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# Management of irrigation water and nutrient demands of tomato (*Lycopersicon esculentum* Mill.) using urban treated wastewater from a pilot-scale horizontal subsurface flow system constructed wetland in Sicily (Italy)

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ABSTRACT

The reuse of treated wastewater (TWW) in crop irrigation is an advanced and rational approach to water resource management in agriculture. Results would seem to demonstrate that it could be an extremely important tool in the reduction of freshwater (FW) consumption in agriculture, at the same time helping to increase crop yields through the transfer of nutrients required for crop growth. In arid and semi-arid areas of the Mediterranean, constructed wetlands can play a key role in the treatment and reuse of wastewater due to their multifunctional nature. The aim of this study was to manage water and nutrient requirements of tomato (*Lycopersicon esculentum* Mill.) using TWW from a pilot-scale horizontal subsurface flow system (HSSFs) in a comparative study with traditional agronomic management methods. Research was carried out in 2015 at a pilot-scale HSSFs test area in West Sicily. Tomato plant plots were irrigated with both FW and with TWW from 2 planted-units and an unplanted-unit. Results showed that the pilot system was efficient in the treatment of wastewater and FW saving was high: approx. 90 $m^3$ of water per t of total tomato yield. The TWW affected the productive, biometric and qualitative parameters of the tomato fruits considerably. The increase in total tomato fruit yield using TWW compared to FW was found to be 4 t $ha^{-1}$ regarding water from unit A, 6 t $ha^{-1}$ from unit B, and 7 t $ha^{-1}$ from unit C of the HSSFs. *Escherichia coli* concentrations were not always within the threshold limits required by Italian law concerning the reuse of TWW in irrigation. Maximum microbial contamination was found in the fruit skin (106 CFU 100 $g^{-1}$ on average) and in those fruits which were in contact with bare soil. No significant variation of soil pH was found but an increase in organic matter content and salinity was recorded in TWW-irrigated plots. Results confirm that TWW *can* provide an additional source of water and fertilizer in areas where FW supply is limited.

*Keywords:* Phytoremediation; Freshwater saving; N, P, K supply; Agricultural crop; Irrigation and fertilization

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## 1. Introduction

The reuse of treated wastewater (TWW) in irrigation is a highly developed approach to the use of water resources in agriculture and a widespread practice in many European countries in the Mediterranean region [1–7], mostly as a result of water scarcity for irrigation purposes in arid and semi-arid areas [8]. The Agricultural sector is responsible for the overwhelmingly greatest consumption of water [7–9] and it has been forced to introduce a number of water-saving measures, primarily in response to a marked drop in rainfall [10]. The reuse of TWW, therefore, is seen as an extremely important initiative when attempting to reduce freshwater (FW) consumption in agriculture. Treated wastewater is widely regarded as an alternative to FW sources traditionally consumed in crop irrigation, and the rational use of wastewater could contribute to satisfying that water demand whilst leading to significant savings in FW. [11–14]. The reuse of TWW would, therefore, reduce competition for water between the various sectors [15] and ensure higher-quality water is available to address more urgent needs, such as that of drinking water. The reuse of TWW in irrigation can also help increase yields through the transfer of nutrients required for crop growth [16–21]. This could supplement or replace nutrient management programmes, leading to financial savings for the farmer. The treatment of wastewater can also lead to a number of ecological and environmental benefits primarily linked to reducing surface and groundwater withdrawals and to lessening water pollution caused by the release into the environment of untreated wastewater [22]. Experience gained in other countries has highlighted a number of issues surrounding the reuse of wastewater in irrigation [23,24] and the importance of legislation in defining health and hygiene regulations [25,26]. In Italy, microbiological threshold concentrations concerning the reuse of TWW are regulated by Ministerial Decree no. 185/2003. Compared to guidelines [27] provided in other countries, these threshold levels are considered highly restrictive and limit the use of TWW in agriculture considerably. Furthermore, no distinctions are made in this Decree between irrigation of food or non-food crops [28], or between crops requiring restricted or unrestricted irrigation [29]. Apart from legislation, the reuse of TWW in irrigation depends upon the purification efficiency of any given wastewater treatment system, which must ensure high water quality standards if intended for reuse in crop irrigation. Traditional technologies found in conventional treatment systems do not always meet legislative requirements as they do not perform all treatments needed to ensure high water quality. In arid and semi-arid areas of Italy, [30] remark that (in those area where agriculture relies heavily on irrigation) constructed wetlands (CW) can play a key role in the treatment and reuse of wastewater due to their multifunctional nature. These systems can be integrated into traditional wastewater treatment works and can be used to complete the purification process of wastewater from different water streams, as shown by various studies in Italy [31–35]. Tomato (*Lycopersicon esculentum* Mill.) is not only highly-suited to TWW irrigation but is also one of the most important crops for the Mediterranean area as a major dietary component in many Mediterranean countries. The tomato is a spring-summer crop cycle species and constitutes a key element in the human diet due to its remarkable nutritional and health properties: it is a valuable source of nutrients, such as folate, vitamin C and potassium, as reported by various authors. [36,37] and is also rich in lycopene: a powerful natural antioxidant [38–40]. Research on this species in recent years has focused on the effects of irrigation with TWW on tomato yield and quality. From an agronomic point of view, results from a number of research studies have shown an increase in tomato yields and/or a fall in health and hygiene standards in the fruits, requiring disinfection treatment to reduce microbial contamination [3,7,29,41,42]. However, there appears to be very little in international literature on nutrient balance calculation in tomato fertilization through the reuse of TWW from CWs as an alternative to traditional nutrient management programmes. The aims of this study were: i) to evaluate the pollutant treatment performance of a pilot horizontal subsurface flow system constructed wetland (HSSFs) and to calculate the partial balance of water; ii) to calculate the N, P, K supply for tomato fertilization using TWW and to evaluate nutrient savings compared to traditional agronomic management methods; iii) to assess the effects of irrigation using urban TWW from a pilot HSSFs on a) the physical-chemical properties of the soil, b) the biometric, productive and qualitative parameters of tomato plants and c) microbial contamination of tomato fruits compared to irrigation with freshwater.

## 2. Materials and methods

### 2.1. Experimental site

Tests on the reuse of urban wastewater for irrigation of tomato (*Lycopersicon esculentum* Mill.) were carried out in 2015 in the experimental area of the pilot HSSFs in Piana degli Albanesi, a rural community (6000 inhabitants) in the West of Sicily (37°59′56″40 N–13°16′50″16 E, 740 m a.s.l.). The climate in the area is humid with a mean annual rainfall of about 800 mm, mainly distributed between October and April. With reference to time series 2002–2015, the average annual temperature was 15.5°C, average maximum temperature was 20.1°C and average minimum temperature 10.6°C. The summer drought was severe and the dry period fell between June and September; rainfall occurring mostly during spring and winter. The soil type in the area is sandy clay loam (Aric Regosol, 54% sand, 23% silt and 23% clay) with a pH content of 7.9, organic matter (OM) content of 1.91%, electric conductivity (EC) of 0.52 dS $m^{-1}$, total calcareous of 5.81%, active calcareous of 3.71%, total Kjeldahl nitrogen (TKN) of 1.30 g kg, assimilable phosphorus (P) of 18.11 ppm, assimilable potassium (K) of 152.20 ppm, and magnesium (Mg) and sodium (Na) content of 138.31 and 84.78 ppm, respectively [14,21].

### 2.2. Description of the pilot HSSFs

The system was designed by the Department of Agricultural and Forestry Sciences at the University of Palermo (Italy) in 2004 and was located downhill from the town's sewage plant (Fig. 1). The system included 3 separate parallel units (A, B and C) each 33 m long and 1 m

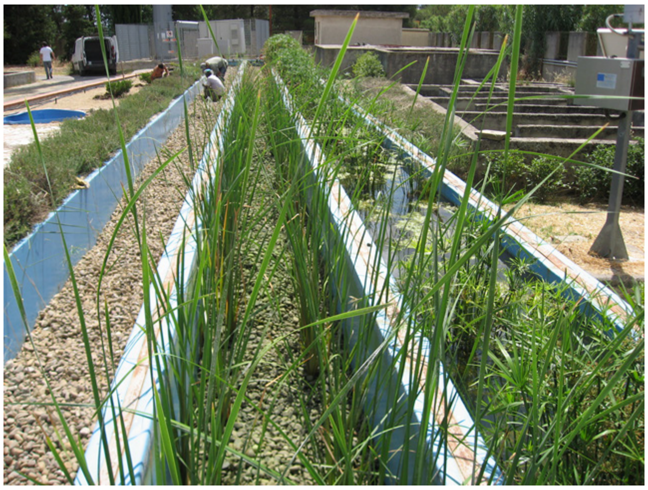

Fig. 1. A view of pilot-scale HSSF system located in Piana degli Albanesi (Sicily, Italy).

wide, providing a total surface filter bed area of 99 $m^2$ (Fig. 2). Filter bed depth was 0.5 m to allow for greater root development and to create a larger rhizosphere. The slope was 1.5%, needed to obtain regular flow. The walls of the three units were made of concrete and the floor was levelled with fine sand. The units were filled with a substrate of evenly-sized 20–30 mm silica quartz river gravel (Si 30.32%; Al 5.23%; Fe 6.87%; Ca 2.79%; Mg 1.01%). Each unit was lined with sheets of IDROEVA. Unit A and B were planted with *Cyperus alternifolius* L (umbrella sedge) and *Typha latifolia* L. (reedmace) respectively, while unit C remained unplanted. The treated urban wastewater from the outflow tank of the municipal sewage plant was initially fed into a reinforced storage tank. This water was pumped through a 1 m wide perforated pipe into each of the three units to ensure even distribution of the wastewater throughout the filter bed section, reducing the risk of hydraulic short-circuiting. In each unit, the pipe was placed 10 cm from the surface of the substrate. A timer-controlled pumping system ensured the homogeneous distribution of the wastewater in each unit. A flow meter measured the flow inlet in each unit. Pumping was continuous throughout the day without variations in time. A filter grill was fitted between the tanks and the substrate in order to avoid blockage at the outflow tanks located downhill from the 3 units. The outflow wastewater flowed downhill into three 64 $m^3$ storage tanks, one for each unit, which were connected to three drip irrigation systems (one for each tank) and used to irrigate tomato. A separate FW tank was also connected to a drip irrigation system and used for the tests. The units operated under the same hydraulic conditions and were tested under an hydraulic loading rate (HLR) of 12 cm $d^{-1}$.

### 2.3. Urban wastewater analysis

Urban wastewater samples were taken twice monthly from April to September 2015, amounting to a total of 12 times. The samples were collected at the inflow (0 m) and at the outflow (33 m) of each unit. A litre of wastewater was collected from each of the two points during each sampling. There was only one influent sampling point for each unit. The influent sample was taken close to the pipe while the effluent sample was collected at the mouth of the outflow pipe. The influent and effluent samples were instantaneous samples. Hourly sampling always occurred at the same time, at 9:00 a.m. in the morning. The pH value (± 0.01 pH), electrical conductivity (EC) (% 0.05 of value), temperature (T) and dissolved oxygen levels (DO) (% 0.05 of value) levels were determined directly on site at the time of sampling using a portable Universal meter (Multiline WTW P4), in compliance with the calibration protocol for each of the four parameters. Using Italian water analytical methods [43], total suspended solids (TSS) (± 0.1, mg $L^{-1}$), biochemical oxygen demand ($BOD_5$) (± 0.09, mg $O_2$ $L^{-1}$), chemical oxygen demand (COD) (± 0.09, mg $O_2$ $L^{-1}$), total Kjeldahl nitrogen (± 0.1, mg N $L^{-1}$), ammonia nitrogen ($NH_4$–N) (± 0.1, mg $NH_4$ $L^{-1}$), total phosphorus (TP) (± 0.1, mg P $L^{-1}$), sodium (± 0.09, mg Na $L^{-1}$), potassium (± 0.08, mg K $L^{-1}$), calcium (Ca) (± 0.08, mg Ca $L^{-1}$), magnesium (± 0.09, mg Mg $L^{-1}$), and chloride (Cl) (± 0.09, mg Cl $L^{-1}$) levels were determined in laboratory. Total coliform (TC), faecal coliform (FC), faecal streptococci (FS), *Escherichia coli* (*E. coli*) and

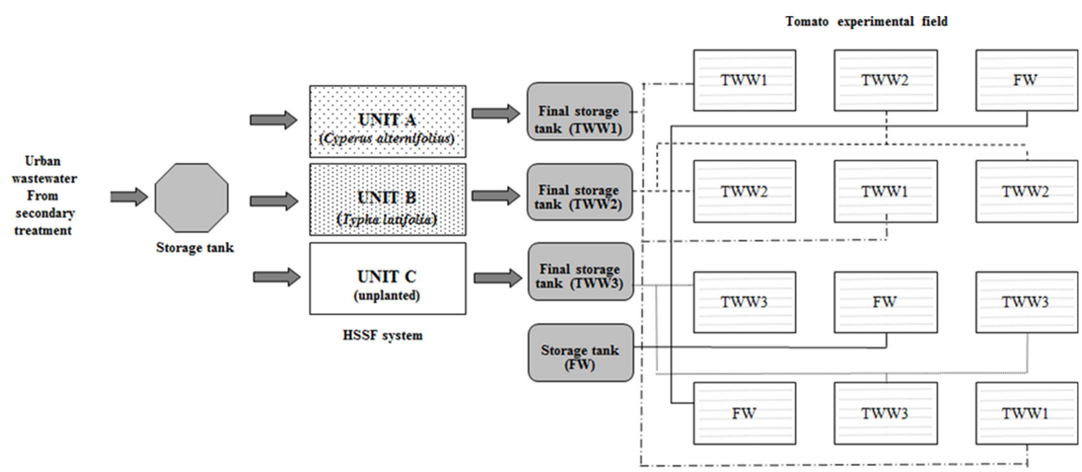


Fig. 2. Layout of pilot-scale HSSF system in Piana degli Albanesi (Sicily, Italy).

*Salmonella* spp. levels were determined by membrane filter technique, based on standard methods for water testing [44]. Helminth egg level was determined according to [45] guidelines. Removal efficiency (RE) of a pilot HSSFs was based on pollutant concentrations and was calculated according to IWA [46]:

$$RE = \frac{C_i - C_0}{C_i} * 100 \quad (1)$$

where $C_i$ and $C_0$ are the mean concentrations (mg/L) of the pollutants in the influent and effluent.

### 2.4. HSSFs water balance

The FAO Penman-Monteith method was used to calculate $ET_0$ (reference evapotranspiration) [47]. The Penman-Monteith equation was used to calculate daily $ET_0$ (mm/d) based on microclimate data taken from an automatic weather station belonging to the Sicilian Weather and Climate Service located near to the pilot system.

$$ET_0 = \frac{0.408\Delta\left(R_n - G\right) + \gamma\left(\frac{900}{T} + 273\right)u_2\left(e_s - e_a\right)}{\Delta + \gamma\left(1 + 0.34u_2\right)} \quad (2)$$

where $R_n$ is net radiation at the crop surface (MJ m$^2$/d), G is soil heat flux density (MJ m$^2$/d), $T$ is average air temperature (°C), $u_2$ is wind speed at 2 m height (m/s), $e_s$ is the saturation vapour pressure (kPa), $e_a$ is the actual vapour pressure (kPa), $e_s - e_a$ is the saturation vapour pressure deficit (kPa), Δ is the slope of the vapour pressure curve (kPa/°C), γ is the psychrometric constant (kPa/°C). The $ET_0$ values were calculated using the cool-season turfgrass *Festuca arundinacea* Schreb. The water balance for each unit was determined separately every 10 d from April to November 2015. This period was chosen according to the growth dynamics of the species.

For the planted units, an estimate of the water balance was calculated, in agreement with IWA [46], using the following equation:

$$Q_o = Q_i + \left(P - ET_c\right)A \quad (3)$$

where $Q_o$ = wastewater outflow rate (m$^3$/d), $Q_i$ = wastewater inflow rate (m$^3$/d), $P$ = precipitation rate (mm/d), $ET_c$ = crop evapotranspiration (mm/d), $A$ = wetland top surface area (m$^2$).

For the unplanted unit, the water balance was calculated using the following equation: $Q_o = Q_i + (P - ET_{con})A$, where $ET_{con}$ = evapotranspiration from unplanted control (mm/d). The amount of water at the inflow and outflow of each unit was determined using a volumetric flow meter. Rainfall was determined with a pluviometer. $ET_c$ was estimated using Eq. (3)

$$ET_c = Q_i + P(A) - Q_o$$

$ET_{con}$ was estimated using Eq. (3):

$$ET_{con} = Q_i + P(A) - Q_o$$

### 2.5. Description of the experimental open field and main cultivation practices

An experimental open field of tomato was set up close to the pilot HSSFs. Tomato cultivar "Incas" was used for the tests and it was transplanted in the third 10-d-period of April 2015. The plant density was 2.2 plants m$^{-2}$. The between-plant distance on the row was 30 cm and the inter-row distance was 150 cm. The single plot size was 50 m$^2$ leading to 112 plants per plot. The experimental field was equipped with four drip irrigation systems, one for each irrigation treatment. For each irrigation treatment, the tomato plot was replicated three times. Each module therefore irrigated a total of 336 tomato plants. Drippers in the irrigation system were located 30 cm apart. Irrigation was applied from April to June twice a week for 1 h and from July to September twice a week for 3 h, due to differences in rainfall distribution and tomato plant evapotranspiration rates and intensity between these two periods. This irrigation schedule was applied both for TWW and FW. Tomato water needs were defined by the differences between the amount of water lost by evapotranspiration and rainfall rates. Crop evapotranspiration ($ET_c$) of tomato was calculated according to Doorenbos and Pruitt [48] using the equation: $ET_c = K_c\, ET_0$, where $K_c$ is the crop coefficient of tomato and $ET_0$ is the reference evapotranspiration (Penman-Monteith equation). During the crop cycle, FW irrigated-plots received 80 kg N ha$^{-1}$, 130 kg $P_2O_5$ ha$^{-1}$ and 120 kg $K_2O$ ha$^{-1}$ through fertigation. In TWW irrigated-plots, we estimated the amount of N, $P_2O_5$ and $K_2O$ supplied by irrigating with TWW which should be taken into consideration for the commonly-used fertilization programme of tomato based on previous analyses. Both for FW and TWW, the Sodium Adsorption Ratio (SAR) was calculated according to the following formula:

$$\text{SAR} = \frac{\left(\text{Na}^+\right)}{\sqrt{\frac{1}{2}\left(\text{Ca}^{2+}\right) + (\text{Mg}^{2+})}} \quad (4)$$

Pest and weed control was carried out according to traditional management practices. Tomato fruits were hand harvested at full red stage of maturity from the first 10-d in August to the third 10-d in September at weekly intervals.

### 2.6. Crop measurements

The marketable and unmarketable fruits were counted and weighted. The marketable yield (MY), the number of marketable fruits per plant (MYP), the unmarketable yield (UMY) and the number of unmarketable fruits per plant (UMYP) were calculated. The main biometric and qualitative parameters of tomato were successfully determined in laboratory on a sample of 20 marketable fruits from each plot. Fruit diameter (D) was expressed as relationship between the equatorial and longitudinal diameter of the fruit. Fruit colour (Co) was evaluated using a spectrophotometer and the a*/b* ratio was calculated to describe the color changes of tomato fruits. Fruit soluble solids content (SSC) was determined using a refractometer to determine the °Brix. Dry matter content (DM) was calculated by drying the fruits in a ventilated oven at 70°C. The titratable acidity (TA) of tomato juice was also measured with a free acid neutralization. Fruits in 300 g samples were harvested a week before irrigation from each plot to determine the microbial contamination. Samples were successfully washed with sterile water. Fruit skin was removed and homogenized with sterile water by a stomacher. The same procedure was carried out for fruit flesh. Then serial 10-fold dilutions were made within the same medium. In both of two fruit parts, faecal coliforms, faecal streptococci and *E. coli* were measured, using membrane filtration techniques [44]. Salmonella protocol was made according to the methodology shown by Giammanco et al. [49]. All the analyses were carried out at Corissia Research Centre of Palermo.

### *2.7. Soil analysis*

Soil parameters were: pH, electrical conductivity (EC), organic matter (OM), total nitrogen (TKN), assimilable phosphorus (P), assimilable potassium (K), active calcareous (active $CaCO_3$), magnesium (Mg) and sodium (Na) content. Soil measurements were carried out only in the topsoil (0.30 m) close to the rhizosphere of tomato. Before transplanting, three soil samples were randomly collected from each replicate and analysed. At the end of the test, one soil sample was collected from each subplot for each replicate and analysed. Soil samples were air dried, ground and sieved to pass through a 2-mm sieve screen and then analyzed for chemical and physical characteristics. The samples were analysed for pH and EC in the ratio of 1:2 dry soil: water extract, pH was determined with a calibrated pH-meter (±0.01), EC with a calibrated conductivimeter (% 0.05 of value), OM with the Walkley and Black method [50] (±0.01, %), TKN by the Kjeldahl procedure [51] (±0.02, g $kg^{-1}$), assimilable P by the Olsen method [52] (±0.02, ppm) and active calcareous using the Drouineau method [53] (±0.01, %). K (±0.08, ppm), Mg (±0.09, ppm) and Na (±0.09, ppm) contents were determined by atomic absorption spectrophotometer. All the analyses were carried out at Corissia Research Centre of Palermo.

### *2.8. Climatic data*

Data on rainfall, temperature and potential evapotranspiration were collected from a meteorological station belonging to the Sicilian Agro-Meteorological Information Service situated close to the pilot HSSFs. The station was synchronized with GMT in order to operate using synoptic forecast models. It was equipped with a MTX datalogger (model WST1800) and various sensors: wind speed sensor MTX (model Robinson cup VDI with an optoelectronic transducer), global radiation sensor (model Philipp Schenk – 8102 thermopile pyranometer) to measure cumulative direct and diffuse solar irradiance, temperature sensor MTX (model TAM platinum PT100 thermoresistance with anti-radiation screen), relative humidity sensor – MTX (model UAM with capacitive transducer with hygroscopic polymer films and antiradiation screen), rainfall sensor MTX (model PPR with a tipping bucket rain gauge), and leaf wetness sensor MTX (model BFO with PCB). This equipment provided data on the wind speed (m/s), minimum daily relative moisture levels (%), average daily soil temperature (°C), average daily air temperature (°C), total daily solar irradiance ($MJ/m^2$), total daily rainfall-frequency [days mm > 1] (%), and rainy days per year [days mm > 1] (%). Furthermore, using the Penman–Monteith equation, the potential evapotranspiration (PET) was calculated.

### *2.9. Experimental design and statistical analysis*

A randomized complete block design [54] was used with three replications to test irrigation with four treatment levels: 1) irrigation with FW; 2) irrigation with TWW from a *Cyperus alternifolius* planted-unit; 3) irrigation with TWW from a *Typha latifolia* planted-unit; 4) irrigation with TWW from an *unplanted*-unit. Statistical analysis was performed with the software SPSS for Windows and included analysis of variance (one-way ANOVA). The difference between means was carried out using the Tukey test. All the representative values were presented using mean ± standard error calculations.

## 3. Results and discussion

### *3.1. Removal of pollutants in the pilot-scale HSSFs*

Tables 1–2 show the pollutant removal levels of the pilot HSSFs obtained during testing carried out from April to September 2015. The pH value at the inflow pipe was somewhat alkaline; this alkalinity increased at the outflow for both of the planted units, however, levels were even higher at the outflow of the unplanted unit. In the unplanted unit, the absence of vegetation stimulated greater atmospheric aeration in the substrate and, in some cases, the growth of algae with consequences on the hydrogenization of the water, as found by DeBusk and DeBusk [55]. Differences were also found regarding EC when comparing the planted units with the unplanted unit. The EC was found to be higher in the planted units; the highest level being observed at the outflow of the reedmace-unit. This was due to ET processes which determined a greater loss of water and, therefore, an increase of the solute in the solution, as described by Leto et al. [28]. No significant differences in DO levels were found between the planted units despite differences in the root apparatus of the two macrophytes; DO remained under 1.25 mg $L^{-1}$, consistent with values found in other HSSFs [46]. At the outflow, the chemical-physical pollutant levels were found to be lower in both of the planted units compared to the unplanted unit. This was mainly due to the influence of vegetation on pollutant removal rates. The direct uptake of the nutrients by the plants and the direct degradation of pollutants by the microorganisms determined the improvement in TWW quality , as stated by Stottmeister et al. [56]. Pollutant removal efficiencies were higher in the planted units compared to the unplanted unit. When comparing only the planted units, TSS, $BOD_5$, COD, TKN, $N\text{-}NH_4$ and TP removal levels were higher in the reedmace-unit due to greater adaptability of the species to the climate and substrate conditions of the study area, and also due to greater plant and root density in reedmace. Gersberg et al. [57], not finding any significant differences between planted and unplanted beds, claim that TSS removal can be attributed to the gravel size of the substrate. In our research, however, the differences between the planted and unplanted

Table 1
Variation of pH, T, DO and ECw in the pilot units from April to September 2015. Average (± standard error), minimum and maximum values are shown ($n$ = 12)

| Parameters | Main inlet | Unit A | | Unit B | | Unit C | | Threshold values for Italian Ministerial Decree 185/2003 |
|---|---|---|---|---|---|---|---|---|
| | | *Cyperus alternifolius* | | *Typha latifolia* | | Unplanted | | |
| | | Outlet | Variation (%) | Outlet | Variation (%) | Outlet | Variation (%) | |
| pH | 7.55 ± 0.05 | 7.58 ± 0.03 | –1.3 | 7.72 ± 0.03 | –1.3 | 8.15 ± 0.03 | –8.0 | 6–9.5 |
| | (7.21–7.70) | (7.42–7.78) | | (7.50–7.90) | | (8.00–8.43) | | |
| T (°C) | 22.3 ± 0.2 | 22.5 ± 0.1 | –0.9 | 22.4 ± 0.1 | –0.4 | 22.7 ± 0.1 | –1.8 | – |
| | (20.9–23.1) | (21.9–23.2) | | (21.8–23.1) | | (22.0–23.5) | | |
| EC (µS $cm^{-1}$) | 5398.9 ± 12.9 | 673.4 ± 5.0 | –24.7 | 706.2 ± 7.1 | –30.8 | 567.9 ± 14.5 | –5.2 | 3000 |
| | (492.3–634.2) | (657.8–701.2) | | (676.8–759.1) | | (511.2–672.3) | | |
| DO (mg $L^{-1}$) | 1.35 ± 0.03 | 0.99 ± 0.01 | 30.8 | 0.98 ± 0.03 | 23.1 | 1.25 ± 0.03 | 7.7 | – |
| | (1.10–1.50) | (0.90–1.10) | | (0.80–1.20) | | (1.13–1.42) | | |

units were marked and this demonstrates that the interaction of the macrophyte root systems with the substrate and microorganisms influences the TSS reduction process to a greater extent than the substrate alone. This consideration is consistent with the findings of Gagnon et al. [58], who highlight that, in a constructed wetland the presence of plants enhances microbial density/activity which, in turn, greatly influences pollutant removal. $BOD_5$ and COD removal rates stayed within a range consistent with previous HSSFs studies using TWW. As Iamchaturapatr et al. [59] report, in CWs the macrophytes use phosphorous as an essential element and their root tissues contain phosphorous. In our research, the lower phosphorous removal rate in the planted units was mainly due to the granular saturation of most of the substrate sorption sites. The adsorption capacity of these sites in HSSFs can be used to obtain significant phosphorous removal rates, although adsorption would seem to decrease over time, according to Vymazal [60]. Nitrogen removal efficiency was lower than organic matter removal in all the units due to low oxygen levels in the system, as reported by Vymazal [61]. The planted units produced higher removal levels for TKN and N–$NH_4$ compared to the unplanted unit and this was consistent with other authors [62,63]. In particular, Vymazal and Krőpfelová [64] highlight the fact that the planted units outperform the unplanted ones in as much as the rhizosphere contributes to the development of the microbe community, providing a valuable source of carbon compounds through root exudates and releasing oxygen through the roots. Ammonia nitrogen levels in the TWW at the inflow were not high, and, hence, given also the limited oxygen levels in the substrate, it is reasonable to assume that incomplete transformation of ammonia nitrogen into nitrites and nitrates occurred and, therefore, low nitrification. The planted units did not show high removal rates for metals Ca, K, Mg and Na. No great differences in metal removal rates were found between the two planted-units: findings which were consistent with literature. Vymazal and Švеha [65], for example, reported Ca, K, Mg and Na removal as averaging only 1.4 %, 10.6 %, 6.1 % and 7.4 %, respectively, in two HSSF CWs in the Czech Republic; the authors claim that HSSFs were not effective in the retention of these metals. Kadlec and Wallace [66] provide an explanation of why there is little change in alkali metal concentrations from inlet to outlet in a CW. As sustained by these authors, calcium concentration does not change significantly in a CWs because there is generally an excess of Ca in wastewaters. High concentration levels of magnesium are not affected when wastewaters flow through the CWs because Mg concentration of wastewaters almost always exceeds the requirements for plant growth. Moreover the dissolved sodium content of wastewaters does not change significantly from inlet to outlet in a CWs due to low Na demand of plant species. On a microbiological level (Table. 3), the three units showed marked differences for all the parameters in the study. The treated wastewater at the inflow and outflow pipes did not contain *Salmonella* spp. or helminth eggs. Both of the planted units produced pathogen levels which were lower at the outflow than in the unplanted unit. In particular, better aerobic conditions in the planted units, due to atmospheric air circulation and the translocation of oxygen from the macrophyte root system, facilitated production of a greater bacterial biofilm and promoted pathogen load removal compared to the unplanted unit, as claimed by El-Khateeb et al. [67]. In our research, pathogen removal efficiency was high for each parameter in the study and consistent with international literature: *Escherichia coli* removal was 87.7 % in the umbrella sedge-unit and 90.4 % in the reedmace-unit. However, the average values of the chemical and microbiological parameters at the outflow of the pilot HSSFs were not all within the legal limits as stipulated by the Italian Ministerial Decree 185/2003 regarding the reuse of treated wastewater for irrigation purposes. The age and the hydraulic conditions of the pilot HSSFs affected TP and $NH_4$–N concentrations at the outflow of planted and unplanted units. *E. coli* levels were not always within legally acceptable limits, despite the system's high RE. In fact, for a considerable number of TWW samples collected in April and May, *E. coli* levels (up

Table 2
Main chemical and physical composition of the urban wastewater from the inflow and outflow of the pilot units. Removal efficiency (RE) from April to September 2015. Average (± standard error), minimum and maximum values are shown ($n$ = 12)

| Parameters | Main inlet | Unit A | | Unit B | | Unit C | | Threshold values for Italian Ministerial Decree 185/2003 |
|---|---|---|---|---|---|---|---|---|
| | | *Cyperus alternifolius* | | *Typha latifolia* | | Unplanted | | |
| | | Outlet | RE (%) | Outlet | RE (%) | Outlet | RE (%) | |
| Colour | NP* | NP | – | NP | – | NP | – | – |
| Odour | NU** | NU | – | NU | – | NU | – | – |
| Coarse matter | – | – | – | – | – | – | – | – |
| TSS (mg$^{-1}$) | 30.0 ± 0.7 | 9.8 ± 0.2 | 67.3 | 9.0 ± 0.2 | 69.9 | 22.7 ± 0.5 | 24.2 | 10 |
| | (26.7–34.1) | (8.7–11.4) | | (8.1–10.5) | | (21.1–28.2) | | |
| $BOD_5$ (mg $O_2$ L$^{-1}$) | 24.2 ± 0.9 | 7.7 ± 0.3 | 68.1 | 7.3 ± 0.3 | 69.8 | 13.0 ± 0.2 | 44.4 | 20 |
| | (20.3–30.2) | (6.2–10.8) | | (6.0–10.2) | | (12.1–15.2) | | |
| COD (mg $O_2$ L$^{-1}$) | 50.4 ± 3.4 | 12.0 ± 0.7 | 75.8 | 10.9 ± 0.7 | 78.1 | 30.8 ± 1.9 | 38.3 | 100 |
| | (35.8–74.2) | (8.3–18.5) | | (8.7–17.4) | | (21.2–47.4) | | |
| TKN (mg N L$^{-1}$) | 18.0 ± 0.7 | 9.1 ± 0.1 | 48.7 | 8.4 ± 0.2 | 53.0 | 15.6 ± 0.4 | 12.4 | 15 |
| | (14.5–23.1) | (8.1–10.7) | | (7.1–10.1) | | (14.0–19.2) | | |
| N-$NH_4$ (mg $NH_4$ L$^{-1}$) | 13.5 ± 0.3 | 7.4 ± 0.2 | 44.7 | 6.5 ± 0.2 | 52.0 | 10.7 ± 0.2 | 20.2 | 2 |
| | (11.8–16.2) | (6.6–8.7) | | (5.0–7.6) | | (9.6–11.9) | | |
| TP (mg P L$^{-1}$) | 7.4 ± 0.07 | 4.7 ± 0.07 | 36.2 | 4.5 ± 0.05 | 39.2 | 7.0 ± 0.05 | 4.8 | 2 |
| | (7.0–7.9) | (4.4–5.1) | | (4.2–4.7) | | (6.7–7.3) | | |
| Cl (mg Cl L$^{-1}$) | 125.4 ± 0.5 | 115.4 ± 0.6 | 8.1 | 114.2 ± 0.7 | 9.1 | 123.5 ± 0.4 | 1.7 | 250 |
| | (121.5–129.1) | (109.4–119.1) | | (110.2–119.8) | | (120.3–125.2) | | |
| Ca (mg Ca L$^{-1}$) | 80.3 ± 0.6 | 61.5 ± 0.8 | 23.3 | 57.8 ± 0.6 | 28.1 | 72.2 ± 0.2 | 10.1 | – |
| | (76.1–82.3) | (58.4–66.4) | | (55.9–62.1) | | (70.6–73.3) | | |
| K (mg K L$^{-1}$) | 94.4 ± 1.1 | 71.3 ± 0.7 | 24.4 | 68.1 ± 0.5 | 27.8 | 84.4 ± 0.7 | 10.6 | – |
| | (89–101) | (68.4–77.9) | | (66.6–74.1) | | (81.2–88.1) | | |
| Mg (mg Mg L$^{-1}$) | 23.9 ± 0.4 | 21.0 ± 0.2 | 12.1 | 20.7 ± 0.3 | 13.2 | 22.5 ± 0.4 | 5.9 | – |
| | (22.1–27.9) | (19.9–22.9) | | (18.9–22.3) | | (20.2–25.2) | | |
| Na (mg Na L$^{-1}$) | 154.2 ± 1.7 | 139.4 ± 0.2 | 9.6 | 138.3 ± 0.6 | 10.3 | 150.1 ± 1.7 | 2.7 | – |
| | (143.2–161.4) | (137.7–140.2) | | (136.3–143.2) | | (139.3–158.4) | | |

to 100 CFU 100 ml$^{-1}$) exceeded Italian legal limits. Furthermore, great variability in FC levels (from 1 × $10^4$ to 2 × $10^4$ CFU 100 ml$^{-1}$) was also determined in the TWW samples at the outflow.

### 3.2. HSSFs water balance

Trends for maximum air temperature, minimum air temperature, average air temperature, solar radiation and total rainfall are shown in Fig. 3. Between April and September 2015, average air temperature trends were consistent with ten-year averages. Maximum average air temperature was 32.2°C in the first 10 d of August and minimum average air temperature was 4.7°C in the first 10 d of April. Rainfall was highly concentrated in May and September. In the summer period, average monthly rainfall was 11.5 mm. Relative humidity trends were similar to ten-year averages due to similar temperatures and rainfall. Greatest total solar radiation was recorded in the third 10 d of June at 28.2 MJ/m$^2$, while lowest was recorded in the third 10-d of September at 14.9 MJ/m$^2$. Climate conditions in the area significantly influenced ET processes and, consequently, water balance in the pilot HSSFs. The results of water balance showed different water use for the reedmace-unit and umbrella sedge-unit compared to the unplanted unit. In the test period, $ET_c$ in the planted units was found to be much higher than $ET_0$ and $ET_{con}$ (Fig. 4). For the reedmace-unit, average 10-d $ET_{typ}$ ranged between 36.0 mm/d (third 10 d of July) and 2.2 mm/d (first 10 d of April). For the umbrella sedge-unit, maximum average 10-d $ET_{cyp}$ (34.0 mm/d) was recorded in the second 10 d of July, whilst minimum average 10-d results (1.9 mm/d) were obtained in the mid 10 d of April. Considering cumulative ET (Fig. 5), the reedmace-unit was found to have higher (3920.1 mm) rates than the umbrella sedge-unit (3318.1 mm). If we com-

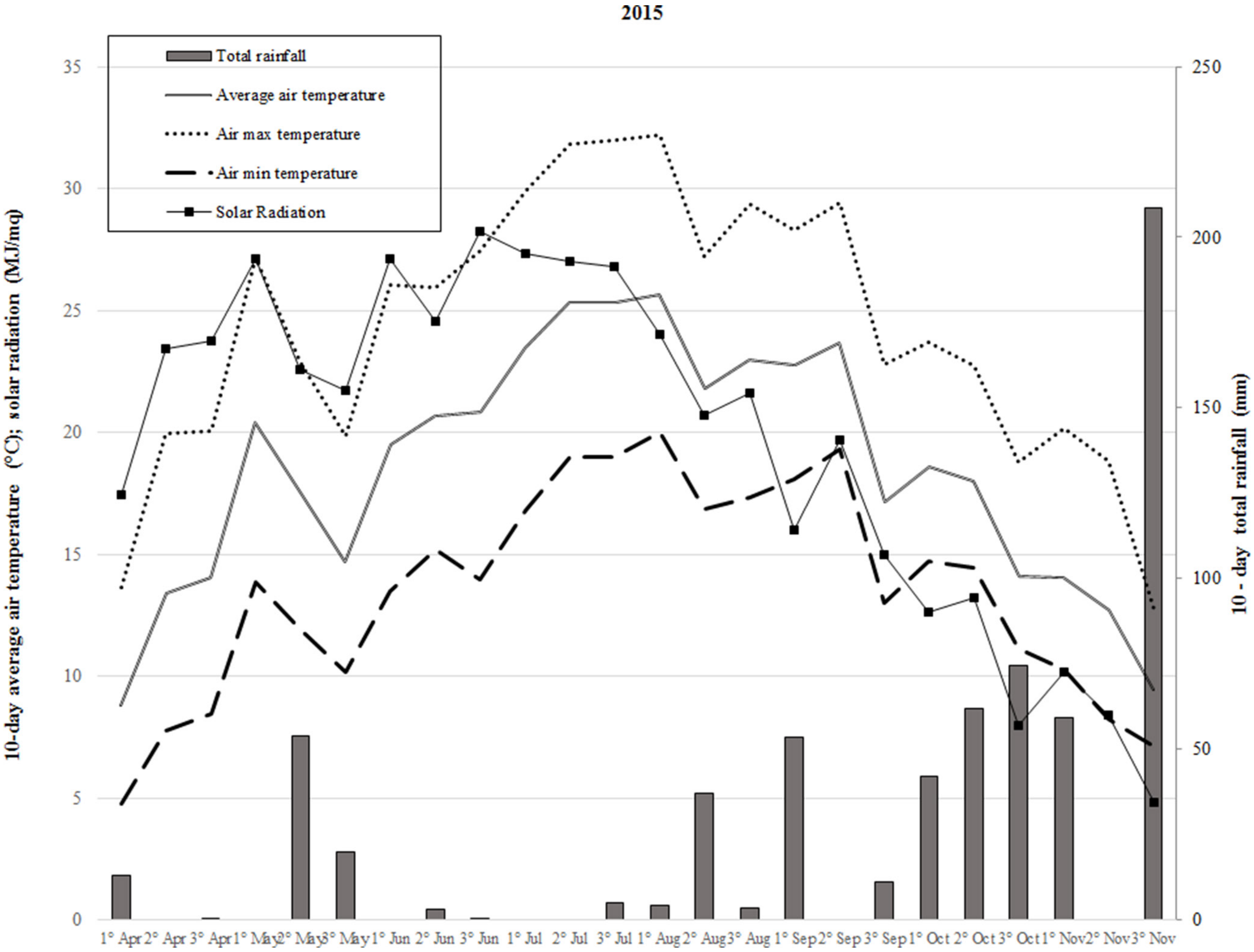


Fig. 3. Trends of 10-d minimum, maximum and average air temperature, solar radiation, and total rainfall during the test period.

pare cumulative $ET_c$ rates from the two planted units with cumulative $ET_{con}$ (853 mm), we find that there was a significant increase in ET for the planted units, which highlights the effect that vegetation has on water loss in a CWs with TWW in continuous movement. In Fig. 6, $Q_0$ trends relative to $Q_i$, cumulative ET and total rainfall are shown. We did not observe great differences in average 10-d $Q_o$ between the two planted-units. In the reedmace-unit, average 10-d $Q_o$ was found to be 50.3 $m^3$, while in the umbrella sedge-unit it was found to be 51.5 $m^3$. As $Q_i$ was constant for all of the 10-d periods in the study period (60 $m^3$/10 d), water loss was on average 9.7 $m^3$/10 d in the reedmace-unit and 8.5 $m^3$/10 d in the umbrella sedge-unit. For both the planted units, the highest water loss was recorded in July due to higher $ET_c$ values for the same period. Despite identical growth, climatic and hydraulic conditions throughout the system, greater water loss occurred in the reedmace-unit, mainly due to greater growth of reedmace compared to umbrella sedge (average leaf surface and foliar density), as sustained by [68]. It is important to highlight that reedmace consumed more water and the amount of TWW available for irrigation purposes was, therefore, lower than that at the umbrella sedge-unit outflow. In arid and semi-arid regions of the Mediterranean, the high water loss levels due to ET must not be undervalued, however, unquestionably, CWs represent an innovative approach which could guarantee continuity for crop irrigation, notwithstanding the large water losses during summer months.

### 3.3. FW and TWW characteristics

The chemical and microbiological characteristics of TWW and FW used in this study are shown in Table 4. On average, TWW had higher EC, OM, N, P, K and other alkali metal values than FW. When comparing the TWW and FW in the test period, the lowest variations in nutrient and salt concentrations were found from June to August. During summer, growth rates of the two macrophytes were higher than in other seasons; this greatly improved pollutant removal rates in the planted units, reducing chemical pollutant levels at the outflow considerably. By comparing TKN levels in the effluents from the planted units between May and September, we observed a decrease in nitrogen in TWW due to plant uptake and microbial nitrogen processes. Knowledge of the chemical characteristics

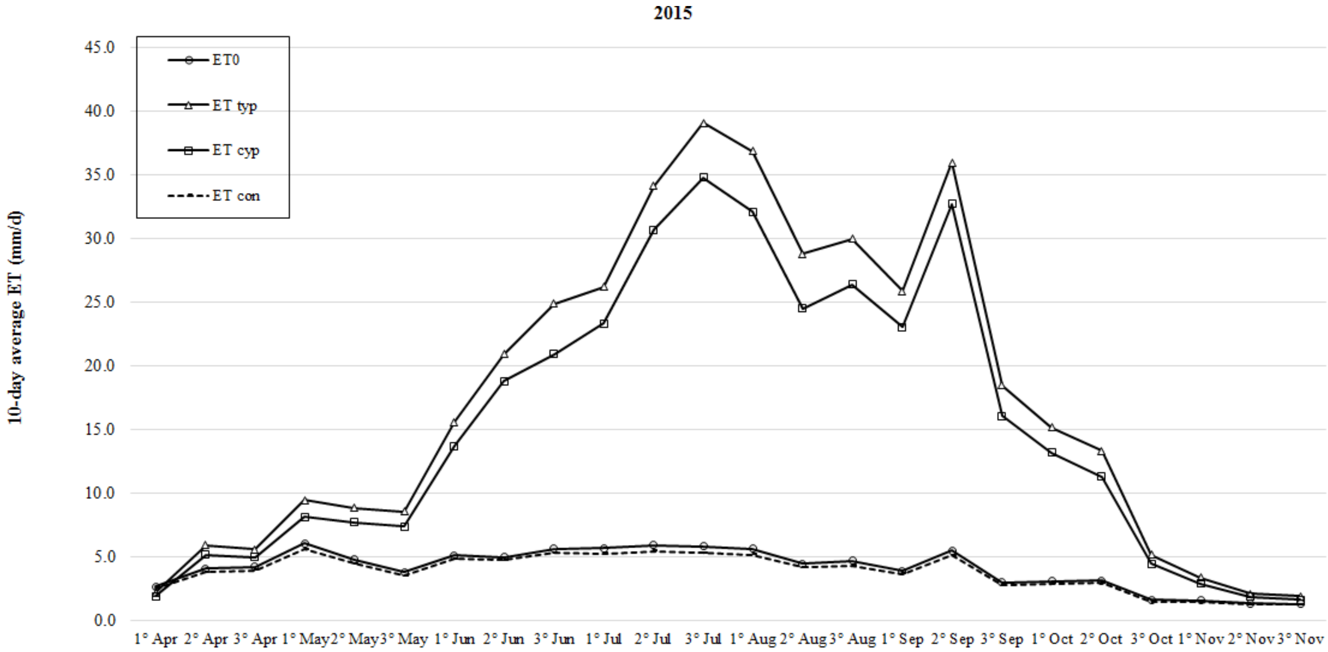


Fig. 4. 10 d- average ET0, ETcon, ETtyp and ETcyp.

of the TWW is essential when irrigating crops frequently consumed raw, such as tomato. Recent research has highlighted the effects of nutrients on yield and quality of tomato and it is clear that some of these nutrients – contained in the TWW – can play an important role in tomato production. As stated by Flores et al. [69], nitrogen affects the tomato growth and increases the fruit weight and yield. Dorais et al. [37] sustain that potassium is involved in metabolic and transport processes, charge balances and generation of turgor pressure in the cells; it can affect fruit shape and fruit acid concentration, as stated by Adams [70]. Magnesium is a major constituent of the chlorophyll molecule and an enzyme activator for a number of energy transfer reactions. Calcium is a major constituent of cell walls and affects fruit quality and yield [71]. K, Mg, and Ca are indispensable nutrients for tomato production and deficiencies of these elements usually occur due to undersupply or competition, thus decreasing growth, yield, and

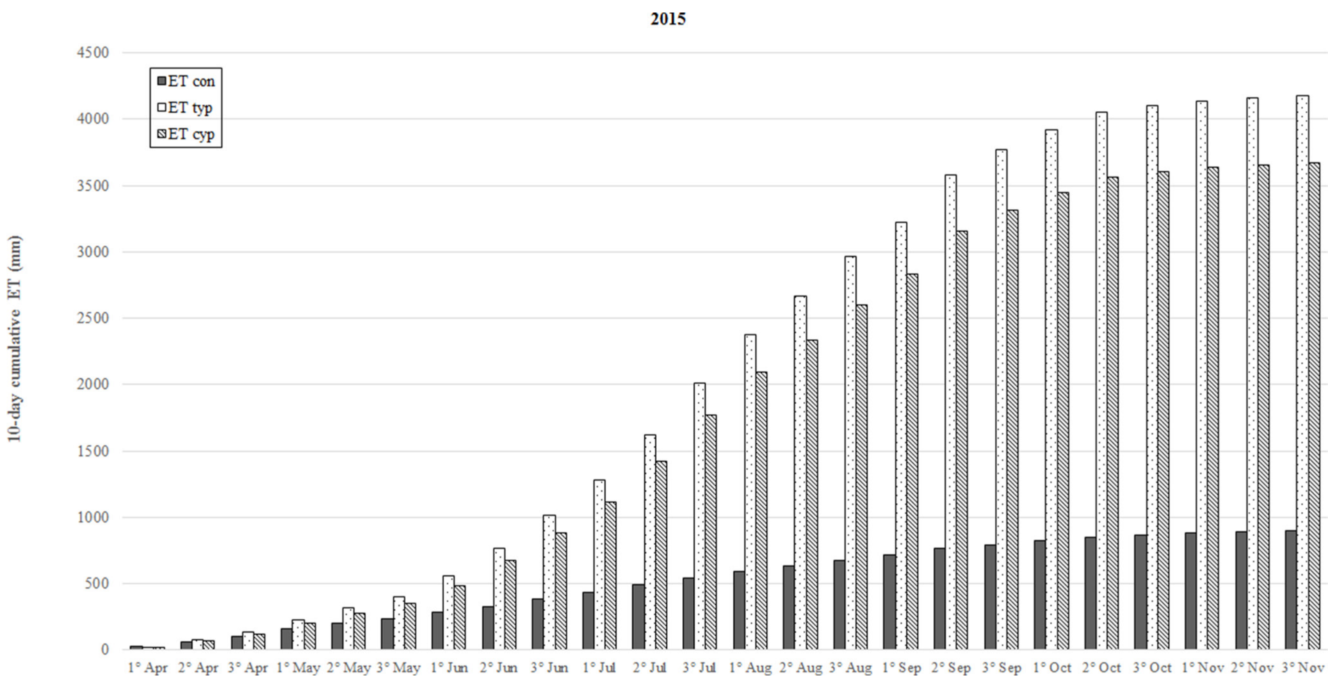


Fig. 5. 10-d cumulative evapotranspiration (ETcon, ETtyp and ETcyp).

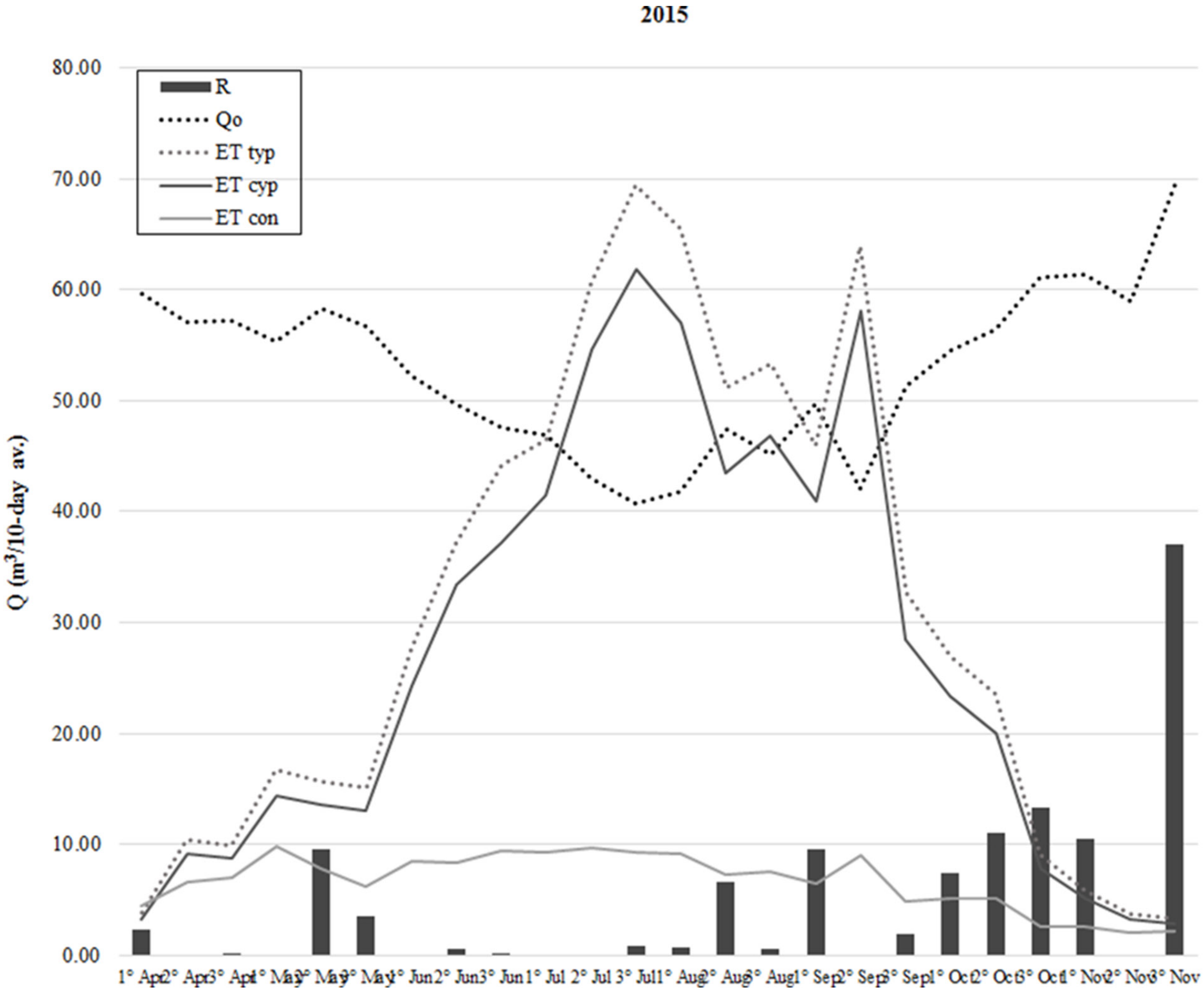


Fig. 6. $Q_0$ trends relative to $Q_i$, cumulative ETcon, cumulative ETtyp, cumulative ETcyp and total rainfall.

Table 3
Main microbiological composition of the urban wastewater from the inflow and outflow of the pilot units. Removal efficiency (RE) from April to September 2015. Average (± standard error), minimum and maximum values are shown (*n* = 12)

| Parameters | Main inlet | Unit A | | Unit B | | Unit C | | Threshold values for Italian Ministerial Decree 185/2003 |
|---|---|---|---|---|---|---|---|---|
| | | *Cyperus alternifolius* | | *Typha latifolia* | | Unplanted | | |
| | | Outlet | RE (%) | Outlet | RE (%) | Outlet | RE (%) | |
| Log10 (CFU 100 ml⁻¹) | | | | | | | | |
| TC (CFU 100 ml⁻¹) | 4.33 ± 0.01 | 3.43 ± 0.02 | 87.3 | 3.27 ± 0.01 | 91.2 | 3.99 ± 0.01 | 54.2 | – |
| | (4.24–4.37) | (3.30–3.51) | | (3.19–3.30) | | (3.95–4.00) | | |
| FC (CFU 100 ml⁻¹) | 4.13 ± 0.01 | 3.50 ± 0.02 | 79.3 | 3.26 ± 0.05 | 87.1 | 3.95 ± 0.03 | 40.6 | – |
| | (4.12–4.26) | (3.38–3.63) | | (3.05–3.62) | | (3.86–4.11) | | |
| FS (CFU 100 ml⁻¹) | 3.86 ± 0.01 | 3.19 ± 0.01 | 78.5 | 3.07 ± 0.02 | 83.5 | 3.60 ± 0.01 | 44.5 | – |
| | (3.84–3.91) | (3.14–3.27) | | (2.99–3.25) | | (3.57–3.65) | | |
| *E. coli* (CFU 100 ml⁻¹) | 3.05 ± 0.03 | 2.14 ± 0.02 | 87.7 | 2.02 ± 0.03 | 90.4 | 2.87 ± 0.05 | 38.7 | 10 (80% of samples) and 100 (maximum value point) |
| | (2.88–3.20) | (2.00–2.26) | | (1.91–2.25) | | (2.43–3.00) | | |
| *Salmonella* spp. (CFU 100 ml⁻¹) | Absent | Absent | | Absent | | Absent | | – |

Table 4
Chemical and microbiological composition of freshwater and treated wastewater that were applied for tomato irrigation. Average (± standard error) values are shown ($n$ = 12)

| Parameters | Freshwater | Treated wastewater from *Cyperus alternifolius*-planted unit | Treated wastewater from *Typha latifolia*-planted unit | Treated wastewater from unplanted-unit |
|---|---|---|---|---|
| pH | 7.01 ± 0.01 | 7.63 ± 0.03 | 7.72 ± 0.03 | 8.12 ± 0.03 |
| EC (µS cm$^{-1}$) | 279.1 ± 1.7 | 673.4 ± 5.0 | 706.2 ± 7.1 | 568.0 ± 14.5 |
| BOD5 (mg $O_2$ L$^{-1}$) | 1.4 ± 0.4 | 7.7 ± 0.3 | 7.3 ± 0.3 | 13.0 ± 0.2 |
| COD (mg $O_2$ L$^{-1}$) | 2.1 ± 0.8 | 12.0 ± 0.7 | 10.9 ± 0.7 | 30.8 ± 1.9 |
| TSS (mg L$^{-1}$) | Not detected | 9.8 ± 0.2 | 9.0 ± 0.2 | 22.7 ± 0.5 |
| $NO_3$-N (mg N L$^{-1}$) | 0.3 ± 0.1 | 2.1 ± 0.1 | 2.2 ± 0.3 | 4.0 ± 0.2 |
| TP (mg P L$^{-1}$) | 0.52 ± 0.22 | 4.71 ± 0.07 | 4.51 ± 0.05 | 7.03 ± 0.05 |
| Cl (mg Cl L$^{-1}$) | 20.1 ± 0.7 | 115.4 ± 0.6 | 114.2 ± 0.7 | 123.5 ± 0.4 |
| Ca (mg Ca L$^{-1}$) | 21.3 ± 0.9 | 61.5 ± 0.8 | 57.8 ± 0.6 | 72.2 ± 0.2 |
| K (mg K L$^{-1}$) | 3.3 ± 1.2 | 71.3 ± 0.7 | 68.1 ± 0.5 | 84.4 ± 0.7 |
| Mg (mg Mg L$^{-1}$) | 15.2 ± 1.1 | 21.0 ± 0.2 | 20.7 ± 0.3 | 22.5 ± 0.4 |
| Na (mg Na L$^{-1}$) | 10.2 ± 0.7 | 139.4 ± 0.2 | 138.6 ± 0.6 | 150.1 ± 0.7 |
| *E. coli* (CFUs 100 ml$^{-1}$) | 1.1 ± 0.3 | 2.14 ± 0.02 | 2.02 ± 0.03 | 2.87 ± 0.05 |
| TC (CFU 100 ml$^{-1}$) | 1.2 ± 0.1 | 3.43 ± 0.02 | 3.27 ± 0.01 | 3.99 ± 0.01 |
| FC (CFU 100 ml$^{-1}$) | 1.3 ± 0.2 | 3.50 ± 0.02 | 3.26 ± 0.05 | 3.95 ± 0.03 |
| FS (CFU 100 ml$^{-1}$) | 1.6 ± 0.7 | 3.19 ± 0.01 | 3.07 ± 0.02 | 3.60 ± 0.01 |
| *Salmonella* (CFU 100 ml$^{-1}$) | Absent | Absent | Absent | Absent |

quality of tomato [72]. However, an excess supply of nutrients to the fruit, such as calcium, can negatively affect fruit appearance and shelf life, as reported by Hao and Papadopoulos [73]. The interpretation of water quality for tomato irrigation (Table 5) was made by using the guidelines [74]. Observing the nutrient contents in the effluents of the pilot HSSFs, we found that the concentrations of ammonium and nitrates were not always below the recommended guidelines while the concentrations of sodium and chloride caused severe problems for irrigation uses. The average values of EC for FW (0.28 dS m$^{-1}$), TWW from umbrella sedge-unit (0.67 dS m$^{-1}$), TWW from reedmace unit (0.71 dS m$^{-1}$) and TWW from unplanted unit (0.57 dS m$^{-1}$) were not critical for tomato growth. All the values of EC may be, in fact, considered with none degree of restriction on use for irrigation according to the recommended guidelines. With

Table 5
General guidelines for interpretation of water quality for crops irrigation (Westcot and Ayers, 1985)

| Item | | Minor problems | Increasing problems | Severe problems |
|---|---|---|---|---|
| *Salinity* | | | | |
| *EC (water) | (mmhos cm$^{-1}$ or dS m$^{-1}$) | < 0.75 | 0.75–3.0 | > 3.0 |
| *Specific ion toxicity* | | | | |
| Sodium | (meq L$^{-1}$) | < 3 | > 3–9 | > 9 |
| Chloride | (meq L$^{-1}$) | < 4 | 4–10 | > 10 |
| Boron | (mg L$^{-1}$) | < 0.75 | 0.75–2.0 | > 2.0 |
| *Miscellaneous effects* | | | | |
| Ammonium-N ($NH_4$–N) | (mg L$^{-1}$) | < 5 | 5–30 | > 30 |
| Nitrate–N ($NO_3$–N) | (mg L$^{-1}$) | < 5 | 5–30 | > 30 |
| Bicarbonates ($HCO_3$) | (meq L$^{-1}$) | < 1.5 | 1.5–8.5 | > 8.5 |
| pH | | | normal range 6.0–8.4 | |

*EC = electricak conductivity

concerning to microbiological characteristics of water, in our research, FW had on average much lower values of *E. coli*, TC, FC and FS than TWW from planted and unplanted units. However contamination level with TC, FC and FS in all TWW was enough high. At outlet of pilot HSSFs, a high removal level of pathogens was observed but the concentration levels of most pathogens were not acceptable in terms of law for crops irrigation. Then the need of additional disinfection treatments to improve the quality of TWW for irrigation purposes was evident.

### 3.4. N, P and K supply using TWW

The agronomic management of N, P and K in the FW and TWW-irrigated plots is shown in Table 6. In the FW-irrigated plots, we managed the tomato plants with a fertilization programme in widespread use, applying granular fertilizers and using fertigation during the crop cycle. However, in the TWW-irrigated plots, we exploited the nutrient content in the TWW to integrate the tomato N, P and K requirements. At the outflow of the TWW-planted units, we found similar N, P and K concentrations. This was mainly due to similar nutrient removal efficiencies of the two HSSFs planted-units. The two macrophytes presented similar nutrient uptakes and the interaction between plants, microorganism and medium determined the same effect in the two planted units. It was undoubtedly these similar conditions which determined no significant differences in N, P and K rates between the TWW-irrigated plots. For N, an additional application of nitrogenous fertilizer was made between April and June in order to sustain good plant growth; this was not necessary from July to September due to excess nitrogen in the TWW. An additional application of potassium fertilizer was made only in April both for FW and TWW-irrigated plots in order to avoid any negative variation in fruit shape and fruit acid concentration. From May to September, we exploited the excess amount of potassium in the TWW to satisfy the K demand for tomato. With regards to P, in the TWW-irrigated plots from the *planted* units, we integrated the amount of P in TWW

Table 6
Agronomic management of nitrogen, phosphorus and potassium fertilization programme of tomato FW-irrigated plots and TWW-irrigated plots

| Fertilizers (kg ha$^{-1}$ month of growth$^{-1}$) | FW-irrigated plots | TWW-irrigated plots (1) | TWW-irrigated plots (2) | TWW-irrigated plots (3) |
|---|---|---|---|---|
| *Nitrogen (N)* | | | | |
| April | 20.0 | 18.9 | 18.8 | 17.9 |
| May | 20.0 | 15.4 | 15.1 | 11.3 |
| June | 20.0 | 15.6 | 15.5 | 11.6 |
| July | 10.0 | 0.0 (+3.25)* | 0.0 (+3.20)* | 0.0 (+13.40)* |
| August | 10.0 | 0.0 (+2.15) | 0.0 (+0.65) | 0.0 (+11.15) |
| September | 0.0 | 0.0 (+20.92) | 0.0 (+17.65) | 0.0 (+31.50) |
| Total Nitrogen | 80.0 | 49.8 | 49.3 | 40.7 |
| *Phosphorus ($P_2O_5$)* | | | | |
| April | 50.0 | 49.5 | 49.5 | 49.2 |
| May | 20.0 | 17.8 | 17.6 | 16.5 |
| June | 20.0 | 17.7 | 17.5 | 16.5 |
| July | 20.0 | 13.1 | 13.0 | 9.1 |
| August | 10.0 | 3.0 | 3.3 | 0.0 (+0.65)* |
| September | 10.0 | 0.6 | 0.1 | 0.0 (+5.07) |
| Total Phosphorus | 130.0 | 101.6 | 100.8 | 91.2 |
| *Potassium ($K_2O$)* | | | | |
| April | 20.0 | 11.8 | 11.3 | 10.4 |
| May | 20.0 | 0.0 (+18.20)* | 0.0 (+15.55)* | 0.0 (+23.75)* |
| June | 20.0 | 0.0 (+15.15) | 0.0 (+13.40) | 0.0 (+24.05) |
| July | 20.0 | 0.0 (+80.45) | 0.0 (+79.90) | 0.0 (+101.80) |
| August | 20.0 | 0.0 (+83.80) | 0.0 (+80.50) | 0.0 (+102.35) |
| September | 20.0 | 0.0 (+133.90) | 0.0 (+135.70) | 0.0 (+167.20) |
| Total Potassium | 120.0 | 11.8 | 11.3 | 10.4 |

(1) TWW from *Cyperus alternifolius*-planted unit; (2) TWW from *Typha latifolia*-planted unit; (3) TWW from unplanted-unit. *Extra nutrients content in the TWW.

with granular phosphorus fertilizer throughout the entire test period. This was not deemed necessary for the TWW-irrigated plots from the *unplanted* unit: a commonly-used P fertilization was made only in the month of April. The N, P and K supply using TWW highlights the fact that irrigation with TWW also provides combined fertilization for tomato due to the N, P and K supplies. In particular, for TWW-irrigated plots from HSSFs *planted* units, combined fertilization allowed on average a saving of 30 kg N $ha^{-1}$, 30 kg $P_2O_5$ $ha^{-1}$ and 108 kg $K_2O$ $ha^{-1}$ in comparison with commonly-used N, P and K fertilization programme for tomato. This fertilizer saving was more pronounced in the TWW-irrigated plots from HSSFs *unplanted* unit due to the absence of macrophytes. These results confirm that irrigation with TWW can decrease or even remove the need for mineral fertilization whilst maintaining high productive and qualitative performance of tomato plants, in accordance with international literature [3,20,29,41]. If we consider that the use of TWW from an HSSFs constructed wetland also leads to savings in freshwater consumption compared to traditional crop management, it is clear that this is an excellent way to manage the irrigation and fertilization of tomato in arid and semiarid regions in particular.

### *3.5. Effects of TWW irrigation on soil*

In Table 7, the chemical characteristics of the FW irrigated-soils and TWW-irrigated soils are reported. In the topsoil (0.30 m) of the experimental plots, the effects of TWW application were not significant on soil pH. No significant differences in pH were recorded between TWW-irrigated plots and FW-irrigated plots. The short duration of TWW application (six months) was doubtless the main reason for these results. This negligible effect of TWW on soil pH has been reported in other studies. Rusan et al. [17] highlighted that a two, five and ten year-period of TWW application on several forage crops did not have significant effects on soil pH. Likewise, the OM content in the topsoil of the FW and TWW-irrigated plots was not significantly different: short-term application of TWW did not allow for significant accumulation of OM in the topsoil. Moreover, the same authors reported that the effects of TWW application on topsoil OM are highly correlated to the amount of organic matter in the wastewater. In our research, the application of TWW to soils with not high water-holding capacity did not contribute significantly to the accumulations of salts in the topsoil.

The increase in salinity in the soil is attributed directly not only to the chemical and physical properties of the soil but also to the original level of total dissolved salts in the TWW [16,17]. With regard to the total dissolved salt content, differences found between the treatments were not significant: short-term application of TWW did not determine a significant effect on soil salinity. Dissolved salts tend to accumulate more in the deeper soil layers than in the topsoil due to the leaching process, as indicated by various authors [16,75,76]. In our research, electrical conductivity varied from 0.61 to 0.58 dS $m^{-1}$ in the TWW irrigated-plots while the average EC value was 0.62 dS $m^{-1}$ in the FW-irrigated plots. With regard to N, P and K concentrations in the soils, no significant differences were found between the treatments. Concerning soil nutrients, Na was of great interest due to its negative effects on soil properties. As highlighted by Turgeon [77], an excess of Na in the soils displaces divalent cations, such us Ca and Mg, and soil structure deteriorates. The greater the sodium percentage on exchanges sites, therefore, the more the soil permeability decreases. Sodium adsorption ratio (SAR) characterizes soils affected by dissolved salts. In our research, the SAR calculated for both of the HSSFs *planted* units differed only slightly after passing through the system. Consequently, at the outflow of the HSSFs, Na concentration in the TWW did not decrease significantly. However, as regards the TWW from both of the HSSFs planted units, average SAR values (3.91 meq $L^{-1}$ for the TWW-umbrella sedge unit; 3.93 meq $L^{-1}$ for TWW-reedmace unit) remained below levels which may negatively affect the soil properties (SAR > 10). In our research, despite higher Na concentration in the TWW compared to the FW, we did not observe significant differences between the treatments. The agronomic conditions of the experimental field and the short-term irrigation period did not cause a significant accumulation of sodium in the topsoil and a displacement of calcium and magnesium in the structural aggregates of the soil.

Table 7
Organic and inorganic content of nutrients in FW irrigated-soils and TWW irrigated-soils. Average (± standard error) values are shown

| | pH | EC (dS $m^{-1}$) | OM (%) | TKN (g $kg^{-1}$) | TP (ppm) | Active $CaCO_3$ (%) | K (ppm) | Mg (ppm) | Na (ppm) |
|---|---|---|---|---|---|---|---|---|---|
| FW | 7.89 ± 0.01$^A$ | 0.62 ± 0.01$^A$ | 1.93 ± 0.01$^A$ | 1.69 ± 0.01$^A$ | 18.21 ± 0.02$^A$ | 3.79 ± 0.01$^A$ | 151.81 ± 0.03$^A$ | 135.11 ± 0.05 $^A$ | 84.11 ± 0.38 $^A$ |
| TWW (1) | 7.91 ± 0.01$^A$ | 0.58 ± 0.01$^A$ | 1.94 ± 0.01$^A$ | 1.72 ± 0.02$^A$ | 18.33 ± 0.01$^A$ | 3.80 ± 0.01$^A$ | 153.14 ± 0.36$^A$ | 141.72 ± 0.31 $^A$ | 91.77 ± 0.38 $^A$ |
| TWW (2) | 7.89 ± 0.01$^A$ | 0.61 ± 0.01$^A$ | 1.94 ± 0.02$^A$ | 1.71 ± 0.01$^A$ | 18.22 ± 0.01$^A$ | 3.79 ± 0.01$^A$ | 152.02 ± 0.05$^A$ | 139.31 ± 0.27 $^A$ | 89.21 ± 0.56 $^A$ |
| TWW (3) | 7.90 ± 0.01$^A$ | 0.61 ± 0.01$^A$ | 1.93 ± 0.01$^A$ | 1.73 ± 0.01$^A$ | 18.22 ± 0.01$^A$ | 3.78 ± 0.01$^A$ | 151.91 ± 0.33$^A$ | 142.21 ± 0.32 $^A$ | 92.11 ± 0.32 $^A$ |

Means sharing the same superscript are not significantly different from each other according to the Tukey test (P≤ 0.05).

^FW: freshwater-irrigated soils; TWW (1): treated wastewater-irrigated soils from Cyperus alternifolius-planted unit; TWW (2): treated wastewater-irrigated soils from Typha latifolia-planted unit; TWW (3): treated wastewater-irrigated soils from unplanted unit.

### *3.6. Effects of FW and TWW irrigation on yield and quality of tomato fruits*

The effects of FW and TWW on tomato crop yield are shown in Table 8. The results relate to a number of harvest dates from August to September. No significant differences in yield were recorded between FW-irrigated plants and TWW-irrigated plants. MY was on average higher (72.28 t $ha^{-1}$) in TWW-irrigated fruits than in FW-irrigated fruits (69.40 t $ha^{-1}$), although not markedly. This was mainly due to the higher UMY for FW-irrigated fruits (3.82 t $ha^{-1}$) than for TWW-irrigated fruits from HSSFs planted units (3.75 t $ha^{-1}$). Our findings were in agreement with results obtained by various authors [3,27] who found that MY obtained from TWW-irrigated plants was on average higher than FW-irrigated plants. On the contrary, other authors [3,20] found that the use of TWW determined an increase in MY compared to FW irrigation. However, it is also important to note that, in the same study, Aiello et al. [3] observed the use of irrigation with TWW produced both an increase and decrease of MY in two different tomato cultivars. We might say that the different constitution of a tomato cultivar significantly influences the MY of tomato even when irrigated with the same type of water. In Table 9, the effects of FW and TWW irrigation on qualitative parameters of tomato fruits are reported. The pH of the fruits was significantly affected by the different irrigation treatments. The highest pH (4.60) was recorded in the FW-irrigated fruits whilst the lowest (4.48) in the TWW-irrigated fruits from the HSSFs *unplanted* unit. pH values of the fruits were always within the range of 4, typical of a number of tomato cultivars. The effect of different irrigation treatments has also been confirmed in other studies [20,29,41,78] and this highlights that the quality of irrigation water can determine variations in tomato fruit pH. As described by Garcia and Barret [79], pH influences the effectiveness of thermal processes during industrial processing and, consequently, the fruit quality. When comparing the fruit soluble solids content, no significant differences were found between the irrigation treatments; however, the highest value (4.82° Brix) was obtained for FW-irrigated fruits. These results were in agreement with other studies [20,29,31] who stated that the SSC values were lower in TWW-irrigated fruits. Several studies report SSC values of tomato fruits lower and/or higher than our values. These differences are mainly due to the cultivar used and/or climate and soil conditions, as claimed by Sgherri et al. [80] and Mahajan and Singh [81]. Young et al. [82] affirm that a range of other factors may influence the solid content of tomato fruits, such as the number of fruits, the rate of assimilate export from leaves, the rate of import of assimilates by the fruit and the fruit carbon metabolism. Regarding the other qualitative parameters, fruit dry matter was between 5.39% (TWW-irrigated plants from the unplanted unit) and 5.51% (FW-irrigated plants) but it was not significantly affected by the different irrigation treatments. As claimed by Gatta et al. [20], the value of SSC and DM can significantly affect the tomato canning and processing industry as the higher

Table 8
Effects of the FW and TWW irrigation on productive parameters of the tomato fruits. Average (± standard error) values are shown ($n$ = 4)

| Treatments | Productive parameters | | | | |
|---|---|---|---|---|---|
| | Total yield (t $ha^{-1}$) | Marketable yield | | Unmarketable yield | |
| | | Total (t $ha^{-1}$) | Per plant (kg $plant^{-1}$) | Total (t $ha^{-1}$) | Per plant (kg $plant^{-1}$) |
| FW | 69.40 ± 1.15 $^{A}$ | 65.58 ± 0.97 $^{A}$ | 2.95 ± 0.13 $^{A}$ | 3.82 ± 0.07 $^{A}$ | 0.16 ± 0.01 $^{A}$ |
| TWW (1) | 72.28 ± 0.93 $^{A}$ | 68.43 ± 1.07 $^{A}$ | 3.07 ± 0.03 $^{A}$ | 3.84 ± 0.08 $^{A}$ | 0.17 ± 0.05 $^{A}$ |
| TWW (2) | 73.42 ± 0.45 $^{A}$ | 69.73 ± 0.40 $^{A}$ | 3.17 ± 0.04 $^{A}$ | 3.67 ± 0.05 $^{A}$ | 0.17 ± 0.01 $^{A}$ |
| TWW (3) | 74.21 ± 0.36 $^{A}$ | 69.62 ± 0.23 $^{A}$ | 3.15 ± 0.05 $^{A}$ | 3.47 ± 0.05 $^{A}$ | 0.21 ± 0.02 $^{A}$ |

Means sharing the same superscript are not significantly different from each other according to the Tukey test (P ≤ 0.05).
^FW: freshwater irrigation; TWW (1): treated wastewater irrigation from *Cyperus alternifolius*-planted unit; TWW (2): treated wastewater irrigation from *Typha latifolia*-planted unit; TWW (3): treated wastewater irrigation from unplanted unit.

Table 9
Effects of the FW and TWW irrigation on qualitative parameters of the tomato fruits. Average (± standard error) values are shown ($n$ = 4)

| Treatments | Qualitative parameters | | | | | |
|---|---|---|---|---|---|---|
| | pH | SSC (° Brix) | TA (g 100 $mL^{-1}$) | DM (%) | D (cm) | Co (a*/b*) |
| FW | 4.69 ± 0.02$^{A}$ | 4.82 ± 0.02$^{A}$ | 0.27 ± 0.02$^{A}$ | 5.51$^{A}$ | 1.18$^{A}$ | 2.55$^{A}$ |
| TWW (1) | 4.52 ± 0.03$^{B}$ | 4.76 ± 0.01$^{A}$ | 0.25 ± 0.01$^{A}$ | 5.45$^{A}$ | 1.22$^{A}$ | 2.53$^{A}$ |
| TWW (2) | 4.53 ± 0.01$^{B}$ | 4.79 ± 0.02$^{A}$ | 0.24 ± 0.02$^{A}$ | 5.42$^{A}$ | 1.21$^{A}$ | 2.55$^{A}$ |
| TWW (3) | 4.48 ± 0.01$^{B}$ | 4.74 ± 0.03$^{A}$ | 0.25 ± 0.01$^{A}$ | 5.39$^{A}$ | 1.18$^{A}$ | 2.57$^{A}$ |

Means sharing the same superscript are not significantly different from each other according to the Tukey test ($P$ ≤ 0.05).
^FW: freshwater irrigation; TWW (1): treated wastewater irrigation from *Cyperus alternifolius*-planted unit; TWW (2): treated wastewater irrigation from Typha latifolia-planted unit; TWW (3): treated wastewater irrigation from unplanted unit.

the value of SSC, the lower the energy needed to evaporate the water from the tomatoes during industrial food processing. Titratable acidity of fruit was not significantly affected by the different irrigation treatments. The content of TA was between 0.24 g 100 mL$^{-1}$ (TWW-irrigated plants from HSSFs reedmace unit) and 0.27 g 100 mL$^{-1}$ (FW-irrigated plants). Concerning fruit diameter, the influence of the different irrigation treatments on D was not significant. This may be explained by the fact that the irrigated-plots received the same amounts of N, P and K albeit with differing methodologies.

### *3.7. Effects on TWW irrigation on microbial contamination of tomato fruits*

The effects of TWW irrigation on the microbial contamination of tomato fruits are shown in Table 10. Microbial contamination was found to differ in the two parts of the fruit. Fruit flesh was uncontaminated whereas fruit skin was greatly contaminated by faecal coliforms, faecal streptococci and *E. coli*. No Salmonella contamination was found for either fruit skin or flesh. Maximum fruits skin contamination levels were found in September for FC (415 CFU 100 g$^{-1}$ on average) and for FS (835 CFU 100 g$^{-1}$ on average), and in August for *E. coli* (114 CFU 100 g$^{-1}$ on average). The different contamination levels in the two harvest months were probably due to different microbiological concentration levels in the TWW at the outflow of the HSSFs units in August and September. The most contaminated harvested fruits were those in contact with the bare soil and this was in agreement with previous studies [3,29,42]. As sustained by Bastos and Mara [83], the increased soil moisture from TWW irrigation can extend bacterial survival or allow for bacterial re-growth, whilst Irénikatché Akponikpè et al. [42] claim that the slightly rough surface of the tomato fruit facilitates microbial contamination, especially in fruits which touch the soil. The fact that we found high microorganism content in the fruit skin was also due to contamination from direct contact with TWW, as affirmed by Al-Lahman et al. [41]. The same authors highlight that weather conditions, in terms of moisture, solar radiation and temperature, can determine an increase in microbial contamination on fruit skin. In our research, the average FC and FS contamination levels of the fruit skin were enough to require careful consideration and humans could be exposed to health risks when tomato fruits are consumed raw, in accordance with WHO guidelines [27]. Under these conditions, it is evident that the use of TWW from HSSFs units does not guarantee high quality water in terms of microbiological content for the irrigation of crops consumed both raw and cooked, such as tomato. A number of authors propose solutions to avoid pathogen contamination of TWW-irrigated fruits. Drechsel et al. [84], for example, sustain that the microbial contamination risk in tomato fruits can be minimized if TWW irrigation is timed to allow for an adequate interval between irrigation and fruit harvest. We would say, however, that additional disinfection measures must be carried out post-treatment with HSSF constructed wetlands in order to further eliminate any kind of health risk linked to TWW irrigation. Moreover, following WHO recommendations [27], we deem suitable for irrigation with TWW only those vegetables which will be cooked.

## 4. Conclusions

TWW from CWs represent an important source of water and fertilizer for the irrigation and fertilization of agricultural crops. CWs contribute to large freshwater and fertilizer savings in the agronomic management of crops compared to irrigation and fertilization programmes in widespread use. The benefits for farmers are significant both in environmental and economic terms. In this study, irrigation with TWW did not affect soil pH nor did it contribute to significant accumulations of organic matter and dissolved salts in the topsoil. The productive and qualitative characteristics of tomato were not significantly affected by irrigation with TWW compared to FW. Similar yields and tomato fruit quality were obtained from both FW and TWW-irrigated plants. In TWW-irrigated plants, microbial contamination of the fruits differed in the different parts of the fruits. Fruit flesh was uncontaminated whilst pathogen contamination was detected in the fruit skin. Peak microbial contamination was observed in fruits which were in contact with the bare soil. High levels of microbial contamination highlight the fact that CWs may not satisfy national and international guidelines concerning the use of TWW in agricultural crop irrigation. Additional disinfection techniques for TWW are, therefore, needed in order to avoid any kind of health risk for humans. From an agronomic point of view, our findings show that TWW from CWs can be used both as a nutrient source and water supply for crop irrigation, especially in areas with high FW shortage. However, TWW-irrigated vegetables, such as tomato, should be disinfected and cooked well before being consumed.

Table 10
Microbial contamination of TWW-irrigated tomato fruits during the harvest period. Average values are shown.

| Microbial parameters (CFU 100 g$^{-1}$) | Period | | | |
|---|---|---|---|---|
| | August | | September | |
| | Fuit skin | Fruit flesh | Fuit skin | Fruit flesh |
| Faecal coliforms | 310 | 0 | 415 | 0 |
| Faecal streptococci | 720 | 0 | 835 | 0 |
| *Escherichia coli* | 114 | 0 | 98 | 0 |
| *Salmonella* | Absent | Absent | Absent | Absent |

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