>
<td>10.6</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td><em>E. coli</em> (CFU 100 mL(^{-1}))</td>
<td>2×10(^{0})</td>
<td>1×10(^{2})</td>
<td>1×10(^{3})</td>
<td>1×10(^{5})</td>
<td>2×10(^{3})</td>
<td>1×10(^{3})</td>
<td>6×10(^{1})</td>
<td>6×10(^{1})</td>
</tr>
<tr>
<td><em>EF</em> (CFU 100 mL(^{-1}))</td>
<td>Absent</td>
<td>1×10(^{0})</td>
<td>1×10(^{2})</td>
<td>1×10(^{4})</td>
<td>1×10(^{2})</td>
<td>1×10(^{3})</td>
<td>5×10(^{4})</td>
<td>5×10(^{4})</td>
</tr>
<tr>
<td><em>Salmonella</em> (CFU 100 mL(^{-1}))</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

*Reuse limits for irrigation.*

*Reuse limits for secondary urban effluents treated by lagoons or constructed wetlands.*

*E. coli*: *Escherichia coli*; *EF*: *Enterococcus faecalis*; FC: Faecal coliforms.

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**Figure 3** | Evolution of the emission uniformity (EU) during the monitoring.
scenario, in order to reach a median design risk for rotavirus infection of $10^{-3}$ pppy, considering a 2–5 log unit reduction due to rotavirus die-off between the last irrigation and consumption.

According to the WHO Guidelines (2006), wastewater reclaimed by the CW system could be used for tomato crop irrigation by implementing some post-treatment health protection control measures. For example, up to 5 log unit of microbial reduction could be achieved by washing the produce with clean water, disinfection and peeling. The application of these post-treatment health-protection control measures assures the required log units reduction of *E. coli* for the tolerable rotavirus infection risk.

No indications is given, either in Italian or international legislation, about the maximum allowable concentration of *EF*, which is widely recognized, together with *E. coli*, as a useful indicator for contamination, because of its resistance to disinfection and environmental factors and its ability to survive for long periods in the environment (Tallon *et al.* 2005; Salgot *et al.* 2006; Palese *et al.* 2009). Considerable variability in the level of *EF* was observed in the TWW during the experimental periods, with concentrations ranging from 0 to $5 \times 10^4$ CFU 100 mL$^{-1}$ during 2007 and 2009. Such wide variability depends mainly on changes in the efficiency of the conventional WW treatment plant supplying the CW unit.

*Salmonella* was never detected in TWW samples (Table 3) or in the soil and fruit samples collected during the six years of the trials.

### Hygienic quality of tomato crops and soil

Table 4 reports the average hygienic quality of tomato crops washing solution evaluated over the years of the trial. By

![Figure 4](https://iwaponline.com/wst/article-pdf/67/1/89/441375/89.pdf)

Table 4 Average microbiological quality of tomato fruits over the years of trial

<table>
<thead>
<tr>
<th>Year</th>
<th>Sampling procedure $^a$</th>
<th><em>E. coli</em> (CFU 100 g$^{-1}$)</th>
<th><em>EF</em> (CFU 100 g$^{-1}$)</th>
<th><em>Salmonella</em> (CFU 100 g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>1</td>
<td>$4 \times 10^2$</td>
<td>$5 \times 10^2$</td>
<td>Absent</td>
</tr>
<tr>
<td>2005</td>
<td>1</td>
<td>$1 \times 10^2$</td>
<td>$2 \times 10^3$</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>1</td>
<td>$4 \times 10^2$</td>
<td>$4 \times 10^3$</td>
<td>$1 \times 10^3$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>1</td>
<td>1</td>
<td>$1 \times 10^4$</td>
<td>$2 \times 10^3$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>1</td>
<td>$6 \times 10^1$</td>
<td>$5 \times 10^1$</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>1</td>
<td>$6 \times 10^1$</td>
<td>$4 \times 10^2$</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$: Samples of tomato fruits in contact with soil/plastic mulch.

2: Samples of tomato fruits not in contact with soil/plastic mulch. *E. coli*: *Escherichia coli*; *EF*: *Enterococcus faecalis*.
analysing the microbiological data it is not easy to determine if such contamination was due to the fruits’ contact with TWW, to an environmental pollution or to an accidental contamination occurring during sampling. The first possibility could be realistic during 2004 and 2005, because we operated in the worst-case condition (tomato fruits sampled near the drippers). During 2006–2009 period, the fruits sampled not in contact with soil/plastic mulch showed a very weak *E. coli* contamination (60 CFU 100 g⁻¹), which fell within the quality recommendations (≤100 CFU g⁻¹ *E. coli* for pre-cut fruits and vegetable ready to eat) established by the European Commission (Regulation 2073/2005, *European Commission 2005*). Previous studies carried out by *Bastos & Mara (1995)* found contamination levels on radishes and lettuces of the order of 10³–10⁴ *E. coli* per 100 g of product, using for irrigation an effluent within limits set by WHO (*1989*). *Minhas et al. (2006)* reported contamination for different type of vegetables irrigated with WW, with FC count between 2 × 10⁵ and 9 × 10⁵ MPN 100 g⁻¹. In addition, *Minhas et al. (2006)* highlighted the importance of the exposure of the edible parts of the plants to solar radiation to reduce pathogen bacteria less resistant to environmental conditions. *EF* concentrations were generally not negligible, suggesting that the found contamination could depend on the contact with TWW.

During 2004–2006 monitoring period, the analyses on soil samples collected between 0.1 and 0.4 m from the surface level evidenced a not negligible microbial content. In particular, a mean *E. coli* content of about 3 × 10³ CFU 100 g⁻¹ was found, with a decrease of about 3 log units along the examined soil profile. *EF* concentrations were found in all investigated soil columns layers, with a mean of about 1 × 10³ CFU 100 g⁻¹. During 2007–2009 monitoring period, the concentrations of *E. coli* measured in the soil sampled were very low. No *Salmonella* contamination was recorded.

TWW irrigation generally caused a disturbed layer at a soil depth of 0.3 m, exhibiting changes in physical properties (increase in bulk density). The investigated layer shows reduced soil porosity, translation of pore size distribution towards narrower pores and a consequent decrease in permeability.

**Crop yield evaluation**

The results of TWW reuse for vegetable cultivation were different according to crop and cultivation seasons (*Table 5*). Differences in crop production features between the trials may be related to the harvest operation modalities. Between the different tomato varieties analysed in the study in 2004, the genotype Missouri was more suitable for TWW. The

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**Table 5** | Crop yield parameters evaluated during the monitoring periods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Water quality</th>
<th>Soil cover</th>
<th>Water quality</th>
<th>Soil cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>TWW</td>
<td>FW</td>
<td>Significance</td>
<td>Mulching film</td>
<td>Bare soil</td>
</tr>
<tr>
<td>2004 MY</td>
<td>104³</td>
<td>n.s.</td>
<td>57</td>
<td>103</td>
</tr>
<tr>
<td>2005 MY</td>
<td>105³</td>
<td>a</td>
<td>57</td>
<td>103</td>
</tr>
<tr>
<td>2006 MY</td>
<td>104³</td>
<td>a</td>
<td>57</td>
<td>103</td>
</tr>
<tr>
<td>2007 MY</td>
<td>104³</td>
<td>a</td>
<td>57</td>
<td>103</td>
</tr>
<tr>
<td>2008 MY</td>
<td>104³</td>
<td>a</td>
<td>57</td>
<td>103</td>
</tr>
<tr>
<td>2009 MY</td>
<td>104³</td>
<td>a</td>
<td>57</td>
<td>103</td>
</tr>
</tbody>
</table>

n.s.: not significant. MY: marketable yield; UMY: unmarketable yield.

*P* < 0.05. *P* < 0.01. *P* < 0.001.

*During 2004 MY was determined just on selected tomato fruit samples, corresponding to five fruits for each section (see Figure 1); during 2005–2009 MY was determined on the whole fruit harvest within each section (see Figure 2).
marketable total yield (mean of 60 t ha$^{-1}$ during the trials) was significantly ($P < 0.05$) higher for TWW-irrigated tomatoes in three of the six available years (even though in 2008 tomato plants were affected by Phytophthora virus). The unmarketable production (mean of 30 fruits m$^{-2}$) was significantly ($P < 0.05$) lower for TW-irrigated tomatoes in three of the six available years.

The use of plastic mulching resulted in a MY increase with respect to bare soil. In 2009, the adoption of SSP laterals (MONO) resulted in a significant improvement of MY (+21%) that was related to the increase in marketable fruits (+18%) and mainly to the decrease in unmarketable fruits (−30%). Finally, from the agronomic point of view, the use of tertiary-treated municipal WW was shown to be suitable for the cultivation of vegetable crops. The obtained qualitative and yield results were slightly influenced by water quality. However, the different TWW quality characteristics during the years of trial require further physical and chemical characterization analyses to optimize the reuse scenario.

CONCLUSIONS

Despite increasing pressure to make more efficient use of water resources, irrigation of food crops with reclaimed water still remains a contentious issue, primarily because of the hygienic risks arising from infectious disease. The debate is complicated by the fact that reuse scenarios can vary substantially with respect to WW treatment level, irrigated crops, sampling procedures, etc. The presented study, based on a six-year monitoring program, showed that municipal TWW, reclaimed according to natural treatment systems (i.e. CW system) may be successfully used, under specific experimental conditions, to irrigate and grow tomato crops. In particular, the risk assessment analysis, carried out by applying the QMRA model according to WHO (2006) procedures, highlighted that by applying the post-treatment health-protection control measures (such as product washing, disinfection, peeling and/or the natural pathogen die-off after last irrigation), the acceptable rotavirus infection risk was generally preserved, although E. coli content of TWW was often over the limits set by the Italian law.

The hygienic quality of the product was generally preserved, although E. coli content of TWW was often over the limits set by the Italian law. Contamination by enteric bacteria (i.e. Enterococcus faecalis), not considered by the guidelines for TWW reuse in agriculture, varied considerably. The TWW distribution along the irrigation seasons significantly affected soil hygienic features especially in the soil top layer. However, a soil quality recovery was observed during the winter periods. No significant contamination was found on tomato fruits not in contact with soil and plastic mulching, whereas a not negligible E. coli content was recorded under the worst-case condition of fruit growth in contact with the irrigated soil. Such concerns suggest that long-term safe reuse of municipal TWW for irrigation of tomato crops should be supported by guidelines which take into account more suitable indicators for the assessment and monitoring of microbiological quality of TWW, soil and products.

Finally, the work herein presented on TWW reuse in an agricultural context suggests the importance of linking the experimental analysis carried out by laboratory procedures with in situ measurement campaigns in order to optimize the field experiment and define a sustainable environmental scenario.

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