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Treated agro-industrial wastewater irrigation of tomato crop: Effects on qualitative/quantitative characteristics of production and microbiological properties of the soil

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ABSTRACT

A comparative study was carried out to evaluate the effects of two water irrigation sources on the quality and microbiological safety of tomato plants and fruit, and on the microbiological soil properties: irrigation with groundwater (GW) and with treated agro-industrial wastewater (TW). In a field experiment in southern Italy (Apulia region), the physico-chemical characteristics of the irrigation waters and the fruit quality parameters were determined. *Escherichia coli*, fecal *Enterococci* and *Salmonella* spp. were also monitored in the irrigation waters, tomato plant and fruit, and root-zone soil. Bacteriological analysis for total heterotrophic counts (THCs) were determined for plant, fruit, and soil samples. The irrigation water source did not significantly affect yield quantitative traits. However, with GW, the marketable fruit yield was higher than with TW (~82 vs. ~79 Mg ha^{-1}, respectively). For both irrigation treatments, the most important qualitative parameters that characterize the processing tomato fruit (i.e., dry matter content, pH, soluble solid content, color parameters) were in agreement with reports in the literature. For the microbiological results, the mean levels of *E. coli* and fecal *Enterococci* were 4408 and 3804 CFU 100 ml^{-1}, respectively, for TW (above the Italian guidelines for TW re-use). For the tomato plant and fruit, no *E. coli* isolated in either, and fecal coliforms and THC were not influenced by the irrigation waters ($P > 0.05$). Total bacterial enumeration by quantitative PCR was lower in soil irrigated with GW, than TW (3.69 vs. 4.02, $\times 10^6$, respectively). Moreover, soil microbial community patterns substantially differed between the two water treatments. These data show that while fecal indicators are not affected, the community composition and dynamics of the whole bacterial population in soil is influenced by the different qualities of these waters used for irrigation.

1. Introduction

The re-use of wastewater in agriculture is gaining wider acceptance in many parts of the world. It represents an agronomic option that is increasingly being investigated and taken up in regions with water scarcity, growing urban populations, and rising demand for irrigation water (Meli et al., 2002; FAO, 2011). Many irrigated areas around the world are experiencing water shortages due to several factors, such as climate change and surface and groundwater pollution. Water scarcity poses serious economic, social and even political concerns in all of its aspects. Under these circumstances, treated wastewater use can help to mitigate the damaging effects of local water deficits (FAO, 2010).

Treated wastewater not only offers an alternative water irrigation source, but also the opportunity to recycle plant nutrients (Chen et al., 2008). Its application might ensure the transfer of fertilizing elements, such as nitrogen (N), phosphorous (P), potassium (K^+), organic matter, and meso-nutrients and micro-nutrients, into agricultural soil (WCED, 1987). Hence, wastewater nutrients can contribute to crop growth, although there is a need for their periodic monitoring, to avoid any imbalance in the nutrient supplies, which might cause excessive vegetative growth, uneven plant and/or fruit maturity, and/or reduced qualitative/quantitative aspects of yields (Pedrero et al., 2010).

Treated wastewater can also be a source of pathogenic organisms and potentially hazardous chemical substances, such as enteric bacteria and viruses, salts, heavy metals, and surfactants.

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These might then accumulate in the soils, with unfavorable effects on crop quality and productivity, and on the ecological soil conditions (Siebe and Cifuentes, 1995; Chen et al., 2008). One of the major concerns with wastewater re-use is the risk of the transfer of pathogenic microorganisms that represent a potential risk to human health if they enter the food chain (Al-Lahham et al., 2003; Salgot et al., 2006; Toze, 2006; Palese et al., 2009). Indeed, many studies have shown that microbiological contamination can be a major issue for the re-use of treated agricultural wastewater (Rubino and Lonigro, 2008; Lopez et al., 2010; Patterson et al., 2011; Vivaldi et al., 2013). To maximize the benefits and at the same time, to minimize the risks related to the use of treated wastewater, international policies and uniform legislative frameworks should be adopted.

In Italy, the agricultural use of reclaimed wastewater (municipal and agro-industrial) is regulated by Ministerial Decree no. 185/2003. With regard to microbiological contamination levels in particular, this Decree has defined some significantly lower threshold values (e.g., *Escherichia coli*, <10 CFU 100 ml^{-1} in 80% of the samples) than those included in international guidelines. These threshold values can be considered highly restrictive, because the risk of contamination has been reported to be low when contamination of irrigation water does not exceed 1000 CFU ml^{-1} (WHO, 2006; Blumenthal et al., 2000).

Studies have been carried out in southern Italy relating to treated urban wastewater re-use for the irrigation of crops (Pollice et al., 2004; Lonigro et al., 2007; Lopez et al., 2007). These have included wastewater with microbiological contamination levels higher than those required by the current legislation, and they have indicated the opportunity to increase the threshold values in the Italian guidelines. Therefore, there is the need for further studies to better define acceptable microbiological contamination levels of different sources of irrigation water as used on different crops. These should also take into account wastewater treatment, irrigation methods, and cultivation practices.

The majority of the studies conducted on wastewater applications in agriculture have focused mainly on reclaimed urban effluents. The aim of the present study was to determine the effects of secondary treated agro-industrial wastewater on tomato crop performance. In particular, the objectives of the study were: (i) to evaluate the effects of the wastewaters on qualitative and quantitative aspects of tomato crop production; (ii) to assess the impact of the wastewaters on the microbiological contamination of tomato fruit and the microbiological soil properties.

2. Materials and methods

2.1. Field characteristics and agronomic conditions

This field trial was carried out with the tomato (*Solanum lycopersicum* L.; formerly *Lycopersicon esculentum* Mill.) cultivar 'Manyla' (Semillas Fitò, Spain) during the growing season of 2012 (April to August). It took place in an agricultural area in the Foggia district (Stornarella: 41° 15′N, 15° 44′E; altitude, 154 m a.s.l.) of the Apulian region in southern Italy, on a site belonging to the Fiordelisi agricultural and food manufacturing company, which produces and processes vegetables. The tomato plants were grown under a net house structure, covered with an anti-hail net, in six identical 15 m × 30 m plots that were located near to the company wastewater treatment plant.

The experimental area is characterized by a Mediterranean climate, with long-term mean annual rainfall of 590 mm, which is mainly distributed from October to April (Caliandro et al., 2005). The mean monthly main climate parameters recorded during the trial are reported in Table 1. These were measured by a weather station near to the experimental area, and stored on a nearby data-logger (Campbell Scientific, USA). The mean maximum and mean minimum temperatures during the growing season were 34.5 °C and 8.5 °C, respectively, and the total rainfall was 108.4 mm, of which about 62% (67.0 mm) occurred in the first month of the growing season.

The trial was carried out in a clay loam soil (United States Department of Agriculture classification), with a field capacity (−0.03 MPa) of 30.5% dry weight (dw), a wilting point (−1.5 MPa) of 15.9% dw, and a bulk density of 1.41 Mg m^{-3}. The main characteristics of the soil layer of the experimental site (0–60 cm) are as follows: sand, 40.1%; loam 32.5%; clay 27.4%; organic matter 1.6%; Olsen P_2O_5, 80.1 mg kg^{-1}; Ac-extractable K_2O, 730 mg kg^{-1}; total N, 0.8‰ (Kjeldahl); mineral NO_3–N, 4.75 mg kg^{-1}; mineral NH_4–N, 7.50 mg kg^{-1}; pH 7.9; electrical conductivity, 0.49 dS m^{-1}.

The tomato seedlings were transplanted into the plots on April 12, 2012, 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 collar and iron wires arranged longitudinally in the direction of the plant rows, and fixed to the upper part of the nethouse, at 2.5 m from the ground.

During the cropping season, standard agronomic practices for tomato crops in the area were performed. The soil was subsoiling to a depth of 45 cm, and before transplanting, its surface was milled. Pre-transplanting fertilization was applied to the soil by distributing 35 kg ha^{-1} N and 70 kg ha^{-1} P_2O_5. Throughout the crop cycle, 75 kg ha^{-1} N and 100 kg ha^{-1} P_2O_5 were added through fertirrigation. Pest and weed control were performed according to local management practices.

The tomato fruit were hand harvested at full stage maturity. Four harvestings were performed from June to August, on the days after transplanting of: 82 (HD_1), 96 (HD_2), 110 (HD_3) and 124 (HD_4).

2.2. Treatments and experimental design

Two experimental irrigation treatments were applied to the tomato plants: irrigation with groundwater (GW), and irrigation with treated agro-industrial wastewater (TW). The GW was from a water source that is commonly applied for crop irrigation in the experimental area. The TW used in this study was taken from the wastewater treatment plant that purifies all of the wastewater produced by the company during their industrial processing of vegetables (i.e., tomatoes, egg plants, courgettes, peppers). It is an activated sludge wastewater treatment plant that produces an annual volume of effluent of approximately of 46,500 m^3. The incoming wastewaters undergo a preliminary treatment through a 6-mm sieve screen, to separate out the coarse organic waste. The effluent water then goes into an equalization tank, for the secondary biological treatment. At the end of this phase, the wastewater is clarified in a secondary settler, and the sludge is separated out. For the present study, part of the treated wastewater not subjected to chlorine treatment was directed into the experimental area through a 100-mm diameter PVC pipe, and stored in a 3000-l tank; subsequently, it was used for the tomato irrigation.

The experiment was laid out in a randomized complete block design with the two irrigation treatments each replicated three times (Fig. 1). A drip irrigation system was used for the crop irrigation. This comprised a single pipe, with drippers at a 2 l h^{-1} flow rate spaced every 40 cm, and it was arranged in the middle of each paired row. Except for the first irrigation that was designed for the rooting and establishment of the plants, the following irrigations were performed with each water treatment every time the available soil moisture was depleted to the threshold value of 40% (Allen et al., 1998). This irrigation scheduling took into account continuous measurements of volumetric soil water content changes at

Table 1
Main climatic parameters recorded during the growing season of the tomato crops (2012).

Month	Climatic parameter[a]						
	T_{min} (°C)	T_{max} (°C)	RH_{min} (%)	RH_{max} (%)	P (mm)	Ws ($m\,s^{-1}$)	E_v (mm)
April	8.5	20.1	51.7	95.6	67.0	2.30	86.9
May	11.6	25.0	36.6	82.8	28.0	2.42	137.5
June	17.9	33.0	27.3	71.1	0.0	2.72	197.9
July	20.8	34.4	30.6	77.1	8.4	2.43	195.3
August	20.2	34.5	29.4	81.4	5.0	2.10	176.5
Growing season	**15.8**	**29.4**	**35.1**	**81.6**	**108.4**	**2.40**	**794.1**

[a] T_{min}, T_{max}, monthly minimum, maximum air temperature; RH_{min}, RH_{max}, monthly minimum, maximum relative air humidity; P, total precipitation; Ws, monthly mean wind speed; E_v, total "class A" pan evaporation.

the effective rooting depth (soil layer depths: 0–10, 10–20, 20–30, 30–40, 40–50 cm), using frequency domain reflectometry probes (EasyAG, Sentek Sensor Technologies), which were installed in each plot prior to the crop transplanting. At each irrigation, the soil water content of each plot was increased to field capacity. The amount of irrigation water applied to the tomato crop during the whole crop cycle was 4957 $m^3\,ha^{-1}$, with the water volume at each irrigation varying from 100 $m^3\,ha^{-1}$ to 300 $m^3\,ha^{-1}$, depending on the growth stage of the crop.

2.3. Water, soil, plant and fruit sampling

GW and TW samples were collected at monthly intervals throughout the crop irrigation period, to characterize the physico-chemical and microbiological properties of these irrigation waters. Three samples of the GW and TW irrigation sources were collected (Fig. 1) in sterile 1000-ml glass bottles, and transported to the laboratory in refrigerated bags. The samples collected were kept in a refrigerator at +4 °C, and examined within 24 h of their collection.

Soil samples were collected in triplicate from the GW and TW plots, six times throughout the cropping season. All of the soil samples were taken from a 30 cm layer in each plot, from under the drippers, and they were air-dried, crushed, and passed through a 2 mm sieve before the chemical analysis. Tomato plant samples were collected at the same time, in triplicates for each experimental treatment.

The fruit sampling was performed at each of the four harvesting dates (about two days after irrigation crop) in an experimental plot of 20 m^2, by picking all of the mature fruit. The plants and harvested fruit were transported immediately to the laboratory for the chemical and microbiological analyses.

2.4. Water, soil and fruit chemical analysis

2.4.1. Water chemical analysis

The irrigation water samples were analyzed in triplicate, according to the Italian standard methods (APAT IRSA-CNR, 2003), which refer to the common international methods (APHA-AWWA-EF, 2005). The analysis included the physico-chemical parameters of pH, electrical conductivity (EC_w; $dS\,m^{-1}$), total suspended solids (TSS; $mg\,l^{-1}$), biological oxygen demand over 5 days (BOD_5; $mg\,l^{-1}$), chemical oxygen demand (COD; $mg\,l^{-1}$), ammonium-nitrogen (NH_4–N; $mg\,l^{-1}$), nitrate-nitrogen (NO_3–N; $mg\,l^{-1}$), nitrite-nitrogen (NO_2–N; $mg\,l^{-1}$), phosphorus (PO_4-P; $mg\,l^{-1}$), sodium (Na^+; $mg\,l^{-1}$), calcium (Ca^{2+}; $mg\,l^{-1}$), magnesium (Mg^{2+}; $mg\,l^{-1}$), potassium (K^+; $mg\,l^{-1}$), carbonates (CO_3^{2-}; $mg\,l^{-1}$), bicarbonates (HCO_3^-; $mg\,l^{-1}$), sulphate (SO_4^-; $mg\,l^{-1}$), sodium adsorption ratio (SAR) and water hardness ($mg\,l^{-1}$ $CaCO_3$).

The pH was measured using a GLP 22+ pH & Ion Meter (CRISON INSTRUMENTS, Spain) and the electrical conductivity with a GLP 31+ EC-Meter (CRISON INSTRUMENTS, Spain). The Na^+, Ca^{2+}, Mg^{2+}, and K^+ levels were determined by ion-exchange chromatography

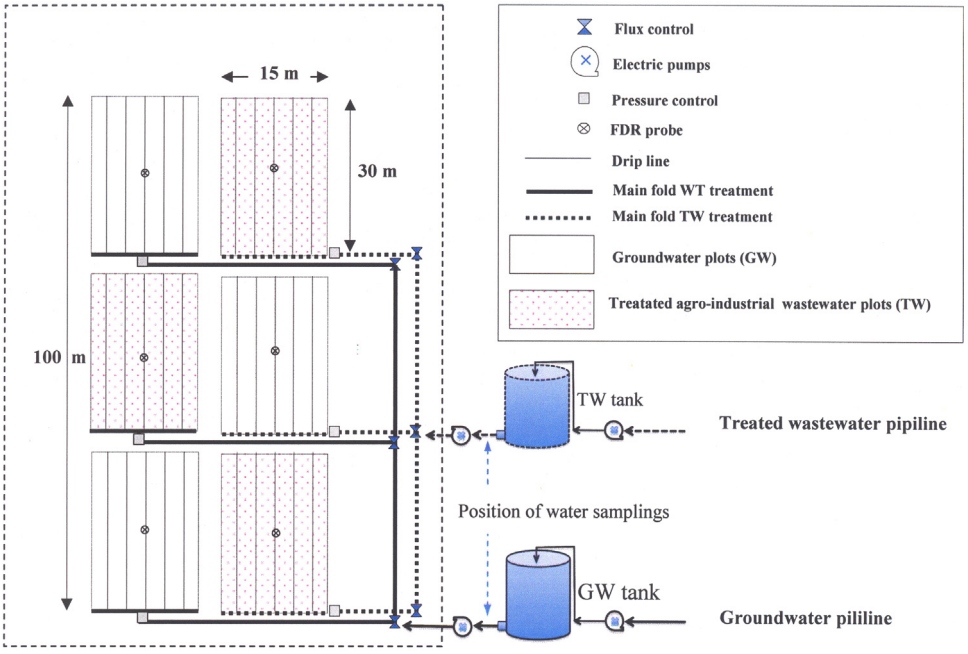

Fig. 1. Layout of the experimental field and the irrigation systems.

(Dionex ICS-1100, Dionex Corporation, Sunnyvale, CA, USA). The TSS was determined after filtration of the water samples through 0.45-μm-pore-size (47-mm-diameter) nitrocellulose membranes (Whatman, Maidstone UK), using a vacuum system. The SAR was calculated using the formula (with concentrations in meq l^{-1}) (Richards, 1954): $SAR = (Na^+)/[(Ca^{2+} + Mg^{2+})/2]^{1/2}$.

2.4.2. Soil chemical analysis

The soil electrical conductivity and pH were measured on 1:2 (w/v) and 1:2.5 (w/v) aqueous soil extracts, respectively. The available phosphorus was determined using the sodium bicarbonate method (Olsen et al., 1954), and the total organic carbon (TOC) was determined by oxidation with potassium dichromate titration of $FeSO_4$, according to Walkley and Black (1934). The soluble NO_3–N and NH_4–N were determined according to Keeney and Nelson (1982).

All of the soil chemical parameters were used together with the soil microbiological characteristics for multivariate analysis, to determine the effects of the two soil treatments (i.e., GW, TW) on the dynamics of the bacterial communities.

2.4.3. Yield and fruit qualitative analysis

During the harvest, the marketable and discarded fruit were counted and weighted, to estimate the different components of the tomato yield: total yield (TY; Mg ha^{-1}), marketable yield (MY; Mg ha^{-1}), marketable fruit per plant (MYP; kg $plant^{-1}$), nonmarketable yield (NMY; Mg ha^{-1}), and nonmarketable fruit per plant (NMYP; kg $plant^{-1}$). On a sample of 10 marketable fruit from each plot, the following parameters were measured: mean diameter (equatorial and longitudinal diameter) (D; cm), soluble solids content of the flesh (SSC; °Brix), titratable acidity (TA; g citric acid 100 ml^{-1} fresh juice) (AOAC, 1995), dry matter content (DM; % fruit fresh matter) (AOAC 1990), a^*/b^* ratio (CI) (Jiménez-Cuesta et al., 1981), and Ca^{2+}, Na^+, Mg^{2+}, K^+ and nitrate NO_3^- content.

The color parameters were measured using a CM-700d spectrophotometer (Minolta Camera Co. Ltd., Osaka, Japan), as the CIELAB coordinates (i.e., L^*, a^*, b^*) on four randomly selected areas of the fruit surface. Here, only the a^*/b^* ratio is reported, which represents an index that describes well the color changes of tomato fruit (Francis and Clydesdale, 1975; Favati et al., 2009).

The anion and cation contents were determined by ion-exchange chromatography (Dionex ICS-1100, Dionex Corporation, Sunnyvale, CA, USA). The anions were extracted from 0.5 g dried and ground samples, with 50 ml 3.5 mmol l^{-1} Na_2CO_3 and 1.0 mmol l^{-1} Na_2HCO_3, and they were measured using an IonPac AG14 pre-column and an IonPac AS14 separation column. The data are expressed as mg 100 g^{-1} fresh weight (fw). For the cations, 1.0 g dried and ground samples was use for the ash in a muffle furnace at 550 °C, and then digested in 20 ml 1.0 mol l^{-1} HCl in boiling water (99.5 ± 0.5 °C), for 30 min. The resulting solution was filtered, diluted, and analyzed using an IonPac CG12A guard column and an IonPac CS12A analytical column. The data are expressed as mg 100 g^{-1} fw (Renna et al., 2013).

2.5. Microbiological analysis

The GW and TW samples were analysed *E. coli* and fecal *Enterococci* enumeration, by the membrane filtration method. Triplicate aliquots of 100, 10, 1.0 and 0.1 ml of each water sample were filtered through 0.45-μm-pore-sized (47-mm-diameter) nitrocellulose membranes (Whatman, Maidstone UK). For *E. coli* enumeration, the membranes were placed onto tryptone bile agar with X-glucuronide (TBX agar; Oxoid, Basingstoke, UK) and incubated at 37 °C for 24 h. For fecal *Enterococci* enumeration, the membranes were placed onto Slanetz & Bartley Agar (Oxoid, UK), and incubated at 37 °C for 48 h. The same water samples were also analyzed for *Salmonella* spp., with their detection performed according to procedure UNI EN ISO 19250:2013.

The bacteriological analysis of the soil, plant and fruit samples included determination of *E. coli*, fecal coliforms, and total heterotrophic counts (THCs). These analyses were conducted by the spread plate method, as follows: 25.0 g of each sample was weighed and added to 225.0 ml buffered peptone water, homogenized in a stomacher for 180 s, and stored at room temperature for 30 min to allow bacterial cell recovery. Then serial 10-fold dilutions in buffered peptone water were spread onto plates containing TBX for *E. coli*, C-EC agar (Biolife) for fecal coliforms, and tryptic soy agar for THC. The plates were incubated under different incubation temperatures (and times): 37 °C for *E. coli* (24 h), 44 °C for fecal coliforms (48 h), and 22 °C for THC (72 h).

2.5.1. Quantification of total soil eubacteria by quantitative PCR

DNA extractions from triplicate soil samples irrigated with either GW or TW were performed with Powersoil DNA isolation kit (MoBio, Ca USA), following the manufacturer protocol. The DNA was eluted in 100 μl elution buffer, and visualized on ethidium bromide stained 1% agarose gels after electrophoresis, to assess the yield and quality of the extracted DNA. The DNA was quantified with an Eon microplate spectrophotometer (Biotek, Winooski, VE, USA) before further analyses.

Quantitative-PCR (q-PCR) analysis with universal primers targeting the bacteria was performed to determine the total eubacteria population in the soil. The primers and probes used were designed in previous studies (Nadkarni et al., 2002). Amplification and detection were performed using an AB 7300 real-time PCR system (Applied Biosystems, Foster city CA, USA), with a final reaction volume of 25.0 μl, which contained 100 nM of each primer, 150 nM probe, and 2× Taqman Fast Advanced master mix (Applied Biosystems). The cycling program was: 40 cycles of 15 s at 95 °C and 60 s at 60 °C. Conversion of the 16S rRNA gene copy numbers to cell numbers was carried out considering that bacteria have an average of four copies per cell of the 16S rRNA gene.

2.5.2. Soil microbial community analysis by automated ribosomal intergenic spacer analysis

To compare the soil microbial community compositions under the two different treatments (soil irrigated with GW and TW), automated ribosomal intergenic spacer analysis (ARISA) was applied. Internal transcribed spacer (ITS) regions of the soil microbial DNA were amplified using the primers ITSF (5′-GTCGTAACAAGGTAGCCGTA-3′) and ITSReub (5′-GCCAAGGCATCCACC-3′), labelled with 6-FAM, according to the chemical and thermal amplification protocol of Cardinale et al. (2004). The three replicates of six PCR samples from the soils (T_0, T_1, T_2, T_3, T_4, T_5) were sent to STAB Vida Lda (Caparica, Portugal) for the capillary electrophoresis. Peak Scanner Software 1.0 (Applied Biosystems) was used to analyze the fragment data. The T_0 and T_1 soil samples were taken 1 week before transplanting and 15 days after transplanting (just before starting the GW and TW irrigation), respectively, and T_2, T_3, T_4 and T_5 were collected at intervals of about one month (42, 69, 96 and 124 days from transplanting, respectively). To obtain the output matrix, each fragment size detected by ARISA was converted to the nearest integer value, and these were subsequently aligned according to their peaks, against the rounded sizes of the fragments, using the Microsoft-Excel macro Treeflap (Rees et al., 2004). The matrix was normalized and root-square transformed for the statistical analysis.

2.6. Statistical analysis

The measured data from each of the continuum variables relating to the qualitative/quantitative traits of the tomato fruit were

processed statistically using analysis of variance (ANOVA). When significant effects were detected ($P \leq 0.05$), mean multiple comparisons were performed according to Tukey's tests. With reference to the analyzed qualitative parameters, the Bartlett test confirmed the homogeneity of the variance among the harvest data, so a combined statistical analysis was performed later. Furthermore, the variables related to the qualitative parameters of the tomato fruit were jointly considered in a multivariate approach, and statistically processed for canonical variates analysis (CVA), with the two experimental factors (water irrigation treatment and harvest data) as the discriminating sources (Cooley and Lohnes, 1971; Sadocchi, 1981; Gittins, 1985; Podani, 2007). Before performing the CVA, the values of each variable were correctly standardized. The first two canonical variates accounted for the larger part of the data variability, and these were considered for further data interpretation. The CVA is represented graphically in a biplot, which considers both the canonical standardized scores (corresponding to each multivariate experimental datum) and the vectors (originating from the centroids and recording the canonical standardized coefficients). Finally, the ARISA data matrix and the standardized soil parameters were analyzed using canonical correspondence analysis (CCA). The ANOVA and CVA were performed using the JMP software package, version 8.1 (SAS Institute Inc., Cary, NC, USA). The CCA was performed using the PAST software in its default settings (Hammer et al., 2001). All of the graphical representations were carried out using the SigmaPlot software (Systat Software, Chicago).

3. Results and discussion

3.1. Irrigation water properties

Table 2 shows the means of the physico-chemical characteristics of GW and TW measured during the experimental trial. These analyses show some specific differences in their compositions. TW was characterized by higher N (especially as NH_4-N), P, Mg^{2+}, and K^+ than GW, which represent important nutrients for improving plant growth, soil fertility and crop yield. TW also showed higher organic matter content than GW (as indicated by BOD_5 and COD; Table 2).

Table 2
Means of the main physico-chemical parameters measured during the experimental period for the groundwater (GW) and the treated agro-industrial wastewater (TW) used for the tomato irrigation.

Water parameter	Irrigation treatment		Significance
	GW	TW	
Ph	7.63 ± 0.10	7.76 ± 0.09	ns
EC (dS m^{-1})	0.69 ± 0.05	2.18 ± 0.12	*
TSS (mg l^{-1})	3.26 ± 0.34	16.21 ± 2.24	*
NH_4-N (mg l^{-1})	0.04 ± 0.00	0.39 ± 0.10	*
NO_3-N (mg l^{-1})	29.06 ± 1.67	1.20 ± 0.23	*
NO_2-N (mg l^{-1})	0.02 ± 0.01	0.07 ± 0.02	*
PO_4-P (mg l^{-1})	0.10 ± 0.01	0.29 ± 0.02	*
BOD_5 (mg l^{-1})	9.33 ± 1.03	21.58 ± 1.62	*
COD (mg l^{-1})	18.44 ± 1.62	39.73 ± 2.78	*
Na^+ (mg l^{-1})	33.53 ± 0.54	219.85 ± 6.05	*
Ca^{2+} (mg l^{-1})	52.82 ± 3.23	85.27 ± 1.24	*
Mg^{2+} (mg l^{-1})	8.90 ± 0.20	10.25 ± 0.12	*
K^+ (mg l^{-1})	9.35 ± 0.16	41.17 ± 1.96	*
CO_3^{2-} (mg l^{-1})	171.50 ± 5.32	193.67 ± 11.89	ns
HCO_3^- (mg l^{-1})	257.89 ± 2.85	254.23 ± 21.57	ns
SO_4^- (mg l^{-1})	30.17 ± 1.30	31.84 ± 0.85	ns
SAR	1.13 ± 0.03	5.99 ± 0.18	*
Hardness (mg l^{-1} $CaCO_3$)	168.57 ± 7.79	255.20 ± 3.54	*

Data are means ± standard errors for each analyzed trait, determined on 15 samples for each irrigation water treatment (1 sample per water treatment ×3 replicates ×5 sampling dates).
* Statistically significant at $P \leq 0.05$; ns, not significant.
For abbreviations, see main text.

These higher nutrient levels in TW compared to GW indicate that this TW can provide an important source of plant nutrients for the soil, and can contribute to crop growth.

However, of note, the NO_3–N of GW was significantly higher (29.06 mg l^{-1}) than for TW (1.20 mg l^{-1}) (Table 2). This elevated NO_3–N in GW appears to be due to an important nitrate contamination problem of the aquifer in the study area, where the intensive agricultural activity has led to the common and diffuse practice of extensive nitrogen fertilizer application to the various crops. This elevated NO_3–N content in GW represents an important source of nutrient for the plants, but generally it is not taken into account by the farmers when applying fertilizers. The resulting nitrogen surplus in the soil is then particularly exposed to the risk of leaching, thus increasing the environmental problem of nitrate pollution.

TW also showed higher Na^+, Ca^{2+}, Mg^{2+}, SAR, EC, and TSS than GW (Table 2). If the SAR is related to the EC (Ayers and Westcot, 1985) of TW, it appears that there is no limit to its agricultural application, and there would be no reduction in its rate of infiltration into the soil. Both GW and TW were alkaline, with a higher pH for GW than TW (although not significantly higher). The other parameters analyzed showed similar levels in GW and TW, and in general, the values for these main physico-chemical water properties met the Italian standards for wastewater re-use (Ministerial Decree no. 185/2003).

3.2. Effects of irrigation water on qualitative/quantitative traits of tomato yield

Table 3 gives the ANOVA data with reference to the influence of the water irrigation treatments on the productive traits of the tomato crop. These traits are related to the combined harvest dates (i.e., the cumulative yields).

For GW, MY was higher (82.88 Mg ha^{-1}), although not significantly so, than for TW (79.05 Mg ha^{-1}). This appears to be mainly due to the higher NMY for TW (6.26 Mg ha^{-1}) with respect to GW (4.66 Mg ha^{-1}). The MY recorded at the end of the tomato crop cycle is roughly in agreement with results obtained in other experimental trials carried out in other Italian regions (Aiello et al., 2007). The MY obtained from a tomato crop (variety 'Missouri') irrigated with fresh water by Aiello et al. (2007) was higher than that irrigated with wastewater. On the contrary, in the same study, for a different tomato variety (i.e., 'Incas'), the use of urban wastewater irrigation produced an increase in MY compared to the same tomato variety irrigated with fresh water (Aiello et al., 2007). Under the experimental conditions of the present study, this cultivar 'Manyla' tomato crop showed a lower yield than for this other study. This might be due to differences in the genetic constitution of the cultivars used, or to the type of treated wastewater applied and the pedo-environmental conditions of the cultivation area.

Table 4 shows the means for the qualitative traits of the tomato yield with respect to the two experimental factors, the water irrigation treatments and the harvest data. The irrigation treatments and the harvest data did not show any significant effects in their interactions. The different water irrigation treatments significantly ($P \leq 0.05$) affected the pH of the tomato fruit, with a higher pH for GW (4.54) compared to TW (4.35). This pH parameter is very important, because it can strongly influence the effectiveness of thermal processes carried out on tomato fruit during their industrial transformation (Garcia and Barret, 2006). The pHs in the present study are comparable to those of another study related to the influence of irrigation and organic fertilization on fruit quality for tomato (Madrid et al., 2009); this other study showed a pH from 4.32 to 4.56, with the higher value in the fertilizer treatments (compared to the nontreated control).

The crop DM and SSC were not different between the GW and TW irrigation treatments (7.52% vs. 7.44%; 5.73 vs. 5.53 °Brix;

Table 3
Influence of the groundwater (GW) and the treated agro-industrial wastewater (TW) used for the tomato irrigation on some of the quantitative traits of the tomato fruit.

Treatment	Quantitative trait[a]				
	Total yield	Marketable yield		Nonmarketable yield	
	(Mg ha^{-1})	Total (Mg ha^{-1})	Per plant (kg plant^{-1})	Total (Mg ha^{-1})	Per plant (kg plant^{-1})
GW	87.54 ± 10.37[a]	82.88 ± 9.47[a]	3.06 ± 0.29[a]	4.66 ± 0.89[a]	0.17 ± 0.05[a]
TW	85.32 ± 5.01[a]	79.05 ± 4.76[a]	2.93 ± 0.15[a]	6.26 ± 0.61[a]	0.23 ± 0.03[a]
Significance	ns	ns	ns	ns	ns

Data are means ± standard error, as measured from 162 plants (54 plants per plot ×3 replicates).
ns, not significant.
[a] Means followed by the same letters in each column are not significantly different ($P \leq 0.05$; Tukey tests).

respectively; Table 4). These data are in agreement with Turhan and Seniz (2009), who reported DM ranging from 4% to 7%, and SSC ranging from 3.3 °Brix to 5.5 °Brix for 33 genotypes of tomatoes cultivated in the Mediterranean region. However, our data here are lower than in another study (Sgherri et al., 2008), where for the Cherry tomato 'Dulcito RZ' grown in greenhouses, DM and SSC were 10% to 12% and 9 °Brix to 10 °Brix, respectively. However, in studies on the effects of irrigation on productivity and fruit quality of tomatoes produced under different water regimes, Patanè et al. (2011) and Favati et al. (2009) reported lower DM and SSC than in the present study. These differences will mainly be due to the different genotypes used (Sgherri et al., 2007, 2008), and the environmental drought (Mahajan and Singh, 2006; Soraya et al., 2001) and climate conditions (e.g., temperature, CO_2 concentration, light conditions). High DM and SSC might have important positive implications for the tomato canning and processing industry (Richardson and Hobson, 2006; Favati et al., 2009), as it is well known that tomatoes with high SSC improve the processing efficiency, as less energy is needed to evaporate the water from the tomatoes when producing paste, concentrated juice, and dried or semi-dried tomatoes.

For the D, CI and TA parameters (Table 4), there were no significant differences between the TW and GW treatments, and these mean data are in agreement with other studies for similar tomato genotypes (Madrid et al., 2009; Mahajan and Singh, 2006).

Among the mineral components of the tomato fruit (i.e., Ca^{2+}, Mg^{2+}, K^+, Na^+, NO_3^-), only the Na^+ and NO_3^- content showed differences with respect to water irrigation factors (Table 4). The Na^+ content of the fruit was higher for TW than GW (11.05 vs. 9.15 mg 100 g^{-1}, respectively); this is probably related to the higher content of Na^+ in TW compared to GW (see Table 2). Finally here, the NO_3^- content in the tomato fruit was higher for the GW treatment (1.32 mg 100 g^{-1}) than for TW (0.92 mg 100 g^{-1}), which is in agreement with the higher NO_3–N in GW, and it is also much lower than that defined in the European guidelines (Reg. CE n. 1881/2006; Reg. UE n. 1258/2011). Except for the Ca^{2+} and Na^+ contents, our data are in agreement with the mineral data for tomato fruit reported in other studies (Suárez et al., 2007; Guil-Guerrero and Rebolloso-Fuentes, 2008).

For the harvest data experimental factor, only the means of the D, TA, pH and Na^+ parameters were significantly different. Across all of the harvest data, D had a mean that ranged from 3.82 cm to 5.04 cm, and was significantly higher for HD_1 than for the rest of the harvest dates (HD_{2-4}). This can be explained by the position of the tomato fruit on the plant during the first harvest (HD_1). Indeed, our data are in agreement with the consolidated literature, in which it has been stated that the size and shape of a fruit can also vary in relation to the position of the fruit within the plant and the sequence of pollination among the flowers (Sawhney and Greyson, 1972; Bohner and Bangerth, 1988). In particular, it has been shown that the first fruit on the first truss is generally larger in size than the rest, and that it can also be multilocular, which further supports the relationship between locule number and fruit size.

The TA fruit content was between 0.4 g 100 ml^{-1} and 0.19 g 100 ml^{-1} across the harvest dates, with a significant difference between the first two harvest dates (HD_1 vs. HD_2). The TA for HD_3 and HD_4 (0.32 vs. 0.30 g 100 ml^{-1}, respectively) were not different. The pHs were within the range of 4.36 to 4.60, at HD_1 and HD_4, respectively.

For the ionic components of the fruit, the Na^+ content gradually increased with the later harvest dates, from 7.21 mg 100 g^{-1} to 14.46 mg 100 g^{-1} for HD_1 and HD_4, respectively. As already indicate above, this increase in Na^+ is probably due to the high Na^+ in the water irrigation treatments (and particularly for TW) and will parallel the progressive Na^+ accumulation in the soil.

3.2.1. Canonical analysis on the qualitative composition of the tomato fruit

The ANOVA results reported above show the effects of experimental factors on each qualitative variable separately. A multivariate approach analysis allowed us to integrate these data to evaluate which of the qualitative variables (considered simultaneously) contributes most to the group differences (experimental factors). According to the CVA, the eleven original qualitative variables related to the qualitative traits of the tomato fruit were reduced to two canonical variates that represent 76.2% of the total data variability: 53.1% for the first (CAN_1), and 23.1% for the second (CAN_2) (Table 5). On the basis of the large amount of overall variability explained by CAN_1, this can be considered as a 'multivariate qualitative index' of the tomato fruit.

To correctly interpret the relationships between canonical variates (CAN_j) and the original variables (V_i), it is important to recall that the CAN_j are linear combinations of the original variables and that the canonical coefficients maximize the discrimination among the experimental factors considered using canonical coefficients. The original variable V_i with the largest standardized canonical coefficient has, indeed, the strongest impact on the canonical variates CAN_j.

CAN_1 was positively affected by pH and NO_3^- content (scores, 0.975 and 0.589, respectively), and negatively affected by the D and a^*/b^* ratio (CI) parameters (scores, −1.167 and −0.493, respectively) (Table 5). The other V_i contributed to a limited extent to defining CAN_1 (low canonical scores). CAN_2, instead, was mainly defined by the Na^+ content (score, 0.856), and to a different extent by pH and D of the fruit (scores, −1.051 and −0.505, respectively).

Coherent information can also be derived from the correlation matrix (Table 6) between the 'old' original variables (V_i) and the 'new' canonical variables (CAN_j). The higher the correlation coefficient between V_i and CAN_j, the stronger the influence that V_i has on CAN_j. These considerations can be easily seen using biplot graphs (Fig. 2) by considering the length and orientation of the 'vectors'. The biplots represent the effects of the discriminating experimental factors (irrigation water treatment and harvest date) on the qualitative characteristics of the tomato fruit.

Among the original qualitative variables considered in the CVA, those that affected CAN_1 (pH, NO_3^-, D) and CAN_2 (Na^+, D, pH)

Table 4
Effects of water irrigation treatment, as the groundwater (GW) and the treated agro-industrial wastewater (TW), and harvest collection data (HD_{1-4}), and their factorial combination, on some of the qualitative traits of the tomato fruit.

Parameter	Qualitative traits[a]						Anion and cation content (mg 100 g^{-1} fresh matter)				
	DM	D	CI	SSC	TA	pH	Ca^{2+}	Mg^{2+}	K^+	Na^+	NO_3^-
	(% FM)	(cm)	(a^*/b^* ratio)	(°Brix)	(g 100 ml^{-1})						
Water irrigation treatment											
GW	7.44 ± 0.11[a]	4.22 ± 0.32[a]	1.03 ± 0.03[a]	5.73 ± 0.12[a]	0.31 ± 0.02[a]	4.54 ± 0.04[a]	8.63 ± 0.54[a]	7.25 ± 0.63[a]	225.3 ± 9.5[a]	9.15 ± 0.94[b]	1.32 ± 0.10[a]
TW	7.52 ± 0.21[a]	4.44 ± 0.28[a]	0.97 ± 0.04[a]	5.53 ± 0.12[a]	0.30 ± 0.03[a]	4.35 ± 0.04[b]	9.33 ± 0.62[a]	7.07 ± 0.33[a]	225.4 ± 6.0[a]	11.05 ± 0.92[a]	0.92 ± 0.06[b]
Significance	ns	ns	ns	ns	ns	*	ns	ns	ns	*	*
Harvest data											
HD_1	7.19 ± 0.11[a]	5.04 ± 0.10[a]	0.93 ± 0.06[a]	5.40 ± 0.05[a]	0.40 ± 0.01[a]	4.36 ± 0.02[c]	10.43 ± 0.88[a]	7.22 ± 0.66	213.1 ± 17.8[a]	7.21 ± 0.51[c]	1.04 ± 0.08[a]
HD_2	7.60 ± 0.21[a]	4.63 ± 0.07[b]	1.01 ± 0.02[a]	5.60 ± 0.13[a]	0.19 ± 0.02[c]	4.48 ± 0.03[b]	9.31 ± 1.03[a]	6.45 ± 1.07	218.6 ± 8.8[a]	7.93 ± 0.73[c]	1.18 ± 0.14[a]
HD_3	7.24 ± 0.31[a]	3.82 ± 0.08[d]	1.07 ± 0.04[a]	5.85 ± 0.14[a]	0.32 ± 0.01[b]	4.43 ± 0.02[bc]	8.45 ± 0.61[a]	7.40 ± 0.47	228.4 ± 4.6[a]	10.82 ± 0.71[b]	1.12 ± 0.14[a]
HD_4	7.98 ± 0.21[a]	4.21 ± 0.06[c]	1.17 ± 0.05[a]	5.68 ± 0.27[a]	0.30 ± 0.02[b]	4.70 ± 0.03[a]	7.73 ± 0.32[a]	7.88 ± 0.53	241.5 ± 6.7[a]	14.46 ± 0.66[a]	1.14 ± 0.20[a]
Significance	ns	***	ns	ns	***	**	ns	ns	ns	***	ns
Water irrigation treatment × Harvest data											
Significance	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Data are means ± standard error, as measured from 30 marketable fruits (10 fruit per plot, ×3 replicates).
* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$; ns, not significant.
[a] Means followed by the same letters in each column are not significantly different ($P \leq 0.05$; Tukey tests).
For abbreviations, see main text.

Table 5
Standardized coefficients (scores) for the first two canonical variates (*CANj*), considering the qualitative properties of the tomato fruit. The corresponding percentages of accounted variation are also reported.

Original variable (*Vi*)	Standardized canonical coefficients	
	CAN_1	CAN_2
Mean diameter (D)	−1.167	−0.505
a^*/b^* ratio (CI)	−0.493	0.199
Soluble solids content of the flesh (SSC)	0.001	0.098
pH of the flesh	0.975	−1.051
Titratable acidity (TA)	−0.137	0.343
Dry matter (DM)	0.057	−0.074
Nitrate content (NO_3^-)	0.589	−0.226
Sodium content (Na^+)	−0.101	0.856
Potassium content (K^+)	−0.012	−0.113
Magnesium content (Mg^{2+})	−0.272	0.101
Calcium content (Ca^{2+})	0.187	0.070
Percentage explained variation	53.1	23.1
Percentage cumulative variation	53.1	76.2

Table 6
Correlation matrix (Pearson coefficients) between the quality parameters of the tomato fruit and the two canonical variables extracted (CAN_1 and CAN_2).

Original variables (*Vi*)	Canonical variables	
	CAN_1	CAN_2
Mean diameter (D)	−0.84**	−0.42*
a^*/b^* ratio (CI)	−0.39	−0.12
Soluble solids content of the flesh (SSC)	0.40	0.06
pH of the flesh	0.64**	−0.61*
Titratable acidity (TA)	−0.29	0.32
Dry matter (DM)	0.24	−0.24
Nitrate content (NO_3^-)	0.41*	−0.39
Sodium content (Na^+)	−0.36	0.55*
Potassium content (K^+)	−0.09	−0.11
Magnesium content (Mg^{2+})	−0.26	0.16
Calcium content (Ca^{2+})	0.30	0.15
CAN_1	1	0
CAN_2	0	1

* $P \leq 0.05$.
** $P \leq 0.001$.

Fig. 2. Canonical analysis of the qualitative parameters of the tomato fruit using the harvest data (HD_j) and the water irrigation treatments (GW, TW) as the discriminant variables. For abbreviations, see main text. Horizontal and vertical bars indicate standard errors of three replicates.

Table 7
Enumeration of the bacterial indicators in the water, soil, plant and fruit samples according to the groundwater (GW) and the treated agro-industrial wastewater (TW) irrigation.

Source	Irrigation Treatment		Significance
Bacterial indicator	GW	TW	
Water (CFU 100 ml^{-1})			
E. coli	7	4400	**
Fecal *coliforms*	9	3800	**
Soil (CFU g^{-1})			
E. coli	n.d.	n.d.	
Fecal *Enterococci*	1230	1360	ns
Total heterotrophic counts	3.69×10^6	4.02×10^6	ns
Soil q-PCR (cells g^{-1})			
Total eubacteria	4.86×10^7	16.2×10^7	*
Plant (CFU g^{-1})			
E. coli	n.d.	n.d.	
Fecal *Enterococci*	150	183	ns
Total heterotrophic counts	18,400	16,400	ns
Fruit (CFU g^{-1})			
E. coli	n.d.	n.d.	
Fecal *Enterococci*	173	237	ns
Total heterotrophic counts	5500	17,800	ns

* $P \leq 0.05$.
** $P \leq 0.01$.
ns, Not significant n.d., not detected.

were the same ones that better discriminated between the experimental factors (irrigation water treatment and harvest date), in agreement with the ANOVA results (see Table 4). In particular, Na^+ content allowed discrimination of the irrigation treatment with TW, while NO_3^- content and pH of the fruit allow identification of the irrigation treatment with GW. Thus, from the GW to TW water treatments, there is a marked increase in the Na^+ content and a corresponding decrease in both pH and NO_3^- content.

Considering the first canonical axis, the high D (low CAN_1) discriminates between the first two harvest dates (HD_1 vs. HD_2), while pH identifies the third harvest date (HD_3). Finally, the last harvest date (HD_4) is characterized by a lower D (i.e., its position is distant from D; Fig. 2). The other quality traits (DM, CI, SSC, Ca^{2+}, Mg^{2+}, K^+) showed low contributions to the discrimination of the experimental groups (irrigation water treatment and harvest date), considering the length of their 'vectors'.

In general, the results of the multivariate analysis show that pH and NO_3^- allow better differentiation between the two water irrigation treatments, while D and pH allow better differentiation between (most of) the harvest dates.

3.3. Effects on microbial indicators

With the aim of evaluating the safety aspects related to the use of these different qualities of irrigation water for tomato crop irrigation, several microbial analyses were conducted, as reported in Table 7. For TW, the mean *E. coli* and fecal *Enterococci* counts were 4400 and 3800 CFU 100 ml^{-1}, respectively. These are well above the Italian limits for treated wastewater re-use (Ministerial Decree no. 185/2003). These data are in agreement with the objective of the present study, as we intentionally provided an input of non-chlorinated treated wastewater to also be able to evaluate any effects on the soil and plant microbiological quality.

GW was almost free of fecal indicators, with 7 CFU 100 ml^{-1} and 9 CFU 100 ml^{-1} for *E. coli* and fecal *Enterococci*, respectively. *Salmonella* spp. was not isolated in any of the water samples. In that case the microbiological parameters are in line with the above cited legislation limits.

For the irrigated soil, the level of fecal coliform counts was almost identical for GW treatment and TW treatment. The total heterotrophic plate counts (THC) showed a lower level in the soil irrigated with TW than GW (4.02×10^6 and 3.69×10^6, respectively), although this was not significant ($P > 0.05$). *Salmonella* spp. was not isolated in any of these water samples, and thus here the microbiological parameters are in line with the above-cited legislation limits.

When q-PCR was applied, the total bacterial level of TW (16.2×10^7 cells g^{-1}) was significantly higher than GW (4.86×10^7 cells g^{-1}) ($P \leq 0.05$), Moreover the q-PCR count estimated the total (viable and nonviable) bacterial populations in the soil as >1 log CFU g^{-1} than the viable cell count. The marked differences between the bacterial cultivation method (THC) and the molecular method (q-PCR) in assessing the total bacterial population arises from the higher sensitivity of the q-PCR, as a narrower range of the total bacterial population can be cultivable on synthetic media. *E. coli* was not isolated in any of the soil samples, independent of the water used for irrigation.

The data related to fecal coliforms and THC in the soil are in agreement with Benami et al. (2013), who assessed soil irrigated with treated 'gray' water and with fresh water. Other studies have reported that the level of fecal indicators in soil irrigated with raw or treated wastewater can significantly differ from that with freshwater application (Malkawi and Mohamad, 2003; Travis et al., 2010). It also needs to be considered that in the study of Malkawi and Mohamad (2003) there were no fecal coliforms recorded in the soil before the irrigation with fresh water, and thus sources other than water can affect this indicator. With Travis et al. (2010), the levels of fecal coliforms in soil irrigated with untreated or treated gray water was always below 100 CFU g^{-1}.

Considering that *E. coli* was not isolated from any of the soil samples in the present study, it is possible that the die-off, or at least the loss of cultivability, of this important indicator in the present field study occurred faster than previously reported (Lang et al., 2007; Van Elsas et al., 2011). However, it is more likely that the levels of viable and cultivable *E. coli* under these given environmental conditions were below the sensitivity of the method applied (10^2 CFU g^{-1}) (Samarajeewa et al., 2010). Analogous data were obtained for the plant and fruit here, as no *E. coli* was isolated from either, and the levels of fecal coliforms and THC were relatively low and not influenced by the water used for irrigation ($P > 0.05$). These data are in agreement with other studies (Cirelli et al., 2012), in which only fruit samples (tomato and eggplant) directly in contact with the soil where contaminated by fecal bacteria. Wood et al. (2010) showed that the decline in *E. coli* on the surface of spray-irrigated spinach was considerably rapid (3–5 log reduction in 72 h, and no isolation after 6 days). Another study reported that in the summer months, which are characterized by higher sunlight exposure of the crops, there was a more rapid decay of both the indicator and pathogenic microorganisms (Sidhu et al., 2008). In the present study, there was no particular increase in the microbial contamination for the TW-treated tomato crops. We would thus argue that

the good microbial quality of tomato fruits, (no *E. coli*, very low fecal coliforms, no *Salmonella* spp.), can be seen as the positive consequence of several factors: among the principal ones, the drip irrigation system, the summer month with increased UV radiation exposure of fruit and leaves surfaces. Also, the interval time between irrigation and sampling may have contributed to reduce the effect of treated wastewater on the microbial load of fruits.

3.3.1. Effects on soil microbial communities

Apart from the possible contamination by fecal microorganisms, our aim was also to determine the short-term impact of irrigation with wastewater on the soil microbial communities of this tomato

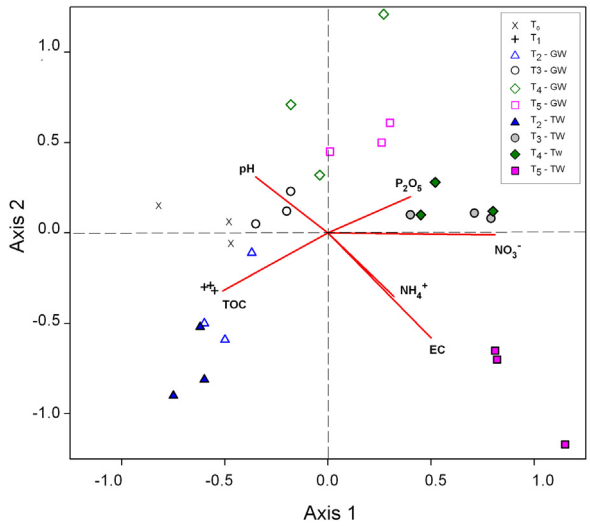

Fig. 3. Canonical correspondence analysis base on the ARISA matrix and the chemical soil parameters. For abbreviations, see main text. T_0, sampling before transplanting; T_1, sampling 15 days after transplanting and before the water irrigation treatments; T_2, T_3, T_4 and T_5, sampling at 42, 69, 96 and 124 days after transplanting, respectively.

cropping system. The CCA of the ARISA matrix show many interesting aspects related to the dynamics of the bacterial communities in the soil (Fig. 3).

There was a partial separation of the T_0 samples (soil sampled 1 week before transplanting) from the later time points. The T_1 (15 days after transplanting, but before starting GW and TW irrigation) and T_2 (42 days after transplanting; 27 days of GW or TW irrigation) time points clustered together, with the T_0 samples nearby. Both the T_1 and T_2 time points and T_0 were affected by TOC, but not by ammonia or phosphorous. The partial shift from T_0 to the T_1/T_2 clusters might indicate a rhizosphere effect on the soil microbial community, and that this effect is stronger than the irrigation methodology. Hence, this would mean that in the early stages of cultivation, the root exudates of tomato plant are the more important factor in shaping the bacterial communities, with respect to the water treatments used. Indeed, root exudates are known to contain compounds that can exert stimulatory and inhibitory influences on rhizosphere microbial community structure and composition (Dennis et al., 2010; Hartmann et al., 2009).

Even if soil pH is generally considered a major factor in controlling the soil microbial diversity and composition across a wide range of habitats (Fierer and Jackson, 2006), in the present study, pH did not appear to be an important contributor to the microbial community structures, which is also in agreement with other studies on tomato cropping systems (Buyer et al., 2010).

After almost a month of GW or TW irrigation, at T_2, the first separation between the GW and TW samples is seen, which suggests that these different irrigation waters do affect the microbial communities, and even after this relatively short irrigation period. From T_3 (69 days after transplanting; 54 days of GW or TW irrigation), there is a clear and significant separation between GW and TW, and this separation becomes stronger as time progresses. The TW samples show the greater effects, which are evidently correlated to nitrogen (both as ammonia and nitrates), phosphorous and the water conductivity.

The diverging ARISA patterns of the microbial communities after >30 days of irrigation with these different water sources (i.e., from T_2) is in agreement with other studies. Mosse et al. (2012), suggested that it takes >16 days for the effects of the water application on the soil microbial community to become apparent. Despite the strong differences in the fecal indicators in the GW and TW waters and the marked shift of the soil microbial communities, the microbiological quality of the final product (and of the plant) were not significantly different. In a recent study, Telias et al. (2011) investigated the bacterial community diversity and variation on the surface of tomato fruit by culture-independent methods (i.e., pyrosequencing). They found that even if water with very different microbial characteristics is used for long periods of spraying of the tomato surfaces, this did not have any significant impact on the bacterial composition of the tomato fruit surface. Another study aimed to evaluate the potential transfer of enteric bacteria from the soil to the plants by soil splash created by rain-sized droplets (Monaghan and Hutchison, 2012). In this case, both the transfer and survival of artificially inoculated human pathogens occurred, even if the persistence was considerably reduced during the summer months. In the present study, notwithstanding the high input of enteric bacteria into soil, when TW was used, the microbiological quality of final product was not compromised.

4. Conclusions

In the present study, we wanted to evaluate the use of agro-industrial wastewater in a closed circle system where an agriculture and food manufacturing company produces and processes tomatoes. The aim was to determine whether TW from the company can be used for tomato cropping without compromising the quality and safety of the final product.

Our data have revealed many interesting aspects: (i) the yields of the tomato fruit irrigated with TW were not significantly different from those obtained when the crop was irrigated with GW; (ii) for both the GW and TW irrigation treatments, the most important morpho-qualitative parameters of the processing tomato fruit (i.e., dry matter content, pH, soluble solid content, color parameters) were in agreement with those reported in the literature; and (iii) tomatoes microbial quality was very good for all the thesis stated, even when treated agro-industrial wastewaters were used. That was made possible by combining an accurate control of irrigation treatments with good agricultural practices.

Even if all of the fecal indicators monitored were well over the threshold of the Italian legislation limit for irrigation re-use of TW, the possible die-off of *E. coli* in the soil and the low levels of total coliforms in the soil and plants are of particular interest. To a broader extent, the community composition and the dynamics of the whole bacterial population in the soil was affected by the different qualities of the GW and TW that were used for the tomato crop irrigation.

The present study focused on a comprehensive multidisciplinary approach for the assessment of product quality and safety during a single crop cycle, to evaluate the short-term effects on the use of TW from the food industry. These data are encouraging, even though they are based on a relatively short period of observation. Further studies are being planned to determine the mid-to-long-term effects in the same experimental field.

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References

Aiello, R., Cirelli, G.L., Consoli, S., 2007. Effect of reclaimed wastewater irrigation on soil and tomato fruits: a case study in Sicily (Italy). Agric. Water Manage. 93, 65–72.

Al-Lahham, O., El Assi, N.M., Fayyad, M., 2003. Impact of treated wastewater irrigation on quality attributes and contamination of tomato fruit. Agric. Water Manage. 61, 51–62.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotraspiration guidelines for computing crop water requirements. In: Irrigation and Drainage Paper No. 56. Food and Agriculture Organisation of the United Nations (FAO), Rome.

APAT, IRSA-CNR, 2003. Metodi analitici per le acque. Manuali e linee guida. APAT, IRSA-CNR.

APHA, AWWA, WEF, 2005. Standard Methods for the Examination of Water and Wastewater, XX ed. American Public Health Association, Washington, DC.

Ayers, R.S., Westcot, D., 1985. Water quality for agriculture. In: FAO Irrigation and Drainage Paper No. 29. Food and Agriculture Organisation of the United Nations (FAO), Rome.

Benami, M., Gross, A., Herzberg, M., Orlofsky, E., Vonshak, A., Gillor, O., 2013. Assessment of pathogenic bacteria in treated graywater and irrigated soils. Sci. Total Environ. 458–460, 298–302.

Blumenthal, U.J., Mara, D.D., Peasey, A., Ruiz-Palacios, G., Scott, R., 2000. Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. Bull. World Health Organ. 78 (9), 1104–1116.

Bohner, J., Bangerth, F., 1988. Cell number, cell size and hormone levels in semi-isogenic mutants of Lycopersicon pimpinellifolium differing in fruit size. Physiol. Plant. 72, 316–320.

Buyer, J.S., Teasdale, J.R., Roberts, D.P., Zasada, I.A., Maul, J.E., 2010. Factors affecting soil microbial community structure in tomato cropping systems. Soil Biol. Biochem. 42, 031–841.

Caliandro, A., Lamaddalena, N., Stelluti, M., Steduto, P., 2005. Caratterizzazione agro-ecologica della Regione Puglia in funzione della potenzialità produttiva. Progetto ACLA. Ideaprint, BARI, Italy, ISBN: 2-85352-339-X.

Cardinale, M., Brusetti, L., Quatrini, P., Borin, S., Puglia, A.M., Rizzi, A., Zanardini, E., Sorlini, C., Corselli, C., Daffonchio, D., 2004. Comparison of different primer sets for use in automated ribosomal intergenic spacer analysis of complex bacterial communities. Appl. Environ. Microbiol. 70, 6147–6156.

Chen, W., Wu, L., Frankenberger Jr., W.T., Chang, A.C., 2008. Soil enzyme activities of long-term reclaimed wastewater-irrigated soils. J. Environ. Qual. 37, 36–42 (September–October Supplement).

Cirelli, G.L., Consoli, S., Licciardello, F., Aiello, R., Giuffrida, F., Leonardi, C., 2012. Treated municipal wastewater reuse in vegetable production. Agric. Water Manage. 104, 163–170.

Cooley, W.W., Lohnes, P.R., 1971. Multivariate Data Analysis. Wiley, New York, NY.

Decree of Ministry for Environment, 2003. Decree of Ministry for Environment, No. 185, 12/06/2003, Gazzetta Ufficiale, No. 169, Rome July 23, 2003. Italian Technical Guidelines for Wastewater Reuse.

Dennis, P.G., Miller, A.J., Hirsch, P.R., 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol. Ecol. 72, 313–327.

FAO, 2010. The wealth of waste: The economics of wastewater use in agriculture. In: FAO Water Report No. 35. Water Development and Management Unit, Food and Agriculture Organization of the United Nations, ISBN 978-92-5-106578-5.

FAO, 2011. The State of the World's Land and Water Resources for Food and Agriculture. The Food and Agriculture Organization of the United Nations And Earthscan, ISBN 978-1-84971-326-9 (hdk).

Favati, F., Lovelli, S., Galgano, F., Miccolis, V., Di Tommaso, T., Candido, V., 2009. Processing tomato quality as affected by irrigation scheduling. Sci. Hortic. 122 (November (4)), 562–571.

Fierer, N., Jackson, R.B., 2006. The diversity and biogeography of soil bacterial communities. Proc. Natl. Acad. Sci. U.S.A. 103, 626–631.

Francis, F.J., Clydesdale, F.M., 1975. Food Colorimetry: Theory and Applications. AVI Publ. Co., Westport, CT, pp. 477.

Garcia, E., Barret, D.M., 2006. Evaluation of processing tomato from two consecutive growing seasons: quality attributes, peelability and yield. J. Food Process. Preserv. 30, 20–36.

Gittins, R., 1985. Canonical Analysis. A Review with Application in Ecology. Stringer, Berlin.

Guil-Guerrero, J.L., Rebolloso-Fuentes, M.M., 2008. J. Food Compos. Anal. 22, 123.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 1–9.

Hartmann, A., Schmid, M., van Tuinen, D., Berg, G., 2009. Plant-driven selection of microbes. Plant Soil 321, 235–257.

Lang, N.L., Bellett-Travers, M.D., Smith, S.R., 2007. Field investigations on the survival of *Escherichia coli* and presence of other enteric micro-organisms in biosolids-amended agricultural soil. J. Appl. Microbiol. 103, 1868–1882.

Lonigro, A., Rubino, P., Brandonisio, O., Spinelli, R., Pollice, A., Laera, G., 2007. Vegetable crops irrigation with tertiary filtered municipal wastewater. Plant Biosyst. 141 (2), 275–281.

Lopez, A., Pollice, A., Laera, G., Lonigro, A., Rubino, P., 2010. Membrane filtration of municipal wastewater effluents for implementing agricultural reuse in Southern Italy. Water Sci. Technol. 625, 1121–1128.

Lopez, A., Pollice, A., Laera, G., Lonigro, A., Rubino, P., Passino, R., 2007. Reuse of membrane filtered municipal wastewater for irrigating vegetable crops. In: Proc. of the International Conference on "Water Saving in Mediterranean Agriculture & Future Research Needs, Valenzano (Bari) 14/17 febbraio 2007. Vol. 2, pp. 181–189.

Jimenez-Cuesta, M., Cuquerella, J., Martinez–Javaga, J.M., 1981. Determination of color index for citrus fruits degreening. Proc. Int. Soc. Citriculture 2, 750–753.

Keeney, D.R., Nelson, D.W., 1982. Nitrogen inorganic forms. In: Methods of Soil Analysis. Part 2, second ed. Agron Monogr 9 ASA and SSSA, Madison, WI, USA, pp. 643–698, 1982.

Madrid, R., Barba, E.M., Sanchez, A., Garcia, A.L., 2009. Effects of organic fertilizers and irrigation level on physical and chemical quality of industrial tomato fruit (cv. Nautilis). J. Sci. Food Agric. 89, 2608–2615.

Mahajan, G., Singh, K.G., 2006. Response of greenhouse tomato to irrigation and fertigation. Agric. Water Manage. 84, 202–206.

Malkawi, H.I., Mohamad, M.J., 2003. Survival and accumulation of microorganisms in soils irrigated with secondary treated wastewater. J. Basic Microbiol. 43, 47–55.

Meli, S., Porto, M., Belligno, A., Bufo, S.A., Mazzatura, A., Scopa, A., 2002. Influence of irrigation with lagooned urban wastewater on chimica and microbiological soil parameters in a citrus orchard under Mediterranean condition. Sci. Total Environ. 285, 69–77.

Monaghan, J.M., Hutchison, M.L., 2012. Distribution and decline of human pathogenic bacteria in soil after application in irrigation water and the potential for soil-splash-mediated dispersal onto fresh produce. J. Appl. Microbiol. 112, 1007–1019.

Mosse, K.P.M., Patti, A.F., Smernik, R.J., Christen, E.W., Cavagnaro, T.R., 2012. Physico-chemical and microbiological effects of long- and short-term winery wastewater application to soils. J. Hazard. Mater. 201–202, 219–228.

Nadkarni, M.A., Martin, F.E., Jacques, N.A., Hunter, N., 2002. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology 148, 257–266.

AOAC, 1990. Official Method of and Analysis. Official Method of Analysis. Association of Official Analytical Chemists, Washington, DC (No. 934.06).

AOAC, 1995. Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists, Washington, DC, pp. 16.

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of Available Phosphorus in Soil by Extraction with Sodium Bicarbonate, 939. USDA Circular, Washington, DC, pp. 1–19.

Palese, A.M., Pasquale, V., Clano, G., Figliuolo, G., Masi, S., Xiloyannis, C., 2009. Irrigation of olive groves in Southern Italy with treated municipal wastewater: effects on microbiological quality of soil and fruits. Agric. Ecosyst. Environ. 129, 43–51.

Patanè, C., Tringali, S., Sortino, O., 2011. Effect of deficit irrigation on biomass, yield, water productivity an fruit quality of processing tomato under semi-arid Mediterranen climate conditions. Sci. Hortic. 129, 540–596.

Pedrero, F., Kalavrouziotis, I., Alarcón, J.J., Koukoulakis, P., Asano, T., 2010. Use of treated municipal wastewater in irrigated agriculture—review of some practices in Spain and Greece. Agric. Water Manage. 97, 1233–1241.

Podani, J., 2007. Analisi ed esplorazione multivariata dei dati in ecologia e biologia. Liguori, Napoli, Italy.

Pollice, A., Lopez, A., Laera, G., Rubino, P., Lonigro, A., 2004. Tertiary filtered municipal wastewater as alternative water source in agriculture: a field investigation in Southern Italy. Sci. Total Environ. 324, 201–210.

Patterson, S.R., Ashbolt, N.J., Sharma, A., 2011. Microbial Risks from wastewater irrigation og salad crops: a screening-level risk assessment. Water Environ. Res. 73 (6), 667–672.

Rees, G.N., Baldwin, D.S., Watson, G.O., Perryman, S., Nielsen, D.L., 2004. Ordination and significance testing of microbial community composition derived from terminal restriction fragment length polymorphisms: application of multivariate statistics. Antonie van Leeuwenhoek 86, 339–347.

Reg. CE n. 1881/2006 del 19/12/2006 in materia di "Tenori massimi di alcuni contaminanti nei prodotti alimentari".

Reg. UE n. 1258/2011 del 2 December 2011. GU L 320/15.

Renna, M., Gonnella, M., Giannino, D., Santamaria, P., 2013. Quality evaluation of cook-chilled chicory stems (*Cichorium intybus* L., Catalogna group) by conventional and sous vide cooking methods. J. Sci. Food Agric., http://dx.doi.org/10.1002/jsfa.6302, Epub 2013 Aug 2.

Richardson, C., Hobson, G.E., 2006. Compositional changes in normal and mutant tomato fruit during ripening and storage. J. Sci. Food Agric. 40, 245–252.

Richards, L.A., 1954. Diagnosis and Improvement of Saline and Alkali Soils. USDA Agriculture Handbook 60, Washington DC.

Rubino, P., Lonigro, A., 2008. Municipal treated wastewater irrigation: microbiological risk evaluation. Ital. J. Agron. 1 (1), 119–124.

Sadocchi, S., 1981. Manuale di analisi statistica multivariata per le scienze sociali. Franco Angeli Editore, Milano, Italy, pp. 274.

Salgot, M., Huertas, E., Weber, S., Dott, W., Hollender, J., 2006. Wastewater reuse and risk: definition of key objectives. Desalination 187, 29–40.

Samarajeewa, A.D., Glasauer, S.M., et al., 2010. Evaluation of Petrifilm EC method for enumeration of *E. coli* from soil. Lett. Appl. Microbiol. 50 (5), 457–461.

Sawhney, V.K., Greyson, R.I., 1972. Fruit size increase in tomato following application of gibberellic acid. J. Am. Soc. Hortic. Sci. 97, 589–590.

Sgherri, C., Navari-Izzo, F., Pardossi, A., Soressi, G.P., Izzo, R., 2007. The influence of diluted seawater and ripening stage on the content of antioxidants in fruits of different tomato genotypes. J. Agric. Food Chem. 55, 2452–2458.

Sgherri, C., Kadlecova, Z., Pardossi, A., Navari-Izzo, F., Izzo, R., 2008. Irrigation with diluted seawater improves the nutritional value of cherry tomatoes. J. Agric. Food Chem. 56, 3391–3397.

Sidhu, J.P., Hanna, J., Toze, S.G., 2008. Survival of enteric microorganisms on grass surface irrigated with treated effluent. J. Water Health. 6 (2), 255–262.
Siebe, C., Cifuentes, E., 1995. Environmental impact of wastewater irrigation in central Mexico: an overview. Int. J. Environ. Health Res. 5, 161–173.
Soraya, G., Nadia, B., Cherubino, L., Christian, G., 2001. Tomato fruit quality in relation to water and carbon fluxes. Agronomie 21, 385–392.
Suárez, M.H., Rodríguez, E.M.R., Romero, C.D., 2007. Food Chem. 104, 489.
Telias, A., White, J.R., Pahl, D.M., Ottesen, A.R., Walsh, C.S., 2011. Bacterial community diversity and variation in spray water sources and the tomato fruit surface. BMC Microbiol. 11, 81.
Toze, S., 2006. Reuse of effluent water-benefits and risks. Agric. Water Manage. 80, 147–159.
Travis, M.J., Wiel-Shafran, A., Weisbrod, N., Adar, E., Gross, A., 2010. Greywater reuse for irrigation: effect on soil properties. Sci. Total Environ. 408, 2501–2508.
Turhan, A., Seniz, V., 2009. Estimation of certain chemical constituents of fruits of selected tomato genotypes grown in Turkey. Afr. J. Agric. Res. 4, 1086–1092.
UNI EN ISO 19250:2013. Water quality-Detection of *Salmonella* spp. (ISO 19250:2010).
Van Elsas, J.D., Semenov, A.V., Costa, R., Trevors, J.T., 2011. Survival of *Escherichia coli* in the environment: fundamental and public health aspects. ISME J. 5, 173–183.
Vivaldi, G.A., Camposeo, S., Rubino, P., Lonigro, A., 2013. Microbial impact of different types of municipal wastewaters used to irrigate nectarines in Southern Italy. Agriculture. Ecosyst. Environ. 181, 50–57.
Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining organic carbon in soils: effect of variations in digestion conditions and of inorganic soil constituents. Soil Sci. 63, 251–263.
WCED, 1987. Our Common Future. World Commission on Environmental and Development. Oxford University Press, Oxford, UK, pp. 1987.
Wood, J.D., Bezanson, G.S., Gordon, R.J., Jamieson, R., 2010. Population dynamics of *Escherichia coli* inoculated by irrigation into the phyllosphere of spinach grown under commercial production conditions. Int. J. Food Microbiol. 143, 198–204.
World Health Organization (WHO), 2006. Guidelines for the Safe Use of Wastewater, Excreta and Greywater, Volume 4. Excreta and Greywater Use in Agriculture. World Health Organization, Geneva.