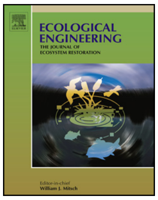
Ecological Engineering 87 (2016) 295–304

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng



Water balance and pollutant removal efficiency when considering evapotranspiration in a pilot-scale horizontal subsurface flow constructed wetland in Western Sicily (Italy)

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ARTICLE INFO

Article history:
Received 15 December 2014
Received in revised form 21 October 2015
Accepted 18 November 2015
Available online 17 December 2015

Keywords:
Constructed wetlands
Water balance
Evapotranspiration
Crop coefficient
Pollutant removal efficiency
Cyperus alternifolius
Typha latifolia

ABSTRACT

In constructed wetland systems (CWs) for wastewater treatment, the performance of the system is affected by evapotranspiration (ET). This study shows the results of a series of water balance and pollutant removal efficiency analyses taken from a pilot horizontal-subsurface flow system (HSSFs) in the West of Sicily (Italy). The system comprised three separate units, one planted with *Cyperus alternifolius* L., one planted with *Typha latifolia* L. and an unplanted unit. The system was fed with urban wastewater from an activated-sludge wastewater treatment plant. The aims of the study were to determine water balance and pollutant removal rates when considering evapotranspiration in two root emergent macrophytes in typically Mediterranean climate conditions. ET values were calculated by determining three components of a simplified water balance model without taking subsurface and surface water into consideration. Crop coefficient values were estimated using the FAO 56 method. Removal efficiency (RE) of a pilot HSSFs was calculated using both inflow and outflow pollutant concentrations and mass loads. Biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) were the main pollutants examined. The *T. latifolia*-unit was found to have higher cumulative evapotranspiration rates (3579 mm) than the *C. alternifolius*-unit (3142 mm). Water-use efficiency (WUE) for *C. alternifolius* (0.66 g/L) and *T. latifolia* (0.75 g/L) was somewhat low on average compared to traditional open-field crops. Percent removal was calculated using mass loads was on average higher than that determined using concentrations for both the planted and unplanted units. Further knowledge on water losses could provide useful information when designing CWs. The estimate of ET is highly important for arid areas, especially where the water at the outflow of the CWs is required for reuse.

1. Introduction

In constructed wetland systems (CWs) designed for the treatment of wastewater for irrigation purposes, an estimation of the evapotranspiration (ET) rates is of primary importance. ET is a highly complex process which can be defined as the amount of water lost in the atmosphere due to the evaporation of water from the soil and/or open water, and water loss due to transpiration from plant tissue (Allen et al., 1998; Mitsch and Gosselink, 2007; Kadlec and Wallace, 2009). Research shows that ET is the greatest cause of water loss in various types of wetlands. Pedescoll et al. (2013) noted that ET is the only process through which CWs can lose water, as the bed of the wetland is isolated from the underlying soil by a waterproof sheet thereby stopping water loss from soil infiltration. Evapotranspiration is highly susceptible to meteorological variables. Allen et al. (1998) observed that solar radiation is the main climatic variable affecting ET. Yu et al. (2002) reported that solar radiation is the most sensitive variable and wind speed the least sensitive variable as regards ET estimation, and that relative humidity has a reverse effect on ET estimates: as the former rises the latter will fall. In addition to meteorological variables, various authors agree that the plant growth stage is the factor which affects evapotranspiration rates in CWs to the greatest extent (Headley et al., 2012; Pedescoll et al., 2013; Tuttolomondo et al., 2015). ET is, therefore, higher during the summer months

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http://dx.doi.org/10.1016/j.ecoleng.2015.11.036

when plants are at their vegetative and production maximum, and lower in the winter months when vegetative activity slows. Pedescoll et al. (2013) noted that plant height and leaf density substantially affect the amount of water lost to ET in that, as shown in a number of studies (Pauliukonis and Schneider, 2001; Bialowiec et al., 2014), CWs vegetated by tall plants with a high leaf area index are also those with the highest ET rates. Mitsch and Gossellink (2007) noted that the rate of water loss is directly proportional to the difference between vapour pressure at the leaf surface and that in the overlying air. Knowledge on typical ET rates of species used in treatment units and variations in ET rates throughout the year is extremely important when designing CWs owing to ET dynamics. Grismer et al. (2001) noted that high ET can change the flow of water within a CWs. Kadlec and Wallace (2009) commented that ET considerably affects treatment performance levels in CWs. Ávila et al. (2013) stated that ET affects the redox conditions in the system, thereby affecting the pollutant removal. Beebe et al. (2014) claim that increased evapotranspiration seems to have a damaging effect on pollutant removal efficiency. The authors report that differences in ET attributed to various factors in CWs, such as plant selection or climatic conditions, can lead to incorrect evaluation of treatment performance when using previously determined removal rate coefficients. ET can be a huge disadvantage in those arid or semi-arid areas where treated wastewater can be reused for irrigation in agriculture (Green et al., 2006; Leto et al., 2013a,b). In these areas, ET can affect the amount of water available at the outflow of the CWs. This paper shows the results of tests carried out on a pilot horizontal sub-surface flow system (HSSFs) planted with *Cyperus alternifolius* and *Typha latifolia*, located in the West of Sicily (Italy) under typically Mediterranean climate conditions. The main aims of the study were: (1) to quantify the evapotranspiration rate of the two macrophytes based on climate conditions and on the growth stage of the macrophytes, (2) to provide an estimate of the crop coefficient values for the two macrophytes for the various growth stages, (3) to determine the water use efficiency for the two macrophytes (4) to determine water balance based on crop evapotranspiration rates and (5) to determine pollutant removal rates.

2. Materials and methods

2.1. Test site

Tests were carried out in the two years 2013–2014 on the pilot HSSFs in Piana degli Albanesi, a rural community (6000 inhabitants) in the West of Sicily (37°59′56“40N–13°16′50″16E, 740 m a.s.l.). The climate of the area is humid with a mean annual rainfall of approx. 800 mm, mainly distributed between October and April. With reference to time series 2002–2013, the annual average temperature was 15.1 °C, average maximum temperature was 19.7 °C and average minimum temperature was 10.9 °C. The summer drought was severe and the dry period fell between June and September (Fig. 1).

2.2. Description of the pilot system

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. 2). The system included 3 separate parallel units (A, B and C) each 33 m long and 1 m wide, providing a total surface filter bed area of 99 m^2 (Fig. 3). 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 3 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. Units A and B were planted with *C. alternifolius* L and *T. latifolia* L., respectively and unit C was left 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, thereby reducing the risk of hydraulic short-circuiting. The pipe was placed 10 cm from the surface of the substrate in each unit. A timer-controlled pumping system ensured homogeneous distribution of the

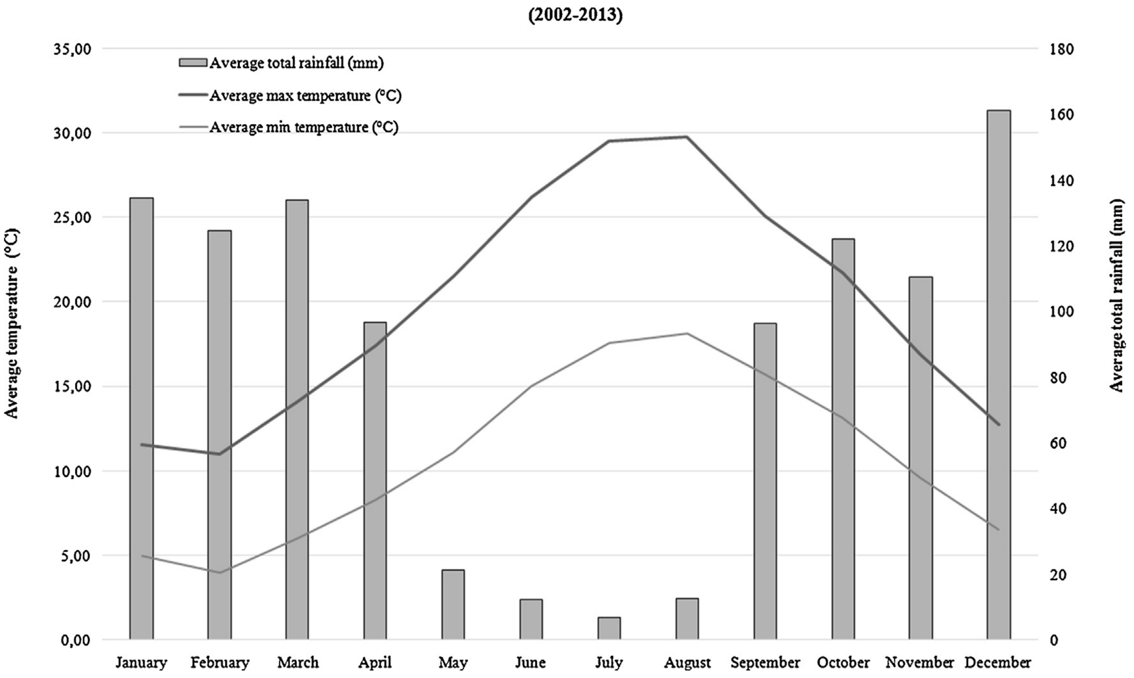

Fig. 1. Time series of average total rainfall, average maximum termperature and average minimum temperature from 2002 to 2013.

Fig. 2. A view of HSSF pilot system located downhill from the sewage plant in *Piana degli albanesi* (Sicily, Italy).

wastewater and the inflow was measured by a flow meter in each unit. Pumping was continuous throughout the day without variations in time. The outflow tanks, located downhill from the 3 units, were installed with a filter grill between the tanks and the substrate in order to avoid blockage. The outflow wastewater ran downhill into a 64 m^3 storage tank, which was connected to a sprinkler system and used to irrigate the surrounding area. The units operated under the same hydraulic conditions and were tested under a hydraulic loading rate (HLR) of 12 cm/d.

2.3. Plant material

Mother plants of *C. alternifolius* were obtained from a nursery in 2011 and propagated using cuttings. Fertigation was applied to increase plant vegetative vigour. The rooted stems were planted in unit A in March 2012 at a density of 5 stems/m^2. *T. latifolia* plants were collected from natural wetland areas near to the pilot site and the rhizomes were used for propagation in a small nursery at a constant moisture level. The rhizomes were planted in unit B in March 2012 at a plant density of 4 rhizomes/m^2. In November 2012, plants were cut back to a height of 50 cm above gravel bed height.

2.4. Plant biomass analysis

Plant height, stem density and fresh and dry weight of the aboveground plant parts (leaves and stems) were used to determine plant growth. Measurements were taken from April to November 2013. Plant height was determined fortnightly by measuring the maximum height of 10 plants selected randomly from the initial, the middle and the end sections of each unit. Maximum height was measured from the surface of the filter bed to top leaf insertion and only plants in good vegetative and phytosanitary condition were selected. Stem density was determined randomly on an area of 1 m^2 for each unit.

During 2013, four crop growth stages (Allen et al., 1998) were identified:

- initial stage: from 'greenup' to the beginning of stem elongation;
- crop development stage: from stem elongation to initial flowering;
- mid-season stage: from flowering to initial canopy senescence;
- late-season stage: from canopy senescence to plant harvest.

In November 2013, the plants were cut back once again to a height of 50 cm above gravel bed height. On this date, the fresh aboveground (stems and leaves) plant parts were determined only on a representative sample of 10 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.

2.5. Water balance

The FAO Penman–Monteith method was used to calculate ET$_0$ (Allen et al., 1998). 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.

$$\mathrm{ET}_0 = \frac{0.408\Delta(R_\mathrm{n} - G) + \gamma(900/T + 273))u_2(e_\mathrm{s} - e_\mathrm{a})}{\Delta + \gamma(1 + 0.34u_2)} \tag{1}$$

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), $e_\mathrm{s} - e_\mathrm{a}$ 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.

Water balance for each unit was determined separately every 10 days from April to November 2013. This period was chosen according to the growth dynamics of the two species. For the planted units, an estimate of the water balance was calculated, in agreement with International Water Association (2000), using the following equation:

$$Q_\mathrm{o} = Q_i + (P - \mathrm{ET}_\mathrm{c})A \tag{2}$$

where, Q_o = output wastewater flow rate (m^3/d), Q_i = wastewater inflow rate (m^3/d), P = precipitation rate (mm/d), ET$_\mathrm{c}$ = crop evapotranspiration (mm/d), A = wetland top surface area (m^2). Q_i was kept constant during the tests at 60 m^3/d. For the unplanted unit, water balance was calculated using Eq. (2).

The amount of water at the inflow and outflow of each unit was determined using a volumetric flow meter. Rainfall was

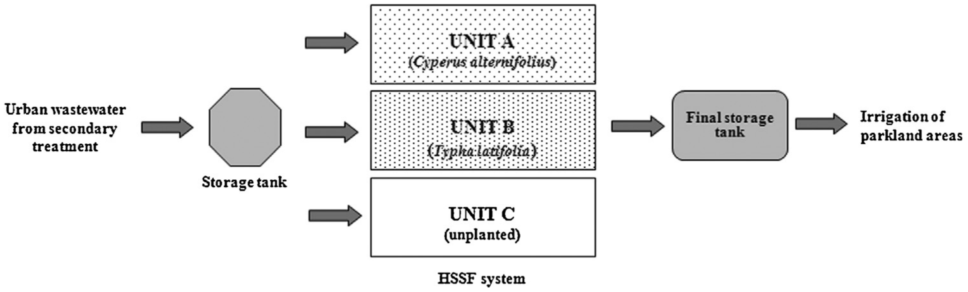

Fig. 3. Layout of pilot-scale HSSF system in *Piana degli albanesi* (Sicily, Italy).

determined with a pluviometer. ET_c and ET_{con} were estimated using Eq. (2).

The K_c values for *C. alternifolius* and *T. latifolia* were calculated, in agreement with Jensen et al. (1990) and Allen et al. (1998), using the equation:

$$K_c = ET_c/ET_0 \tag{3}$$

Crop coefficients were calculated every 10 days in 2013 for each growth stage of the two macrophytes.

2.6. *Water use efficiency (WUE)*

Mengel and Kirkby (2001) defined water use efficiency as the amount of plant matter produced per unit of water consumed. This parameter (g/L) was calculated, for each vegetated unit, using the ratio between above-ground biomass dry weight produced in a year (g/m^2) and the total volume of water lost via ET (L/m^2) in the same period. This parameter was calculated immediately following harvest in November 2013 and allowed us to compare relative water consumption between the planted units under identical hydraulic conditions but vegetated with different species.

2.7. *Removal efficiency (RE)*

Urban wastewater samples were taken monthly during the period April–September 2014, amounting to a total of 6 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 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. Only biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) levels were determined, using Italian water analytical methods (APAT and IRSA-CNR, 2004).

Removal efficiency of a pilot HSSFs was calculated using both concentrations and mass loads. Removal efficiency based on concentrations was calculated according to an International Water Association recommended equation (2000):

$$\mathrm{RE} = \frac{C_i - C_0}{C_i}100 \tag{4}$$

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

Removal efficiency based on mass loads was calculated according to the Bialowiec et al. equation (2014):

$$\mathrm{RE} = \frac{(C_iQ_i) - (C_0Q_0)}{(C_iQ_i)}100 \tag{5}$$

where, $C_i \times Q_i = M_i$ (mg) and $C_0 \times Q_0 = M_0$ (mg) are the inflow and outflow mass loads, respectively. C_i and Q_i were identical in each unit. The two equations of removal efficiency were compared under the hydraulic loading rate (HLR) of 12 cm/d. Correlations between ET and examined pollutants were calculated.

2.8. *Statistical analysis*

An estimate of variability in the data populations of crop coefficients and urban wastewater chemical and physical parameters was determined using mean ± standard deviation calculations. The software MINITAB Release 14 for Windows was used.

3. Results

3.1. *Microclimatic conditions*

Trends for the main meteorological parameters during the test period are shown in Fig. 4. Between April and November 2013, average air temperature trends were consistent with 10-year averages. Maximum average air temperature was 31.2 °C in the first 10-days of August and minimum average air temperature was 4.6 °C in the final 10-days of November 2013. Rainfall was highly concentrated between September and November. In the summer

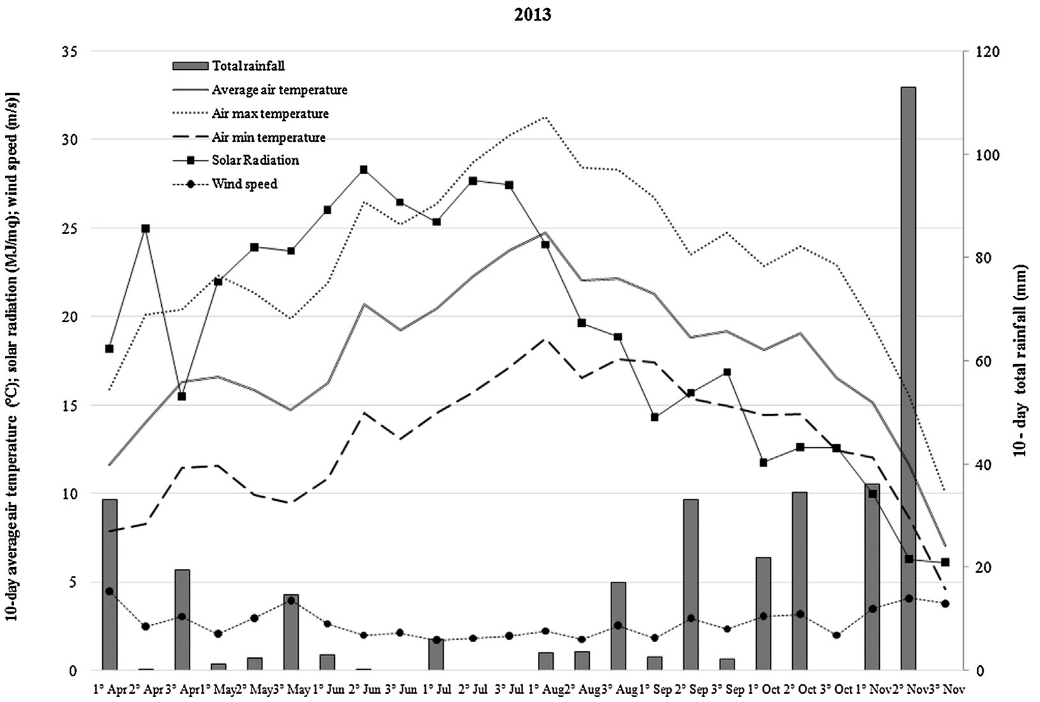

Fig. 4. Trends of 10-day minimum, maximum and average air temperature, solar radiation, wind speed and total rainfall during the test period.

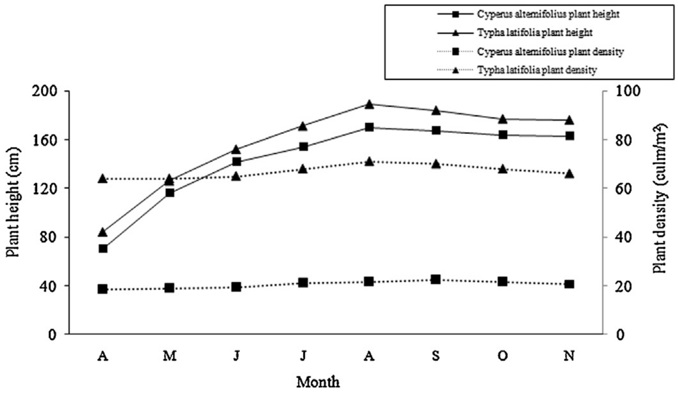

Fig. 5. *Cyperus alternifolius* and *Typha latifolia*: plant height trend and plant density.

period, average monthly rainfall was 17.8 mm. The most cumulative rainfall event was recorded in the second 10-day period of November (113 mm). Maximum daily average relative humidity was 93.8% in August. Minimum daily average relative humidity was 33.5% in April. Average total solar radiation was 18.6 MJ/m^2. The highest total solar radiation was recorded in the first 10 days of June at 28.3 MJ/m^2 while the lowest was in the third 10 days of November at 6.1 MJ/m^2. In the test period, average wind speed was 2.7 m/s. The highest average wind speed was determined in the first 10 days of May (4.5 m/s) while the lowest in the first 10 days of July (1.7 m/s).

3.2. Plant growth and biomass production

During the test period, plants reached maximum plant growth during the summer months, at the same time as high temperatures and relative humidity levels. Both species reached maximum height in August at 189 cm for *T. latifolia* (on average 157.3 ± 35.9 cm) and 170 cm for *C. alternifolius* (on average 143.2 ± 34.5 cm) (Fig. 5). Plant cover of the two species was high in both units at 95% for *T. latifolia* and 89% for *C. alternifolius*. Average culm/stem density during the test period was 41 ± 2.7 culms/m^2 for *T. latifolia* and 67 ± 2.6 stems/m^2 for *C. alternifolius*. Fig. 6 shows the evolution of the main growth stages of the two species, according to Allen et al. (1998). The flowering stage for *C. alternifolius* occurred between June and July, whilst full senescence for the above-ground biomass (stems and leaves) occurred mid-October. As regards *T. latifolia*, flowering occurred during July while full senescence of the above-ground parts occurred at the beginning of November. For both species, vegetative activity fell sharply at the onset of winter when air temperatures dropped. Above-ground dry matter production (leaves and culms) was found to be different for the two species due to morphological characteristics of the above-ground plant parts and due to differing cover density. In November 2013, *T. latifolia* produced above-ground biomass production (2697 g/m^2) which was greater than that of *C. alternifolius* (2082 g/m^2).

3.3. Water balance

Water balance results showed different water use for the *T. latifolia*-unit and *C. alternifolius*-unit compared to the unplanted

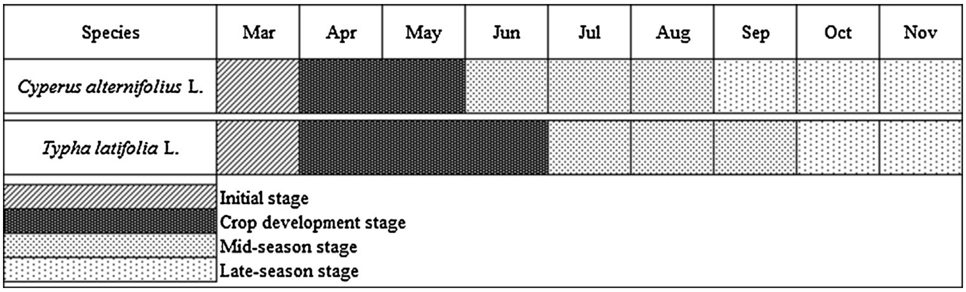

Fig. 6. Evolution of the main growth stages of *Cyperus alternifolius* L. and *Typha latifolia* L.

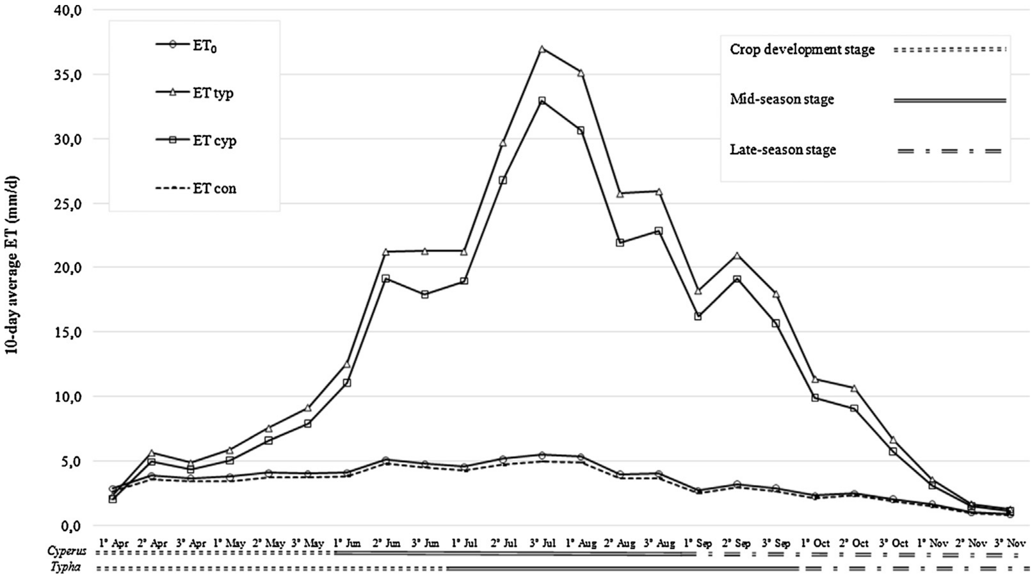

Fig. 7. 10 day- average ET_0, ET_{con}, ET_{typ} and ET_{cyp}.

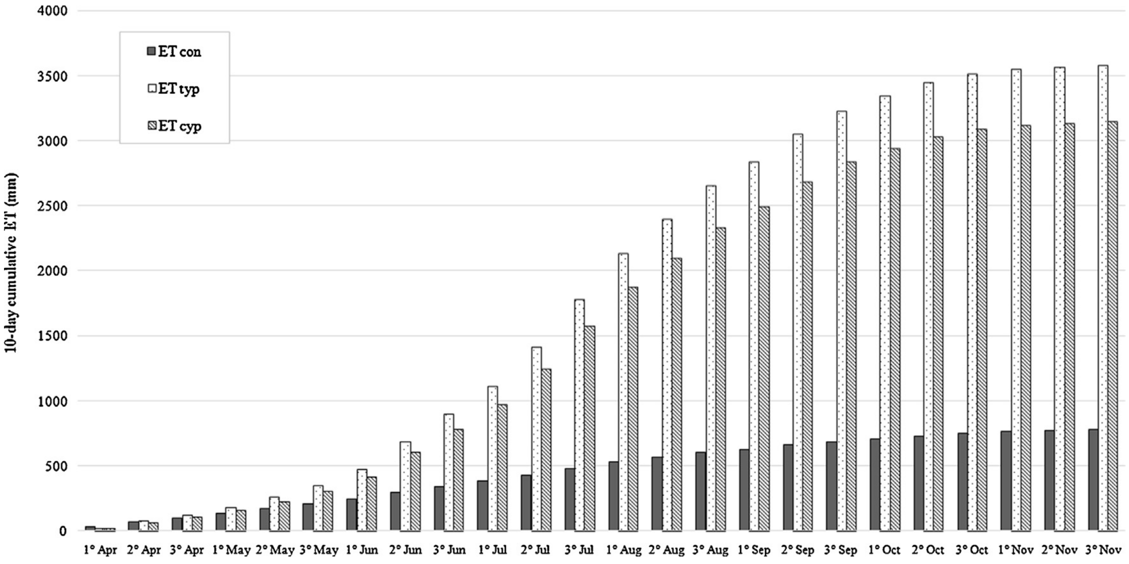

Fig. 8. 10-day cumulative ET_0, ET_{con}, ET_{typ} and ET_{cyp}.

unit. From April to November, ET_c values in the planted units were found to be different and much higher compared to ET_0 and ET_{con} (Fig. 7). As regards the *T. latifolia*-unit, average 10-day ET_{typ} ranged between 37.0 mm/d (3rd 10 days of July) and 1.3 mm/d (3rd 10 days of November). For the *C. alternifolius*-unit, maximum average 10-day values for ET_{cyp} (33.0 mm/d) were found in the 1st 10 days of August whilst minimum average 10-day values (1.1 mm/d) were obtained in the 3rd 10 days of November. Seasonal trends of average ET_{typ} and ET_{cyp} 10-day values were found to be very similar to each other. Taking into consideration the different growth stages of the two macrophytes, ET_c values of both species showed the same trend, with a constant 10-day and monthly increase. At the beginning of the mid-season stage, ET_c values increased rapidly in both planted units, reaching maximum values. In this phase, during which maximum vegetative growth of the two species was also reached, ET_{typ} was found on average to be higher than ET_{cyp}. At the end of the mid-season stage, which coincided with initial apical senescence of the two macrophytes, a progressive fall in ET_c values was found. During this stage, the *T. latifolia*-unit produced average ET_c values which were even higher than the *C. alternifolius*-unit. During the late-season stage, with the approach of the plant biomass harvesting period, minimum ET_c values were recorded. In November, ET_{typ} in the *T. latifolia*-unit fell by 91.4%, compared to average ET_{typ} obtained in the summer months. ET_{cyp} in the *C. alternifolius*-unit, however, fell by 90.2% compared to the summer average. These percentages showed evidence of a relationship between a fall in vegetative activity and a reduction in evapotranspiration activity of the two species. Considering cumulative evapotranspiration (Fig. 8), the *T. latifolia*-unit was found to have higher (3579.9 mm) rates than the *C. alternifolius*-unit (3146.6 mm). If we compare cumulative ET_c rates from the two planted units with cumulative ET_{con}, we find that there was a substantial increase in ET rates for the planted units, which highlights the effect that vegetation has on water loss in a CWs with water in continuous movement. Table 1 shows K_c values for the two species during each of the growth stages. K_c values for both of the species in the test varied during the course of the year with noticeable differences between the various stages of the growth cycle. Higher K_c values were recorded for *T. latifolia* than for *C. alternifolius*, with average values ranging between 0.8 (1st 10 days of April) and 6.8 (3rd 10 days of July). If we compare K_c values for the two species during the various growth stages, it is clear that the greatest differences were found during crop development (3.84 ± 1.79 *T. latifolia* and 3.39 ± 1.62 *C. alternifolius*) and mid-season stage (6.51 ± 0.16 *T. latifolia* and 5.71 ± 0.23 *C. alternifolius*) coinciding with maximum ET_c values. If we take a closer look at average 10-day K_c values of the two species, we see that K_c varies monthly, and highest values were found during the summer months, when the plants had the highest vegetative growth, and lowest values were found during the autumn months, during above-ground biomass senescence (Fig. 9). In terms of percentage, the greatest K_c increase was recorded during crop development, when the growth rate of the two species was greater than during other stages. Regarding WUE, *T. latifolia* was found to have a higher WUE value (0.75 g/L) than *C. alternifolius* (0.66 g/L) due to greater above-ground dry matter production compared to total water loss via ET. In Fig. 10, Q_0 trends relative to Q_i, cumulative ET and total rainfall are shown. As Q_i was constant for all of the 10-day periods in the study period, water loss was on average 6.5 m^3/10 days in the *T. latifolia*-unit and 5.6 m^3/10 days in the *C. alternifolius*-unit. Greater water loss found in the two vegetated units during the summer months was mostly due to higher ET_c values for the same period. Taking the different growth stages into consideration, greatest water loss in the two vegetated units occurred during crop development stage and mid-season stage, whereas least loss occurred during late-season stage.

Table 1
Cyperus alternifolius and *Typha latifolia* crop coefficient (K_c) in the main growth stages.

Stage	K_c			
	Cyperus alternifolius L.		*Typha latifolia* L.	
	μ	σ	μ	σ
Initial stage	1.05	0.30	1.20	0.34
Crop development stage	3.39	1.62	3.84	1.79
Mid-season stage	5.71	0.23	6.51	0.16
Late-season stage	2.55	1.24	2.95	1.45

μ = means; σ = standard deviation.

3.4. Removal efficiency of pollutants

Results from pollutant removal level tests carried out on a pilot HSSFs from April to September 2014 are shown in Table 2. Removal efficiency of BOD_5 and COD based on concentrations and mass loads was on average higher in the *T. latifolia*-unit than the *C. alternifolius*-unit. For the *T. latifolia*-unit, RE of BOD_5 calculated

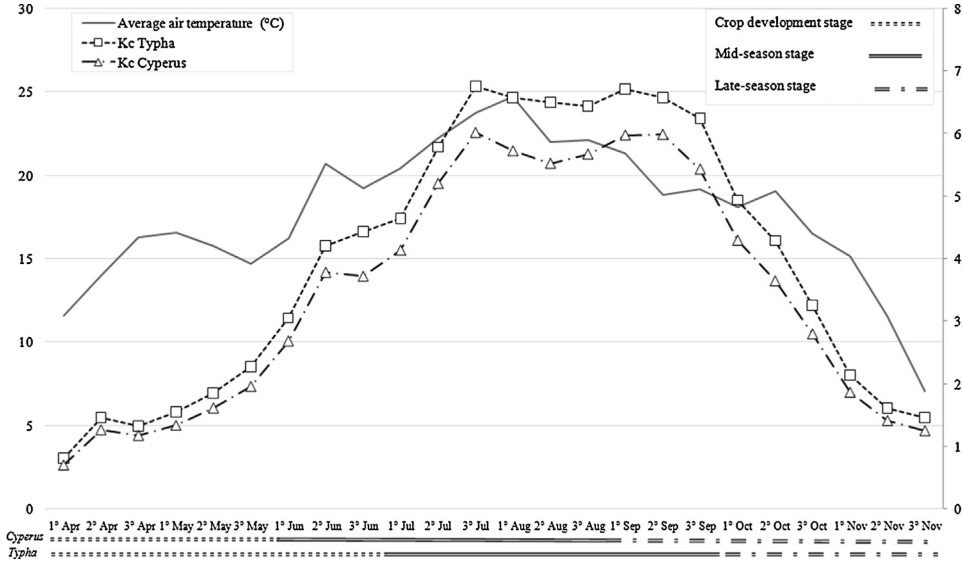

Fig. 9. 10-day average K_c trends for *Cyperus alternifolius* and *Typha latifolia* and average air temperature.

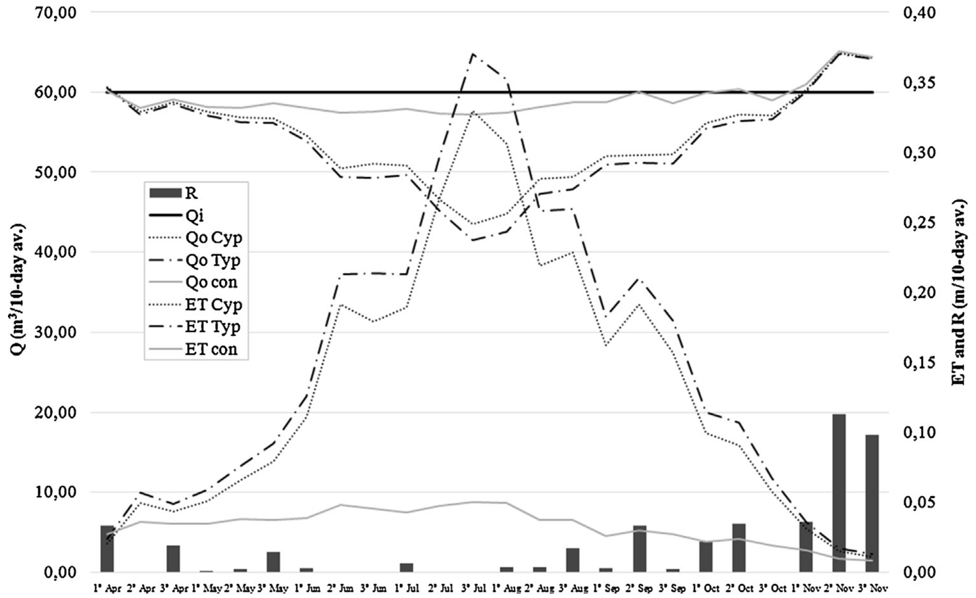

Fig. 10. Q_0 trends relative to Q_i, cumulative ET_c and total rainfall.

Table 2
BOD_5 and COD concentrations of the urban wastewater from the inflow and outflow of the three units. Removal efficiency of a pilot HSSFs from April to September 2014. Six-months average values (±standard deviation) are shown ($n = 6$).

Parameters	Treatments								
	Cyperus alternifolius-unit			*Typha latifolia*-unit			Unplanted-unit		
	HLR 12 mm/d	Concentration RE (%)	Mass load RE (%)	HLR 12 mm/d	Concentration RE (%)	Mass load RE (%)	HLR 12 mm/d	Concentration RE (%)	Mass load RE (%)
BOD_5									
BOD_{5i} (mg/L)	26.8 ± 4.5			26.8 ± 4.5			26.8 ± 4.5		
BOD_{50} (mg/L)	10.4 ± 2.1	60.5 ± 8.9		9.0 ± 1.1	65.5 ± 7.4		17.2 ± 2.1	35.3 ± 3.9	
$M(BOD_5)_i$ (g)	160.7 ± 27.1			160.7 ± 27.1			160.7 ± 27.1		
$M(BOD_5)_0$ (g)	55.4 ± 10.3		65.5 ± 5.5	47.1 ± 4.2		70.7 ± 3.8	100.5 ± 12.3		37.4 ± 3.4
COD									
COD_i (mg/L)	54.5 ± 14.8			54.5 ± 14.8			54.5 ± 14.8		
COD_0 (mg/L)	19.1 ± 2.6	63.6 ± 5.9		16.3 ± 2.9	69.3 ± 4.5		27.9 ± 7.8	48.7 ± 3.1	
$M(COD)_i$ (g)	326.7 ± 88.7			326.7 ± 88.7			326.7 ± 88.7		
$M(COD)_0$ (g)	101.8 ± 15.4		68.8 ± 5.8	84.6 ± 19.5		74.0 ± 1.7	163.1 ± 46.2		50.1 ± 3.1
ET									
ET (mm/d)	14.2 ± 8.3			16.1 ± 9.4			3.7 ± 0.7		

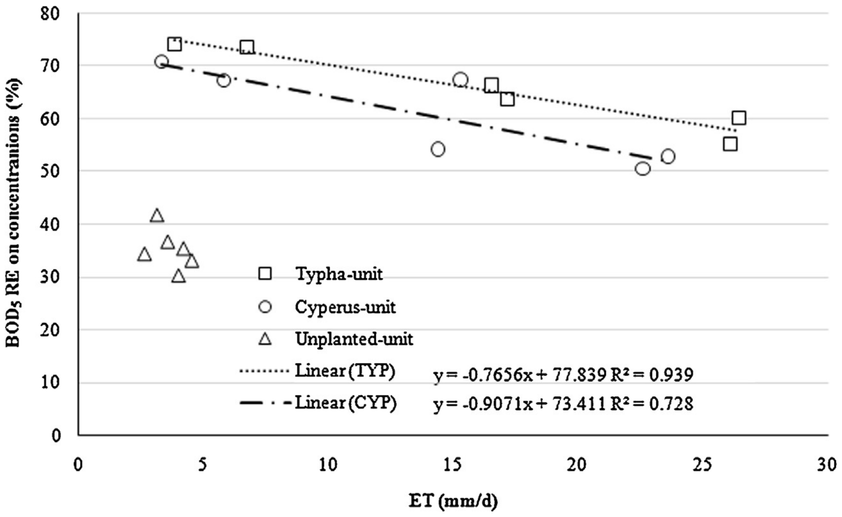

Fig. 11. Correlations between ET and BOD_5 removal efficiency based on concentrations in planted and unplanted units.

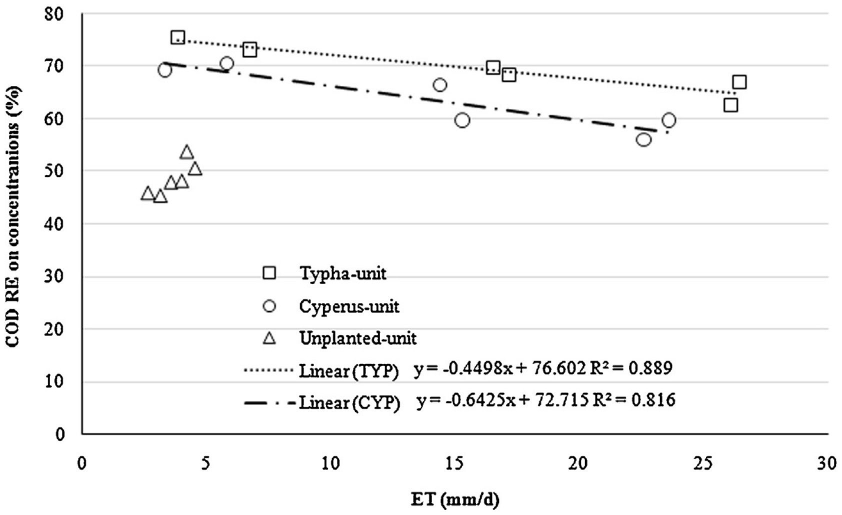

Fig. 12. Correlations between ET and COD removal efficiency based on concentrations in planted and unplanted units.

using concentrations was 65.5 ± 7.4, whereas the removal rate based on mass loads was 70.7 ± 3.8. The two planted-units were found to have higher RE rates than the unplanted-unit for both methods of RE calculation. For the unplanted-unit RE of COD based on mass loads was 50.1 ± 3.1, which was much lower than RE calculated for the *T. latifolia*-unit (74.0 ± 1.7) and the *C. alternifolius*-unit (68.8 ± 5.8) using the same method. Figs. 11 and 12 show correlations between ET and RE based on BOD_5 and COD concentrations. Regarding both pollutants, it is worth noting that when ET fell below 10 mm/d, RE did not decrease as quickly as when ET rose above 10 mm/d, for both the planted units. Our research showed that when ET reached average values of over 20 mm/d (summer months 2014), water loss increased notably and increases in BOD_5 and COD concentrations in the final effluent were also observed. This resulted in a decrease of apparent RE.

4. Discussion

Research showed that, under Mediterranean climate conditions, ET_c rates in a pilot HSSF system with continuous flow wastewater were higher than ET_{con} and ET_0. A comparison of the planted and unplanted units highlighted the fact that, under identical environmental and hydraulic conditions, vegetation represented the factor which affected water loss to the greatest extent in CWs. Despite the fact that this research did not include a comparison of different CWs or variable technical parameters, we are able to say, however, that leaf transpiration of the macrophytes greatly affects the amount of water at the outflow of CWs, independent of the design of the chosen system. Transpiration is greater the wider the leaf, the greater the number of leaves per plant, the taller the plant and the greater the plant density. As a consequence, above all in arid and semi-arid regions, where the main aim of wastewater treatment is to provide water for use in irrigation, ET dynamics must be taken into consideration carefully when designing a system. In our research, ET was more intense during the summer months, when temperatures and solar radiation, in particular, peaked. During this season, excessive stomata aperture increased the flow of water from plant tissue to the atmosphere. We found that greatest water demand occurred during mid-season stage, when more favourable climate conditions aided growth. *T. latifolia* was found to have higher cumulative ET_c than *C. alternifolius* due to the greater leaf width and an increase in above-ground and below-ground biomass production. Relative to average ET_c values of the two planted units, we also need to take into consideration the effect that the size of the system has on water loss. Kadlec and Wallace (2009) state that increases in ET_c, which are found in many HSSFs, are greater the smaller the system, especially when these systems are located in small towns, as was the case in our research. The results obtained, however, were comparable to those from other CWs located in Mediterranean areas under similar climate conditions (Borin et al., 2011; Milani and Toscano, 2013). This shows how different growth conditions in CWs can greatly influence ET dynamics, independent of the design and the size of the system. In particular, it is important to note that, in a CWs where there is a continuous flow of water, the use of an inert substrate can affect ET dynamics as, unlike agricultural soils (e.g., clay soils), adhesion of water molecules to the gravel particles is very low; their matrix potential being minimum. Despite the different environmental conditions at the test location compared to open-field conditions, and the use of 1-year plants, K_c trends for both species were found to be similar to those found in open field crops during the various growth stages. Furthermore, there was an increase during the initial growth stages, which peaked during the mid-season stage. As Allen et al. (1998) and Papaevangelou et al. (2012) note, it is possible to establish a strong correlation between variations in morphological characteristics of the species and variations in K_c values. As a consequence, when the plants enjoy the best vegetative conditions, in terms of height, stem density and leaf number, in correspondence to favourable climate conditions, ET rates are higher. With comparing this study to the results of other studies (Pedescoll et al., 2013; Beebe et al., 2014), the estimation of crop coefficients for *C. alternifolius* and *T. latifolia* can be considered as new knowledge given that calculations were made throughout the various growth stages of the two macrophytes. The crop coefficients estimated in this research can also be used to predict ET_c from pilot-scale HSSF systems located in other Mediterranean areas where ET_0 is known. WUE rates for both macrophytes were lower than WUE for *Phragmites australis*, one of the most frequently used macrophytes in CWs in Europe and Asia, as reported by Vymazal (2011). In the East of Sicily, in a pilot HSSFs operating under Mediterranean climatic conditions, Milani and Toscano (2013) found an average WUE rate of 2.27 g/L for *P. australis.* When comparing the WUE rates of the two macrophytes with those of *P. australis* found by Milani and Toscano (2013) in Sicily, we could claim that WUE rates of *C. alternifolius* and *T. latifolia* are lower than those of *P. australis*. In Spain, Pedescoll et al. (2013), when comparing the WUE rates of *Typha angustifolia* and *P. australis*, reported that water losses in the CWs planted with *T. angustifolia* were higher than in the CWs planted with *P. australis* but that *T. latifolia* was more efficient in water use. As a consequence, it is not easy to establish which of the species is best in WUE terms as the final result is affected by a number of factors, such as the physiological properties of each of the species, and the amount of above-ground biomass and leaf area. These latter have been found to vary with climatic conditions and agronomic/engineering experimental conditions, as confirmed by Zhou and Zhou (2009). If we compare WUE rates from open field crops, such as wheat and barley (Corbeels et al., 1998; Kermanian et al., 2005) with those from our research, we find that WUE rates for both macrophytes were lower than open-field crop WUE averages. According to Headley et al. (2012), the low WUE of the two macrophytes is due to physiological differences, such as the lack of adaptation capacity for water conservation, as water was continuously available (ET was not limited by water availability). Above-ground biomass production for the two macrophytes was found to be greater for *T. latifolia* than for *C. alternifolius*. Furthermore, as *T. latifolia* WUE rates were higher in the test year, we can assume that increases in above-ground biomass are directly proportional to increases in WUE. A relationship such as this is highly significant in agronomic terms as it affects crop choice in CWs. It is also important to remember that, in areas where the reuse of treated wastewater is a priority, macrophytes with low WUE and ET rates are more useful. In our study, ET was found to be constantly high throughout the test period, which was limited to the spring and summer months. As ET values substantially increased, a rise in the water loss from the system was determined together with a decrease in apparent RE of organic compounds in the system. The reason for the unexpected decrease in removal efficiency can be explained when considering plants, aerobic microbial communities, temperature and dissolved oxygen concentration. In constructed wetlands, removal of dissolved organic compounds is mainly due to aerobic microbial activity, a process which is greatly affected by water temperature and dissolved oxygen concentration. During the summer months, the rise in temperatures can determine a decrease of dissolved oxygen concentration in the water. Macrophytes are able to transfer oxygen from shoots to roots but only a limited amount of oxygen is diffused in the root-zone. Moreover, plant roots and aerobic microbial communities compete for limited dissolved oxygen concentration in the water due to the respiration process. Consequently, in summer, when dissolved oxygen concentration falls sharply, microbial activity often decreases together with the removal of dissolved organic compounds. Kadlec and Reddy (2001) noted that, in constructed wetlands, regarding the influence of temperature on organic carbon removal, the performance of the system decreases as temperature increases. Stein and Hook (2005) stated that pollutant removal may be less effective, equally effective or more effective in winter than summer mainly due to seasonal variations in plant growth, temperature, microbial activity and root-zone oxidation. The same authors highlighted that the interactions between plants and seasonal variations in temperature, dissolved oxygen concentration and other factors can significantly influence the RE in CWs, therefore, in summer, when temperatures rises sharply together with ET, a decrease in apparent RE of organic compounds can be expected. The removal efficiency of the HSSFs produced values which were extremely different depending upon the method of calculation of the RE, that is to say whether based on pollution concentrations or mass loads. When we compared the two methods of calculation, we found that, RE of the two pollutants based on mass loads was higher than RE based on concentrations. This seems to be due to a greater capacity of the mass loads calculation to evaluate specific contaminant RE as this calculation even takes into consideration water flow variations determined by ET at the inflow and outflow. In accordance with Bialowiec et al. (2014), our research highlighted the need to calculate RE based on mass loads, as it provided us with a more complete picture of the purifying process of the HSSFs. This method of calculation allowed us to evaluate the RE of the system more accurately, examining the differences in concentration of the pollutants at the inflow and outflow of the system and, at the same time, estimating the influence of ET on the system water loss. This is of particular interest in arid and semi-arid areas where evaluation of the purification capacity of CWs must include an estimate of evapotranspiration—which represents the main cause of water loss from the system. If we compare the results of our study with those of Bialowiec et al. (2014), large differences were found due to differences in the experimental system, wastewater and quantities and retention times of the wastewater. Bialowiec et al. (2014), using a lysimeter as a model of vertical subsurface flow system (VSSFs), noted that there is a strong correlation between ET and removal efficiency based on concentration levels but no significant correlation between ET and COD removal efficiency based on mass loads. Furthermore, these authors also point out that as ET and HLR increase, differences in the two methods of RE calculation are great in terms of R; the high values being obtained for these two parameters using mass loads calculations. In our research, with constant HLR (12 cm/d) and a constant daily load applied continuously throughout the day (24 h), we found that only when ET was very high (similar to levels recorded in the summer months) did we find any appreciable difference between RE using the two different calculation methods. However, when ET fell significantly, due to a fall in the maximum values of the climate variables (temperature and solar radiation in particular), the pollutant removal efficiency, calculated using both methods, was found to be similar. Nonetheless, as it is most important that CWs are designed for the reuse of wastewater functions as its best during the spring-summer months (when water demand for irrigation rises) it is obvious that the removal efficiency calculation using mass loads is more accurate and provides us with more valuable information. This method is, therefore, favoured in the management of CWs.

5. Conclusions

The research highlights the fact that, in constructed wetlands for wastewater treatment, evapotranspiration is the most important factor in the water balance of the system, affecting the amount of water available at the outflow of the system. Determining ET is fundamental when designing systems—especially in arid and semi-arid areas where the need to minimize water losses for irrigation is a primary concern. It is also important to take ET into consideration when calculating the removal efficiency of pollutants in the system. Several factors significantly affect RE, but when ET rises considerably, a decrease in RE of organic compounds (BOD_5 and COD) can be expected, as was found in our research. Although a correlation is apparent, there is no influence or causation relating to changes in ET on RE. RE is mostly calculated based on initial and final pollutant concentrations. However, this method of calculation is incomplete as it does not take into consideration variations in water flow rates due to ET. The best way to calculate RE seems to be the method which uses mass loads: it was found to give greater information on the treatment performance of HSSFs, especially when ET is very high. We believe that the results of this research further knowledge on the use of *T. latifolia* and *C. alternifolius* in an HSSF system regarding evapotranspiration rates, including knowledge on crop coefficients throughout the various growth stages, on data concerning water use efficiency by the species, and on the pollutant removal efficiency of the system. In particular, as water use dynamics of the two macrophytes is highly dependent upon morphological, physiological, hydraulic and climatic factors, it would be useful to define a standardized methodology for CWs for the characterization of water use by species in order to reduce variability in data found in literature and to allow for data comparison.

Acknowledgements

The authors would like to thank the Sicilian Regional Ministry of Food and Agricultural Resources, the Corissia Research Centre and the University of Palermo (D71D04000000008) for having provided the funds for the study. A special thanks also goes to Branwen Hornsby for her continuing support to the research and linguistic contribution.

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