

Effects of treated agro-industrial wastewater irrigation on tomato processing quality

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Abstract

This study was designed to determine the qualitative and microbiological impact of two different sources of irrigation water on tomato fruit: groundwater (GW), as the control, and treated agro-industrial wastewater (SW). The mean tomato fruit quality parameters of dry matter, weight, diameter, colour index, pH, soluble solids content, titratable acidity, Ca²⁺ and Na⁺ content were not significantly affected by the different water treatments. Conversely, NO₃⁻ contents was significantly higher with GW use, than with SW (2.21 vs 1.62 mg 100 g⁻¹, respectively; P≤0.05). The microbial quality of the tomato fruit was not significantly different across the GW and SW treatments, with no *Salmonella* spp. isolated from any of the fruit, and the faecal indicators always below 10 CFU g⁻¹. These data show that agro-industrial treated wastewater can be used for irrigation for industrial tomato production once the long-term effects on the agroecosystem have been defined.

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Introduction

The use of wastewater for irrigation is increasingly being considered as a technical solution to minimise soil degradation, to restore the nutrient content of the soil, and to increase water supplies to agriculture. Treated wastewater is normally used for irrigation purposes in countries suffering from water shortages, to narrow the gap between supply and demand. The application of treated wastewater to croplands is an attractive option also for wastewater disposal, particularly as it might improve the physical properties and fertility of the soil (Pomares *et al.*, 1984). This is due to the content of nitrogen (N), phosphorus (P), organic matter and other trace elements in wastewater, which thus represents a good source of nutrients for plant growth and promotion of harvest yield and quality (Benitez *et al.*, 2001). This fertiliser effect of wastewater can provide reduced costs in terms of nitrogen and phosphorus mineral fertiliser use, and might also improve the quantitative aspects of crop yield (Wild and Jones, 1991). On the other hand, wastewater might contain undesirable chemical constituents and pathogens that can have negative environmental and health impacts (Papadopoulos, 1995).

Some studies have investigated the influence of irrigation with treated wastewater on tomato crop yields, but there remains the need to better define the full effects on the fruit quality parameters and the acceptable levels of biological contamination (Aiello *et al.*, 2007; Christou *et al.*, 2014; Gatta *et al.*, 2014). For this purpose, the present study was designed to evaluate the effects of two water irrigation sources, as the groundwater (GW) control, and treated agro-industrial wastewater (SW), on the main quality parameters and microbiological safety of processing tomato fruit.

Materials and methods

Experimental field and cultivation practices

The field trial was carried out with the tomato (*Lycopersicon esculentum* Mill.) cultivar *Manyla* (Semillas Fitò, Spain) during the growing season of 2013 (April to September), in an agricultural area of the Apulian region in southern Italy (Stornarella: 41° 15' N, 15° 44' E; altitude, 154 m asl). The tomato plants were grown under a net-house structure, and were covered with an anti-hail net. The experimental site was near to the Fiordelisi agricultural and food manufacturing company, which produces and processes vegetables.

The investigation was carried out in a clay loam soil, with a field capacity (-0.03 MPa) of 31.9% dry weight (dw), a wilting point (-1.5 MPa) of 15.3% dw, and a bulk density of 1.38 Mg m⁻³.

The tomato seedlings were transplanted into the plots on 5 April, 2013, in mulched paired rows (40 cm apart) spaced at 250 cm, with the plants at a distance of 30 cm apart along each single row. The final

plant density was 2.7 plants m⁻². The plants were grown in a vertical setting, using nylon threads disposed between plants collars, and iron wires arranged longitudinally in the direction of the plant rows, and fixed to the upper part of the net-house, at 2.5 m from the ground.

Two experimental irrigation treatments were applied to the tomato plants, relating to the source of their irrigation water: irrigation with GW (control) and SW. The SW used in this study was taken from the secondary wastewater treatment plant that purifies all of the wastewater produced by the Fiordelisi agricultural and food manufacturing company during their industrial processing of vegetables.

The experiment was laid out as a randomised complete block design, with the two irrigation treatments each replicated three times. A drip irrigation system was used for crop irrigation. The irrigation scheduling was based on continuous measurements of volumetric soil water-content changes at the effective rooting depth, using frequency domain reflectometry probes (EasyAG, Sentek Sensor Technologies, Stepney SA, Australia). The irrigation was performed every time the available soil moisture was depleted to the threshold value of 40% (Allen *et al.* 1998), and at each irrigation, the soil water content of each plot was increased to field capacity. The soil fertilising and pest and weed control were performed according to local management practices, and were the same across the two experimental systems.

Four harvests (HD) were carried out, from July to September, at: 97 (HD₁), 118 (HD₂), 137 (HD₃) and 160 (HD₄) days after transplanting, each on an experimental plot of 20 m², by picking all of the mature fruit.

Water and tomato fruit analysis

The GW and SW samples were collected at monthly intervals, to characterise their physico-chemical and microbiological properties. These samples were analysed in triplicate, according to the international methods of the American Public Health Association, the American Water Works Association, and the Water Environment Federation (APHA, AWWA, WEF, 2005). The physico-chemical characterisation included the analysis of: pH, electrical conductivity (EC_w; dS m⁻¹), total suspended solids (TSS; mg L⁻¹), biological oxygen demand over 5 days (BOD₅; mg L⁻¹), chemical oxygen demand (COD; mg L⁻¹), ammonium-nitrogen (NH₄-N; mg L⁻¹), nitrate-nitrogen (NO₃-N; mg L⁻¹), nitrite-

nitrogen (NO₂-N; mg L⁻¹), phosphorus (PO₄-P; mg L⁻¹), sodium (Na⁺; mg L⁻¹), and calcium (Ca²⁺; mg L⁻¹).

For the microbiological parameters, the GW and SW samples were initially analysed for *Escherichia coli* using a membrane filtration method, and for *Salmonella* spp. using the ISO 19250:2013 procedure (ISO, 2013). The bacteriological analysis of the plant and fruit samples included determination of *E. coli*, faecal coliforms, and total heterotrophic counts (THCs). These analyses were conducted by the plate-

Table 1. Main physico-chemical and microbial parameters related to the two irrigation water types considered.

| Parameter | Irrigation treatment | | Significance |
|---|----------------------|-------------|--------------|
| | GW | SW | |
| Main physico-chemical characteristics | | | |
| NH ₄ -N (mg L ⁻¹) | 0.05±0.01 | 2.00±0.52 | ** |
| NO ₃ -N (mg L ⁻¹) | 22.95±1.00 | 0.66±0.15 | *** |
| PO ₄ -P (mg L ⁻¹) | 0.13±0.01 | 0.38±0.04 | ** |
| COD (mg L ⁻¹) | 18.68±1.03 | 36.97±3.00 | * |
| BOD ₅ (mg L ⁻¹) | 10.70±1.25 | 23.70±2.00 | * |
| Electrical conductivity (dS cm ⁻¹) | 0.87±0.01 | 3.07±0.20 | *** |
| pH | 7.75±0.05 | 7.84±0.08 | ns |
| Total suspended solids (mg L ⁻¹) | 3.70±2.28 | 17.63±4.17 | ** |
| Na ⁺ (mg L ⁻¹) | 33.47±0.51 | 305.74±6.87 | *** |
| Ca ²⁺ (mg L ⁻¹) | 79.83±1.41 | 80.50±2.20 | n.s. |
| Main microbial characteristics (CFU 100 mL⁻¹) | | | |
| <i>E. coli</i> | 0 | 1560 | ** |
| Faecal coliforms | 17 | 3094 | ** |
| <i>Salmonella</i> spp. | n.d. | n.d. | |

GW, groundwater; SW, secondary agro-industrial wastewater. *, Statistically significant at P≤0.05; **, statistically significant at P≤0.01; ***, statistically significant at P≤0.001. n.s., not significant; n.d., not detected.

Table 2. Main qualitative traits of the tomato fruit analysis related to the water irrigation treatments and the harvest date.

| Experimental factor | Qualitative parameter of the tomato fruit | | | | | | | | | |
|--|---|-------------------------|------------------------|-------------------------|------------------------|-----------------------|------------------------------|--|---|--|
| | DM (% FM) | W ^m (g) | D ^m (cm) | CI | pH | SSC (°Brix) | TA (g 100 mL ⁻¹) | Ca ²⁺ (mg 100 g ⁻¹ FM) | Na ⁺ (mg 100 g ⁻¹ FM) | NO ₃ ⁻ (mg 100 g ⁻¹ FM) |
| Harvest date | | | | | | | | | | |
| HD ₁ | 6.83±0.20 ^a | 78.03±1.73 ^a | 4.99±0.03 ^a | 1.29±0.02 ^a | 4.43±0.03 ^a | 6.3±0.10 ^a | 0.45±0.08 ^a | 29.53±3.71 ^b | 18.07±0.95 ^b | 1.57±0.32 ^b |
| HD ₂ | 7.36±0.12 ^a | 75.56±2.81 ^a | 4.91±0.05 ^a | 1.04±0.01 ^{bc} | 4.40±0.01 ^a | 6.3±0.12 ^a | 0.29±0.04 ^b | 49.95±4.05 ^{ab} | 19.67±0.92 ^b | 2.88±0.35 ^a |
| HD ₃ | 6.92±0.17 ^a | 62.08±2.73 ^b | 3.49±0.04 ^b | 1.03±0.02 ^c | 4.51±0.02 ^a | 5.6±0.08 ^b | 0.28±0.02 ^b | 60.77±7.44 ^a | 21.97±0.69 ^b | 1.55±0.42 ^b |
| HD ₄ | 8.34±0.77 ^a | 50.89±1.96 ^b | 3.21±0.11 ^c | 1.17±0.03 ^{ab} | 4.44±0.03 ^a | 5.3±0.19 ^b | 0.19±0.01 ^c | 54.61±9.22 ^a | 31.50±1.62 ^a | 1.96±0.18 ^{ab} |
| Significance | n.s. | *** | *** | *** | n.s. | *** | *** | * | *** | * |
| Irrigation treatment | | | | | | | | | | |
| GW | 7.42±0.42 ^a | 68.57±3.86 ^a | 4.18±0.10 ^a | 1.12±0.03 ^a | 4.47±0.02 ^a | 5.9±0.15 ^a | 0.31±0.03 ^a | 54.97±1.82 ^a | 22.80±1.82 ^a | 2.21±0.13 ^a |
| SW | 7.30±0.26 ^a | 64.70±3.50 ^a | 4.12±0.09 ^a | 1.13±0.04 ^a | 4.45±0.01 ^a | 5.8±0.17 ^a | 0.30±0.03 ^a | 42.47±1.62 ^a | 23.97±1.43 ^a | 1.62±0.32 ^b |
| Significance | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | * |
| Harvest date × irrigation treatment | | | | | | | | | | |
| Significance | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |

^{ab}Means followed by the same letters in each column are not significantly different (P≤0.05; Tukey tests). Data are means±standard error, from 30 marketable fruit (10 fruit per plot, ×3 replicates). DM, dry matter; FM, fresh matter; W^m, mean weight; D^m, mean diameter; CI, colour index; SSC, soluble solids content; TA, titratable acidity; Ca²⁺, Na⁺, NO₃⁻, calcium, sodium and nitrate contents; HD₁, HD₂, HD₃ and HD₄, 97, 118, 137 and 160 days after transplanting; GW, groundwater; SW, treated agro-industrial wastewater; n.s., not significant. *P≤0.05; *** P≤0.001.

count method on tryptone bile X-glucuronide agar, *E. coli* agar, and tryptone soya agar, respectively (Allen *et al.*, 2004; Wood *et al.*, 2010). Moreover, the tomato plant and fruit samples were also analysed for *Salmonella* spp.

After the harvesting on the four harvest dates (HD₁₋₄), a sample of 10 marketable fruit from each plot from each harvest date were measured for the following: mean weight (W_m ; g), mean diameter (equatorial and longitudinal diameter) (D_m ; cm), soluble solids content of the flesh (SSC; °Brix), pH, titratable acidity (TA; g citric acid 100 mL⁻¹ fresh juice; AOAC 1995), dry matter content (DM; % fruit fresh matter), a*/b* ratio (colour index; CI) (Jiménez-Cuesta *et al.*, 1981; Favati *et al.*, 2009), and Ca²⁺, Na⁺ and NO₃⁻ contents. The Na⁺, Ca²⁺ and NO₃⁻ levels were determined by ion-exchange chromatography (Dionex ICS-1100, Dionex Corporation, Sunnyvale, CA, USA).

Statistical analysis

The data are expressed as means±standard error. The data were processed for statistical analysis by unpaired t-tests (water qualitative parameters, bacterial indicators) or by ANOVA procedures (tomato fruit parameters), and differences are considered significant at $P \leq 0.05$. For the qualitative parameters of the tomato fruit, Bartlett tests confirmed the homogeneity of the variance among the harvest data, so a combined statistical analysis was performed.

Results and discussion

Water qualitative traits

Table 1 given the means of the physico-chemical and microbial characteristics of the GW and SW analysed during the experimental trial. Except for NO₃-N, higher values were observed in the SW than the GW, in agreement with other studies (Cirelli *et al.*, 2012; Lopez *et al.*, 2010). Compared to GW, SW was characterised by higher NH₄-N and PO₄-P, which are strongly related to soil fertility and plant growth, as well as by higher organic matter content (as indicated by the TSS, COD and BOD₅), Na⁺ and EC_w. In contrast, the GW was characterised by significantly higher NO₃-N than the SW (22.95 mg L⁻¹ vs 0.66 mg L⁻¹). This will be due to the extensive nitrogen fertiliser application in the study area (Gatta *et al.*, 2014), which leads, in turn, to consistent nitrate leaching and diffuse aquifer pollution. Similar pH and Ca²⁺ levels were observed for the GW and the SW.

The microbiological qualities of the two irrigation waters were significantly different (Table 1). Indeed, *E. coli* and *Salmonella* spp. were not isolated in any of the GW samples, while faecal coliforms were very low in the GW (17 CFU 100 mL⁻¹). The SW was characterised by 1560 CFU 100 mL⁻¹ and 3094 CFU 100 mL⁻¹ for *E. coli* and faecal coliforms, respectively. The SW samples were not, however, positive for *Salmonella* spp. (Table 1). These SW microbial indicators were typical of secondary effluent that is not subjected to disinfection.

Qualitative traits and microbial pollution of the tomato plants and fruit

The mean qualitative traits of the tomato fruit in terms of the water irrigation treatments and the harvest date are reported in Table 2. Neither of the experimental factors considered (harvest date, irrigation treatment) showed any significant effects in their interactions.

For the qualitative parameters of the tomato fruit that were analysed, only NO₃⁻ content showed significant differences in terms of the water irrigation treatments. The NO₃⁻ content of the tomato fruit was significantly higher for the treatments with the GW (2.21 mg 100 g⁻¹) than the SW (1.62 mg 100 g⁻¹). This will be related to the higher NO₃-N con-

Table 3. Microbial counts (by plate counts) of the bacterial indicators for the tomato plants and fruit.

| Bacterial indicator | Irrigation treatment | |
|-------------------------------|----------------------|-----------------|
| | GW | SW |
| Plants (CFU g ⁻¹) | | |
| <i>E. coli</i> | <10 | <10 |
| Faecal coliforms | <10 | <10 |
| Total heterotrophic count | 5941±1134 | 3408±985 |
| <i>Salmonella</i> spp. | n.d. | n.d. |
| Fruit (CFU g ⁻¹) | | |
| <i>E. coli</i> | <10 | <10 |
| Faecal coliforms | <10 | <10 |
| Total heterotrophic count | 13,450±5554 | 978,325±599,019 |
| <i>Salmonella</i> spp. | n.d. | n.d. |

GW, groundwater; SW, treated agro-industrial wastewaters; n.d., not detected. Data are means±standard errors for each analysed trait. For plants: 1 sample per water treatment × 3 replicates × 6 sampling dates; for fruit: 1 sample per water treatment × 3 replicates × 4 sampling dates. None of these data were significantly different between the irrigation treatments (t test).

tent in the GW than the SW (Table 1). However, it should be noted that the NO₃⁻ content in the tomato fruit remained well below the limits of the European guidelines (European Commission, 2011). The mean values of the remaining qualitative parameters (*i.e.*, DM, W_m , D_m , CI, pH, SSC, TA, Ca²⁺, Na⁺) were in agreement with the data from other studies (Mahajan and Singh, 2006; Favati *et al.*, 2009; Madrid *et al.*, 2009).

In terms of the harvest date as an experimental factor, only the DM and pH were not significantly different. Here, the W_m and D_m ranged from 78.03 g to 50.89 g, and from 4.99 cm to 3.21 cm, respectively, with the highest values for HD₁. These data can be explained-considering that the size and shape of a fruit can vary in relation to its position on the plant. Generally, the first fruit of the first truss are larger in size than the rest (Sawhney and Greyson, 1972).

The CI showed the lowest values for HD₃ (1.03) and HD₂ (1.04); these are probably related to the different levels of radiation that will have been received by the tomato fruit during the cycle. The SSC and TA decreased significantly from the first (HD₁) to the last (HD₄) harvest dates, with decreases from 6.3 to 5.3 °Brix and from 0.45 to 0.19 g 100 mL⁻¹, respectively. In general, in the course of the trial, the SSC and TA were consistent with data from other field experiments on tomato (Richardson *et al.*, 2006; Madrid, 2009).

The Na⁺ content of the tomato fruit increased to the last harvest date, varying from 18.07 mg 100 g⁻¹ (HD₁) to 31.50 mg 100 g⁻¹ (HD₄), and thus showing a 40% increase from HD₁ to HD₄. This increase in Na⁺ was probably due to the high Na⁺ contents in the irrigation water source (particularly in the SW), and consequently in the soil (data not shown). A similar behaviour was observed for the Ca²⁺ content.

The plant analysis (including the shoots and leaves) were characterised by faecal indicators below the sensitivity threshold of the count method, and THC was in the range of 10³ CFU g⁻¹ for all samples, without significant differences between the GW and SW data (Table 3). Also, the tomato fruit microbial quality was not significantly different between the GW and SW treatments, as *Salmonella* spp. were not isolated in any of the samples, and the faecal indicators were all below 10 CFU g⁻¹ (Table 3). THC in the tomato fruits were in the range of 10⁴ CFU/g, without significant differences between GW and SW. The higher level with respect to the plant (leaf and shoot) is due to the maturation of the fruit and the higher level of sugars on tomato skin that could benefit the epiphytic bacteria (Mercier and Lindow, 2000).

Our results are in agreement with other recent studies that have shown low impact for the microbial contamination of tomato crops irrigated by treated secondary wastewaters (Cirelli *et al.*, 2012; Christou *et*

al., 2014; Gatta *et al.*, 2014). This will be due to several factors, such as: the drip irrigation system and mulching system adopted, and the effects of UV radiation exposure on the fruit and leaf surfaces.

Conclusions

The principal objective of this researcher was to evaluate the use of agro-industrial wastewater in a closed circle system where a food manufacturing company produces and processes tomatoes. More specifically, the study was aimed at examining the impact of treated agro-industrial wastewaters, used for irrigation of tomato crop, on quality and safety of the fruits.

The main results can be summarised as follows: i) the most important morpho-qualitative parameters of processing tomato fruit (*i.e.*, W_m and D_m of the fruit, TA, SSC, DM, pH, CI) for the GW and SW irrigation treatments are in agreement with the literature; and ii) despite higher levels of faecal indicators in the SW, the microbiological quality of the harvested fruit was not affected.

Our research showed that irrigation of tomato plants with agro-industrial treated wastewater did not negatively affect the main quality parameters of the tomato fruits. However, we will continue to monitor the effects of treated wastewater use on fruit quality in terms of the long-term effects of this irrigation source application on the agroecosystem.

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