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Growth of *Arundo donax* L. and *Cyperus alternifolius* L. in a horizontal subsurface flow constructed wetland using pre-treated urban wastewater—a case study in Sicily (Italy)

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ABSTRACT

Constructed wetland systems for wastewater treatment represent an excellent opportunity for the recovery of water resources in those areas subject to prolonged periods of water shortage. This paper presents a study of the efficiency of a pilot horizontal subsurface flow system using pre-treated urban wastewater from a small town in the West of Sicily. The pilot system had a total surface area of $100\,m^2$ with two units. Unit A was planted with *Arundo donax* L. and unit B with *Cyperus alternifolius* L. The HLR was $10\,cm\,d^{-1}$. The main objectives of research were to evaluate the growth and biomass yield of two macrophytes and to determine the removal efficiency of physical–chemical and microbiological parameters. The results showed excellent organic pollutant removal (BOD_5 70–72%, COD 61–67%), whilst macronutrient removal was found to be lower (TKN 47–50%, TP 43–45%). Pathogen load removal was found to be approximately 90%, but *Escherichia coli* concentrations at outflow were not within Italian legislative limits. Giant reed showed higher nitrogen content in the biomass (an average $28.9 \pm 1.8\,g\,m^{-2}\,year^{-1}$ and $63.8 \pm 1.8\,g\,m^{-2}\,year^{-1}$ for the aboveground and belowground parts, respectively) than umbrella sedge. The treated wastewater was used to irrigate parkland areas.

Keywords: *Arundo donax* L.; *Cyperus alternifolius* L.; Horizontal subsurface flow; Sicily; Urban wastewater

1. Introduction

In recent years, a fall in the rainfall levels and an increase in the air temperatures in Italy have had a significant impact on water resources in the southern regions of Italy, creating long periods of water shortage, and highlighting the precariousness of the water supply system [32]. The agricultural sector, which consumes over 50% of the water supply, has been hit particularly hard by the fall in water supply with major effects on crops as a consequence. The reuse of non-conventional water, wastewater in particular, seems to be one of the most attractive prospects for sustainable water management in the sector for a number of reasons, amongst which are making of a significant impact on reducing global water consumption, reducing pollution in water bodies, improving economic conditions for farmers and reserving better quality water for human consumption [39]. The use of constructed

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wetland systems (CWs) may play an important role in the treatment and in the reuse of agricultural wastewaters [7], particularly in those areas where agriculture is highly dependent upon irrigation. There are a number of international studies of interest which demonstrate the potential of CWs in the agricultural sector, for example, research on the efficacy of a horizontal subsurface flow (H-SSF) system treating wastewater from a dairy farm [16] and wastewater treatment in the wine-growing sector [40,41]. Studies on olive mill wastewater [21,48] and livestock wastewater [25], were also proved of interest as the results demonstrated a significant reduction in organic, mineral and microbiological loads independent of the type of system and/or the plant species used in the systems. Despite numerous examples of possible applications of CWs in Mediterranean countries [35], they still continue to be relatively unknown in Sicily, to some extent due to cultural, economic and legislative reasons. In the East of Sicily, studies were carried out to evaluate the efficiency of an H-SSF system in the treatment of urban wastewater to be reused in the irrigation of olive groves, with results showing a significant drop in key pollutants [5,11]. A study carried out on the effects of irrigating tomatoes with reclaimed urban wastewater was particularly interesting, as it showed an increase in crop yields and low levels of *Escherichia coli* in the fruits [3]. Within the agricultural sector, little attention has been paid to the reuse of non-conventional water on parkland for sports and leisure activities. If we exclude studies carried out on the irrigation of turfgrass with treated wastewater [10,20], there is little data available in literature for this sector, probably due to the health risks, though minimal, associated with microorganisms which are considered dangerous to human health.

The aim of this study was to verify the efficiency of a pilot H-SSF constructed wetland system that is used to "polish" urban wastewater for use in the irrigation of sports and leisure parkland. During the study, the removal efficiency and the agronomic behaviour of two macrophytes in typical Mediterranean climate conditions were evaluated. These macrophytes have never been used in the constructed wetland test trials in Sicily, and the results from the treatment of wastewater using these species are new for Sicily and for most Mediterranean countries.

2. Materials and methods

2.1. Test site

Tests were carried out in the four years from 2007–2010 on the pilot H-SSF system in Raffadali, a small rural community in the West of Sicily (37°24′N—1°05′E, 378–446 m a.s.l.). The system was designed by the Department of Agri-Environmental Systems at the University of Palermo (Italy) and has been operational since 2000. It was previously used to treat the other types of wastewater.

2.2. Description of the pilot system

The pilot H-SSF system was constructed with two parallel units (A and B) each 50 m long and 1 m wide providing a total surface filter bed area of 100 m^2. Unit A was planted with *Arundo donax* L. and unit B with *Cyperus alternifolius* L. (Fig. 1), respectively. The system was fed with pre-treated urban wastewater from the standard sewage treatment system (activated sludge) in Raffadali which effectuated primary and secondary treatment of the wastewater. The units operated under the same hydraulic conditions and were tested under a hydraulic loading rate (HLR) of 10 cm d^{-1}. The depth of the two beds was 0.50 m with a water depth of 0.30 m and a 2% slope necessary to ensure regular flow and to avoid water stagnation.

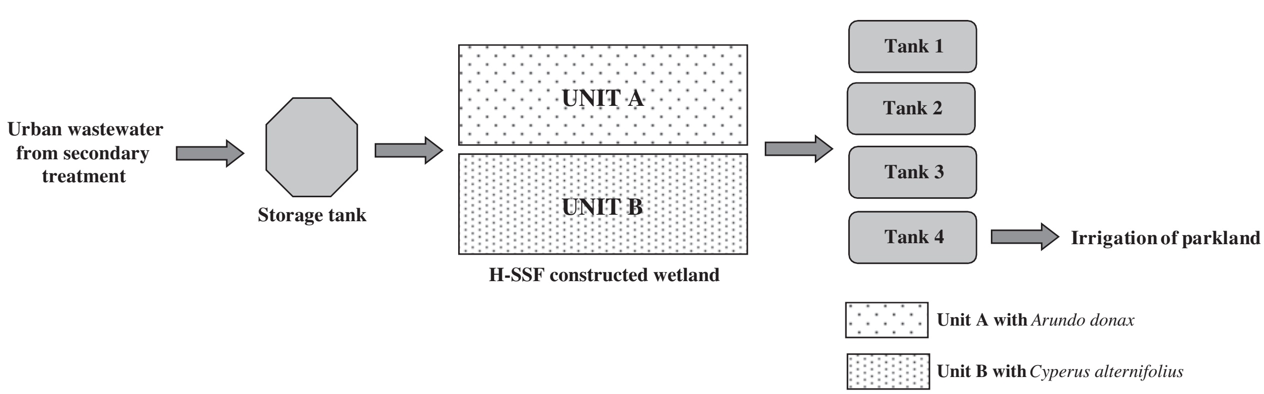

Fig. 1. Layout of pilot-scale H-SSF constructed wetland in Raffadali (Sicily, Italy).

The walls of the units were made of tufa blocks and mortar, and were filled with a substrate of evenly sized 30 mm gravel medium to facilitate hydraulic conductivity. The silica quartz river gravel was washed carefully to ensure it was free of sand, soil and other deposits.

The urban wastewater from the municipal treatment plant was fed initially into a 15 m^3 waterproofed, vibrated cement storage tank. The tank was equipped with a litre gauge and an outlet valve for the periodic cleaning of solid sediments. It was also equipped with a submerged electric pump and timer, in order to regulate times and duration of wastewater flow into the two units.

The urban wastewater was fed into the two pilot units through a perforated polyvinylchloride pipe. The treated wastewater was then fed into a system of four interconnected tanks of 5 m^3 each.

The last of these tanks was used to supply water to a sprinkler system to irrigate turfgrass populated by *Festuca arundinacea* Schreb. and *Cynodon dactylon* (L.) Pers. The wastewater was fed into the system immediately after planting it with the two macrophytes.

2.3. Plant species material

In 2007, *Arundo donax* L. plants were collected from natural wetland areas and the rhizomes used for propagation in a small nursery. The rhizomes were planted in unit A in February 2008 with a plant density of four rhizomes m^{-2}.

Mother plants of *Cyperus alternifolius* L. were obtained from a nursery in 2007 and propagated using cuttings. The rooted stems were planted in unit B in February 2008 with a plant density of five stems m^{-2}.

Phenological stage identification was carried out through observations on the plants based on specific descriptors from the time the wastewater was released into the units. Phenological data were obtained every two weeks using the BBCH classification scale [18].

Plant growth analysis was carried out by determining plant height and through an examination of the plant biomass. The data were analysed from the start of the second year (2009) on one-year-old plants so as to allow adequate root system development within the two units.

Plant height was determined fortnightly by measuring the maximum height of the five plants selected randomly from each unit. Maximum height was measured from the surface of the filter bed up to the top leaf insertion and only on plants in good vegetative and phytosanitary condition [9].

In November 2009, the plants were cut back to a height of 50 cm above the gravel bed. The fresh aboveground (stems and leaves) and belowground (roots and rhizomes) weights were determined from a representative sample of five plants from each unit. The biomass dry weight was then calculated by drying the collected plant material in an oven at 62°C for 72 h. This process was repeated following the next cutting, after approximately 12 months. Nitrogen levels in the above and belowground biomass parts were measured using a CHN analyser, in full compliance with plant biomass basic analysis standards.

2.4. Urban wastewater analysis

Sampling of the urban wastewater was carried out during the period June 2009–November 2010 on a monthly basis. Altogether 18 samples were taken. The samples were collected at the inflow (0 m) and at the outflow (50 m) of the two units. One litre of wastewater was collected from each of the two points at each sampling.

The pH value, electrical conductivity (ECw), temperature (T) and dissolved oxygen (DO) levels were determined directly on site using a portable Universal meter (Multiline WTW P4), following calibration protocol for each of the four parameters being studied.

Using Italian analytical methods for water [29], total suspended solids (TSS), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total phosphorus (TP), chloride (Cl^-) and fat and oil levels were determined. Total coliform (TC), faecal coliform (FC), faecal streptococci (FS), *Escherichia coli* (EC) and *Salmonella* spp. levels were determined by membrane filter methods, based on standard methods for water testing [1].

The percentage concentration decrease efficiency was evaluated according to the IWA recommended equation (2000) [6,30]: $E = C_i - C_0/C_i \times 100$, where E = percentage concentration decrease efficiency (%), C_i = inlet concentration (mg/L) and C_0 = outlet concentration (mg/L).

2.5. Statistical analysis

An estimate of variability in the data populations was determined using mean ± standard error calculations. The software MINITAB Release 14 for Windows was used.

3. Results and discussions

3.1. Plant growth analysis and biomass production

Arundo donax L. and *Cyperus alternifolius* L. were chosen as the plant components in the pilot units due to the need to use macrophytes which were known to adapt well, were resistant and had a good phyto-extractive capacity and tolerance to high wastewater loads [8]. *Cyperus alternifolius* L., although not native to Sicily, was chosen as it had a positive visual impact, an important factor as the system was situated inside parkland. Another reason for choosing the species was to test growth behaviour in a CW of a non-native species–new in Sicily although wellknown as a tropical species.

The use of *Phragmites australis* L., considered to be the most commonly used macrophyte in the world of constructed wetland systems [12,45], was discounted as it had no degree of innovation as many studies in Southern Europe have already been carried out regarding biomass yields and purification capacity.

In the study area, temperature trends were consistent with the 10-year average, with values never falling below zero. Maximum average temperatures were 22.2℃ and minimum average temperatures were 13.5℃ for both years. The most significant rainfall events were recorded in the second 10-day period of January 2010 (98 mm). Dry periods were observed between May and August in both the years (Fig. 2). Growth of the two macrophytes was affected by the climate during the testing period. The correlation between plant growth and average temperatures was positive in both years. As an example, correlations between giant reed growth and average temperatures in 2009 and 2010 are shown in Fig. 3(a) and (b), respectively.

During the tests, the plants reached maximum vegetative growth during the summer months, which coincided with high temperatures. Both species reached maximum height in 2010 with the maximum values ranging between 229 cm (*Arundo donax* L.) and 190 cm (*Cyperus alternifolius* L.) (Fig. 4). Propagation methods used in the study affected growth rates of the species, as confirmed by previous studies. In Sicily studies have shown that, for giant reed, the use of the rhizomes ensured good root establishment and produced good biomass levels from as early as first year, compared to plant material propagated by alternative means, such as by culm cuttings [13]. Conversely, it was found in Uganda that, as far as the purification process was concerned, the use of cuttings was the best propagation system for umbrella sedge, in terms of growth and biomass [37].

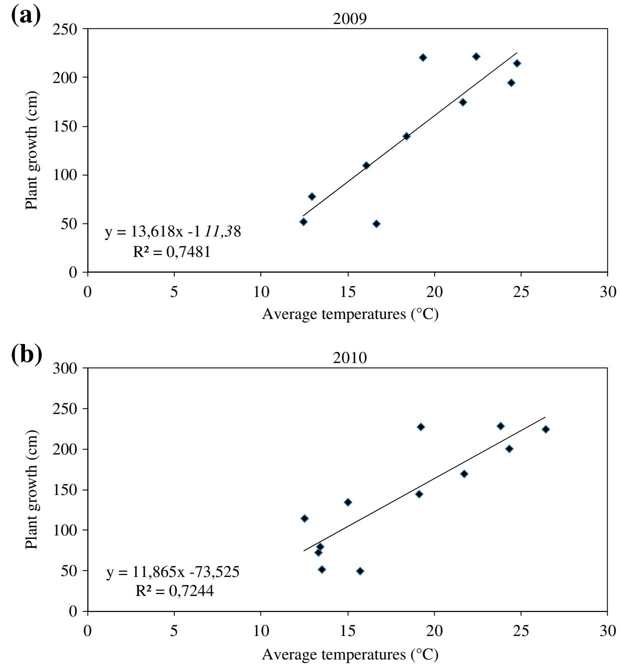

Fig. 3. Correlation between *Arundo donax* growth and average temperatures in 2009 and 2010.

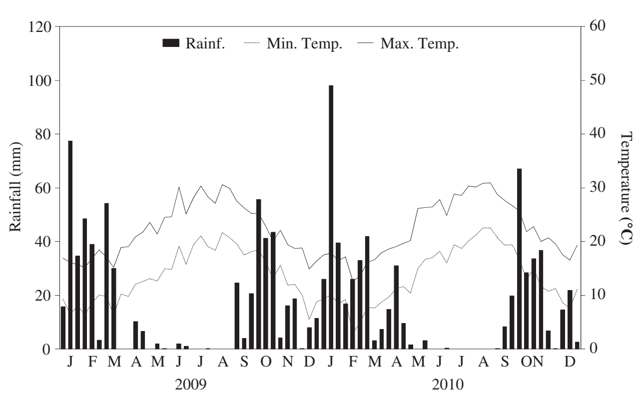

Fig. 2. Rainfall and temperatures during the tests period.

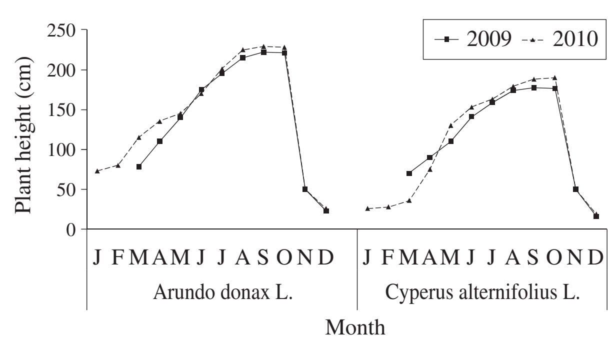

Fig. 4. *Arundo donax* and *Cyperus alternifolius*: plant height trend (2009-2010).

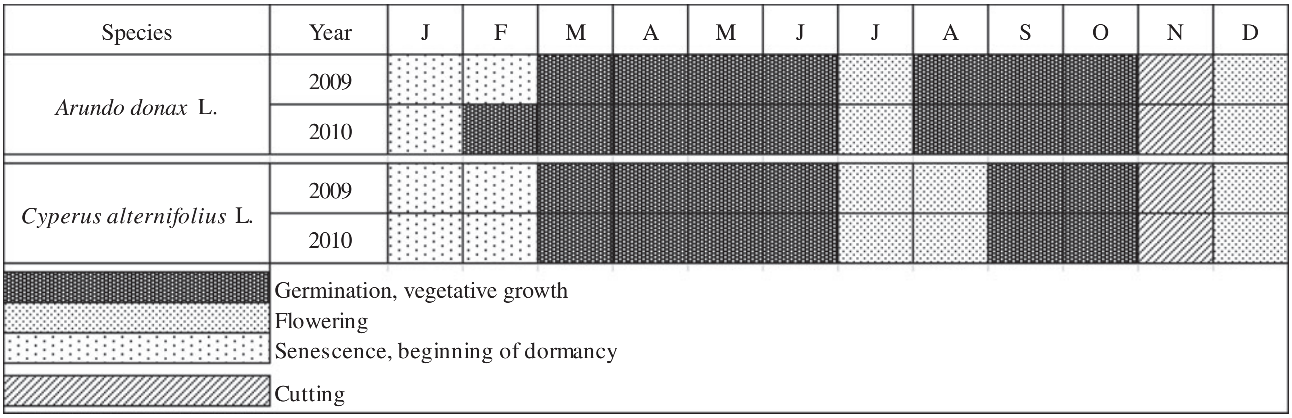

Fig. 5. Evolution of the main phenological stages of *Arundo donax* and *Cyperus alternifolius* determined by extended BBCH Scale.

Phenological analysis showed that the flowering stage took place during the summer for both species. Following autumn harvesting, the plants grew back quickly starting from the belowground parts, but did not reach flowering and ripening stages. Vegetative growth slowed down sharply during the autumn-winter period with the fall in temperatures and began again when temperatures rose in the spring (Fig. 5). Minimum temperatures at the test area were around an average of 13°C for both years, thereby reducing the length of the dormant stage considerably for both species typical of hot-temperate areas.

In Fig. 6 the results of above and belowground biomass and nitrogen content of giant reed and umbrella sedge are shown, respectively. The average dry matter for the aboveground parts of the giant reed (leaves and stems) for the two years 2009–2010 was $3{,}950 \pm 150$, and $8{,}000 \pm 100\,\mathrm{g\,m^{-2}}$ $\mathrm{year^{-1}}$ for the belowground parts (roots and rhizomes). The average

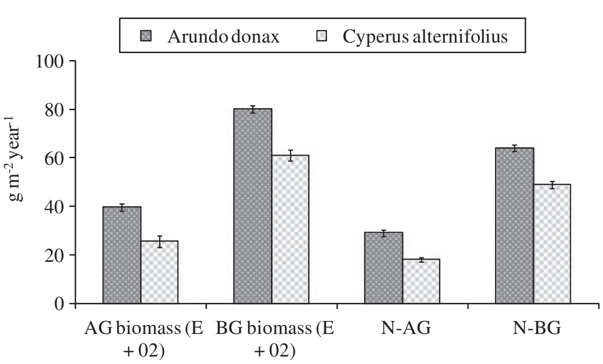

Fig. 6. Aboveground (AG) and belowground (BG) biomass and nitrogen content (N) of *Arundo donax* and *Cyperus alternifolius*. Bars indicate standard error of the means.

culm density for the two years was 18 culms $\mathrm{m^{-2}}$. The results of the study were consistent with other studies carried out in different climatic areas. In an H-SSF system in Australia [28], the wet weight yields of the aboveground parts of the giant reed were $15.0 \pm 3.4\,\mathrm{kg}$, with an estimated dry matter production of approx. 125 t $\mathrm{ha^{-1}}$ $\mathrm{year^{-1}}$. The authors of this study also made a comparison with *Phragmites australis* and found that the giant reed was significantly more productive, taking into consideration the same growth season, number of harvestings and harvesting period. This higher aboveground biomass production level of the giant reed compared to *Phragmites australis* was also found by [47] in China, using a vertical subsurface flow (V-SSF) system planted with various species native to subtropical regions and typical of the Monsoon climate area. In a study using a free water surface (FWS) system on the Island of Crete, although biomass production levels as such were not determined, [33] observed rapid coverage capacity of the wetland area by *Arundo donax* and a significantly higher level of competitiveness of the species compared to the other macrophytes in the wetland. These results show that the main advantage of using *Arundo donax* over other macrophytes in constructed wetlands is its higher biomass production levels and the possibility of using it for secondary purposes. On this point, in a study aimed at evaluating the phenological, biometric and production characteristics in open field conditions of 39 *Arundo donax* clones gathered from various Southern Italian areas, it was found that the species was particularly promising for energy production and cellulose paste [14]. The use of giant reed for energy purposes was highlighted by some Italian researchers [4,34], who noted that the yields and energy balance of the giant reed in terms of GJ $\mathrm{ha^{-1}}$, were found to be better and higher than other biomass species in varying agronomic treatments, such as fertilization and irrigation.

The average dry matter production for the aboveground parts of the umbrella sedge for the two years 2009–2010 was 2,550 ± 250 and 6,100 ± 200 g m^{-2} $year^{-1}$ for the belowground parts. The average stem density for the two years was 97 stems m^{-2}. The biomass levels obtained were lower than those found in literature, probably due to the higher adaptability of the species to tropical and subtropical climates; studies on the use of this species in constructed wetlands were mainly carried out in tropical and subtropical countries and there we do not seem to have any data available for the Mediterranean area. In Uganda, in a horizontal flow constructed wetland (substrate free) planted with *Cyperus papyrus* L., higher above and belowground biomass levels were found by [26], possibly due, in part, to the different size of the system, but mainly due to the different climate conditions, which significantly affected the growth of the species. A study in China [15] using a simulated V-SSF system planted with *Cyperus alternifolius* L. and using various substrates, found significant seasonal changes in the aboveground biomass levels in all the treatments of the study, thereby confirming the influence of temperature on the growth rates of the species, independent of the wastewater concentration levels.

Differences in the biomass production levels of the two macrophytes led to important consequences concerning the removal of the principal components of the wastewater and the absorption of the macronutrients. In particular, the higher above and belowground production levels of *Arundo donax* L. favoured greater absorption and a greater level of macronutrient translocation compared to *Cyperus alternifolius* L. Average nitrogen levels in the aboveground parts were found to be 28.9 ± 1.8 for giant reed and 18.2 ± 1.3 g m^{-2} $year^{-1}$ for umbrella sedge, whilst in the belowground parts, average nitrogen levels were found to be 63.8 ± 1.8 g m^{-2} $year^{-1}$ for giant reed and 48.7 ± 2.1 g m^{-2} $year^{-1}$ for umbrella sedge. This shows that giant reed was able to remove a greater quantity of nutrients per area unit of the pilot system and also explains the difference in biomass production.

Similar results were obtained in Japan by [43], who observed that the unit planted with *Canna generalis* and *Phragmites australis* performed better in NH_4-N, NO_3-N, NO_2-N, TN and TP removal than those planted with *Cyperus alternifolius*. However, another study [36] found that units planted with *Cyperus papyrus* showed a lower PO_4^{2-} removal rate compared to those units planted predominantly with *Phragmites mauritianus*. It was also found that wetlands planted with *Cyperus papyrus* produced higher NH_4-N and TP removal rates compared to those planted with *Miscanthidium violaceum* [26]. These results highlight the fact that the choice of species is highly influential upon the purification efficiency of a phyto-wastewater purification system but that the adaptability of the species to its environmental and management conditions are crucial, albeit a consideration which is not universally accepted by all researchers.

3.2. Removal efficiency of pollutants in the pilot units

Results of physical–chemical variation and contaminant removal levels of urban wastewater are shown, respectively, in Table 1 and Table 2. Some of the pollutants (i.e. COD) found in the urban wastewater at the inlet of the pilot H-SSF system had been significantly removed through secondary activated sludge treatment. However, the sludge did not remove all the

Table 1
Variation of pH, T, DO and ECw in the pilot units from June 2009 to November 2010. Two-year average (± standard error), minimum and maximum values are shown

Parameters	Inflow	Outflow unit A	Outflow unit B	Variation unit A	Variation unit B	Threshold values for Italian Ministerial Decree 185/2003
pH	7.5 ± 0.1 (6.3–8.2)	7.1 ± 0.1 (6.0–7.8)	7.1 ± 0.1 (6.1–7.5)	5.3 (1.5–8.9)	5.1 (1.4–9.8)	6–9.5
T (°C)	15.0 ± 0.1 (14.7–15.9)	14.7 ± 0.1 (14.2–15.6)	14.7 ± 0.1 (14.1–15.3)	2.1 (0.7–3.4)	2.5 (1.3–3.4)	–
DO (mgL^{-1})	1.4 ± 0.1 (1.0–1.7)	1.0 ± 0.1 (0.9–1.4)	1.1 ± 0.0 (0.8–1.4)	21.1 (8.3–30.8)	23.7 (12.5–29.4)	–
ECw (μS cm^{-1})	506.5 ± 46.2 (225–927)	371.8 ± 36.5 (154–669)	366.9 ± 35.8 (134–647)	27.2 (21.1–36.5)	27.6 (17.1–42.5)	3,000

Table 2
Main chemical and physical composition of the urban wastewater from the inflow and outflow of the pilot units. Removal efficiency of the pilot units from June 2009 to November 2010. Two-year average (± standard error), minimum and maximum values are shown ($n = 18$)

Parameters	Inflow	Outflow unit A	Outflow unit B	Removal efficiency unit A	Removal efficiency unit B	Threshold values for Italian Ministerial Decree 185/2003
Colour	NP*	NP	NP	–	–	–
Odour	NU**	NU	NU	–	–	–
Coarse mater						
	39.3 ± 2.4	8.8 ± 0.6	10.4 ± 0.6	76.7	72.6	10
TSS (mg L^{-1})	(24–57)	(6.1−15.9)	(7.0−14.7)	(63.7–85.3)	(62.9–83.3)	
	27.7 ± 0.8	7.6 ± 0.4	8.3 ± 0.4	72.5	69.8	20
BODs (mg O_2 L^{-1})	(21.9–32.3)	(5.1–12.7)	(6.3–14.7)	(61.6–81.0)	(54.1–80.5)	
	56.8 ± 2.9	18.0 ± 0.7	21.1 ± 0.9	67.5	61.5	100
COD (mg O_2 L^{-1})	31.4–75.8	(11.6–24.2)	(11.2–28.1)	(55.8–75.9)	(42.2–82.1)	
	19.1 ± 0.9	9.4 ± 0.6	10.0 ± 0.7	49.9	47.1	15
TKN (mg N L^{-1})	(12.7–25.5)	(6.1–16.9)	(6.8–17.7)	(33.7–64.4)	(21.3–62.8)	
	3.4 ± 0.2	1.8 ± 0.1	1.9 ± 0.1.	45.1	43.4	2
TP (mg P L^{-1})	(2.1–4.9)	(1.2–2.5)	(1.1–3.0)	(30.3–55.3)	(22.2–61.5)	
	26.1 ± 0.3	22.6 ± 0.4	22.5 ± 0.3	13.2	14.3	250
Chloride (mg Cl L^{-1})	24.0–28.2	(18.4–24.7)	(20.1–24.2)	(5.6–27.6)	(7.1–23.3)	
	1.7 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	25	25.3	10
Oil and fat (mg L^{-1})	(1.1–2.5)	(0.7–1.7)	(0.9–2.1)	(14.3–36.4)	(13.7–38.9)	

*Not perceptible.
**Not unpleasant.

pollutants as required by Italian Ministerial Decree 185/2003 governing the reuse of wastewater in agricultural irrigation, because it did not perform the tertiary treatment that was required for the complete treatment of wastewater. The lack of tertiary treatment was due to both original design and management reasons. Design constraints were related to old policies on wastewater management in Sicily whose aims were to feed pretreated wastewater back into watersheds, lagoons or rivers. These policies did not provide the reuse of wastewater in irrigation and assumed that the wastewater fed back into the watersheds would be further treated by exploiting the removal capacity of the aquatic ecosystem.

The management reasons depended on high costs of installation and maintenance of most common tertiary treatments in the sewage treatment system and often poor results of systems in Sicily. New Italian policies on wastewater management in agriculture, particularly the Italian Ministerial Decrees 152/1999, 185/2003 and 152/2006, which focus on wastewater reuse in irrigation and in water-saving methods, have been poorly used in Sicily, mostly because sewage treatment systems do not perform tertiary treatments and, therefore, cannot achieve the objectives set by these policies. In our study, these problems were overcome using a CW, and interactions between plants, soils and microorganisms were used in order to significantly remove the organic and mineral pollutants of pre-treated urban wastewater. The construction of a CW was chosen instead of improving the activated sludge performances because of the main benefits of a CW (as listed in international literature). CWs are typically inexpensive to build and maintain, require little or no energy to operate, show high flexibility in wastewater and pollution levels and are viewed as an environmentally friendly technology. The environmental and ecological benefits of CW were determinant in the choice of CW because it was built in an urban park.

At outlet no coarse matter was found in the wastewater and odours were not unpleasant, as defined by Italian law. The pH values at the inlet were moderately alkaline throughout the sampling phase, stabilizing at neutral at the outflow, considered optimal for the growth of the two species. The limited fall in pH value in an H-SSF system is due to the production of CO_2 caused by the decomposition of plant residues, by the removal of various components of the wastewater retained in the root area and, to a lesser extent, by the nitrification of ammonia [19]. It was observed that most H-SSF systems manage to buffer wastewater which is slightly acid or basic at the inflow and bring the pH values back to near neutral, which would seem to confirm the results found in our pilot plant [22].

ECw values fluctuated significantly during the sampling period with a variation of 27%. Despite the fact that evapotranspiration caused water losses, which increased the solute concentrations, the fall-in observed electrical conductivity which could be due to micro and macroelement uptake mechanisms by the plant and bacteria, and to adsorption by the root apparatus, by the medium and by undecomposed plant residues [30]. DO levels at the outflow of the CWs were equal to 1.0 mg L^{-1}, consistent with values found in other H-SSF systems [30]. Moreover, in contrast to Figures reported by Kaseva [24], they did not vary with a decrease in the wastewater temperature.

TSS removal efficiency was found to be almost identical in the two units. TSS concentrations at different stages are shown in Fig. 7(a). The TSS removal percentages were consistent with values found in literature and can be explained by filtration and sedimentation mechanisms at work in the *medium* and in the root system of the plants [12]. The high removal rate found in the two units allowed the wastewater to flow easily throughout the study without causing blockages or preferential flow channels. Throughout the two test years, the wastewater did not come to the surface nor did hydraulic short-circuiting occur.

BOD_5 removal efficiency varied from 72.5 (unit A) to 69.8% (unit B). COD removal efficiency at the outlet varied from 67.5 (unit A) to 61.5% (unit B). BOD_5 and COD concentrations at different stages are shown in Fig. 7(b) and (c), respectively.

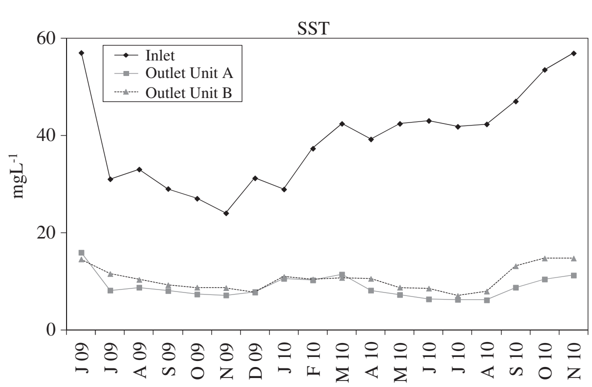

Fig. 7(a). Total suspended solids concentrations at different stages.

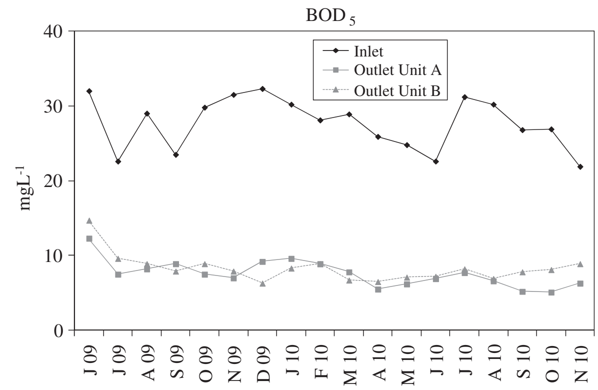

Fig. 7(b). Biochemical oxygen demand (5 days) concentrations at different stages.

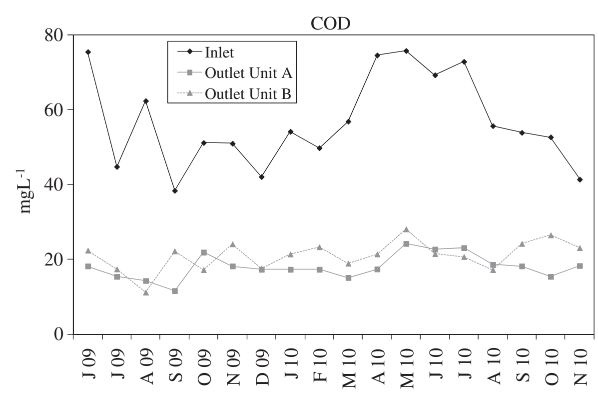

Fig. 7(c). Chemical oxygen demand concentrations at different stages.

In general, organic matter removal efficiency stayed within a range consistent with previous H-SSF system studies using urban wastewater, and was facilitated mainly by the high root density of the two macrophytes. Following the examination of various H-SSF systems operating in different countries around the world [44], BOD_5 and COD removal efficiency was respectively determined as 85 and 75%. Part of the oxygen released by the macrophyte roots and rhizomes is used to aid organic matter aerobic biodegradation through heterotrophic bacteria [8]. Based on the pilot system constructional and functional characteristics, such a quantity cannot be considered as sufficient to guarantee a high removal level. Based on these considerations, we may conclude that the high organic matter removal efficiency in the two units was due to additional anaerobic biodegradation processes

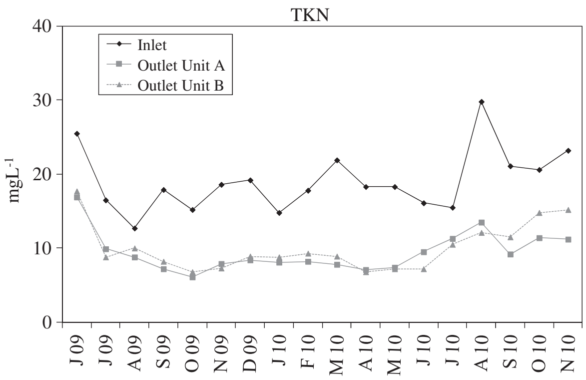

Fig. 7(d). Total Kjeldahl nitrogen concentrations at different stages.

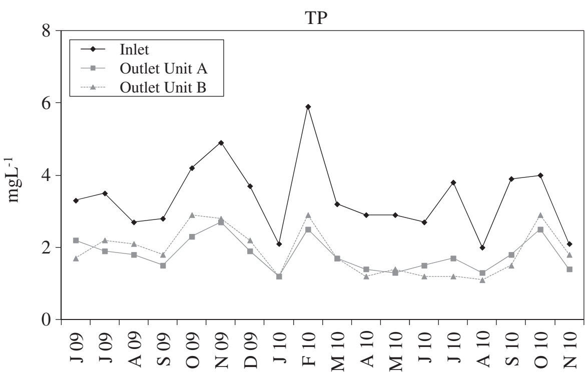

Fig. 7(e). Total phosphorus concentrations at different stages.

carried out by localised bacteria around the root system of the two macrophytes and the substrate particles. This is also confirmed by previous studies on H-SSF systems. It was not, however, possible to make a comparison with the BOD background concentrations recorded by [23] for H-SSF systems as the parameter in our study was determined in five days.

TKN and TP removal efficiency results were found to be similar in unit A and B. The concentrations at different stages are shown in Fig. 7(d) and (e).

In an H-SSF system, plant absorption processes, precipitation processes with various cations (Ca^{2+}, Fe^{2+} and Al^{3+}) around the gravel particles, and adsorption processes play a significant role in phosphorous removal [22]. Phosphorous removal was never found to be particularly high (lower than 50%) during the study, probably due to a range of factors, such as the gradual filling of the sorption sites by the plant root systems, by the non-regular harvesting of the plants, by the presence of undecomposed plant material around the substrate surface and by the adsorption properties intrinsic to the substrate itself [26]. Consistent with international literature, in the future we would expect to see a further reduction in phosophorous removal mostly due to the gradual saturation of most of the substrate sorption sites where these processes were active. Other studies show that phosphorous removal, in the long term, is also limited regarding the burial capacity of the phosphorous biogeochemical cycle in a constructed wetland [23] and that phosphorous removal is efficient only in the initial working stages of an H-SSF system, immediately after the introduction of the wastewater, even in lower plant density conditions, and when the gravel adsorption processes are highly active [31].

As regards nitrogen removal, it was found to be lower compared to organic matter removal due to the low oxygen levels in the system which hindered ammonia nitrification significantly: a process which most of the literature maintains, is the most important organic nitrogen removal mechanism. The influence of oxygen levels on the intensity of the nitrification process is maintained by [46], who observed a reduced or absent nitrification rate at dissolved oxygen levels of below $0.5\,\text{mg L}^{-1}$. The contribution of the two macrophytes in nitrogen removal was important in terms of total nitrogen levels in the above and belowground biomass. However, this represents only a fraction of all the nitrogen removed, if we consider above all that the harvesting times and nitrogen accumulation times in the biomass were not contemporary [12,44].

Cl^- levels remained more or less constant throughout the sampling phase; removal efficiency was found to be 13.2% and 14.3%, respectively, at the outlet of unit A and B. Fat and oil removal efficiency did not exceed 25.3%.

Bacteria load removal was particularly effective right from the initial sampling stages (Table 3). Maximum removal levels were expressed in log units and were found to be 1.95 (88.9%) for TC, 1.94 (86.4%) for FC, 1.93 (85.0%) for FS and 1.94 (87.4%) for EC. No *Salmonella* spp. was reported either in the inflow or the outflow water of the CWs. Consistent with previous studies, the high bacteria removal capacity of the pilot system could be attributed to a combination of physical, chemical and biological mechanisms [44] such as filtration and adsorption, chemical oxidation, sedimentation, predation by nematodes and protists, and viral and bacterial activity [8,38]. In Italy, the concentration of *Escherichia coli* in the final outflow water is an important microbiological indicator in terms of quality, as cited in the Ministerial Decree 185/2003 governing the reuse of treated wastewater in

Table 3
Main microbiological composition of the urban wastewater from the inflow and outflow of the pilot units. Removal efficiency of the pilot units from June 2009 to November 2010. Two-year average (± standard error), minimum and maximum values are shown ($n = 18$)

Parameters	Inflow	Outflow unit A	Outflow unit B	Removal efficiency unit A (%)	Removal efficiency unit B (%)	Threshold values for Italian Ministerial Decree 185/2003
Log10 (MPN 100 ml^{-1}–CFU 100 ml^{-1})						
Total coliforms (MPN 100 ml^{-1})	4.40 ± 3.2 (4.11–4.69)	3.42 ± 2.4 (3.16–3.81)	3.49 ± 2.4 (3.25–3.87)	1.95 (1.91–1.98)	1.94 (1.89–1.97)	–
Faecal coliforms (MPN 100 ml^{-1})	4.24 ± 2.8 (4.12–4.37)	3.37 ± 2.4 (3.16–3.64)	3.43 ± 2.5 (3.14–3.71)	1.94 (1.89–1.96)	1.93 (1.86–1.96)	–
Faecal streptococci (MPN 100 ml^{-1})	3.96 ± 2.5 (3.86–4.09)	3.14 ± 1.9 (2.93–3.33)	3.21 ± 2.0 (2.96–3.41)	1.93 (1.90–1.95)	1.91 (1.86–1.95)	–
Escherichia coli (*CFU 100 ml^{-1}*)	3.03 ± 1.7 (2.88–3.19)	2.12 ± 1.1 (1.80–2.36)	2.16 ± 1.0 (1.91–2.32)	1.94 (1.88–1.97)	1.93 (1.89–1.96)	10 (80% of samples) and 100 (maximum value point)
Salmonella spp. (MPN 100 ml^{-1})	–	–	–	–	–	–

irrigation. *E. coli* concentrations at different stages are shown in Fig. 8.

During the test period, the microbiological data obtained for *E. coli* (on average 120 CFU 100 ml^{-1} for unit A and 130 CFU 100 ml^{-1} for unit B) were not found to be within these legislative limits (10 CFU 100 ml^{-1} in 80% of the samples and 100 CFU 100 ml^{-1} maximum levels), however, a high microorganism removal capacity was observed, which must be taken into consideration as most conventional treatment systems in Sicily, such as activated sludge or trickling filter systems, demonstrate low removal capacity of these pollutants due to the fact that often not all three treatment processes are effectuated. A factor which may have influenced the non-total removal of the microbiological load was the size of the two wetland units, which may not have been adequate for the microbiological loading rate at the inflow. A possible solution may be that of repeating the process by pumping the water at the outflow through the system again in order to obtain further microbial decontamination. Another interesting hypothesis might be the use of a combined H-SSF-V-SSF system to remove pathogens with higher efficacy, as demonstrated in other Mediterranean areas [2,6,35]. The different retention times of the wastewater–longer in a H-SSF system than a V-SSF one–would determine a change in the general aerobic/anaerobic conditions and affect the

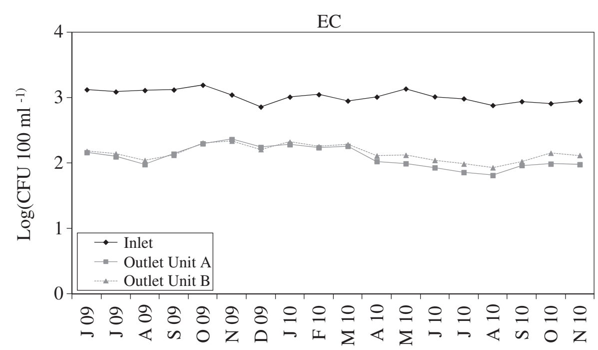

Fig. 8. *Escherichia coli* concentrations at different stages.

chemical oxidation mechanisms regarding pathogens. Microbiological removal results, although not within legal limits according to Italian law, should not necessarily lead to the dismissal of the system as unsuitable for reuse in parkland irrigation for various reasons. If we look carefully at Italian law on the reuse of wastewater in irrigation, we can see that it is excessively restrictive regarding bacteriological contaminant limits of the effluent compared to some Mediterranean countries, where treated wastewater is used in the irrigation of golfing greens. Furthermore, Italian law does not make a distinction in quantitative terms between the irrigation of food and non-food crops, and puts the irrigation of a crop for raw consumption on the same level as the irrigation of a "crop" for recreational or sports use. There is, however, a substantial difference between the two in so far as the law should be restrictive in regulating water for reuse in food crop cultivation and more permissive for reuse in parkland, taking into consideration also the buffer action of the soil. Specific research on the risks involved in using treated wastewater in public parkland carried out in various countries around the world did not show any major contraindications due to the mechanisms with which microorganisms still present in the wastewater are retained by the soil, such as soil surface filtering, sedimentation in the gaps in the ground and adsorption. Finally, if irrigation is carried out using low-impact irrigation systems (subirrigation, for example) or if irrigation is timed to allow an adequate interval between irrigation and use by the public, or if the surface dries quickly before fruition, direct human contact with the microbiological load can be avoided.

On comparison with other H-SSF systems planted with giant reed and umbrella sedge, very similar results were found concerning the removal of the main contaminants under examination, even when varying parameters, such as the initial treatment, daily hydraulic load, wastewater concentration levels and the system size. In Morocco, in an H-SSF system planted partially with giant reed and used for the treatment of domestic wastewater with an initial pretreatment, BOD_5 and COD removal were around 82% with highly significant faecal coliform removal rates [17]. On the Island of Crete, in a hybrid FWS and H-SSF system planted partially with giant reed for the treatment of highway runoff, TN and TP removal were 49 and 60%, respectively, with an 80% higher TSS removal [42]. In an H-SSF system planted with giant reed in Australia [27], BOD and TN removal were 71 and 78%, respectively, using untreated storm water collected from the hard-pan and other surfaces of a dairy processing factory in southwest Victoria. In another study carried out in the same area on a system of nine H-SSF CWs, partially planted with giant reed and used for the recirculation of aquaculture system effluent, the main organic and inorganic contaminant removal level was found to be above average and a 100% *E. coli* removal was determined, allowing for the water to be reused in the irrigation of crops for human consumption [28].

As regards umbrella sedge, little data is available as the use of papyrus sedge is more common, mainly in tropical and subtropical regions. In Europe, the use of the species is highly rare. In a pilot H-SSF system in Uganda with an aerated lagoon planted partially with *Cyperus papyrus* L. and used for the treatment of domestic wastewater, BOD_5 and COD removal levels were found to be 80 and 70%, respectively, with a 99% pathogen load removal [36]. In Japan, in an experimental pilot system made up of 10 acrylic columns with horizontal subsurface flow, it was found that the unit planted with *Cyperus alternifolius* exhibited BOD removal capacity of 94.9%, which was higher than the other species included in the study, whilst the nitrogen and phosphorous removal rates were found to be lower [43].

4. Conclusions

All the chemical and physical values at the outflow were found to be compatible with "threshold limit values" as stipulated by the Italian Ministerial Decree 185/2003 regarding the reuse of treated wastewater in irrigation. The microbiological analysis results were not within legislative limits but the systems showed high capacity in removing microorganisms.

This study did not include testing of the effects of using treated wastewater on turfgrass as this was not the main aim of the study; the study was limited to the reuse of the wastewater in irrigation. No counter health indications were reported in the use of the wastewater leaving the pilot system, in terms of odour in the green areas around the system. Furthermore, the turfgrass species used did not show any anomalous aesthetic characteristics. As regards, the long-term continuous use of wastewater for parkland irrigation, there are a number of issues which need to be addressed, amongst which two seem to be most critical. The first is the plant behaviour in long term, measured in terms of growth and micronutrient accumulation (e.g. sodium) in the plant tissue, and the second is soil response to prolonged irrigation using wastewater.

The two macrophytes used showed good removal efficiency of the main contaminants and this is an excellent result for the research, especially with a view to wider use of the species in Sicily and the Mediterranean area in general. Giant reed, in particular, already known in the energy sector as a high performance lignocellulosic crop, would seem a good choice in the planning of CWs in the Mediterranean area as it is native, shows high adaptability, competiveness and is easily found throughout Sicily. Based on average nitrogen levels found in the above and belowground plant parts during the study, the giant reed showed greater nitrogen absorption capacity than umbrella sedge, despite the fact that nitrogen removal rates in the two units were found to be similar.

This shows that, on the one hand, the species absorb and accumulate nitrogen in different ways, mainly due to having different root apparatus and reserve organ complexity, making the choice of the species fundamental. On the other hand, this capacity, although significant in the treatment process, must be considered in relation to the influent pollutant loading rate. This would, therefore, force us to take into consideration the action of the microorganisms and the substrate and their interactions with the plants. It follows that the next stage of research must be to understand which mechanisms compensate the lower nitrogen absorption capacity of the umbrella sedge leading to homogeneity in nitrogen removal rates of the two units, and, in addition, what kind of agronomic management is necessary in order to improve the nitrogen absorption capacity of the giant reed in order to enforce its role in nitrogen removal in a wastewater treatment system.

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