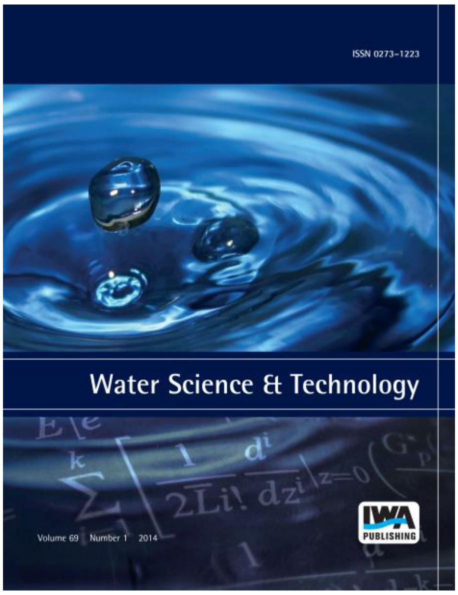

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The use of constructed wetlands for the treatment and reuse of urban wastewater for the irrigation of two warm-season turfgrass species under Mediterranean climatic conditions

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ABSTRACT

Constructed wetlands (CWs) represent low-cost technology for the treatment and reuse of wastewater in urban areas. This study aimed to evaluate the pollutant removal efficiency of a CW system and to assess the effects of irrigation using treated urban wastewater on soil and on two warm-season turf species. The research was carried out in Sicily (Italy) on a pilot-scale horizontal subsurface flow system which was fed with treated urban wastewater following secondary treatment from an activated-sludge wastewater treatment plant. The pilot system was located in an open urban park and comprised two separate parallel planted units. Experimental fields of *Cynodon dactylon* (L.) Pers. and *Paspalum vaginatum* Sw. were set up close to the system and irrigated with both treated wastewater (TWW) and freshwater (FW). Irrigation with TWW did not result in a significant variation in soil pH and soil salinity in the topsoil. The turf species tolerated high sodium levels in the soil due to TWW irrigation. Savings in FW and mineral fertilizers were deemed significant. The results highlight the fact that use of CW systems for the treatment and reuse of wastewater can represent a sustainable way to obtain alternative water resources for turfgrass irrigation in urban areas.

Key words | freshwater saving, horizontal subsurface flow system, irrigation, treated wastewater, warm-season turf species

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INTRODUCTION

In the last 20 years, interest in turfgrass science and culture has increased in the southern Mediterranean region as demonstrated by the number of scientific and technical research activities (Croce *et al.* 2004; Martiniello 2007; Ntoulas *et al.* 2011; Severmutlu *et al.* 2011). The main reasons for this interest are undoubtedly due to the functional, recreational and aesthetic benefits that turfgrass can generate in urban areas. Functional benefits, for example, include soil erosion control, groundwater conservation, organic compound biodegradation, urban heat and temperature dissipation and noise, glare and visual pollution abatement. Recreational benefits include a low-cost surface for outdoor sport and leisure activities, increased physical activity and a unique cushion against impact injuries. The aesthetic benefits include enhanced beauty and attractiveness, improved mental health with a positive therapeutic impact, social harmony and stability, improved work productivity and an overall better quality of life (Beard & Green 1994). In urban areas, high turfgrass quality is generally requested. One of the main agronomic practices that can assure high turfgrass quality is irrigation. Irrigation satisfies water demand and promotes plant root and stem growth. However, water demand in turf species is relatively high during the growth stage and is usually dependent upon the intensity of turfgrass management and on soil and climatic conditions. In recent years, increasing water consumption in metropolitan areas and consecutive drought periods in several areas of the Mediterranean have caused a decrease in water resources, creating long periods of water shortage and highlighting the need to find alternative

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strategies for turfgrass irrigation. In urban areas, the reuse of treated wastewater (TWW) represents one of the most attractive prospects regarding sustainable water management for irrigation purposes due to a number of reasons. These include making a significant impact on reducing global water consumption, reducing pollution in water bodies and retaining better quality water for human consumption, as stated by Leto *et al.* (2013). TWW can also increase crop yields due to the fact the water contains significant levels of organic compounds and inorganic elements, such as nitrogen and potassium, required for crop growth, as claimed by Castro *et al.* (2011). However, the long-term application of TWW for irrigation purposes could have negative effects on the turfgrass and soil. Plant growth may be diminished due to significant accumulations of sodium, particularly in plant tissue whilst the soil pH and salinity could vary in the long-term as a result of high levels of dissolved salts in the topsoil, as noted by Evanylo *et al.* (2010). Consideration must also be given to health risks, though minimal, associated with prolonged TWW irrigation and microorganisms which are considered dangerous to human health, as highlighted by Mañas *et al.* (2012). In many Mediterranean countries, the reuse of TWW in crop irrigation is well documented (Pedrero *et al.* 2010; Barbagallo *et al.* 2014; La Bella *et al.* 2016). However, little attention has been paid to the reuse of TWW for non-food crops, such as turfgrass for sports and leisure activities (Castro *et al.* 2011; Licata *et al.* 2016). Harivandi (2008) affirms that the reuse of TWW for turfgrass irrigation is extremely beneficial for various reasons. The same author highlights the fact that turfgrass can absorb large amounts of nutrients, which are found in greater concentrations in wastewater compared to freshwater (FW); he also points out that most soil- and plant-related problems concerning the use of wastewater may have a lower environmental and economic impact on turfgrass than on food crops. In urban areas, constructed wetland (CW) systems provide low-cost technology for the reuse of TWW for turfgrass irrigation due to the functional and technical characteristics of the system. The aim of this study was: (i) to evaluate the removal efficiency (RE) of a pilot-scale horizontal subsurface flow system (HSSFs) and calculate the water balance, (ii) to assess the effects of irrigation with TWW, on the chemical and physical soil properties when compared to FW irrigation, (iii) to assess the effects of irrigation with TWW on the biometric, productive and qualitative parameters of Bermudagrass (*Cynodon dactylon* (L.) Pers.) and seashore paspalum (*Paspalum vaginatum* Sw.) turfs, compared to FW irrigation.

MATERIALS AND METHODS

Test site

Tests were carried out in 2015 in the experimental area adjacent to the pilot-scale HSSFs in Raffadali, a rural community (13,000 inhabitants) in the west of Sicily (37°24′07″N, 13°31′54″E; 378–446 m a.s.l.). The climate is sub-humid with an average annual rainfall of approximately 650 mm, mainly distributed between October and March. With reference to the time series 2002–2014, the annual average temperature was 17.7 °C, average maximum temperature 23.7 °C and average minimum temperature was 11.8 °C. The summer drought was severe and the dry period fell between June and September.

Description of the pilot HSSFs

The system comprised two separate, parallel units (A and B) each 50 m long and 1 m wide, providing a total surface filter bed area of 100 m^2. The depth of the two units was 0.50 m with a water depth of 0.30 m and a 2% slope. The substrate was made by evenly sized 30 mm silica quartz river gravel (Si 30.02%; Al 5.11%; Fe 6.10%; Ca 2.65%; Mg 1.05%) with a porosity of 35–40%. The two units were built in concrete and lined with an impermeable membrane. Unit A was planted with giant reed (*Arundo donax* L.) and Unit B with umbrella sedge (*Cyperus alternifolius* L.). The system was fed with urban wastewater from an activated-sludge wastewater treatment plant which provided both primary and secondary treatment of the wastewater. Urban TWW from the municipal treatment plant was fed initially into a 15 m^3 waterproofed, vibrated cement storage tank. The period of time that the urban TWW was held in the storage tank was approximately 1 day only. The tank was equipped with a litre gauge and an outlet valve for the periodic cleaning of solid sediments. TWW was pumped through a 1 m wide perforated polyvinylchloride pipe into each of the two units. In each unit, the inlet pipe was placed 10 cm from the surface of the substrate. The pipe was positioned widthwise at the top of the pilot system. The homogeneous distribution of TWW in each unit was ensured through a timer-controlled pumping system. The pumping was continuous throughout the day without variations in time. The outflow TWW was then fed into a tank of 5 m^3 for each unit. Each tank was connected to a sprinkler system and used to irrigate the experimental turf fields (Figure 1). A separate FW tank was also used for the tests. The two units operated under the same hydraulic conditions and were tested using a hydraulic loading rate of 12 cm d^{-1}.

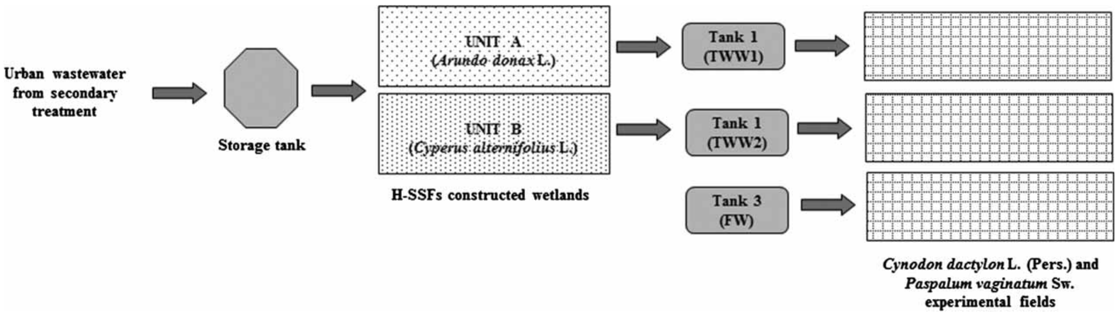

Figure 1 | Layout of pilot-scale HSSF system in Raffadali (Sicily, Italy).

Urban wastewater analysis

A litre of wastewater was collected at each of two points during sampling: at the inflow (0 m) and at the outflow (50 m) of each HSSFs unit. Sampling was carried out twice per month from April to September 2015, amounting to a total of 12 times. 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. The main physical and chemical characteristics of urban TWW were determined using Italian water analytical methods (APAT & IRSA-CNR 2004). Microbiological levels were determined by membrane filter methods, based on standard methods for water testing (APHA *et al.* 1998). RE of the pilot-scale HSSFs was calculated based on pollutant concentrations according to IWA (2000).

Water balance

The FAO Penman–Monteith method was used to calculate reference evapotranspiration (ET_0) (Allen *et al.* 1998), based on microclimate data taken from an automatic weather station belonging to the Sicilian Weather and Climate Service located near to the pilot system. Water balance was determined separately every 10 days from April to November 2015. For the planted units, an estimate of the water balance was calculated using the equation provided by IWA (2000): $Q_o = Q_i + (P - ET_c)\,A$ where Q_o = wastewater outflow rate ($m^3\,d^{-1}$), Q_i = wastewater inflow rate ($m^3\,d^{-1}$), P = precipitation rate (mm d^{-1}), ET_c = crop evapotranspiration (mm d^{-1}), A = wetland top surface area (m^2). The amount of water at the inflow and outflow of each unit was determined using a volumetric flow meter. Crop coefficients were calculated, in agreement with Allen *et al.* (1998), every 10 days in 2015 for each growth stage of the two macrophytes.

Main cultivation practices

Experimental fields of Bermudagrass and seashore paspalum were set up close to the pilot HSSFs. Two vegetative varieties, Tifway and Salam, were used for the tests. Transplanting took place in July 2014. The plots were 3 m^2 and were spaced 50 cm apart. Inter-plot spaces were periodically treated with herbicide (N-(phosphonomethyl) glycine) at 4 kg ha^{-1} $year^{-1}$. The experimental field was equipped with a sprinkler irrigation system. In 2015 irrigation was applied from April to September on average three times per week, both with TWW and FW in order to maintain active growth of the turf. Water requirements of the species were determined by the difference in the amount of water lost due to ET and rainfall rates. The FW-irrigated plots were managed with a widely used fertilization programme. Nitrogen, phosphorus and potassium levels in the TWW were taken into consideration and the resulting fertilization programme for the TWW-irrigated plots adapted as a result. Turfs were mowed by a helicoidal mower and maintained at a mowing height of 30–35 mm. No insecticide and fungicide treatments were carried out during the test period.

Turf and soil analysis

The main biometric, productive and qualitative turf parameters were calculated according to Leto *et al.* (2008). The leaf texture was determined monthly by randomly removing 100 flattened leaves per subplot and measuring the leaf width at a distance of 1 cm from its ligule. The horizontal stem density was calculated in June and September by counting the number of stems in a 50 cm^2 core for each subplot. Turf colour and visual turf quality were determined monthly, based on a visual rating scale, during the vegetative growth stage. The above-ground dry biomass was calculated by removing all plant tissue from the core top and drying the collected material in an oven at 60 °C to constant weight.

A grass sample was taken randomly from each subplot of each irrigation treatment level in June and September. Soil measurements were carried out only in the topsoil (0.30 m) close to the rhizosphere of the plants. Before transplanting, three soil samples were randomly collected from each replication and analysed. At the end of the study, one soil sample was collected from each subplot for each replication and analysed. Total organic carbon (TOC) was determined using the Walkley and Black method, total Kjeldahl nitrogen (TKN) using the Kjeldahl procedure, assimilable phosphorus (P) by the Olsen method and total calcareous using the Drouineau method. K, Mg and Na contents were determined by atomic absorption spectrophotometry. All the analyses were carried out at the Corissia Research Centre in Palermo, Italy.

Experimental design and statistical analysis

A split-plot design for a two-factor experiment was used with four replications. The main plot factor was irrigation with four treatment levels. The subplot factor was species with two treatment levels. Statistical analysis was performed with the package MINITAB release 17.1 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 are presented using mean ± standard error calculations.

RESULTS AND DISCUSSION

Pollutants RE of the pilot HSSFs

Results from pollutant removal rate tests are shown in Tables 1 and 2. In both of the planted units, the pH value at the inflow pipe was already slightly alkaline; this alkalinity increased as the water reached the outflow. The different electrical conductivity (EC) values between the two planted units were probably due to ET processes of the two macrophytes. In the giant-reed unit, ET resulted in greater water loss and, therefore, an increase of the solute in the solution. No significant differences in dissolved oxygen (DO) levels were found between the two macrophytes despite differences in the root apparatus. When comparing the two planted units, removal rates for total suspended solids (TSS), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), TKN and total phosphorus (TP) were higher in the giant-reed unit than umbrella-sedge unit. Giant reed showed a greater level of adaptability to the climate and substrate conditions of the study area, which influenced the pollutant removal potential of the two macrophytes. We observed that, in both the planted units, RE rates for TSS, BOD_5 and COD were higher than those of TKN and TP; these rates remained within limits consistent with findings by other authors for HSSFs. In particular, in the umbrella-sedge unit, the lower plant and root density influenced TSS filter mechanisms, leading to lower levels of sedimentation at the roots and the substrate, TSS therefore passing into the outflow waters. With regard to TP RE, Iamchaturapatr *et al.* (2007) highlight that in CWs the macrophytes can use phosphorous as an essential element for root growth. However, as stated by Maehlum *et al.* (1995), the phosphorus adsorption by the plants would seem to decrease over time in the HSSFs and this is mainly due to the granular saturation of most of the substrate sorption sites. TKN RE was not high due to low oxygen levels in the system. This had a significant effect on the ammonium nitrification process, believed by many researchers to be one of the most important nitrogen removal mechanisms. The two planted units did not show high

Table 1 | Variation of pH, temperature (T), DO and EC in the pilot units from April to September 2015

		Unit A		Unit B		
		Arundo donax		***Cyperus alternifolius***		
Parameters	**Main inlet**	**Outlet**	**Variation (%)**	**Outlet**	**Variation (%)**	**Threshold values for Italian Ministerial Decree 185/2003**
pH	7.3 ± 0.05 (7.2–7.5)	7.7 ± 0.2 (7.2–8.2)	−5.4	7.7 ± 0.9 (7.0–8.4)	−5.4	6–9.5
T (°C)	15.9 ± 0.3 (14.9–16.8)	16 ± 0.1 (15.8–16.3)	−0.6	16.1 ± 0.2 (16.0–16.2)	−1.2	–
EC (μS cm^{-1})	441.5 ± 42.3 (385.5–498.3)	614.5 ± 21.2 (331–898)	−39.2	573.5 ± 23.2 (348–799)	−29.8	3,000
DO (mg l^{-1})	1.2 ± 0.02 (1.1–1.4)	0.9 ± 0.04 (0.5–1.1)	25.0	1.0 ± 0.3 (0.9–1.2)	16.6	–

Average (± standard error), minimum and maximum values are shown (*n* = *12*).

Table 2 | Main chemical and physical composition of the urban wastewater from the inflow and outflow of the pilot units

		Unit A		Unit B		
		Arundo donax		*Cyperus alternifolius*		Threshold values for Italian Ministerial Decree 185/2003
Parameters	Main inlet	Outlet	RE (%)	Outlet	RE (%)	
Colour	NP[a]	NP	–	NP	–	–
Odour	NU[b]	NU	–	NU	–	–
Coarse matter	–	–	–	–	–	–
TSS (mg l^{-1})	34.8 ± 1.6 (25.3–52.1)	10.4 ± 0.2 (9.7–15.2)	69.5	12.2 ± 0.5 (10.1–16.4)	64.5	10
BOD_5 (mg l^{-1})	29.2 ± 0.8 (23.1–36.1)	12.5 ± 0.3 (10.1–15.3)	57.1	13.5 ± 0.4 (10.1–17.4)	54.2	20
COD (mg l^{-1})	56.1 ± 3.2 (39.3–76.3)	14.6 ± 0.3 (13.8–20.3)	72.9	15.6 ± 0.7 (12.4–22.4)	72.0	100
TKN (mg l^{-1})	19.6 ±0.7 (16.1–25.5)	9.0 ± 0.2 (7.8–11.2)	54.0	9.4 ± 0.2 (8.2–10.3)	51.9	15
N-NH_4 (mg l^{-1})	13.4 ± 0.4 (10.7–15.8)	5.4 ± 0.1 (4.7–7.1)	59.7	5.7 ± 0.2 (5.0–8.2)	57.5	2
TP (mg l^{-1})	7.7 ± 0.1 (7.2–8.5)	5.0 ± 0.05 (4.6–5.2)	35.1	4.9 ± 0.05 (4.6–5.3)	36.4	2
Cl (mg l^{-1})	125.8 ± 0.1 (116.1–127.8)	114.9 ± 0.9 (104.2–121.1)	8.8	115.2 ± 0.6 (105.4–118.1)	8.6	250
Ca (mg l^{-1})	80.9 ± 0.4 (78.2–82.8)	58.0 ± 0.5 (55.9–60.1)	28.0	59.7 ± 0.6 (57.1–62.8)	26.0	–
K (mg l^{-1})	92.4 ± 0.6 (90–98)	68.5 ± 0.4 (66.3–74.4)	26.3	73.5 ± 1.2 (68.1–82.2)	21.0	–
Mg (mg l^{-1})	24.2 ± 0.3 (22.1–28.2)	20.3 ± 0.3 (18.5–23.5)	16.4	21.5 ± 0.2 (20.2–24.5)	11.5	–
Na (mg l^{-1})	155.2 ± 1.5 (149.6–157.3)	139.8 ± 0.7 (137.3–144.0)	9.9	144.2 ± 1.6 (135.1–151)	7.0	–

Removal efficiency (RE) from April to September 2015.
Average (± standard error), minimum and maximum values are shown *(n = 12)*.
[a]Not perceptible.
[b]Not unpleasant.

removal levels for metals such as Ca, K, Mg and Na and little difference in removal rates was found between them. For example, Ca RE was found to be 28% and 26% at the outflow of the giant reed and umbrella-sedge units, respectively. Our data were compared with those of international literature and it was found that not all macrophytes have high metal uptake, due to structural damage caused in the plant tissue. On a microbiological level (Table 3), pathogen RE was high for each parameter in the study and consistent with international literature. This was due to a combination of physical, chemical and biological processes carried out by the plants, nematodes, viruses and bacteria, as illustrated by Brix (1997). Improved aerobic conditions in the planted units (due to atmospheric air circulation and the translocation of oxygen from the root system of macrophytes) facilitated production of a more extensive bacteria biofilm and promoted pathogen load removal. In our research 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 TWW for irrigation purposes. More specifically, the concentration levels of *Escherichia coli* were not always within acceptable legal limits. It is evident that there is the need to find a solution to improve the RE of bacteria and respect the legal limits of the law. Ottova *et al.* (1997) claim that the chemical oxidation represents an important mechanism to reduce significantly the concentrations of the pathogens. The use of different retention times in the pilot HSSFs could positively affect the pathogen removal rate, due to change of aerobic/anaerobic conditions of the substrate.

HSSFs water balance

The amount of TWW at the outflow of the system was affected by ET processes. ET can influence the redox conditions in the HSSFs; therefore, increased ET could negatively affect pollutant RE. When the system is subject to a considerable rise in ET, a decrease in apparent RE of organic compounds in particular can be expected, as found by Tuttolomondo *et al.* (2016). Although a correlation between ET and RE is apparent there is no influence or causation relating to changes in ET on RE. However, above all, in arid regions of the Mediterranean, where the main aim of wastewater treatment is to provide TWW for use in irrigation, ET dynamics must be taken into consideration

Table 3 | Main microbiological composition of the urban wastewater from the inflow and outflow of the pilot units

Parameters	Main inlet	Unit A *Arundo donax* Outlet	Unit A RE (%)	Unit B *Cyperus alternifolius* Outlet	Unit B RE (%)	Threshold values for Italian Ministerial Decree 185/2003
Log_{10} (CFUs 100 ml^{-1})						
Total coliforms (CFU 100 ml^{-1})	4.36 ± 0.02 (4.23–4.51)	3.33 ± 0.02 (3.19–3.39)	90.7	3.41 ± 0.03 (3.16–3.54)	88.3	–
Faecal coliforms (CFU 100 ml^{-1})	4.28 ± 0.01 (4.21–4.33)	3.25 ± 0.02 (3.16–3.37)	90.5	3.25 ± 0.01 (3.21–3.30)	89.8	–
Faecal streptococci (CFU 100 ml^{-1})	4.06 ± 0.01 (3.99–4.12)	3.24 ± 0.02 (3.13–3.23)	84.6	3.27 ± 0.02 (3.12–3.26)	87.1	–
Escherichia coli (CFUs 100 ml^{-1})	3.13 ± 0.01 (3.09–3.19)	2.13 ± 0.02 (1.98–2.21)	89.9	2.15 ± 0.01 (2.04–2.21)	89.5	10 (80% of samples) and 100 (maximum value point)
Salmonella spp. (CFU 100 ml^{-1})	–	–		–		–

Removal efficiency (RE) from April to September 2015.
Average (± standard error), minimum and maximum values are shown (*n* = *12*).

carefully when designing an HSSFs. In our research, no significant differences in average 10-day Q_o were observed between the two planted units. In the giant-reed unit, average 10-day Q_o was found to be 49.6 m^3 while in the umbrella-sedge unit it was found to be 51.1 m^3 from April to September (Figure 2). As Q_i was constant for all of the 10-day periods (60 m^3 10-days^{-1}), water losses were on average 10.4 and 8.9 m^3 10-days^{-1} in the giant reed and umbrella-sedge units, respectively. A higher level of water loss was found during the summer months and was mostly due to higher ET_c values for the same period (Figure 3). Despite identical growth, climatic and hydraulic conditions in the system, greater water loss occurred in the giant-reed unit and this was probably due to greater growth of giant reed compared to umbrella sedge. Giant reed consumed more water but used water with greater efficiency than umbrella sedge, also due to a preliminary greater aboveground biomass production. However, despite water losses from ET, a large amount of TWW was obtained at the outflow of both planted units throughout the test period.

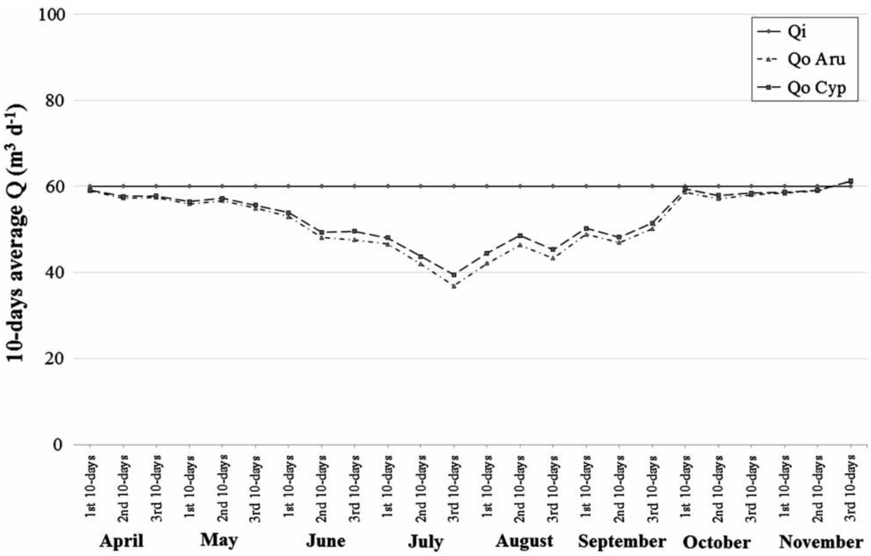

Figure 2 | Q_o and Q_i trends in the study period.

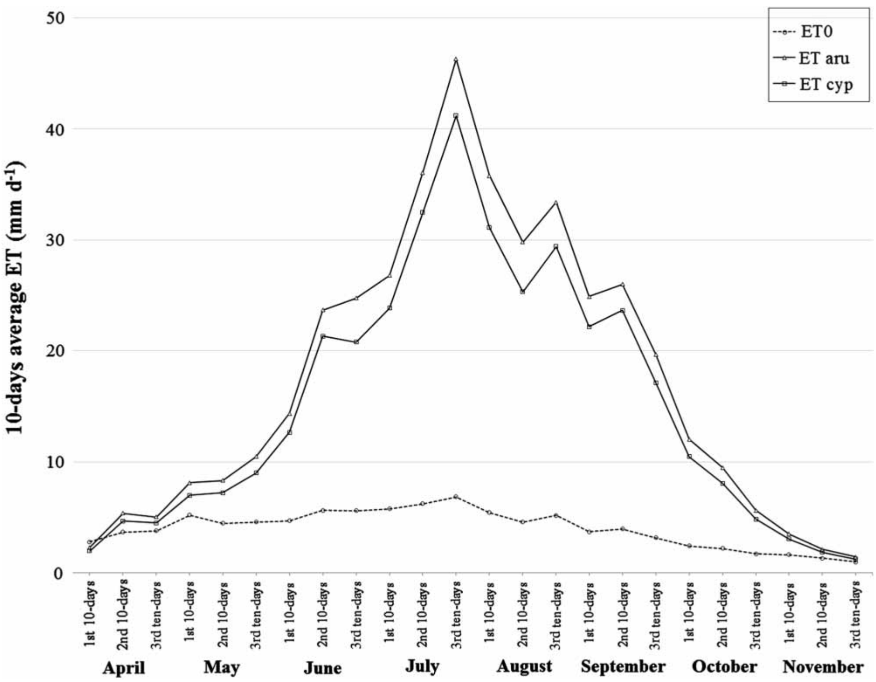

Figure 3 | Ten-day average ET_0, ET_{aru} and ET_{cyp}.

HSSFs enabled wastewaters to be treated and reused, leading to savings in FW and for the FW to be redirected for other purposes. These results highlight that, in arid and semi-arid regions of the Mediterranean, an HSSFs represents a 'green' phytoremediation technology which could guarantee continuous water availability for irrigation, even taking into consideration large water losses during summer months.

FW and TWW characteristics

TWW quality is important for turfgrass irrigation. N, P and K are essential for turf growth while other alkali metals, such as Ca and Mg, affect a number of vital physiological processes within the plants. The suitability of TWW for turf irrigation depends on the type and quantity of dissolved salts and nutrients in the water. When comparing the composition of FW and TWW over the test period, we observed that on average TWW had higher levels of dissolved salts and nutrients than FW (Table 4). This difference in concentrations was greatest from April to June and least during the summer months. During this period, the growth of the two macrophytes was greatest and this had a positive effect on chemical and microbiological pollutant removal rates at the outflow of the HSSFs planted units, thereby reducing concentration levels. With regard to the use of TWW in turf irrigation, Harivandi (2008) claims that turf species may be considered the most suited for TWW irrigation due to their morphological and physiological characteristics. The same author notes that TWW can be used efficiently by turfgrass because it is applied on a frequent and regular basis. However, literature highlights that a high quantity of dissolved salts and nutrients in TWW can limit water uptake by plants and reduce cell turgor, leaf area and a number of other processes, such as photosynthesis, carbohydrate storage and rooting. In order to ensure that the use of TWW in turf irrigation is not problematic, water quality must be evaluated using water quality guidelines for turfgrass irrigation (Ayers & Westcot 1985; McCarty 2001). Observing nutrient content in the effluent of the pilot HSSFs, we found that N concentrations were on average below guideline levels while Na concentrations could be problematic for use in irrigation. Average EC values found for FW (0.29 dS m^{-1}), TWW from giant-reed unit (0.61 dS m^{-1}) and TWW from umbrella-sedge unit (0.57 dS m^{-1}) were not critical for turf growth and could be considered less problematic for use in irrigation, based on the recommended guidelines.

Effects of TWW irrigation on soil

The soil was clay loam (40% sand, 21% silt and 39% clay) with a pH of 7.6, moisture levels of 14.1 g kg^{-1}, TOC of 7.5 g kg^{-1}, EC of 189.2 μS cm^{-1}, total calcareous of 1.3 g kg^{-1}, TKN of 1.2 g kg^{-1}, assimilable P of 31.2 mg kg^{-1},

Table 4 | Chemical composition of FW and TWW that were applied for Bermudagrass and seashore paspalum irrigation

Parameters	FW	TWW from *Arundo donax*-planted unit	TWW from *Cyperus alternifolius*-planted unit
pH	7.31 ± 0.07	7.72 ± 0.22	7.72 ± 0.91
EC (μS cm^{-1})	297.2 ± 0.8	614.5 ± 21.2	573.5 ± 23.2
DO (mg l^{-1})	Not available	0.9 ± 0.04	1.0 ± 0.3
BOD_5 (mg l^{-1})	1.8 ± 0.1	12.5 ± 0.3	13.5 ± 0.4
COD (mg l^{-1})	2.5 ± 0.9	14.6 ± 0.3	15.6 ± 0.7
TSS (mg L^{-1})	Not detected	10.4 ± 0.2	12.2 ± 0.5
NO_3-N (mg l^{-1})	0.3 ± 0.1	1.9 ± 0.7	2.1 ± 0.4
TKN (mg l^{-1})	2.1 ± 1.1	9.0 ± 0.2	9.4 ± 0.2
N-NH_4 (mg l^{-1})	0.9 ± 1.3	5.4 ± 0.1	5.7 ± 0.2
TP (mg l^{-1})	0.9 ± 0.8	5.0 ± 0.05	4.9 ± 0.05
Cl (mg l^{-1})	16.1 ± 0.3	114.9 ± 0.9	115.2 ± 0.6
Ca (mg l^{-1})	24.1 ± 0.3	58.0 ± 0.5	59.7 ± 0.6
K (mg l^{-1})	2.9 ± 1.0	68.5 ± 0.4	73.5 ± 1.2
Mg (mg l^{-1})	12.2 ± 1.4	20.3 ± 0.3	21.5 ± 0.2
Na (mg l^{-1})	11.2 ± 0.2	139.8 ± 0.7	144.2 ± 1.6
Heavy metals	Not available	Not detected	Not detected
SAR (meq l^{-1})	0.5 ± 0.1	4.0 ± 0.9	4.1 ± 1.2

exchangeable K of 530 ppm, exchangeable Mg of 622 ppm, exchangeable Ca of 27.8 ppm and exchangeable Na of 86.0 ppm. The chemical characteristics of the FW-irrigated soils and TWW-irrigated soils are shown in Table 5. The effects of TWW irrigation on soil pH were not significant. It is reasonable to imagine that the short-term tests of TWW irrigation (6 months) did not result in a significant variation in topsoil pH of the TWW-irrigated plots compared to FW-irrigated plots, as confirmed by literature. Castro *et al.* (2011), in a 2-year study of TWW irrigation on *Festuca arundinacea*, found that the pH of the soil profile was not affected by TWW application. In long-term tests (2, 5 and 10 years) regarding a number of forage crops, Rusan *et al.* (2007) claimed that the duration of TWW application did not significantly affect soil pH. In our research, soil TOC increased with TWW irrigation and there was a

Table 5 | pH, EC, TOC, TKN, assimilable P, total calcareous, K, Ca, Mg and Na content in FW-irrigated soils and TWW-irrigated soils

	pH	EC (μS cm^{-1})	TOC (g kg^{-1})	TKN (g kg^{-1})	Assimilable P (mg kg^{-1})	Total $CaCO_3$ (g kg^{-1})	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)
Species										
Tifway (Bermudagrass)	7.63 a	220.11 a	9.28 a	1.27 a	38.25 a	1.33 a	582.03 a	27.96 a	632.06 a	106.91 a
Salam (seashore paspalum)	7.65 a	219.33 a	9.14 a	1.29 a	38.11 a	1.32 a	580.33 a	28.00 a	631.93 a	105.79 a
Irrigation										
FW	7.57 a	192.51 a	7.53 a	1.19 a	31.19 a	1.32 a	533.02 a	27.75 a	622.01 a	88.50 a
TWW (1)	7.65 a	229.02 a	10.27 b	1.29 a	40.08 b	1.33 a	613.50 b	28.01 a	643.02 a	112.28 b
TWW (2)	7.69 a	237.5 a	9.84 b	1.38 a	43.26 b	1.33 a	597.00 b	28.22 a	631.00 a	118.26 b
Species × irrigation	n.s.	n.s.	*	n.s.	*	n.s.	*	n.s.	n.s.	*

Average values are shown.

Means followed by the same letter are not significantly different according to the Tukey test ($P \leq 0.05$).

*Significant; n.s.: not significant. TWW (1): treated wastewater-irrigated soils from *Arundo donax*-planted unit; TWW (2): treated wastewater-irrigated soils from *Cyperus alternifolius*-planted unit.

greater accumulation of organic compounds in the topsoil compared to FW irrigation. Similar results were also found in other studies with varying TWW irrigation duration and this highlights the fact that the effects of TWW on topsoil organic matter are highly correlated to the quantity of organic compounds in the TWW. The greater the concentration levels of TOC in TWW and the longer TWW is used for irrigation, the more the topsoil TOC increases. TWW also represents a precious source of salt addition to soils; however, the effects of TWW on soil salinity vary greatly and are dependent upon a number of reasons, such as initial salt levels in TWW, soil texture and structure, soil drainage, climate conditions and soil management practices. In this study, irrigation tests were carried on a clay loam soil and the specific soil structure greatly influenced the results of TWW irrigation. Clay loam soils are characterized by a high percentage of clay in the texture and high cation-exchange capacity. As a consequence, these soils can hold large amounts of salts in the topsoil. Moreover, in this type of soil, salts tend to accumulate more in the topsoil than in deeper soil layers. In this research, the short-term application of TWW did not result in significant effects on soil salinity despite the high percentage of clay in the soil structure. Soil salinity was on average 230 $\mu S\ cm^{-1}$ in TWW-irrigated plots and 192 $\mu S\ cm^{-1}$ in FW-irrigated plots. With regard to nutrient content, several studies highlight that nutrient accumulation in the topsoil can be attributed to the original nutrient levels in the TWW but that the subsequent actions carried out by the soil, microorganisms and plants should also be considered. In this study, TWW irrigation increased N, P and K concentrations in the topsoil; however, these differences were considered not to be significant only in the case of N content, compared to the FW-irrigated plots, mainly due to plant uptake of N. Of the alkali metals, Na was of primary interest due to its negative effects on soil properties and turf quality. Literature highlights that an excess of Na in the soil can displace divalent cations, such as Ca and Mg, leading to soil structure deterioration. Turgeon (2004) states that macropores are destroyed, micropores dominate, pore continuity declines, water infiltration, percolation and drainage decrease and oxygen status declines. Sodium adsorption ratio (SAR) is an agronomic parameter that is used to evaluate Na concentrations compared to Ca and Mg concentrations in the water. In our research, Na concentrations in TWW at the outflow of the HSSFs did not decrease as well as those of N. However, average SAR values (4.0 ± 0.9 meq l^{-1} for the TWW-giant-reed unit; 4.1 ± 1.2 meq l^{-1} for TWW-umbrella-sedge unit) remained below the values that may negatively affect the soil properties (SAR > 10). Soil quality must be kept under observation especially when irrigation is applied on clay soils and on a long-term basis. In our research, significant differences were observed between the irrigation treatments. In particular, the application of TWW resulted in a significant accumulation of Na in the topsoil of the TWW-irrigated plots.

Effects of TWW irrigation on turfgrass

TWW can affect the yield and quality of turfgrass due to its high nutrient content. Shoot and root growth, shoot density, leaf colour, heat, cold and drought hardiness, recuperative potential, stomatal physiological mechanisms and synthesis of the carbohydrates are the main morphological and physiological parameters which can be affected by the nutrient content of TWW. As stated by Turgeon (2004) and Santos *et al.* (2014), deficiencies in N, P, and K in the water can reduce plant growth, alter leaf coloration, decrease the green area of the leaves, decrease water absorption and retention by plants and reduce the above- and below-ground biomass. In the TWW-irrigated plots in our study, the N, P, and K content present in the TWW was exploited and integrated into the fertilization programme of the two varieties of Bermudagrass and seashore paspalum in order to satify the nutrient demand of the two turf species. N-P fertilizers were also required to sustain plant growth, but an additional application of K fertilizer was not made due to extra K content in TWW (Table 6). Irrigation with TWW reduced the need for fertilizers, maintaining high productive performance of Bermudagrass and seashore paspalum varieties. In particular, for TWW-irrigated plots, it allowed on average a saving of 40 kg N ha^{-1}, 22 kg P_2O_5 ha^{-1} and 238 kg K_2O ha^{-1} in comparison with a commonly used N, P and K fertilization programme for the two turf species. Moreover the use of TWW from an HSSFs also led to a FW saving of approximately 69 $m^3\ t^{-1}$ dry above-ground biomass. When comparing the different irrigation treatments, we did not find significant differences in above-ground biomass yields between FW-irrigated plots and TWW-irrigated plots (Table 7). The two varieties showed significant differences regarding most of the biometric and qualitative parameters. The highest average value of visual turf quality was recorded for Tifway, which had the highest quality ratings both in spring and summer. However, the quality and colour of turfgrass were not affected by the different treatment levels of irrigation, despite the high N content in TWW. This can be explained by the fact that, despite having differentiated fertilization management programmes,

Table 6 | Agronomic management of N, P, and K fertilization programmes of Bermudagrass and seashore paspalum 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)
Nitrogen (N)			
April	50	44.0	44.4
May	50	44.4	44.5
June	50	42.1	41.6
July	50	43.1	42.4
August	50	43.2	43.0
September	50	43.7	43.4
Total nitrogen	300.0	260.4	259.3
Phosphorus (P)			
April	50	47.2	47.1
May	20	17.2	17.2
June	20	15.9	16.0
July	20	15.8	16.0
August	10	6.0	6.3
September	10	6.3	6.1
TP	130.0	108.3	108.7
Potassium (K)			
April	40	0.0 (+0.18)[a]	0.0 (+4.39)[a]
May	40	1.5	0.0 (+2.34)
June	40	0.0 (+15.49)	0.0 (+23.42)
July	40	0.0 (+13.70)	0.0 (+20.34)
August	40	0.0 (+14.43)	0.0 (+15.24)
September	40	0.0 (+16.13)	0.0 (+15.16
Total potassium	240.0	1.5 (+59.90))	0.0 (+80.89)

TWW (1): treated wastewater from *Arundo donax*-planted unit; TWW (2): treated wastewater from *Cyperus alternifolius*-planted unit.
[a]Extra potassium content in the TWW.

both the FW-irrigated plots and TWW-irrigated plots received an equal amount of N. It is reasonable to suppose that the lack of N fertilization in FW-irrigated plots ultimately would have led to a significant decrease in plant growth and green leaf colour, N being an important component of the chlorophyll molecule, as reported by Turgeon (2004). Comparison of the irrigation treatments did not highlight any significant differences in leaf texture and shoot density and this was due to the agronomic test conditions. TWW also contained several alkali metals: Ca, Mg and Na represent the most important metals due to their effects on the soil and turfgrass. Literature highlights that Ca is a vital cell wall constituent of turf species while Mg is a constituent of chlorophyll and is also essential for the maintenance of the green colour of turfgrass and its growth. As claimed by Castro *et al.* (2011), Ca and Mg can have an antagonistic effect with regard to K; therefore, an excess in Ca and Mg levels can produce K deficiency. However, no symptoms of K deficiencies were observed in Bermudagrass and seashore paspalum plants in the TWW-irrigated plots. Concerning Na, Evanylo *et al.* (2010) stated that high Na levels in water (above 200 mg Na l^{-1}) can induce nutrient imbalances and Ca, K, Mg, N and P deficiencies. Castro *et al.* (2011) affirm that the negative effects of Na are, however, less evident in turfgrass due to the fact it is mowed periodically, unlike other crops. In our research, despite the higher Na content in TWW, we did not observe any aesthetic anomalies, such as chlorosis of young and old leaves, in the TWW-irrigated plants for either of the turf species. Seashore paspalum, in particular, can tolerate soil salinity levels (affected by the salinity level of irrigation water) above 20 dS m^{-1}. It is clear that

Table 7 | Biometric, qualitative and productive characteristics of Bermudagrass and seashore paspalum varieties irrigated with FW and TWW

	Leaf texture (mm)	Horizontal stem density (n cm^{-2})	Visual quality (1–9)	Colour (1–9)	Dry above-ground biomass (kg m^{-2})
Species					
Tifway (Bermudagrass)	1.56 a	2.57 b	6.68 b	6.15 b	4.38 b
Salam (seashore paspalum)	2.82 b	1.06 a	5.77 a	5.80 a	3.11 a
Irrigation					
FW	2.11 a	1.77 a	3.77 a	5.87 a	6.31 a
TWW (1)	2.19 a	1.81 a	3.75 a	6.01 a	6.36 a
TWW (2)	2.28 a	1.85 a	3.72 a	6.05 a	6.02 a
Variety × irrigation	n.s.	n.s.	*	*	n.s.

Average values are shown.
Means followed by the same letter are not significantly different according to the Tukey test ($P \leq 0.05$).
*Significant; n.s.: not significant. TWW (1): treated wastewater-irrigated soils from *Arundo donax*-planted unit; TWW (2): treated wastewater-irrigated soils from *Cyperus alternifolius*-planted unit.

long-term application of TWW could significantly increase the Na concentration in the topsoil and, consequently, in the plant tissues. In order to reduce the accumulation of excess Na in the soil, specific agronomic practices are required, such as the periodical application of good quality irrigation water for Na removal. In this study, the microbiological aspects were not considered and the effects of TWW on turf quality, in terms of health risks, were not evaluated mainly due to the short-term application of TWW. Mañas *et al.* (2012) affirm that, when considering the microbiological aspects in TWW-irrigated soils, health risks would undoubtedly be low due to the mechanisms by which microorganisms, still present in the TWW, are retained by the soil, such as soil surface filtering, sedimentation and adsorption. Harivandi (2008) illustrates the way in which turfgrass can contribute to reducing pathogen accumulation on the grass and/or in the soil. It is evident that TWW microbiological parameters must be within the legal limits regarding the reuse of TWW for irrigation purposes.

CONCLUSIONS

Bermudagrass and seashore paspalum are the most widespread warm-season turf species in the Mediterranean region and usually require a high amount of water and fertilizers during their crop cycles. TWW from CWs could represent a source of fertilizer since it contributes to the accumulation of macro- and micronutrients and organic matter in the soil without causing negative effects on soil pH and salinity. This does not mean that the use of TWW can entirely replace the use of mineral fertilizers: in this study, an additional application of N-P fertilizer was deemed necessary in order to sustain suitable plant growth of the two turfgrass species. However, there were evident benefits in terms of fertilizer savings. TWW can be also a negative source of Na. Despite the fact that many crops can suffer from the negative effects this metal can have on both the plant and soil, especially during long-term TWW application, Bermudagrass and seashore paspalum can tolerate high levels of Na even over the long term. As a consequence, it is important to monitor NA concentrations in the soil and to provide adequate agronomic practices where Na content is excessive. TWW provides an additional water source where the supply of FW is limited and also permits the conservation of FW. This topic is of particular importance in the arid and semi-arid areas where water shortage periods are prolonged and water can become a limited natural resource. Although the microbiological aspects have not been investigated in this study, it is evident that the periodic monitoring of Bermudagrass and seashore paspalum turf quality is required during short- and long-term TWW irrigation to avoid microbiological risks to human health, despite the fact that there are various agronomic practices available which can ensure the soil health is maintained.

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