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Agro-industrial wastewater reuse for irrigation of a vegetable crop succession under Mediterranean conditions

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ABSTRACT

In many countries of the Mediterranean region, characterized by frequent drought periods, agricultural production often occurs under water deficiency or conditions that cause the depletion of the existing water resources. In these areas, the reuse of reclaimed wastewater for crop irrigation could contribute to mitigate/decrease water shortage, support the agriculture sector and protect groundwater resources. In 1.5-year field experiments in Southern Italy (Apulia Region), the effects of irrigation with treated agro-industrial wastewater on soil properties, crops yield and qualitative traits of crop products, including their microbiological safety, were assessed. Groundwater (GW), secondary treated wastewater (SW) and tertiary treated wastewater (TW) from an innovative “on-demand” UV disinfection system were used to irrigate tomato and broccoli, cultivated in succession. The three irrigation water sources and the corresponding irrigated soils, plants and crop products were analyzed for the main physico-chemical characteristics, quali-quantitative parameters and fecal indicators. SW and TW showed higher values of the main physico-chemical parameters than GW. SW irrigated soil resulted in a significant increase of NH_4-N, Na^+, SAR, EC (below the threshold value beyond which a soil is defined as saline) during the first tomato crop cycle, and of pH during the broccoli growing season. Irrigation with treated wastewater did not significantly affect the marketable yield nor the qualitative traits of tomato and broccoli crops, except for the Na^+ and $NO_3{}^-$ content (below the threshold levels defined by the European guidelines for vegetables). High levels of *E. coli* (above the Italian limit for reuse), *Fecal coliforms* and *Fecal enterococci* (up to 10^4 CFU $100\,ml^{-1}$) were observed in the SW and, when chlorination was not done, in the TW. Nevertheless, *E. coli* was not isolated from any sample of soil, plant and crop product, probably due to its rapid die-off. Moreover, low concentrations of *Fecal coliforms* and *Total heterotrophic count* were found in plant and crop product. The drip irrigation system used, which avoided the close contact between water and plant, may have contributed to this. Under the conditions applied in this study, the reuse of treated agro-industrial wastewater for irrigation can be considered an effective way to cope with agricultural water shortage in the Mediterranean area.

1. Introduction

A number of Mediterranean countries suffer from water scarcity, which has become severe in recent years due to global climate change causing frequent and long lasting periods of drought. Particularly during the summer, these areas experience severe water supply and demand imbalances. In the last decades, many Italian regions have faced the negative impact of drought and the resource scarcity has mostly penalized agricultural activities, which uses more than 50% of the total available water, while other high priority demands, such as those from civil and industrial sectors, are satisfied (Coppola et al., 2004). Particularly, in Apulia Region (South-Eastern Italy), water shortage has a serious impact on the local economy, mostly based on agriculture. Moreover, the agricultural coastal areas of Apulia Region suffer from relevant phenomena of seawater intrusion into the water table, due to the excessive and often uncontrolled groundwater withdrawals for irrigation (Libutti and Monteleone, 2012). For these reasons, alternative water sources are needed.

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Treated municipal wastewater is the most readily available source of water to meet the increasing demand for crop irrigation. Indeed, in recent years, wastewater recycling in agriculture has gained importance as component of agricultural water supply in several water-scarce countries (Qadir et al., 2007; Pedrero et al., 2010). Wastewater reuse not only provides significant amount of irrigation water, but also contributes to conserve potable resources and reduces the environmental impact related to the effluents discharge into water bodies (Aiello et al., 2007; Pedrero et al., 2010; Agrafioti and Diamadopoulos, 2012). Furthermore, soil application of treated wastewater also constitutes a reliable source of nutrients (especially nitrogen, phosphorus and potassium) and organic matter useful for maintaining the fertility and the productivity of the soil (Meli et al., 2002; Rusan et al., 2007). Treated wastewater use for crop irrigation can improve growth and yield of herbaceous species (Kiziloglu et al., 2008; Bedbabis et al., 2010) and can also enhance the economic benefits for farmers, due to reduced need for fertilizer (Bedbabis et al., 2010; Paranychianakis et al., 2006). However, the chemical composition of wastewater has to be monitored to avoid imbalance in nutrient supply, which may result in excessive vegetative growth, uneven fruit maturity, reduced quality and quantity of yields (Pedrero et al., 2010). Treated wastewater should be used for irrigation under controlled conditions, also to minimize hazards to agricultural products, soil and groundwater from toxic and pathogenic contaminants (Aiello et al., 2007; Qadir et al., 2007). Wastewater may contain a variety of pollutants, such as salts, heavy metals, organic compounds, enteric bacteria and viruses. An excessive accumulation of trace metals, such as Cd, Cu, Fe, Mn, Pb and Zn, in soils through irrigation creates problems for agricultural production (Singh et al., 2004) and leads to metal uptake by crops, so affecting food quality and safety (Khan et al., 2008). One of the crucial issues in the reuse of treated wastewater for crop irrigation is the residual presence of pathogenic microorganism (Rubino and Lonigro, 2008; Petterson et al., 2011) which represents a potential health risk to consumers when they enter in the food chain (Toze, 2006). In Italy, there are strict regulations for reclaimed wastewater reuse (Decree No. 152, 2006, Ministry for Environment), especially for levels of some chemical compounds and for microbial parameters. With regard to microbiological contamination levels, the corresponding guidelines allow unrestricted crop irrigation with a bacteriological effluent quality characterized by less than 10 CFU 100 ml^{-1} of *E. coli* in 80% of samples.

Several experimental evidences underline the effect on soil and yield of reclaimed urban wastewater application for vegetable and fruit crops irrigation in the Mediterranean area (Lonigro et al., 2007; Palese et al., 2009; Disciglio et al., 2014; Lonigro et al., 2015; Gatta et al., 2016). Our research activity focuses instead on treated agro-industrial wastewater reuse in agriculture. The reused agro-industrial wastewater was originated by an agricultural and food manufacturing company, which produces and processes vegetables, in Apulia Region (Southern Italy). Studies about the reuse of wastewater of agro-industrial origin for crop irrigation have been little evaluated and are poorly documented in literature. Recovery of this type of wastewater is instead of particular relevance in the Apulia Region where the economic system is strongly based on irrigated agriculture and characterized by a widespread presence of agro-food industries, whose activity annually releases large quantities of wastewater, usually discharged into torrents or rivers. Moreover, Apulia is one of the European regions most heavily affected by water shortage (Xiloyannis et al., 2002). In this contest, a careful management of agro-industrial wastewater for agricultural purposes represents a useful alternative to conventional water resources, allows to support irrigated agriculture, reduces costs and environmental impact of wastewater disposal. These advantages become even more useful when vegetable processing companies produce wastewater and grow the crops at the same site, as in the case study reported in this work. More specifically, the present paper reports the results of a 1.5-year research on irrigating vegetable crops with this type of water source, in Southern Italy. Two treatment schemes were applied to wastewater and the treated effluents were used for the irrigation of a test-field where processing tomato and broccoli crops were cultivated in close succession. With reference to the experimental trial described here, others papers, reporting the results of the first tomato crop cycle, have been already published (Disciglio et al., 2015; Gatta et al., 2015a,b). Unlike all these cited studies, which had the objectives to evaluate the effects of secondary treated agro-industrial wastewater on different soil and tomato crop yield characteristics, the present paper deals with the irrigation use of an additional treated wastewater type, such as the tertiary treated wastewater, and a longer experimental period, such as the succession of three cropping cycles, in order to assess: (i) the impact of treated wastewater on the main chemical properties of irrigated soil; (ii) the effects of treated wastewater on quantitative and qualitative aspects of crops yield; (iii) the risk of microbiological contamination of irrigated soil and the pathogen health risk of crop products.

2. Materials and methods

2.1. Study area and experimental site

The study was carried out in open field, from April 2012 to September 2013, in the north-west part of the Apulia Region (Southern Italy), at Stornarella (41° 15′N, 15° 44′E; altitude, 154 m a.s.l.), in the Foggia district. The area is characterized by a Mediterranean climate, with air temperatures that drops below 0° in winter and exceed peaks of 40 °C in summer. The long-term average annual rainfall is 590 mm, with precipitations unevenly distributed throughout the year and predominantly concentrated in the period from October to April (Caliandro et al., 2005). During the experimental period, the daily meteorological parameters, such as maximum and minimum air temperature (T_{MAX}, T_{MIN}), maximum and minimum air relative humidity (RH_{MAX}, RH_{MIN}), wind speed (W_S), and total precipitation (P) were recorded. These were measured by a weather station placed close to the experimental field and recorded using a data-logger (Campbell Scientific, USA).

The study area is characterized by a widespread presence of agro-food industries specialized in vegetables processing. The experimental site was located within the Fiordelisi agricultural and food manufacturing company. Fiordelisi's activity includes growing, processing, packaging and marketing of preserved, ready-to-eat vegetables, such as tomato, eggplant, zucchini and pepper. This activity involves the production of large quantities of wastewater that undergoes a purification process before being discharged according to local regulations. To this purpose, the Fiordelisi company is equipped with a wastewater treatment plant (WWTP). The WWTP was upgraded in order to produce effluents suitable for reuse, thus supplying the two types of treated wastewater used for crops irrigation during the experimental period, one produced by the conventional treatment system and the other by the upgraded configuration. The reclaimed water was then used within a closed-circle system where the wastewater produced as a by-product from vegetables processing becomes a water resource for vegetable crops irrigation, according to the criteria of recycling treated wastewater as a component of agricultural water supply, in water-scarce environmental conditions.

In the years 2012 and 2013, the Fiordelisi company produced about 100,000 $m^3 y^{-1}$ of wastewater, mainly composed of washing and processing water (vegetables cooking, steaming, bottles washing, etc.), water from cleaning floors and equipment, and toilet water (5–10%). The wastewater was purified in the WWTP

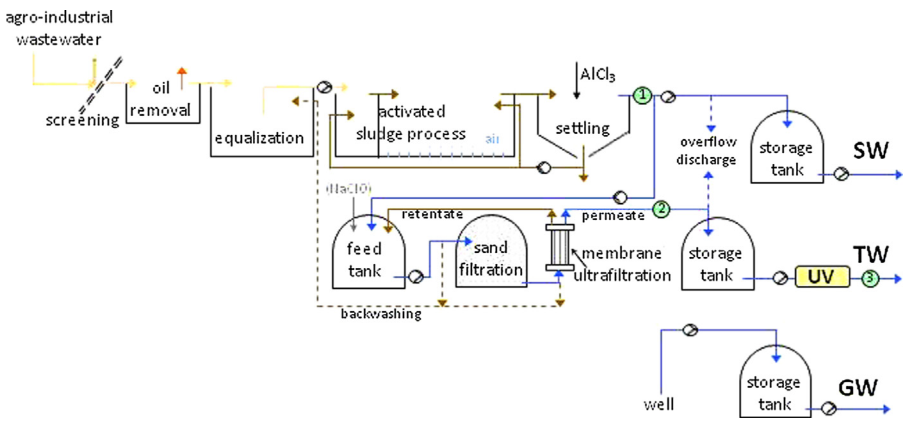

Fig. 1. Configuration of the wastewater treatment plant (WWTP) operated at Fiordelisi company. The scheme shows the production processes of the two different types of reclaimed wastewater (SW and TW), before being used in the experimental field. In the Figure, the supply scheme of GW is also reported.

(Fig. 1) based on the following processes: i) primary treatments, such as screening, oil removal, equalization, and pH adjustment; ii) secondary treatments, such as activated sludge process (anoxic plus aerobic phases) and chemically assisted secondary sedimentation; iii) tertiary treatments, such as chlorination (applied only during the period 01 October 2012–31 March 2013), sand filtration, membrane ultrafiltration (by 8 modules, Hyflux Kristal® 600ET, with a nominal surface of 60 m^2 and a nominal pore size of 0.05 μm), and UV radiation (by 6 mercury-vapor lamps, 200W each). The effluent of the secondary sedimentation is called secondary treated wastewater (SW), whereas the UV outlet is called tertiary treated wastewater (TW). SW and membrane filtered effluent were conveyed to the respective 10 m^3 storage tanks. In order to maximize its effectiveness and save on energy costs, the UV disinfection system was operated in-line with irrigation. Thus the TW was disinfected just before being distributed to the plants ("on-demand" disinfection) (Fig. 1). Periodical backwashing of the sand filter (15 min duration every 8 h of operation) and the ultrafiltration membranes (30 s duration every 45 min of operation) was performed with the membrane permeate. Membranes were also cleaned chemically (with diluted soda and sodium hypochlorite) approximately every 10 days, according to the procedures suggested by the manufacturer. Membrane ultrafiltration was monitored for the operating pressure and the permeate flux.

The experiments were carried out in a clay-loam soil (USDA 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 1.4 Mg m^{-3}. The main physico-chemical properties of the 0–0.60 m soil layer of the experimental field, as characterized before trial started, are: 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, 730.0 mg kg^{-1}; total N, 0.8‰ (Kjeldahl); mineral NO_3-N, 4.8 mg kg^{-1}; mineral NH_4-N, 7.5 mg kg^{-1}; pH 7.9; electrical conductivity, 0.5 dS m^{-1}; Na^+, 856.0 mg kg^{-1}; Ca^{2+}, 4,060.0 mg kg^{-1}; Mg^{2+}, 250.0 mg kg^{-1}; sodium adsorption ratio of soil satured paste extract (SAR), 4.9.

2.2. Crop rotation and agronomic conditions

During the experimental period, three vegetable crops were grown in close succession: processing tomato, broccoli and processing tomato. Tomato (*Lycopersicon esculentum* Mill.), cultivar "Manyla", was transplanted on April 12, 2012 as to the first crop cycle. Broccoli (*Brassica oleracea* L. var. *italica*), ibrid "Partenon" F_1, was transplanted on October 12, 2012. Tomato, cultivar "Manyla", was transplanted on April 5, 2013, as the second crop cycle. The seedlings were transplanted in double rows (40 cm apart) spaced at 250 cm, at a distance of 30 cm along each single row, realizing a final plant density of 2.7 plants m^{-2}. Tomato and broccoli plants were grown under a net house structure, which was covered with an anti-hail net. In particular, tomato plants were grown vertically using nylon threads positioned between the plant collar 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.

During each growing season, the plants were irrigated whenever the soil water deficit in the effective root zone (0–50 cm in depth) was 40% of the total available soil water (Allen et al., 1998). This irrigation threshold was assessed through a continuous monitoring of the volumetric soil water content using probes operating in the frequency domain reflectometry and installed in each plot prior to crops transplanting at 5, 15, 25, 35 and 45 cm soil depth. At each irrigation, the soil water content was increased to field capacity, with a water volume varying from 100 to 400 m^3 ha^{-1}, depending on the crop growth stage. The seasonal irrigation volumes were 4957 m^3 ha^{-1} for tomato crop in 2012, 922 m^3 ha^{-1} for broccoli crop in 2012–2013 and 4070 m^3 ha^{-1} for tomato crop in 2013. A drip irrigation method was used for the three crops. This compriseda single drip line, with drippers at 2 l h^{-1} flow rate, spaced every 40 cm, and placed in the middle of each couple of plant rows. During the two tomato crop cycles, the drip lines were placed under a black plastic mulching film.

All agricultural management practices, including fertilization, weed and pest control applied to the three crops, were performed according to the agronomic techniques commonly adopted by local farmers. Before transplanting, the soil was subsoiled to a depth of 45 cm and its surface was milled. A pre-transplanting fertilization was then applied by distributing 35 kg ha^{-1} N and 70 kg ha^{-1} P_2O_5 before the two tomato cropping cycles started, and 21 kg ha^{-1} N and 35 kg ha^{-1} P_2O_5 before the broccoli cropping cycle started. Throughout each of the two tomato crop cycles, 75 kg ha^{-1} N and 100 kg ha^{-1} P_2O_5 were added through fertirrigation. During the broccoli crop cycle a top-dressing fertilization was applied to the soil by distributing 63 kg ha^{-1} N.

2.3. Experimental layout

During the first tomato crop cycle, two irrigation treatments were applied: irrigation with GW and irrigation with SW. During the broccoli and the second tomato crop cycles a third treatment was also considered: irrigation with TW. The tertiary treatment of wastewater produced by Fiordelisi company was introduced in September 2012, just before the start of the broccoli crop cycle, to produce effluents with lower suspended solids and microbiological pollution.

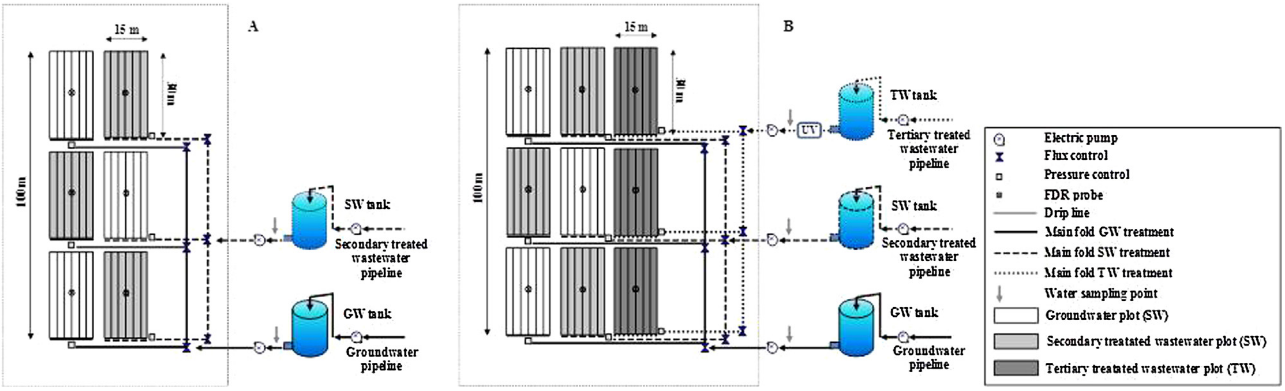

Fig. 2. Layout of the experimental field and irrigation system, during the Tomato 2012 growing season (A); the Broccoli 2012–2013 and the Tomato growing seasons 2013 (B).

GW represents the irrigation source normally used by local farmers for crop irrigation; it was withdrawn from a phreatic well located near the experimental field and stored in a 10 m^3 tank before being used for tomato and broccoli irrigation (Fig. 1). GW, SW and TW were directly pumped from their storage tanks to the drip irrigation system used for plants watering (Fig. 2). As previously mentioned, TW was UV disinfected in-line with the distribution.

The experimental field was arranged according to a complete randomized block design with the irrigation treatments (GW, SW and TW), each one replicated three times for a total of 6 plots for the first tomato crop (2 irrigation treatments × 3 replicates) and 9 plots for the broccoli and the second tomato crops (3 irrigation treatments × 3 replicates) (Fig. 2). Each plot was 450 m^2 (15 m wide × 30 m long) with a sampling area of 20 m^2 (2.5 m wide × 8.0 m long).

2.4. Water, soil, plant and crops product sampling

The raw[1] agro-industrial wastewater produced by Fiordelisi (RW) was collected after the equalization step (Fig. 1). Samples of SW immediately after the settling process (sampling point 1 in Fig. 1), samples of membrane permeate immediately after membrane ultrafiltration (sampling point 2 in Fig. 1) and samples of TW immediately after the UV disinfection (sampling point 3 in Fig. 1), were collected. Sampling was performed monthly, from October 2012 to September 2013. Irrigation water samples (GW, SW and TW) were collected monthly, over the whole irrigation period of each crop, after tank storage and immediately before their inlet in the irrigation system (Fig. 2). At each sampling date, three samples of each water type were collected using sterile 1000-ml glass bottles and transported to the laboratory in refrigerated bags. They were then kept in a refrigerator at +4 °C, and examined within 24 h of collection.

Soil samples were collected in triplicate from the GW, SW and TW irrigated plots, before each crop transplantation and, at monthly intervals, during each crop cycle. All soil samples were taken from a 0–30 cm soil layer (high density root zone), from under the drippers, by using a soil auger. They were air-dried and passed through a 2 mm sieve, before the laboratory analysis.

During each growing season, samples of tomato and broccoli plants were collected at the same time of soil sampling, in triplicates from each experimental plot. Samples of tomato fruits and broccoli heads were collected at each harvesting date, from the sampling area of 20 m^2 of each experimental plot. Plants, tomato fruits and broccoli heads were immediately transported to the laboratory in refrigerated bags and analyzed within few hours from the sampling.

2.5. Water, soil, plant and crops product analysis

All the water samples, both from WWTP and irrigation system, were analyzed according to Standard Methods (APHA-AWWA-EF, 2005) for the physico-chemical and microbiological parameters reported in Table 1. The pH was measured using a GLP 22+ pH & Ion Meter (CRISON INSTRUMENTS, Spain) and the EC with a GLP 31+ EC-Meter (CRISON INSTRUMENTS, Spain). 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 Na^+, Ca^{2+}, Mg^{2+}, and K^+ levels were determined by ion-exchange chromatography (Dionex ICS-1100, Dionex Corporation, Sunnyvale, CA, USA). The SAR was calculated using the formula (with concentrations in $\text{mmol}_c\ l^{-1}$) (US Salinity Laboratory Staff, 1954): $SAR = (Na^+)/[(Ca^{2+} + Mg^{2+})/2]^{1/2}$.

Soil samples from each experimental plot were analyzed for the physico-chemical and microbiological parameters showed in Table 1. The pH was measured in 1:2.5 (w/v) aqueous soil extract and the EC in 1:2 (w/v) aqueous soil extract. The total N was determined by the Kjeldahl method (Bremner, 1996). The NO_3-N and NH_4-N were determined according to Keeney and Nelson (1982). The P_2O_5 was determined by using the sodium bicarbonate method (Olsen et al., 1954). The total organic carbon was detected by oxidation with a potassium dichromate-titration of $FeSO_4$ according to Walkley-Black Method (1947) and the OM was appraised by multiplying the percentage of the organic carbon by the factor 1.724. The Na^+, Ca^{2+}, Mg^{2+} and K^+ were determined in soil saturated paste extracts and analyzed by using Atomic Absorption Spectroscopy – AAS (Perkin-Elmer Atomic Absorption Spectrophotometer – model 2380). The SAR was calculated using the measurements of Na^+, Mg^{2+} and Ca^{2+} concentration ($\text{mmol}_c\ l^{-1}$) in the soil satured paste extract according to Qadir et al. (2001): $SAR = Na^+/[(Ca^{2+} + Mg^{2+})/2]^{1/2}$.

Water samples from WWTP and irrigation system were analyzed for the enumeration of the bacteria listed in Table 1, which are useful indicators of contamination (Tallon et al., 2005; Pourcher et al., 2007). The membrane filtration method was used as follows: 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 44 °C for 24 h. For *Fecal coliforms* enumeration, the membranes were placed onto chromogenic E.coli agar (C-EC agar;

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[1] Wastewater before purification in wastewater treatment plant.

Table 1
Analytical parameters determined on water, soil, plant and crop product, during the Tomato 2012, Broccoli 2012–2013, and Tomato 2013 growing seasons.

Water samples		Soil samples	Crop samples		
from WWTP	from irrigation system		Plant	Product Tomato fruit Quantitative analysis	Broccoli head
PHYSICO–CHEMICAL ANALYSIS					
pH	pH	pH		Total yield –TY (Mg ha^{-1})	TY (Mg ha^{-1})
EC (dSm^{-1})	EC (dSm^{-1})	EC (dSm^{-1})		Total marketable yield – TMY (Mg ha^{-1})	TMY (Mg ha^{-1})
TSS (mg l^{-1})	TSS (mg l^{-1})	Total N (mg kg^{-1})		Marketable yield per plant – YP (kg plant^{-1})	YP (kg plant^{-1})
Total N (mg N l^{-1})	NH_4-N (mg N l^{-1})	NO_3-N (mg kg^{-1})		Total nonmarketable yield – TNMY (Mg ha^{-1})	TNMY (Mg ha^{-1})
PO_4-P (mg P l^{-1})	NO_3-N (mg N l^{-1})	NH_4-N (mg kg^{-1})		Nonmarketable yield per plant – NMYP (kg plant^{-1})	NMYP (kg plant^{-1})
BOD_5 (mg O_2 l^{-1})	NO_2-N (mg N l^{-1})	P_2O_5 (mg kg^{-1})			
COD (mg O_2 l^{-1})	PO_4-P (mg P l^{-1})	Organic matter – OM (%)			
Surfactants (mg l^{-1})	BOD_5 (mg O_2 l^{-1})	Na^+ (mg kg^{-1})			
	COD (mg O_2 l^{-1})	Ca^{2+} (mg kg^{-1})			
	Na^+ (mg l^{-1})	Mg^{2+} (mg kg^{-1})			
	Ca^2 (mg l^{-1})	K^+ (mg kg^{-1})			
	Mg^{2+} (mg l^{-1})	SAR			
	K^+ (mg l^{-1})				
	$CO_3{}^{2-}$(mg l^{-1})				
	$HCO_3{}^-$(mg l^{-1})				
	$SO_4{}^{2-}$ (mg l^{-1})				
	SAR				
	Hardness (mg l^{-1} $CaCO_3$)				
				Qualitative analysis	
				Mean diameter (equatorial and longitudinal diameters) – D (cm)	D (cm)
				a*/b* ratio (Jimenez-Cuesta et al., 1981) – CI	DM (% fresh matter)
				Dry matter content (AOAC, 1990) – DM (% fresh matter)	Ca^{2+} (mg 100 g^{-1} of fresh matter)
				Soluble solids content of the flesh – SSC (°Brix)	Mg^{2+} (mg 100 g^{-1} of fresh matter)
				Titrable acidity (AOAC, 1995) – TA (g citric acid 100 ml^{-1} fresh juice)	K^+ (mg 100 g^{-1} of fresh matter)
				pH	Na^+ (mg 100 g^{-1} of fresh matter)
				Ca^{2+} (mg 100 g^{-1} of fresh matter)	$NO_3{}^-$ (mg 100 g^{-1} of fresh matter)
				Mg^{2+} (mg 100 g^{-1} of fresh matter)	
				K^+ (mg 100 g^{-1} of fresh matter)	
				Na^+ (mg 100 g^{-1} of fresh matter)	
				$NO_3{}^-$ (mg 100 g^{-1} of fresh matter)	
MICROBIOLOGICAL ANALYSIS					
E. Coli (log CFU 100 ml^{-1})	*E. Coli* (log CFU 100 ml^{-1})	*E. Coli* (log CFU g^{-1})	*E. Coli* (log CFU g^{-1})	*E. Coli* (log CFU g^{-1})	*E. Coli* (log CFU g^{-1})
	Fecal coliforms (log CFU 100 ml^{-1})	*FC* * (log CFU g^{-1})	*FC* (log CFU g ml^{-1})	*FC* (log CFU g ml^{-1})	*FC* (log CFU g ml^{-1})
	Fecal enterococci (log CFU 100 ml^{-1})	*THC* ** (log CFU g^{-1})	*THC* (log CFU g^{-1})	*THC* (log CFU g^{-1})	*THC* (log CFU g^{-1})

*Fecal coliforms.
**Total heterotrophic count.

Biolife, Milan, Italy) and incubated at 44 °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.

Microbiological analysis of the soil, plant and product samples from each experimental treatment included the determination of the parameters showed in Table 1. 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. Then serial 10-fold dilutions in buffered peptone water were spread onto agar plates containing TBX for *E. coli*, C-EC agar for *Fecal coliforms*, and tryptic soy agar for *Total heterotrophic count*. The plates were incubated under different incubation temperatures (and times) as follows: 37 °C for *E. coli* (24 h), 44 °C for *Fecal coliforms* (48 h), and 22 °C for *Total heterotrophic count* (72 h).

At each harvesting date, tomato fruits and broccoli heads from each GW, SW and TW irrigated plot were analyzed for quantitative traits. All the marketable and discarded tomato fruits and broccoli heads were counted and weighed respectively, to estimate the yield parameters (Table 1). At the same time, a sample of 10 tomato fruits and a sample of 10 broccoli heads from each plot were transported to the laboratory and immediately analyzed for qualitative traits (Table 1). The color parameters of tomato fruits 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[2] is reported, which represents an index that describes well the color changes of tomato fruit (Francis and Clydesdale, 1975; Favati et al., 2009). The cation and the anion contents of both tomato fruits and broccoli heads were determined by ion-exchange chromatography (Dionex ICS-1100, Dionex Corporation, Sunnyvale, CA, USA).

2.6. Statistical analysis

The data related to the physico-chemical characteristics of the two irrigation waters and the corresponding irrigated soils, the quantitative and qualitative parameters of the crop products, the microbiological properties of the irrigation waters, soils, plants and crop products, collected during the first tomato crop cycle, were analyzed by unpaired *t*-tests. The same data, detected on the three irrigation water sources and the corresponding irrigated soils, plants and crop products, during the broccoli and the second tomato crop cycles, were statistically processed by analysis of variance (ANOVA). When significant effects were detected ($P \leq 0.05$), mean multiple comparisons were performed according to Tukey test. Two different statistical tests were applied because the considered experimental factor (irrigation water) had two levels (irrigation water type: GW and SW) during the first tomato cropping cycle, and three levels (irrigation water type: GW, SW and TW) during the following broccoli and tomato cropping cycles. Therefore, for each experimental data, two and three mean values were statistically discriminated by *t*-test and Tukey test, in the first and second case, respectively. Before being processed, all the microbiological data were logarithmically transformed ($\log 10\ X+1$). When in water, soil, plant and crop product, microbial indicators were not detected the corresponding levels were set equal to zero. The addition of the unit value to X was necessary in order to prevent an impossible solution in case of $X = 0$. All the statistical analyses were performed using the JMP software package, version 8.1 (JMP, 2008).

3. Results and discussion

3.1. Climatic conditions

The average monthly values of the meteorological parameters recorded during the experimental period are reported in Table 2. During the Tomato 2012 and Tomato 2013 growing seasons, mean values of air temperature were similar. Rainfall was higher in 2013 than in 2012, with 43% of total precipitation occurred in August. In 2012, 62% of total precipitation was recorded in the first part of cultivation cycle, in April. During the Broccoli 2012–2013 growing season, rainfall was characterized by an almost regular distribution over the months with a peak in November when 36% of total precipitation occurred.

3.2. Physico-chemical properties of water from wastewater treatment plant

The average values of the main physico-chemical parameters measured in RW, SW and TW from WWTP are reported in Table 3, where also the Italian standards for reuse in irrigation of reclaimed wastewater are showed. RW had low pH, high salinity, and a considerable presence of TSS, organic substances (as demonstrated by the high COD value) and surfactants. The activated sludge process and the following secondary settling effectively removed most suspended solids and organic substances as well as nitrogen and phosphorus, with average removal percentages of 89%, 96%, 86%, 94%, and respectively.

As previously reported, the tertiary treatment of wastewater was introduced in September 2012, before broccoli crop cycle started. The main objective was to remove the residual suspended solids and the microbiological pollution, which represented the main non-compliances for the SW with respect to the standards for reuse in agriculture (Table 3). The influent pressure to the sand filter was maintained between 1.5 and 2.0 bar and the residual pressure in the influent to the membranes was constantly at 0.5–0.6 bar. The average flow produced was $12\ m^3\ h^{-1}$, corresponding to a specific flux of the membranes of $25\ l\ m^{-2}\ h^{-1}$. During the first 6 months (October 2012 – March 2013) of tertiary treatment operation, in correspondence of broccoli cropping cycle, the sand filtration unit was preceded by a chlorination step. This removed completely the monitoring indicator *E. coli*, whose concentration was always below $10\ CFU/100\ ml^{-1}$ in both the influent to the sand filtration unit and in the TW.

From March 2013 the chlorination step was stopped. This allowed to evaluate the effect of the ultrafiltration and UV radiation processes on *E. coli* removal (Fig. 3). A residual presence of suspended solids and *E. coli* was observed in the membrane permeate, indicating a possible leakage for one or more modules. Membrane integrity was then checked through a pressure decay test (ASTM, 2003), which indicated the presence of leakages in 6 modules out of 8. Nevertheless, the concentration of suspended solids in the membrane permeate was on average within the limit for reuse ($10\ mg\ l^{-1}$). Before UV disinfection, which was performed on-line with the irrigation, the TW was stored in the $10\ m^3$ tank (Fig. 1) for a period between 1 and 7 days, depending on the irrigation needs. In the storage tank the concentration of suspended solids tended to increase during this time, possibly due to bacterial re-growth, and reached an average value of $17.5 \pm 9.4\ mg\ l^{-1}$ (Fig. 3). This phenomenon may have limited the effectiveness of the UV disinfection,

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[2] a^*, red/green chromaticity (negative values indicate green, while positive values indicate red); b^*, yellow/blu chromaticity (negative values indicate blue, while positive values indicate yellow). So, the higher the a^*/b^* ratio, the higher the red color of tomato fruit.

Table 2
Main climatic parameters recorded during the Tomato 2012, Broccoli 2012–2013, and Tomato 2013 growing seasons.

Growing season	Tomato 2012						Broccoli 2012–2013						Tomato 2013						
Month	Apr	May	Jun	Jul	Aug	*Seasonal Value*	Oct	Nov	Dic	Jan	Feb	*Seasonal value*	Apr	May	Jun	Jul	Aug	Sept	*Seasonal value*
Climatic parameter[a]																			
T_{MAX} (°C)	20.1	25.0	33.0	34.4	34.5	**29.4**	23.1	17.5	12.1	12.0	11.0	**15.1**	20.7	23.7	28.2	31.2	31.8	27.3	**27.2**
T_{MIN} (°C)	8.5	11.6	17.9	20.8	20.2	**15.8**	12.1	10.1	4.3	3.9	2.7	**6.6**	8.7	11.6	15.1	18.6	19.3	15.7	**14.8**
R_{MAX} (%)	95.6	82.8	71.1	77.1	81.4	**81,6**	99.3	98.9	98.3	98.4	98.2	**98.6**	96.3	97.6	90.7	88.5	88.9	95.6	**92.9**
R_{MIN} (%)	51.7	36.6	27.3	30.6	29.4	**35.1**	57.7	75.7	68.1	61.7	67.2	**68.1**	51.7	47.7	39.6	38.5	41.9	43.8	**43.9**
P (mm)	67.0	28.0	0.0	8.4	5.0	**108.4**	40.4	145.6	68.6	66.0	82.8	**403.4**	40.0	65.1	33.0	20.8	132.8	20.4	**312.1**
W_S (m s^{-1})	2.3	2.4	2.7	2.4	2.1	**2.4**	1.8	1.8	1.8	2.4	3.0	**2.2**	2.4	1.4	1.7	2.2	2.2	2.5	**2.1**
E_V (mm)	86.9	137.5	197.9	195.3	176.5	**794.1**	53.0	25.9	18.8	18.2	27.3	**143.2**	89.6	108.2	114.2	164.3	143.5	99.1	**695.5**

[a] $T_{MAX,}$ T_{MIN}, monthly maximum, minimum air temperature; $R_{MAX,}$ R_{MIN}, monthly maximum, minimum relative air humidity; P, precipitation; W_S, monthly mean wind speed; E_V, total "class A" pan evaporation.

Table 3
Average values of the main physico-chemical parameters measured on raw agro-industrial wastewater after equalization step (RW), secondary treated wastewater after settling process (SW) and tertiary treated wastewater after UV disinfection (TW). The values are compared with the Italian standard for treated wastewater reuse for crop irrigation (Legislative Decree no. 152/2006).

Water type	RW	SW	TW	Limits for irrigation reuse
Parameter				
pH	5.7 ± 0.5	6.8 ± 0.4	7.5 ± 0.5	6–9.5
EC (dS m^{-1})	2.7 ± 1.1	2.8 ± 0.7	2.7 ± 0.5	3.0
TSS (mg l^{-1})	249.1 ± 89.3	26.6 ± 14.6	15.7 ± 4.9	10
Total N (mg l^{-1})	27.7 ± 8.0	3.9 ± 1.9	4.0 ± 1.8	35
PO_4-P (mg l^{-1})	6.4 ± 3.1	0.4 ± 0.4	0.4 ± 0.4	10
BOD_5 (mgO_2 l^{-1})	–	23.1 ± 1.3	22.5 ± 0.4	20
COD (mgO_2 l^{-1})	985.3 ± 321.6	40.6 ± 16.9	37.7 ± 14.5	100
Surfactants (mg l^{-1})	10.3 ± 5.7	0.7 ± 0.2	0.6 ± 0.1	0.5
Escherichia Coli (CFU 100 ml^{-1})	7.7E + 06 *	3.5E + 04*	6.4E + 02*	1.0E + 01*
	3.6E + 07 **	7.0E + 04**	3.0E + 03**	1.0E + 02**

Data are means ± standard errors for each analyzed parameter, determined on 36 samples for each water type (1 sample per irrigation water type × 3 replicates × 12 sampling dates).
*80th percentile.
**Maximum value.

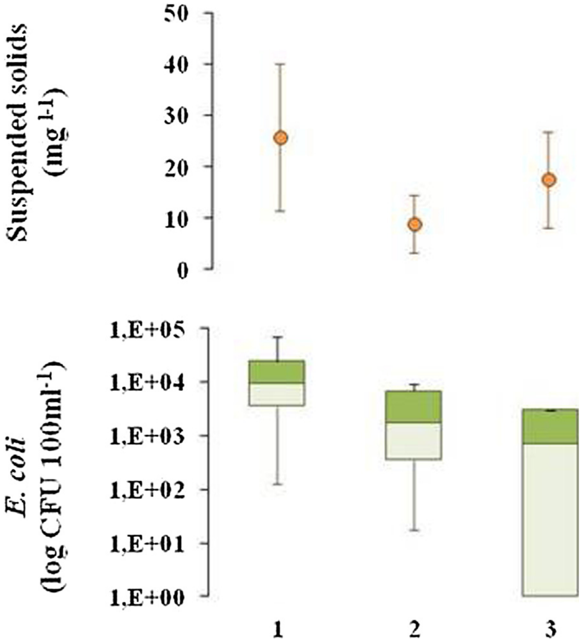

Fig. 3. Concentration of suspended solids and *E. coli* measured in the influent to the filtration unit (1), in the effluent of the membrane ultrafiltration (2), and in the UV outlet (3), from March to September 2013. The locations of sampling points 1–3 are showed in Fig. 1. Vertical bars indicate standard errors (n = 21, 1 sample per water type × 3 replicates × 7 sampling dates).

which is known to decrease with increasing turbidity. Indeed, the removal of the fecal indicator *E. coli* after the UV radiation varied between 0.5 and 3 log units (Fig. 3).

3.3. Physico-chemical properties of irrigation water

Table 4 shows the physico-chemical properties of GW, SW and TW measured during Tomato 2012, Broccoli 2012–2013 and Tomato 2013 growing seasons. The characteristics of the three sources of irrigation water resulted significantly different, during each crop cycle. On the contrary, for each type of irrigation water, very low differences were observed among the crop cycles.

The three water sources were alkaline, with a significantly higher pH for GW than SW and TW only during the Broccoli 2012–2013 growing season. Compared with GW, SW and TW were always characterized by higher values of NH_4-N and NO_2-N, P, K, Ca, and Mg. The presence of these chemical components in treated wastewater is very interesting from an agronomic point of view, since they represent important nutrients for improving soil fertility, plant growth and crop yield (Rodriguez-Liébana et al., 2014). With respect to GW, SW and TW had also a higher content of organic matter, as indicated by BOD_5 and COD values, and significantly higher values of Na^+, EC and SAR. According to Ayers and Westcot (1985), as regards the EC and Na^+ values observed in the two treated wastewaters, their reuse for irrigation can be considered from slightly to moderately restricted; moreover, if the values of SAR are related to the values of EC it follow that their application would not cause a reduction of the infiltration rate into the soil. Also hardness and TSS were significantly higher in the two treated wastewaters than in GW.

During the whole experimental period, GW had significantly higher NO_3-N concentration (29.06, 23.19 and 22.98 mg l^{-1} dur-

Table 4
Average values of the main physico-chemical parameters of water used for irrigation measured over the three growing seasons (Tomato 2012, Broccoli 2012–2013, and Tomato 2013), on groundwater (GW), secondary treated wastewater (SW) and tertiary treated wastewater (TW) used for crops irrigation.

Growing season	Tomato 2012			Broccoli 2012–2013				Tomato 2013			
Irrigation water	GW	SW	Sign[a]	GW	SW	TW	Sign	GW	SW	TW	Sign [a]
Parameter											
pH	7.63 ± 0.10a	7.76 ± 0.09a	ns	8.19 ± 0.08a	7.59 ± 0.07b	7.38 ± 0.07b	***	7.75 ± 0.05a	7.84 ± 0.08a	7.65 ± 0.10a	ns
EC (dS m^{-1})	0.69 ± 0.05b	2.18 ± 0.12a	*	0.81 ± 0.02b	3.05 ± 0.28a	2.85 ± 0.66a	***	0.87 ± 0.01b	3.07 ± 0.20a	2.98 ± 0.20a	***
TSS (mg l^{-1})	3.26 ± 0.34b	16.21 ± 2.24a	*	3.44 ± 0.76b	18.04 ± 5.06a	16.29 ± 6.60a	**	3.70 ± 2.28b	17.63 ± 4.17a	14.88 ± 3.01a	**
NH_4-N (mg l^{-1})	0.04 ± 0.00b	0.39 ± 0.10a	*	0.04 ± 0.01b	0.09 ± 0.02a	0.07 ± 0.01ab	**	0.05 ± 0.01b	2.00 ± 0.52a	2.13 ± 0.47a	**
NO_3-N (mg l^{-1})	29.06 ± 1.67a	1.20 ± 0.23b	*	23.18 ± 0.89a	0.83 ± 0.21b	2.09 ± 0.87b	***	22.95 ± 1.00a	0.66 ± 0.15b	2.05 ± 1.53b	***
NO_2-N (mg l^{-1})	0.02 ± 0.01b	0.07 ± 0.02a	*	0.02 ± 0.01b	0.05 ± 0.01a	0.05 ± 0.01a	**	0.02 ± 0.01b	0.10 ± 0.03a	0.11 ± 0.03a	**
PO_4-P (mg l^{-1})	0.10 ± 0.01b	0.29 ± 0.02a	*	0.17 ± 0.02b	0.41 ± 0.06a	0.49 ± 0.06a	**	0.13 ± 0.01b	0.38 ± 0.04a	0.34 ± 0.06a	**
BOD_5 (mg O_2 l^{-1})	9.33 ± 1.03b	21.58 ± 1.62a	*	6.30 ± 0.60b	23.92 ± 3.02a	22.75 ± 0.33a	***	10.70 ± 1.25b	23.70 ± 2.00a	22.15 ± 1.82a	***
COD (mg O_2 l^{-1})	18.44 ± 1.62b	39.73 ± 2.78a	*	12.98 ± 5.51b	43.13 ± 7.10a	41.62 ± 10.47a	**	18.68 ± 1.03b	36.97 ± 3.00a	37.97 ± 2.32a	***
Na^+ (mg l^{-1})	33.53 ± 0.54b	219.85 ± 6.05a	*	37.10 ± 0.70b	239.01 ± 4.00a	230.43 ± 1.47a	***	33.47 ± 0.51b	305.74 ± 6.87a	316.56 ± 11.16a	***
Ca^{2+} (mg l^{-1})	52.82 ± 3.23b	85.27 ± 1.24a	*	64.64 ± 4.47b	83.99 ± 1.24a	86.45 ± 0.24a	***	74.28 ± 1.41 b	80.50 ± 2.20a	82.66 a ± 2.95a	***
Mg^{2+} (mg l^{-1})	8.90 ± 0.20b	10.25 ± 0.12a	*	9.16 ± 0.12b	10.69 ± 0.30a	11.31 ± 0.16a	***	9.33 ± 0.19b	10.26 ± 0.27a	10.40 ± 0.38a	***
K^+ (mg l^{-1})	9.35 ± 0.16b	41.17 ± 1.96a	*	9.90 ± 0.12b	46.15 ± 3.88a	37.50 ± 0.11a	***	13.37 ± 3.92b	48.02 ± 5.63a	53.65 ± 3.70a	***
${CO_3}^{2-}$ (mg l^{-1})	171.50 ± 5.32a	193.67 ± 11.89a	ns	153.06 ± 6.99a	138.67 ± 9.23a	142.50 ± 6.02a	ns	154.33 ± 1.02a	147.61 ± 2.29a	144.49 ± 2.23a	ns
${HCO_3}^-$ (mg l^{-1})	257.89 ± 2.85a	254.23 ± 21.57a	ns	219.20 ± 10.46a	220.38 ± 16.22a	232.88 ± 16.91a	ns	246.36 ± 7.58a	241.89 ± 30.62a	242.98 ± 29.16a	ns
${SO_4}^-$ (mg l^{-1})	30.17 ± 1.30a	31.84 ± 0.85a	ns	33.12 ± 0.97a	32.50 ± 1.83a	36.24 ± 1.16a	ns	29.39 ± 0.55a	30.98 ± 0.59a	31.09 ± 1.13a	ns
SAR	1.13 ± 0.03b	5.99 ± 0.18a	*	1.15 ± 0.03b	6.52 ± 0.16a	6.27 ± 0.07a	***	0.94 ± 0.02b	8.54 ± 0.25a	8.98 ± 0.33a	***
Hardness (mg l^{-1} $CaCO_3$)	168.57 ± 7.79b	255.20 ± 3.54a	*	199.14 ± 11.43b	253.81 ± 4.2a	252.51 ± 3.21a	***	138.65 ± 4.17b	243.31 ± 6.46a	235.63 ± 8.20a	***

Sign [a], Significance.
Data are means ± standard errors for each analyzed parameter, determined on 15 samples for each irrigation water type (1 sample per irrigation water type x 3 replicates x 5 sampling dates) during the Tomato 2012 and the Broccoli 2012–2013 growing seasons; on 18 samples for each irrigation water type (1 sample per irrigation water type x 3 replicates x 6 sampling dates) during the Tomato 2013 growing season.
*, Statistically significant at $P \leq 0.05$; **, Statistically significant at $P \leq 0.01$; ***, Statistically significant at $P \leq 0.001$; ns, not significant.
Means followed by same letters in each column are not significantly different ($P \leq 0.05$; *t*-test and Tukey test). For abbreviations, see main text.

Table 5
Average values of the soil chemical parameters measured over the three growing seasons (Tomato 2012, Broccoli 2012–2013, and Tomato 2013), on plots irrigated with groundwater (GW), secondary treated wastewater (SW) and tertiary treated wastewater (TW).

Growing season	Tomato 2012			Broccoli 2012–2013				Tomato 2013			
Irrigation treatment	GW	SW	Sign[a]	GW	SW	TW	Sign[a]	GW	SW	TW	Sign[a]
Parameters											
pH	8.30 ± 0.11a	8.06 ± 0.11	ns	8.44 ± 0.07ab	8.48 ± 0.07a	8.29 ± 0.03b	*	8.48 ± 0.10a	8.54 ± 0.11a	8.50 ± 0.11a	ns
EC (dS m^{-1})	0.45 ± 0.11b	1.05 ± 0.11a	***	0.27 ± 0.02a	0.28 ± 0.02a	0.27 ± 0.02a	ns	0.55 ± 0.06a	0.71 ± 0.10a	0.70 ± 0.10a	ns
Total N (%)	0.17 ± 0.01a	0.17 ± 0.01a	ns	0.17 ± 0.01a	0.17 ± 0.01a	0.16 ± 0.01a	ns	0.16 ± 0.01a	0.15 ± 0.01a	0.15 ± 0.01a	ns
NO_3-N (mg/kg)	10.03 ± 0.86a	12.83 ± 0.90a	ns	7.79 ± 0.98a	8.48 ± 0.97a	7.63 ± 0.39a	ns	11.74 ± 0.71a	12.49 ± 1.05a	12.93 ± 1.17a	ns
NH_4-N (mg kg^{-1})	14,71 ± 2.23b	21.23 ± 3.12a	*	10.25 ± 0.71a	10.75 ± 1.00a	10.94 ± 0.66a	ns	10.29 ± 0.71a	10.92 ± 1.42a	12.55 ± 1.93a	ns
P_2O_5 (mg kg^{-1})	147.52 ± 17.01a	181.44 ± 19.59a	ns	117.60 ± 15.15a	105.76 ± 8.22a	94.44 ± 3.72a	ns	126.05 ± 7.65a	139.20 ± 18.67a	170.78 ± 21.12a	ns
OM (%)	1.97 ± 0.07a	1.99 ± 0.08a	ns	1.76 ± 0.10a	1.66 ± 0.08a	1.79 ± 0.05a	ns	1.87 ± 0.12a	1.92 ± 0.09a	2.05 ± 0.08a	ns
Na^+(mg kg^{-1})	705.17 ± 70.38b	1039.33 ± 80.03a	***	885.14 ± 38.32a	978.75 ± 97.60a	955.00 ± 84.20a	ns	1563.67 ± 97.40a	1982.50 ± 211.28a	1522.83 ± 11.46a	ns
Ca^{2+} (mg kg^{-1})	4151.67 ± 49.520a	4196.67 ± 61.39a	ns	4613.33 ± 116.05b	5557.78 ± 186.73a	5287.78 ± 159.72a	***	4898.03 ± 21.17a	4774.09 ± 178.81a	4700.00 ± 77.18a	ns
Mg^{2+} (mg kg^{-1})	290.00 ± 28.36a	306.37 ± 30.05a	ns	293.33 ± 15.12b	365.11 ± 15.37a	336.22 ± 13.18a	***	294.00 ± 10.39a	393.00 ± 56.32a	363.00 ± 38.31a	ns
K^+ (mg kg^{-1})	762.33 ± 30.12a	789.83 ± 46.27a	ns	698.19 ± 21.37a	719.17 ± 24.78a	700.00 ± 20.42a	ns	486.00 ± 41.38a	577.00 ± 24.88a	536.00 ± 40.19a	ns
SAR	4.03 ± 0.28b	5.93 ± 0.28a	***	4.83 ± 0.22a	4.85 ± 0.49a	4.87 ± 0.43a	ns	5.63 ± 0.51a	6.26.43 ± 0.34a	6.09 ± 0.20a	ns

Sign [a], Significance.
Data are means ± standard errors for each analyzed parameter, determined on 18 samples for each experimental plot (1 sample per experimental plot × 3 replicates × 6 sampling dates) during the Tomato 2012 and Tomato 2013 growing seasons; on 15 samples for each experimental plot (1 sample per experimental plot × 3 replicates × 5 sampling dates) during the Broccoli 2012–2013 growing season.
*, Statistically significant at $P \leq 0.05$; **, Statistically significant at $P \leq 0.01$; ***, Statistically significant at $P \leq 0.001$; ns, not significant.
Means followed by same letters in each column are not significantly different ($P \leq 0.05$; *t*-test and Tukey test). For abbreviations, see main text.

ing the three crop cycles, respectively) than the other two water sources. The high concentration of NO_3-N in GW is due to the nitrate contamination of the aquifer, since in the study area intensive agricultural activity has led to extensive, and frequently excessive, nitrogen fertilizer applications to the crops. The resulting nitrogen surplus in the soil is then particularly exposed to the leaching risk, thus increasing the problem of groundwater nitrate pollution (Libutti and Monteleone, 2017). This elevated NO_3-N content in GW represents an important source of nutrient for the plants, but unfortunately it is not always taken into account by farmers in the crop fertilization planning. As regards the other parameters ($SO_4{}^{2-}$, $CO_3{}^{2-}$ and $HCO_3{}^{-}$), they had always similar levels in the three irrigation water sources.

The values for the main physico-chemical water properties for GW, SW and TW met the Italian standards for treated wastewater reuse (Legislative Decree no. 152/2006), except for the TSS and BOD_5 for SW and TW, slightly exceeding the limit set by the legislation (10 mg l^{-1} and 20 mg O_2 l^{-1}, respectively). While a non-compliance for the parameter TSS is common for a secondary settled effluent, in the case of TW, this was due to two factors specific of this case study, i.e. the leakage of ultrafiltration membranes and the bacterial re-growth in the storage tank. Nevertheless, for both SW and TW the concentration of TSS was far below the limit of 50 mg l^{-1} established to avoid clogging problems in drip irrigation systems (Ayers and Westcot, 1985). Concerning the BOD_5, the observed values of this parameter may not be considered a limiting factor for treated wastewater reuse, since they indicate that both SW and TW were a source of organic matter for the irrigated soil. This aspect has been also highlighted by others studies, which proposed to skip the biological processes in the reclamation process in order to save the agronomic potential of organic matter and nutrients contained in the urban wastewater (Lopez et al., 2006; Lonigro et al., 2007; Palese et al., 2009).

3.4. Chemical properties of soil

The main chemical properties of the 0–30 cm soil layer in GW, SW and TW irrigated plots, over the Tomato 2012, Broccoli 2012–2013, and Tomato 2013 growing seasons are reported in Table 5.

Significant differences between GW and SW irrigated plots were observed in some soil chemical properties, during the Tomato 2012 growing season. The use of SW for crop irrigation resulted in a significant increase of NH_4-N, Na^+ and, consequently, of EC and SAR of the soil. These results are consistent with the chemical properties of the irrigation water. Indeed, the higher NH_4-N concentration in SW than in GW (Table 4) resulted in a higher NH_4-N concentration in the corresponding irrigated plots. Even though part of the NH_4-N supplied through irrigation water was readily available for plants growth, most of it was adsorbed to the negative charged clay particle surface of SW irrigated soil (Duan et al., 2010). Similarly, the higher concentration of Na^+ in SW, if compared with GW, resulted in a higher Na^+ concentration in the corresponding irrigated plots. This result is in line with previous studies (Najafi and Nasr, 2009; Mojiri, 2011). Because of the higher Na^+ concentration in SW than in GW, also the EC of the soil in SW irrigated plots was much higher (twice as big) than the values observed in GW irrigated plots and in SW irrigated plots before the experimental period started (0.45 and 0.50 dS m^{-1}, respectively). Rusan et al. (2007) reported similar results. Khai et al. (2008) and Mojiri (2011) stated that the increase of EC in soil irrigated with wastewater was mainly due to the original higher concentration of cations such as Na and K. Nevertheless, EC values observed in SW soil remained below the indicative threshold value (4.00 dS m^{-1}) beyond which a soil is defined as saline (Qadir et al., 2000). Also as regards the SAR of the soil, the significant difference between SW and GW plots was probably due to Na^+ accumulation in the root zone. When SAR is in the range of 12–15, damages to the physical characteristics of the soil may occur (Munshower, 1994). However, the calculated SAR value was clearly below the limit of 15, above which a soil can be considered sodic (US Salinity Laboratory Staff, 1954). Although NO_3-N concentration was higher in GW than in SW, no significant differences between GW and SW plots were observed in terms of NO_3-N content in the 0–30 cm soil. This suggest that the NO_3-N contained in GW was in part utilized by the crop and the possible NO_3-N in excess of plants requirements (so not adsorbed by tomato plants) was likely moved below the0–30 cmsoil layer, due to its high mobility in the soil (Yuan et al., 2000; Shamrukh et al., 2001).

Over the Broccoli growing season 2012–2013, significantly higher pH value was measured in SW than TW irrigated plots (Table 5). In comparison with TW soil, the pH of SW soil raised of 0.2 units to an average value of 8.48. This was likely due to the higher accumulation, although not significantly so, of basic cations, particularly Ca^{2+} and Mg^{2+}, from irrigation water in SW than TW irrigated plots (Table 4). Increase of soil pH by approximately 0.8 units under irrigation with treated wastewater has been reported by Tarchouna et al. (2000). Previously, also Mancino and Pepper (1992) found that irrigation with treated wastewater raised the soil pH by 0.1–0.2 units when compared with irrigation with drinking water. These authors attributed such a pH rise to the high content of basic cations of the wastewater, which raised the alkaline reserve of the soil.

Particularly, the average values for Ca^{2+} and Mg^{2+} in the soil were significantly higher in SW and TW than in GW irrigated plots. This result was consistent with the higher Ca^{2+} and Mg^{2+} concentration of SW and TW than GW (Table 4) and likely indicated that the treated wastewater input for these chemical species was higher than the broccoli plants uptake.

As observed during the previous tomato cycle, also over the broccoli growing season no differences were found in NO_3-N concentration of SW and TW irrigated soils with respect to GW soil. NO_3-N amount in the 0–30 cm soil layer was similar among the three irrigation water sources and lower than the previous crop cycle. This result can be explained considering that during the broccoli cycle the high rainfall, associated with the limited rate of NO_3-N uptake by the plants, contributed to the removal of the NO_3-N, supplied by irrigation water and not utilized by the crop, from the upper to the deeper soil layers by drainage water. A study of Arregui and Quemada (2006) showed that in Mediterranean climate nitrogen leaching easily occurs in the autumn-winter season, when crop nitrogen demand is low, precipitation exceeds crop evapotranspiration and considerable drainage took place along the soil profile. Differently from the previous tomato cycle, there were no significant effects on soil salinity due to use of treated wastewater for broccoli irrigation. Particularly, the EC values measured on SW and TW irrigated soils were very low (0.28 and 0.27 dS m^{-1}, respectively), despite the fact that EC values and Na^+ concentrations in SW and TW were significantly higher than in GW. For the three types of irrigation water, EC in the soil remarkably decreased, not only with respect to the previous crop cycle, but also with respect to the value of EC measured before the start of the experimental period (0.49 dS m^{-1}). As well as for $NO_3{}^-$, this was due to the high rainfall occurred during the broccoli cycle (Libutti and Monteleone, 2012; Monteleone and Libutti, 2012).

Over the tomato growing season 2013, there were no significant effects of the irrigation with treated wastewater (neither SW or TW) on any of the measured chemical parameters of the soil (Table 5). The data observed in the 0–30 cm soil showed similar soil fertility levels for the three irrigation water sources.

Although SW and TW had always higher BOD_5 and COD values than GW, no significant differences were observed among the average values of OM in GW, SW and TW irrigated soils over the three growing seasons. It is possible that a rapid mineralization of the organic material supplied through the irrigation with treated wastewater occurred. Gloaguen et al. (2007) observed a decrease of organic carbon in the soil after the irrigation with treated wastewater and hypothesized that this was related to an increase of the soil microbiological activity, due to the labile carbon supplied by wastewater.

Similarly, for the three crops, there were no significant differences among the average concentrations of total N and P_2O_5 in GW, SW and TW irrigated soils, as observed in the studies of Midrar et al. (2004) and Heidarpour et al. (2007).

3.5. Microbiological properties of water, soil, plant and crops product

The microbiological properties of the three irrigation water sources (GW, SW and TW) divided by growing seasons are reported in Table 6.

GW had a content of *E. coli* below the current Italian threshold of 10 CFU 100 ml^{-1} for wastewater reuse (Legislative Decree No. 152/2006), during the Tomato 2012 growing season and was free of this fecal indicator during the Broccoli 2012–2013 and Tomato 2013 growing seasons. *Fecal coliforms* and *Fecal enterococci* were always detected in GW over the three cropping seasons, but at considerably lower concentration than those observed for the two treated wastewaters. With respect to GW and TW, SW had always a significantly higher concentration of *E. coli* (well above the local limit for reuse), *Fecal coliforms* and *Fecal enterococci*. TW had a very low residual fecal contamination during the Broccoli 2012–2013 growing season, with the total absence of *E. coli* and average levels of 0.20 log CFU 100 ml^{-1} and 0.42 log CFU 100 ml^{-1} for *Fecal coliforms* and *Fecal enterococci*, respectively. A higher average concentration of *E. coli* (2.08 log CFU 100 ml^{-1}), *Fecal coliforms*, and *Fecal Enterococci* (2.20 log CFU 100 ml^{-1} and 1.82 log CFU 100 ml^{-1}, respectively) were observed in TW during the Tomato 2013 growing season. This was due to the membrane failure and, to a minor extent, to the bacterial re-growth occurred in TW storage tank. Because of these two factors, the turbidity of the treated wastewater so increased to a level that hampered the UV disinfection efficiency (Fig. 3). For the three irrigation water types, *Salmonella* spp. was never isolated in any of the analyzed samples, as required by the above cited legislation.

Regarding the microbiological properties of the soil (Table 6), *E. coli* was never isolated in any of the GW, SW and TW soil samples over the three crop cycles, despite the presence of *E. coli* in GW during the Tomato 2012 growing season, in SW during the entire experimental period and in TW during the Tomato 2013 growing season. This could be due to the die-off of this indicator, as reported by several other studies (Lang et al., 2007; Van Elsas et al., 2011). However, it has to be stated that it is not possible to exclude the presence of *E. coli* in the analyzed soil samples, since the sensitivity of the applied method is 100 CFU g^{-1} (Samarajeewa et al., 2010) and a loss of cultivability of *E. coli* may also have led to underestimation. *Fecal coliforms* were isolated in all soil samples but at levels not significantly different among the three irrigation water treatments. Particularly, in GW irrigated soils a high concentration of *Fecal coliforms* was observed over the three growing seasons, if compared with the low presence of these indicators in the corresponding irrigation water. Similar result was obtained in TW irrigated soil during the Broccoli 2012–2013 growing season. This suggests that the presence of *Fecal coliforms* in the soil may be also caused by sources of contamination different from the irrigation water, such as roaming animals, birds and runoff (Mawdsley et al., 1995; Venglovsky et al., 2006).

High level of *Total heterotrophic count*, were observed in all soil samples. These resulted not significantly different among the three irrigation water treatments, during the two Tomato growing seasons. On the contrary, during the Broccoli 2012–2013 growing season, significantly higher level of *Total heterotrophic count* were observed in GW than in TW irrigated soil.

Concerning the microbiological analysis of plant and product of the crops, *E. coli* was not isolated in any of the analyzed samples. Overall, *Fecal coliforms* were found to be lower on the crop products than on the plant and, moreover, their values were not influenced by the water used for irrigation. Also the *Total heterotrophic count*, that accounts for the autochthonous microorganism of soil and the possible input of microorganisms from the irrigation water, was not influenced by the water type, except for the broccoli plants, for which the *total heterotrophic count* resulted higher on SW than on TW irrigated plants. The low levels of microbial indicators on plants and crop products can be explained by a substantial lack of contact between irrigation water, soil, plants and edible parts of the plant. These results are in agreement with the findings of a previous study (Cirelli et al., 2012) that reports the contamination of tomato and eggplant fruits by fecal bacteria only in case of direct contact with the soil irrigated with treated municipal wastewater. As already reported for the soil contamination, also the presence of *Fecal coliforms* on plant and product irrigated with GW during the Tomato 2012 growing season was likely due to other sources of contamination.

3.6. Quantitative and qualitative properties of crops product

Data reported in Table 7 show the quantitative traits of crop products observed over the three considered growing seasons. During the Tomato 2012 growing seasons, marketable yield (MY) was higher, although not significantly so, for GW than SW. As already reported by Gatta et al. (2015a), this appears to be mainly due to the higher nonmarketable yield (NMY) for SW, with respect to GW.

During the Broccoli 2012–2013 growing season, significantly lower values of MY and significantly higher values of NMY were observed for SW and TW than GW treatment (Table 7). There are very few researches in literature about the use of reclaimed wastewater for broccoli crop irrigation, and they focus on the accumulation of macro and micro-nutrients in plant and soil (Kalavrouziotis et al., 2008a, 2008b, 2009). No literature data about the productive response of broccoli crop to irrigation with treated wastewater are available. However, the broccoli yield value observed in the present experimental trial is roughly in agreement with the data obtained by Candido et al. (2001) in a field trial carried out with a conventional irrigation water source in an agricultural area of Southern Italy, similar to that of the present study, and aimed to compare different irrigation methods in order to increase the efficiency of irrigation water.

As during the Tomato 2012, and 2013 growing season, a higher MY for GW with respect to SW and TW treatments was detected, although the differences were not statistically significant. The yields of tomato fruit observed in both the two growing seasons are roughly in agreement with the results obtained by Aiello et al. (2007) in an experimental trial carried out in Southern Italy. They reported a higher MY from the tomato “Missouri” genotype irrigated with freshwater than the same genotype irrigated with treated wastewater. Compared to the yields observed for the “Missouri” genotype in the above mentioned study, the MY obtained in the present study for the “Manila” variety were lower. This might be due to several factors, such as the differences between the genetic constitution of the two cultivars, the characteristics of the treated wastewater, or also the pedo-environmental conditions of the cultivation areas (Gatta et al., 2015a).

Table 6
Enumeration of the bacterial indicators in water, soil, plant and crop product, divided by growing season (Tomato 2012, Broccoli 2012–2013 and Tomato 2013) and irrigation treatment (GW, SW and TW).

Growing season	TOMATO 2012			BROCCOLI 2012–2013				TOMATO 2013			
Irrigation treatment	GW	SW	Sign[a]	GW	SW	TW	Sign[a]	GW	SW	TW	Sign[a]
Parameter											
Water (log CFU 100 ml^{-1})											
E. coli	0.42 ± 0.28b	3.42 ± 0.23a	**	0b	3.48 ± 0.38a	0b	***	0b	1.75 ± 0.69ab	2.08 ± 0.70a	*
Fecal coliforms	0.62 ± 0.39b	4.27 ± 0.11a	**	0.42 ± 0.21b	4.12 ± 0.43a	0.20 ± 0.06b	***	0.65 ± 0.28a	1.64 ± 0.79a	2.20 ± 0.73a	ns
Fecal enterococci	0.48 ± 0.31b	2.90 ± 0.60a	**	0.51 ± 0.35b	3.65 ± 0.62a	0.42 ± 0.20b	***	0.35 ± 0.20a	2.03 ± 0.67a	1.82 ± 0.65a	ns
Soil (log CFU g^{-1})											
E. coli	0a	0a	ns	0a	0a	0a	ns	0a	0a	0a	ns
Fecal coliforms	3.04 ± 0.06a	3.11 ± 0.07a	ns	2.51 ± 0.20a	2.60 ± 0.14a	2.37 ± 0.38a	ns	1.18 ± 0.31a	1.49 ± 0.50a	1.42 ± 0.35a	ns
Total heterotrophic count	6.50 ± 0.14a	6.51 ± 0.14a	ns	4.98 ± 0.11a	4.73 ± 0.10a	3.32 ± 0.38b	***	5.59 ± 0.15a	5.76 ± 0.18a	5.64 ± 0.12a	ns
Plant (log CFU g^{-1})											
E. coli	0	0		0	0	0		0	0	0a	
Fecal coliforms	2.15 ± 0.08a	2.21 ± 0.10a	ns	3.18 ± 0.34a	3.68 ± 0.19a	3.09 ± 0.24a	ns	0a	0a	1.67 ± 1.05a	ns
Total heterotrophic count	4.18 ± 0.07a	4.23 ± 0.08a	ns	6.46 ± 0.16a	6.52 ± 0.13a	5.20 ± 0.08b	*	2.50 ± 0.75a	2.17 ± 0.71a	2.46 ± 0.84a	ns
Product (log CFU g^{-1})											
E. coli	0a	0a	ns	0a	0a	0a	ns	0a	0	0a	ns
Fecal coliforms	2.21 ± 0.13a	2.37 ± 0.12a	ns	1.99 ± 0.34a	1.96 ± 0.26a	1.75 ± 0.52a	ns	0a	0a	8.57 ± 5.53a	ns
Total heterotrophic count	5.10 ± 0.27a	5.16 ± 0.19a	ns	3.65 ± 3.00	3.59 ± 3.34a	3.57 ± 3.36a	ns	1.79 ± 0.64a	1.78 ± 0.86a	2.01 ± 0.69a	ns

Sign [a], Significance.
*, Statistically significant at $P \le 0.05$; **, Statistically significant at $P \le 0.01$; ***, Statistically significant at $P \le 0.001$; ns, not significant.
Data are means ± standard errors for each analyzed parameter, determined on 15 samples for each irrigation water type (1 sample per irrigation water type × 3 replicates × 5 sampling dates) during the Tomato 2012 and the Broccoli 2012–2013 growing seasons; on 18 samples for each irrigation water type (1 sample per irrigation water type × 3 replicates × 6 sampling dates) during the Tomato 2013 growing season.
Means followed by the same letters in each line are not significantly different ($P \le 0.05$; *t*-test and Tukey test). For abbreviations, see main text.

Table 7
Quantitative traits of tomato and broccoli products, divided by growing season (Tomato 2012, Broccoli 2012–2013 and Tomato 2013) and irrigation treatment (GW, SW and TW).

Growing season	Irrigation treatment	Quantitative trait				
		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})
TOMATO 2012	GW	87.54 ± 10.37a	82.88 ± 9.47a	3.06 ± 0.29a	4.66 ± 0.89a	0.17 ± 0.05a
	SW	85.32 ± 5.01a	79.05 ± 4.76a	2.93 ± 0.15a	6.26 ± 0.61a	0.23 ± 0.03a
	Significance	ns	ns	ns	ns	ns
BROCCOLI 2012–2013	GW	9.90 ± 0.23a	9.35 ± 0.21a	0.35 ± 0.01a	0.55 ± 0.02b	0.021 ± 0.001b
	SW	9.16 ± 0.09ab	8.52 ± 0.08b	0.32 ± 0.01b	0.64 ± 0.01a	0.024 ± 0.001a
	TW	8.85 ± 0.35b	8.16 ± 0.32b	0.30 ± 0.01b	0.69 ± 0.03a	0.026 ± 0.001a
	Significance	*	***	***	***	***
TOMATO 2013	GW	86.59 ± 2.75a	81.13 ± 2.21a	3.00 ± 0.08a	5.46 ± 0.55a	0.20 ± 0.02a
	SW	83.94 ± 5.49a	78.57 ± 4.73a	2.91 ± 0.18a	5.37 ± 0.76a	0.20 ± 0.03a
	TW	83.25 ± 4.53a	78.00 ± 3.98a	2.89 ± 0.15a	5.25 ± 0.61a	0.19 ± 0.02a
	Significance	ns	ns	ns	ns	ns

Data are means ± standard error, as measured from 162 tomato plants (54 plants per plot x 3 replicates).
*, Statistically significant at $P \le 0.05$; **, Statistically significant at $P \le 0.01$; ***, Statistically significant at $P \le 0.001$; ns, not significant.
Means followed by the same letters in each column are not significantly different ($P \le 0.05$; *t*-test and Tukey test).

The qualitative traits of crop products are showed in Table 8. Significant differences between the two irrigation water sources (GW and SW) were observed for the pH of fruits during the Tomato 2012 growing season. In particular, a higher pH value in GW than SW treatment was detected. pH is a very important parameter for the evaluation of tomato fruits quality, since it influences the processing thermal condition required to control microbial spoilage and enzyme inactivation, and obtain safe products (Garcia and Barret, 2006). pH values in this study were within the same range (4.32–4.56) obtained by Madrid et al. (2009). This pH range is typical of tomato fruits (Hong et al., 2000) and shows their slightly acid nature. The irrigation water had no effect on titrable acidity (TA) of tomato fruits. Data here presented are very close to the mean acidity of processing tomatoes that is generally of about 0.35% (Garcia and Barret, 2006). Also soluble solids content of the flesh (SSC) values showed no significant difference between the two irrigation water sources; nevertheless, a slightly higher SSC was observed in GW (5.73°Brix) than SW (5.53°Brix). Madrid et al. (2009) and Favati et al. (2009) reported lower SSC values than those observed in the present study. These differences could be due to several factors, among which the tomato genotype used (Sgherri et al., 2007, 2008), the environmental drought (Mahajan and Singh, 2006; Soraya et al., 2001), and the climatic conditions, such as temperature, CO_2 concentration and light. However, high SSC improves the efficiency of tomato fruits processing (Johnstone et al., 2005) and has direct implication for the tomato canning industry (Richardson and Hobson, 2006), because of the minor quantity of energy necessary to evaporate water from the fruits when producing tomato paste or concentrated juice (Favati et al., 2009; Richardson and Hobson, 2006). Higher solid content in fruits is, therefore, a target characteristic, as this would reduce the cost for processing and serves as the basis for fixing the price to be paid to the producer (Turhan and Seniz, 2009). As well as for SSC, also high dry matter content (DM) is a desirable characteristic for the canning tomatoes industry since it improves the quality of the processed product (De Pascale et al., 2001). Results here presented showed DM values not different between GW and SW (7.44 vs 7.52%) and acceptable for industrial processing. Moreover, they are in agreement with the

Table 8
Qualitative traits of tomato and broccoli products, divided by growing season (Tomato 2012, Broccoli 2012–2013 and Tomato 2013) and irrigation water type (GW, SW and TW).

Growing season	Ittigation Treatment	Qualitative trait										
		D	CI	DM	SSC	TA	pH	Anions and cations content (mg 100 g^{-1} fresh matter)				
		(cm)	(a^*/b^* ratio)	(% FM)	(°Brix)	(g 100 ml^{-1})		Ca^{2+}	Mg^{2+}	K$^+$	Na$^+$	NO$_3$$^-$
TOMATO 2012	GW	4.22 ± 0.32a	1.03 ± 0.03a	7.44 ± 0.11a	5.73 ± 0.12a	0.31 ± 0.02a	4.54 ± 0.04a	8.63 ± 0.54a	7.25 ± 0.63a	225.3 ± 9.50a	9.15 ± 0.94b	1.32 ± 0.10a
	SW	4.44 ± 0.28a	0.97 ± 0.04a	7.52 ± 0.21a	5.53 ± 0.12a	0.30 ± 0.03a	4.35 ± 0.04b	9.33 ± 0.62a	7.07 ± 0.33a	225.4 ± 6.00a	11.050.92 ± a	0.92 ± 0.06b
	Significance	ns	ns	ns	ns	ns	*	ns	ns	ns	*	*
BROCCOLI 2012–2013	GW	27.33 ± 1.47a	–	12.56 ± 0.42	–	–	–	46.19 ± 12.72a	13.84 ± 0.83a	348.74 ± 8.54a	4.30 ± 0.46b	25.77 ± 6.51a
	SW	28.23 ± 1.02a	–	13.05 ± 0.85	–	–	–	45.48 ± 1.55a	13.14 ± 0.76a	356.15 ± 12.45a	4.45 ± 0.25b	1.12 ± 0.01b
	TW	28.60 ± 1.20a	–	12.46 ± 0.72	–	–	–	53.90 ± 7.64a	10.95 ± 1.11a	307.15 ± 21.90a	7.64 ± 1.72a	3.97 ± 1.59b
	Significance	ns		ns				ns	ns	ns	***	***
TOMATO 2013	GW	4.19 ± 0.06a	1.13 ± 0.01a	7.66 ± 0.15a	5.96 ± 0.11a	0.31 ± 0.01a	4.46 ± 0.01a	54.79 ± 3.76a	11.05 ± 0.82a	167.90 ± 3.33a	21.13 ± 1.19b	2.22 ± 0.34a
	SW	4.13 ± 0.04a	1.14 ± 0.01a	7.31 ± 0.32a	5.81 ± 0.04a	0.30 ± 0.01a	4.44 ± 0.01a	42.47 ± 0.99b	9.09 ± 0.65a	153.78 ± 13.21a	26.63 ± 1.30a	1.12 ± 0.18b
	TW	4.11 ± 0.06a	1.12 ± 0.01a	6.86 ± 0.06a	5.79 ± 0.12a	0.31 ± 0.01a	4.43 ± 0.04a	44.50 ± 0.86b	9.20 ± 0.24a	136.36 ± 4.90a	25.64 ± 1.093a	1.16 ± 0.16b
	Significance	ns	ns	ns	ns	ns	ns	*	ns	ns	*	*

Data are means ± standard error, as measured from 30 tomato fruits (10 fruits per plot × 3 replicates) and 30 broccoli heads (10 heads per plot × 3 replicates).
*, Statistically significant at $P \leq 0.05$; **, Statistically significant at $P \leq 0.01$; ***, Statistically significant at $P \leq 0.001$; ns, not significant.
Means followed by the same letters in each column are not significantly different ($P \leq 0.05$; *t*-test and Tukey test).

findings of Turhan and Seniz (2009), which observed DM values ranging from 4 to 7%, in a wide field experiment carried out in the Mediterranean basin aiming to compare the quality characteristics of 33 tomato genotypes. Regarding fruits size, no relevant effects of the irrigation water source were observed on mean diameter (D) and color index (CI). The latter, which is commonly used as a quality redness index (brightness of red colour) of tomatoes was found in the range from 0.97 to 1.14. These data were also in agreement with the a*/b* range from 0.95 to 1.21 observed by Batu (2004) on tomato fruits at the red stage of maturity from a greenhouse experiment.

As regards the anions and cations content of fruits, during the Tomato 2012 growing season, Na$^+$ and NO$_3$$^-$ concentrations were significantly influenced by the irrigation with treated wastewater. Na$^+$ was higher in SW than GW (11.05 vs 9.15 mg 100 g^{-1} of fresh matter), likely due to the significantly higher Na$^+$ content in SW, in comparison with GW (Table 4). On the contrary, NO$_3$$^-$ content was significantly higher in GW than SW treatment (1.32 vs 0.92 mg 100 g^{-1} of fresh matter), in agreement with the higher NO$_3$-N concentration of GW (Table 4). However, NO$_3$$^-$ content of tomato fruits were well below the nitrate levels in vegetables as defined by the European guidelines (European Union Regulation N° 1258, 2011), currently adopted in Italy. Except for Ca^{2+} and Na$^+$ contents, the observed results are in agreement with other studies (Guil-Guerrero and Rebolloso-Fuentes, 2009).

According to the data of Table 8, over the Broccoli 2012–2013 growing season, most of the qualitative traits of crop yield were not significantly different among the three irrigation water sources. Although not statistically different, DM and D were respectively higher in SW and TW than GW. On the contrary, for broccoli heads significant differences in Na$^+$ and NO$_3$$^-$ content were observed. Na$^+$ was significantly higher when plants were irrigated with SW and TW than with GW; NO$_3$$^-$ was higher in plants irrigated with GW than in those irrigated with SW and TW. These results clearly reflect the different values of Na$^+$ and NO$_3$$^-$ among the three irrigation water sources (Table 4). Also in this case, the NO$_3$$^-$ content in broccoli heads was below the above mentioned limits for nitrate concentration in vegetables.

During the Tomato 2012–2013 growing season, the qualitative traits measured in tomato fruits confirmed the results obtained in the first tomato crop cycle. For DM, D, CI, SSC, TA, and pH, there were no significant differences among GW, SW and TW water sources. All the obtained values were within the typical range for tomato fruits and also in agreement with the results of other studies, as already reported with respect to the Tomato growing season 2012. As regards the Na$^+$ and Ca^{2+} content of the fruits, higher values were found in Tomato growing season 2013 than in Tomato growing season 2012. Moreover, during the second cycle, the irrigation water significantly affected the Ca^{2+} content of the fruits, which resulted higher for crops irrigated with GW, if compared with those irrigated with SW and TW. Except for Ca^{2+} and K$^+$ contents, these results are in agreement with the mineral content of tomato fruits reported by other authors (Guil-Guerrero and Rebolloso-Fuentes, 2009).

4. Conclusions

This paper presents a 1.5-year field study of reuse of treated agro-industrial wastewater. Secondary treated wastewater (after activated sludge process and sedimentation) and tertiary treated wastewater (after ultrafiltration and UV radiation) were used to irrigate two vegetable species in succession (processing tomato and broccoli) in a closed system where an agri-food company cultivated and processed vegetables and had wastewater as by-product.

The findings of the present study indicate that agro-industrial effluents have the potential to be recycled for irrigation after a tertiary treatment composed of ultrafiltration and “on demand” UV disinfection processes. The yield of tomato and broccoli crops as well as the most important qualitative parameters of tomato fruits (i.e dry matter content, soluble solid content, titratable acidity, pH) and broccoli heads (i.e dry matter content, diameter) were not influenced by the irrigation with treated wastewater.

The microbiological characteristics of the two treated wastewaters were considerably different from the conventional water source (groundwater) and also between each other. Nevertheless, the microbiological quality of the tomato fruits and broccoli heads was not affected by the type of irrigation water used. The die-off of fecal indicators in the soil and the drip irrigation method, by reducing the direct contact between the water and the plant, limited the possible contamination of the crops products. Moreover, this type of irrigation system reduces the amount of irrigation water that needs to be supplied, so avoiding deep water percolation and surface water run-off processes, so it can be recommended for wastewater reuse in irrigation.

The knowledge on the reuse of treated agro-industrial wastewater in irrigation is still scarce and more studies are needed to evaluate the quality of the treated effluent under different conditions, in terms of both industrial processes and wastewater treatments, and the long-term effects on soil and plants. However, the findings presented in this paper suggest that this practice is an effective option to have a useful aternative to conventional water resources in areas where intensive agriculture is present and the sustainability of agricultural sector strongly depends on water availability. Indeed, in the specific case reported, the agro-industrial wastewater reclamation, the recovery and the reuse of the resulting treated water, allowed to profit of about 10,000 m^3 ha^{-1} of water for irrigation of a succession of tomato and broccoli crops, in a time period of 1.5 years, so decreasing the stress on the conventional water resources. In particular, within the context of the experimental trial here reported, this practice could save about 6000 m^3 ha^{-1} of groundwater every year.

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References

AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Washington DC, pp. 1213.

AOAC, 1995. Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists, Washington DC, pp. 1141.

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

American Society For Testing Materials (ASTM), 2003. Standard Practice for Integrity Testing of Water Filtration Membrane System, ASTM D6908-03. ASTM International., West Conshohocken, Pa.

Agrafioti, E., Diamadopoulos, E., 2012. A strategic plan for reuse of treated municipal wastewater for crop irrigation on the Island of Crete. Agric. Water Manage. 105, 57–64.

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.

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration Guidelines for Computing Crop Water Requirements. Food and Agriculture Organisation of the United Nations (FAO) Irrigation and Drainage Paper No 56, Rome.

Arregui, L.M., Quemada, M., 2006. Drainage and nitrate leaching in a crop rotation under different N-fertilizer strategies: application of capacitance probes. Plant Soil 288, 57–69.

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

Batu, A., 2004. Determination of acceptable firmness and colour values of tomatoes. J. Food Eng. 61, 471–475.

Bedbabis, S., Ferrara, G., Ben Rouina, B., Boukhris, M., 2010. Effects of irrigation with treated wastewater on olive tree growth, yield and leaf mineral elements at short term. Sci. Hortic. 126, 345–350.

Bremner, J.M., 1996. Nitrogen-Total. In: Sparks, D.L. (Ed.), Methods of Soil Analysis, Part 3, Chemical Methods. SSSA Book Set: 5. Soil Science Society of America, Madison, WI, pp. 1085–1121.

Caliandro, A., Lamaddalena, N., Stelluti, M., Steduto, P., 2005. Caratterizzazione Agro-ecologica Della Regione Puglia in Funzione Della Potenzialità Produttiva. In: Progetto ACLA, Ideaprint, Bari, Italy, ISBN: 2-85352-339-X.

Candido, V., Cannone, A., Tummolo, G., Posca, G., Bisignano, A., Miccolis, V., 2001. Aspetti produttivi e qualitativi del cavolo broccolo sottoposto a diversi regimi irrigui. In: Atti Del Convegno Conclusivo Progetto OTRIS Ottimizzazione Dell’uso Delle Risorse Idriche, Convenzionali E Non, in Sistemi Colturali Sostenibli, Bari, Italy, 28–29 Novembre (ISBN 88-7427-002-X).

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

Coppola, A., Santini, A., Botti, P., Vacca, S., Comegna, V., Severino, G., 2004. Methodological approach for evaluating the response of soil hydrological behavior to irrigation with treated municipal wastewater. J. Hydrol. 292, 114–134.

De Pascale, S., Maggio, A., Fogliano, V., Ambrosino, P., Retieni, A., 2001. Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. J. Hortic. Sci. Biotechnol. 76, 447–453.

Disciglio, G., Gatta, G., Libutti, A., Tarantino, A., Frabboni, L., Tarantino, E., 2014. Use of treated municipal wastewater on artichoke crop. Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng. 8 (4), 224–230.

Disciglio, G., Gatta, G., Libutti, A., Gagliardi, A., Carlucci, A., Lops, F., Cibelli, F., Tarantino, A., 2015. Effects of irrigation with treated agro-industrial wastewater on soil chemical characteristics and fungal populations during processing tomato crop cycle. J. Soil Sci. Plant Nutr. 15 (3), 765–780.

Duan, R., Sheppard, C.D., Fedler, C., 2010. Short-term effect of wastewater land application on soil chemical properties. Water Air Soil Pollut. 211, 165–176.

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 (4), 562–571.

Francis, F.J., Clydesdale, F.M., 1975. Food Colorimetry: Theory and Applications. AVIPubl. 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.

Gatta, G., Libutti, A., Gagliardi, A., Beneduce, L., Brusetti, L., Borruso, L., Disciglio, G., Tarantino, E., 2015a. Treated agro-industrial wastewater irrigation of tomato crop: effects on qualitative/quantitative characteristics of production and microbiological properties of the soil. Agric. Water Manage. 149, 33–43.

Gatta, G., Libutti, A., Gagliardi, A., Disciglio, G., Beneduce, L., d’Antuono, M., Rendina, M., Tarantino, E., 2015b. Effects of treated agro-industrial wastewater irrigation on tomato processing quality. Ital. J. Agron. 10 (632), 97100.

Gatta, G., Libutti, A., Beneduce, L., Gagliardi, A., Disciglio, G., Lonigro, A., Tarantino, E., 2016. Reuse of treated municipal wastewater for globe artichoke irrigation: assessment of effects on morpho-quantitative parameters and microbial safety of yield. Sci. Hortic. 213, 55–65.

Gloaguen, T.V., Forti, M.C., Lucas, Y., Montes, C.R., Gonçalves, R.A.B., Herpin, U., Melfi, A.J., 2007. Soil solution chemistry of a Brazilian Oxisol irrigated with treated sewage effluent. Agric. Water Manage. 88, 119–131.

Guil-Guerrero, J.L., Rebolloso-Fuentes, M.M., 2009. Nutrient composition and antioxidant activity of eight tomato (Lycopersicon esculentum) varieties. J Food Compos. Anal. 22, 123–129.

Heidarpour, M., Mostafazadeh-Fard, B., Abedi Koupa, J., Malekian, R., 2007. The effects of treated wastewater on soil chemical properties using subsurface and surface irrigation methods. Agric. Water Manage. 90, 87–94.

Hong, J.H., Mills, D.J., Coffman, C.B., Anderson, J.D., Camp, M.J., Gross, K.C., 2000. Tomato cultivation systems affect subsequent quality of fresh-cut fruit slices. J. Am. Soc. Hortic. Sci. 125, 729–735.

JMP, 2008. Statistical Software (Version 8.1). SAS Institute. Inc, Cary, North Caroline (27513).

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

Johnstone, P.R., Hartz, T.K., LeStrange, M., Numez, J.J., Miyao, E.M., 2005. Manging fruit soluble solids with late-season deficit irrigation in drip-irrigated processing tomato production. HortScience 40, 1857–1861.

Kalavrouziotis, I.K., Koukoulakis, P.H., Robolas, P., Papadopoulos, A.H., Pantazis, V., 2008a. Interrelationships of heavy metals macro and micronutriente nad properties of a soil cultivated with Brassica oleracea var. Italica (Broccoli): under the effect of treated municipal wastewater. Water Air Soil Pollut. 190, 309–321.

Kalavrouziotis, I.K., Robolas, P., Koukoulakis, P.H., Papadopoulos, A.H., 2008b. Effects of municipal reclamed wastewater on the macro- and micro-elements satus of soil and of Brassica oleracea var. Italica, and B. oleracea var. gemmifera. Agric. Water Manage. 95, 419–426.

Kalavrouziotis, I.K., Koukoulakis, P.H., Sakellariou-Makrantonaki, M., Papanikolaou, C., 2009. Effects of treated municipal wastewater on the essential nutrients interactions in the plant of Brassica oleracea var. Italica. Desalination 242, 297–312.

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

Khai, N.M., Tuan, P.T., Vinh, C.N., Oborn, I., 2008. Effects of using wastewater as nutrient sources on soil chemical properties in periurban agricultural systems. VNU Journal of Science. Earth Sci. 24, 87–95.

Khan, S., Cao, Q., Zheng, Y.M., Huang, Y.Z., Zhu, Y.G., 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Environ. Pollut. 152 (3), 686–692.

Kiziloglu, M., Turan, M., Sahin, U., Angin, I., Anapali, O., Okuroglu, M., 2008. Effects of wastewater irrigation on soil and cabbage-plant (Brassica oleracea var. capitata cv. yalova-1) chemical properties. J. Plant Nutr. Soil Sci. 170 (1), 166–172.

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. J. Appl. Microbiol. 103, 1868–1882.

Libutti, A., Monteleone, M., 2012. Irrigation management in Mediterranean salt affected agriculture: how leaching operates. Ital. J. Agron. 7 (1), e5, http://dx.doi.org/10.4081/ija.2012.

Libutti, A., Monteleone, M., 2017. Soil vs. groundwater: the quality dilemma. Managing nitrogen leaching and salinity control under irrigated agriculture in Mediterranean conditions. Agric. Water Manage. 186, 40–50, http://dx.doi.org/10.1016/j.agwat.2017.02.019.

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.

Lonigro, A., Montemurro, N., Rubino, P., Vergine, P., Pollice, A., 2015. Reuse of treated municipal wastewater for irrigation in Apulia Region: the IN. E.R.R.A. Project. Environ. Eng. Manage. J. 14 (7), 1665–1674.

Lopez, A., Pollice, A., Lonigro, A., Masi, S., Palese, A.M., Cirelli, G.L., Toscano, A., Pasino, R., 2006. Agricultural wastewater reuse in southern Italy. Desalination 187, 323–334.

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.

Mancino, C.F., Pepper, I.L., 1992. Irrigation of turfgrass with secondary sewage effluent: soil quality. Agron. J. 84, 650–654.

Mawdsley, J.L., Bardgett, R.D., Merry, R.J., Pan, B.F., Theodorou, M.K., 1995. Pathogens in livestock waste, their potential for movement through soil and environmental pollution. Appl. Soil Ecol. 2, 1–5.

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

Midrar, U.H., Khattak, R.A., Puno, H.K., Saleem-Saif, M., 2004. NPK status in effluent irrigated soils of some selected sites of NWFP. Int. J. Agric. Biol. 6 (2), 264–267.

Mojiri, A., 2011. Effects of municipal wastewater on physical and chemical properties of Saline soil. J. Biol. Environ. Sci. 5 (14), 71–76.

Monteleone, M., Libutti, A., 2012. Salt leaching due to rain in Mediterranean climate: is it enough? Ital. J. Agron. 7 (1), e6, http://dx.doi.org/10.4081/ija.2012.e6.

Munshower, F.F., 1994. Practical Handbook of Disturbed Land Revegetation. Lewis Publishers, Boca Raton.

Najafi, P., Nasr, S., 2009. Comparison effects of wastewater on soil chemical properties in three irrigation methods. Res. Crops 10 (2), 277–280.

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

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

Paranychianakis, N.V., Nikolantonakis, M., Spanakis, Y., Angelakis, A.N., 2006. The effect of recycled water on the nutrient status of Soultanina grapevines grafted on different rootstocks. Agric. Water Manage. 81 (1), 185–198.

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.

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

Pourcher, A., Bonnaud, P., Oise, F., Virginie, F., 2007. Survival of faecal indicators and enteroviruses in soil after landspreading of municipal sewage sludge. Appl. Soil Ecol. 35, 473–479.

Qadir, M., Ghaffoor, A., Murtaza, G., 2000. Amelioration strategies for saline soils: a review. Land Degrad. Dev. 11, 501–521.

Qadir, M., Ghafoor, A., Murtaza, G., 2001. Use of saline–sodic waters through phytoremediation of calcareous saline–sodic soils. Agric. Water Manage. 50 (3), 197–210.

Qadir, M., Wichnels, D., Raschid-Sally, L., Singh Minhas, P., Drechsel, P., Bahri, A., McCornick, P., 2007. Agricultural use of marginal-quality water-opportunities and challenges. In: Molden, D. (Ed.), Water for Food; Water for Life. A Comprehensive Assessment of Water Management in Agriculture. Earthscan, 8–12 Camden High Street, London, pp. 425–457.

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

Rodriguez-Liébana, J.A., ElGouzi, S., Mingorange, M.D., Castillo, A., Peña, A., 2014. Irrigation of a Mediterranean soil under field conditions with urban wastewater: effect on pesticide behaviour. Agric. Ecosyst. Environ. 185, 176–185.

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

Rusan, M.J.M., Hinnawi, S., Rousan, L., 2007. Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. Desalination 215, 143–152.

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

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.

Shamrukh, M., Corapcioglu, M., Hassona, F., 2001. Modeling the effect of chemical fertilizers on ground water quality in the Nile Valley Aquifer, Egypt. Groundwater 39 (1), 59–67.

Singh, K.P., Mohan, D., Sinha, S., Dalwani, R., 2004. Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health agricultural, and environmental quality in the wastewater disposal area. Chemosphere 55, 227–255.

Soraya, G., Nadia, B., Cherubino, L., Christian, G., 2001. Tomato fruit quality in relation to water and carbon fluxes. Agronomie 21, 385–392.

Tallon, P., Magagna, B., Lofranco, C., Leung, K.T., 2005. Microbial indicators of fecal contamination in water: a current perspective. Water Air Soil Pollut. 166, 139–166.

Tarchouna, L.G., Merdy, P., Raynaud, M., Pfeifer, H.R., Lucas, Y., 2000. Effects of long-term irrigation with treated wastewater. Part I: Evolution of soil physico-chemical properties. Appl. Geochem. 25, 1703–1710.

Toze, S., 2006. Reuse of effluent water-benefits and risks. Agric. Water Manage. 80, 147–159.

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.

US Salinity Laboratory Staff, 1954. Diagnosis and Improvement of Saline and Alkali Soils. Agriculture Handbook, No. 60. US Department of Agriculture, Washington DC USA.

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. Int. Soc. Microb. Ecol. J. 5, 173–183.

Venglovsky, J., Martinez, J., Placha, I., 2006. Hygienic and ecological risks connected with utilization of animal manures and biosolids in agriculture. Livest. Sci. 102, 197–203.

Walkley, A., Black, I.A., 1947. A critical examination of a rapid method for determining organic matter in soils: effect of variations in digestion conditions and inorganic soil constituents. Soil Sci. 63, 251–264.

Xiloyannis, C., Montanaro, G., Sofo, A., 2002. Water emergency. Proposal in order to contain drought damages in fruit crops [Apulia – Basilicata] (in Italian). Frutticoltura 7–8, 19–27.

Yuan, X.M., Tong, Y.A., Yang, X.Y., Li, X.L., Zhang, F.S., 2000. Effect of organic manure on soil nitrate nitrogen accumulation. Soil Environ. Sci. 9, 197–200.