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Constructed wetlands combined with disinfection systems for removal of urban wastewater contaminants

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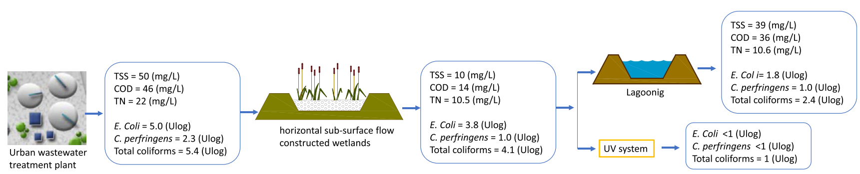
HIGHLIGHTS

- Horizontal sub-surface flow (H-SSF) constructed wetlands (CW) significantly removed urban wastewater contaminants.
- Lagooning, in series with H-SSF CW, provides a high removal of the main microbial groups.
- The combination of H-SSF CW with UV treatment produced the highest effective results.

GRAPHICAL ABSTRACT



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ABSTRACT

The removal efficiency of an urban wastewater treatment plant (WWTP) to obtain an effluent suitable for agriculture reuse was evaluated in a one-year period, taking into account the Italian wastewater limits and the recent European proposal for the minimum requirements water quality for agricultural irrigation. The secondary effluent of WWTP was treated by three full-scale horizontal sub-surface flow (H-SSF) constructed wetlands (CWs), working in parallel, planted with different macrophytes species, and combined with a UV device and a lagooning system running in series.

The H-SSF CW system effectively reduced physico-chemical pollutants and its efficiency was steady over the investigation period, while, *Escherichia coli* densities always exceed the Italian limits required for wastewater reuse in agriculture. The UV system significantly reduced the microbiological indicators, eliminating *E. coli*, in compliance with the Italian regulation, and somatic coliphages, although a variable efficacy against total coliforms and enterococci, especially in winter season, was achieved. Although the lagooning unit provides a high removal of the main microbial groups, it did not reduce physico-chemical parameters. Even if the overall performance target, for the whole treatment chain, met the recent $\log_{10}$ reduction (≥5.0), required by the European Commission, the persistence of enterococci, especially in winter season, poses a matter of concern for public health, for the potential risk to serve as a genetic reservoir of transferable antibiotic-resistance.

1. Introduction

Reclaimed water provides a valid opportunity to supplement water resources, alleviate environmental loads and address the imbalance between water demand and water supply (Lyu et al., 2016). Agricultural reuse is one of the most worldwide significant use of reclaimed water (EPA, 2012) and Constructed Wetlands (CWs) are widely applied as a low-cost alternatives or supplementary systems for wastewater treatment, especially in small and medium communities where low maintenance and easy operation are required (Marzo et al., 2018; Toscano et al., 2013; Vymazal and Kröpfelová, 2009). Irrigation with treated

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wastewater is already implemented in different countries, especially in arid zones and urban areas, such as France, Italy, Spain, Cyprus, Malta, Israel, Jordan, and USA (Pedrero et al., 2010; EPA, 2012; Kalavrouziotis et al., 2015). However, the human and environmental health implications of treated wastewater reuse for agricultural scope poses still some concerns (Phung et al., 2011). In particular, the microbiological parameters represent the biggest threat to municipal wastewater agricultural reuse due to the frequent presence of pathogens in effluents of conventional wastewater treatment plants (WWTPs) (Ghermandi et al., 2007; Calheiros et al., 2017). For each application of treated wastewater reuse, safety criteria must be observed and the potential risks associated with the processes must be defined, according to World Health Organisation guidelines (WHO, 2006). Standard criteria are based on the establishment of threshold values for specific physico-chemical and microbiological parameters. These values must be fulfilled before using treated wastewater for irrigation purpose. In Italy, the use of reclaimed wastewater is regulated by the Ministerial Degree N. 185, 12/06/2003 (Ministry for Environment, 2003) which establishes standard values for fifty-four parameters, many of which are the same required for drinking water (Cirelli et al., 2008). At European level new guidelines for minimum requirements water quality for agricultural irrigation have been recently announced (European Commission, 2018). It sets minimum requirements for treated wastewater from urban WWTPs, referring to four parameters (*Escherichia coli*, BOD_5, TSS and Turbidity), and establishing different water quality for different crop categories and irrigation methods to guarantee the safety use of reclaimed water. Conventional indicator organisms, such as *Escherichia coli*, total and fecal coliforms, are mostly used worldwide (Lyu et al., 2016). However, their presence is not always correlated with the presence of certain pathogens (Saxena et al., 2015) and their reliability has been questioned. In addition, to provide insights into the comprehension on efficiency of natural wastewater (WW) treatment systems, alternative indicators, including enterococci, have been suggested by many researchers (Karpiscak et al., 2001; Stott et al., 2003). Currently, European Commission (2018) proposes to assess the performance target of the treatment chain (in $\log_{10}$ reduction) for some selected indicator microorganisms: F+ specific coliphages, somatic coliphages or coliphages as indicator for pathogenic viruses; *Clostridium perfringens* spores or spore-forming sulfate-reducing bacteria for protozoa, and confirms *E. coli* as indicator for pathogenic bacteria. In order to reduce the microbiological risks associated with the use of treated wastewater in food crop irrigation, additional treatments to WWTP have to be considered (Toscano et al., 2013; Licciardello et al., 2018). Among complementary treatment technologies recently proposed, UV disinfection have attracted an increasing interest (Gomes et al., 2007) to be a fast, safe, and cost-effective process against a wide range of pathogens (Guo et al., 2011; Toscano et al., 2013). In addition, artificial lagoons appear an effective complementary solution (Campos et al., 2002; Oragui et al., 2011) and, thanks to the high microbial inactivation rates, represent an affordable and easy way to produce reclaimed water in small communities, mostly where the norm limits are less restrictive (Mara et al., 1992; Peña et al., 2000).

The aims of the present study were to evaluate the horizontal subsurface flow CWs efficiency in terms of water quality improvement, and to evaluate the efficiency of lagooning and UV disinfection systems, as tertiary treatment processes, in the removal of *E. coli*, total coliform, *Enterococcus* spp., somatic coliphages and *C. perfringens* spores. Moreover, in order to evaluate the seasonal efficiency of the whole system, both physico-chemical and microbiological indicators were monitored for one year period.

2. Materials and methods

2.1. Experimental plant

The present study was carried out in three horizontal sub-surface flow CWs, namely CW1, CW2 and CW3, which receive the secondary effluents of the urban WWTP from San Michele di Ganzaria (37°17′0″ N and 14°26′0″ E). San Michele di Ganzaria is a small community (about 3200 inhabitants, in 2016, as reported by the Italian National Institute of Statistics: www.istat.it) of Eastern Sicily, located in Csa Hot-summer Mediterranean climate, according to Köppen classification (also known as a Mediterranean climate), with a mean annual temperature of 18 °C and mean annual rainfall of 500 mm. Basically two seasons can be distinguished: from April to September (summer, dry and hot, with mean temperatures of 20 °C and mean rainfall of 20 mm/month), and from October to March (winter, wet and cool, with mean temperatures of 11 °C and mean rainfall of 91 mm/month).

CW1, CW2 and CW3 are part of the largest natural WWTP of South Italy that includes four CWs operating in parallel, followed by three wastewater storage reservoirs, realized for tertiary treatment of municipal wastewater aimed at agricultural reuse (Aiello et al., 2016; Castorina et al., 2016). CW1 has been operating for twelve years (since 2006). It has a surface area of about 2000 m^2, is filled with 10–15 mm volcanic gravel, treats a wastewater flow of about 2 L/s and is planted with *Phragmites australis* (about 350 stems per m^2). CW2 has been operating since summer 2012 and presents the same design characteristics as CW1 (area, porous medium, flow rate, vegetation type and density). CW3, also in operation since summer 2012, is the smallest CW (surface area of about 1200 m^2) and is planted with *Typha latifolia*, at a density of four rhizomes per m^2. Its stem density is about 170 per m^2. The main design and operation characteristics of wetland beds are reported in Table 1.

An UV unit and a lagooning system, running in parallel, were designed and connected in series to the CW2 bed to treat part of CW effluent. The type of UV device was selected considered: the flow rate (1–1.5 L/s) to be treat, the wastewater transmittance (60–70%), and the required *E. coli* log reduction (5 Ulog) to achieve the Italian standards for wastewater reuse. UV radiation was applied using LBX 10 (WEDECO), a tubular model with low pressure lamps (80 W, λ = 254 nm) and a reactor volume of 13 l. An automatic wiping system kept the sleeves clean, minimizing manual cleaning effort. For a maximum flow rate of 6 m^3/h the installation provides a minimum dose of 700 J/m^2 to the water with 70% transmittance. However, UV dose varied according to the flow rate (hence, the detention time in the reactor) and the average light intensity (hence, the transmittance of the wastewater) (Amin et al., 2010). The UV unit tested was equipped with a sensor which continuously monitors the UV intensity and with a LC display where was possible to read the UV intensity. If the minimum UV intensity (set to 48 W/m^2) is reached, a visual alarm appears on the display. During the research activity, no alarm message was registered. This means that the minimum dose of 700 J/m^2 declared by the UV producer was always maintained. The flow rate to the UV unit was not continuous and set at 1–1,5 L/s.

The lagooning, a maturation pond, waterproofed by a plastic liner, was designed for pathogen removal. Its storage volume is about 60 m^3 and the maximum depth is 1.0 m. The mean influent flow rate treated by the maturation pond was about 3 m^3/day and the hydraulic retention time (HRT) was about 20 days.

2.2. Sampling points

Influent and treated wastewater samples were collected every 2 or 3 weeks for over one year at: (1) CW influent (i.e., following WWTP); (2) CW1 effluent; (3) CW2 effluent; (4) CW3 effluent; (5) after the lagooning; and (6) after the UV treatment (Fig. 1). All samples were collected in sterile bottles and transported, in refrigerated conditions, to the laboratories of the Department of Agricultural, Food and Environment, University of Catania, for further analyses. The quality of the effluents was compared to the Italian legal limits (Ministerial Decree No. 185/2003) and with European proposal regulation (European Commission, 2018) for wastewater reuse in irrigation (Table 2).

Table 1
Constructed wetland characteristics.

Constructed wetlands	Operation time (year)	Flow rate (m^3/day)	Width (m)	Length (m)	HRT (day)	Area (m^2)	Gravel				Macrophytes planted
							Type	Size (mm)	Nominal porosity	Depth (m)	
CW1	12	240	28.5	70	2,3	2000	Volcanic	8–15	0.47	0.6	*Phragmites australis*
CW2	6	240	28.5	70	2.3	2000					
CW3	6	150	20	60	2.3	1200					*Typha latifolia*

2.3. Microbiological analyses

Influent, after decanting, and effluent samples were subjected to microbiological analyses by membrane filtration method, according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 2006). Briefly, samples were opportunely diluted in a sterile saline solution and 100 mL of each dilution were filtered through a 0.45 μm pore-size sterilized membrane filters (Microfil V, Merk Millipore, Italy). The enumeration of conventional fecal indicator bacteria (i.e., *Escherichia coli*, total coliforms and enterococci) was performed according to the ISO procedures (ISO, 9308-1:2001). The results were expressed as $\log_{10}$ colony forming units (CFU) per unit of volume.

Somatic coliphages were quantified according to the ISO 10705-2:2000 method, incubating samples with appropriate host strain. The results were expressed as $\log_{10}$ of Plaque-Forming Units (PFU) per unit of volume. Spores of *C. perfringens* were quantified according to the ISO 7937:2004 procedure. The analyses were performed in triplicate and the results expressed as $\log_{10}$ colony forming units (CFU) per unit of volume.

2.4. Physico-chemical analyses

Influent and treated wastewater samples were analyzed for physico-chemical parameters including: Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD), Total Nitrogen (TN), Ammonia (NH_4), and Total Phosphorus (TP). Analyses were performed following standard methods (APHA, AWWA, AEF, 2005). COD of CW effluent were evaluated on samples filtered by GF/C Whatmann fiberglass.

2.5. Data analyses

The statistical significance between data of water quality before and after each treatment unit was evaluated by *t*-paired test (Microsoft Office Excel 2010). At UV influent and effluent only the microbiological parameters were compared. The *t*-test was also performed between seasons at the effluent of each treatment unit. Results were taken to be significant at the 5% level ($P = 0.05$). In addition, the XLSTAT statistical software was used to identify clusters at each sampling point and sampling period, in order to visualize correlations between microbial indicators and physico-chemical parameters.

3. Results

3.1. Microbial removal

The microbial removal in CWs (I stage), lagooning system and UV treatment (II stage) was evaluated by monitoring *E. coli*, total coliforms, *Enterococcus* spp., somatic coliphages and *C. perfringens* spores. Results of microbiological mean values are shown in Fig. 2, whereas the reduction throughout the whole monitoring period, in summer and in winter seasons, at the first, and at the second stage, are reported in Table 2. Results indicated that in CW influent the mean values of *E. coli*, total coliforms and enterococci were 5.0, 5.4 and 4.6 log units in 100 mL, respectively, and the mean initial concentration of somatic coliphages and *C. perfringens* spores were 2.9 PFU/mL and 2.3 CFU/mL, respectively (Fig. 2). Considering the first stage of the system and the whole monitoring period (Table 2), the mean reduction for all microbiological indicators were in the range of 0.9 (±1.2)–1.7 (±0.5) Ulog.

In details, for *E. coli*, total coliforms and somatic coliphages, a similar mean reduction (1.2 Ulog, 1.3 Ulog, 1.1 Ulog, respectively) was detected along the continuum, while for enterococci and *C. perfringens* spores a slightly higher average reduction (1.9 Ulog and 1.4 Ulog, respectively) was recorded (Table 2). The P-values between samples collected at the inlet and at the outlet of CWs were always lower than 0.05, revealing a significant reduction of the considered microbiological indicators. Although the CWs showed a high efficiency in microbial reduction, *E. coli* densities, in all CWs effluents (Fig. 2), were always higher than limit for wastewater reuse in agriculture, fixed by Italian legislation (80 percentile equal to 10 CFU/100 mL with a maximum admitted value equal to 100 CFU/100 mL) and higher than EU water quality required for class A (10 CFU/100 mL), class B (100 CFU/100 mL) or class C (1000 CFU/100 mL). No statistical differences between the CW units were observed.

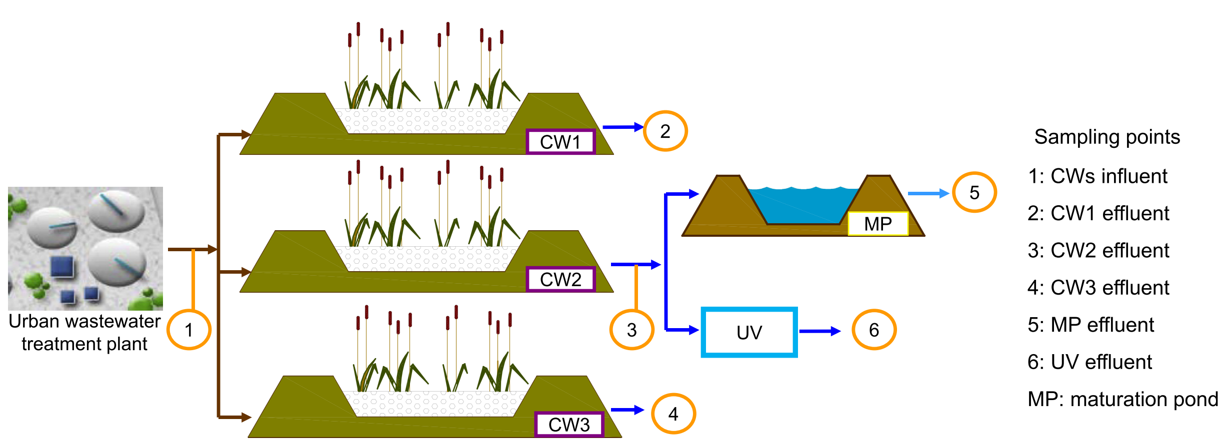

Fig. 1. Layout of experimental plant.

Table 2
Italian standards and European reclaimed water quality for water reuse in agriculture.

Parameters	Italian limits	European guideline	
		Quality requirements	Water quality class
BOD_5 (mg/L)	20	10 25 (according to Council Directive 91/271/EEC)	A[a,e] B[b], C[c] and D[d]
COD (mg/L)	100	–	
TSS (mg/L)	10	10 35 (according to Council Directive 91/271/EEC)	A[a,e] B[b], C[c] and D[d]
NH_4 (mg/L)	2	–	
TN (mg/L)	35	–	
TP (mg/L)	10	–	
Escherichia coli (CFU/100 mL)	10 (80% of samples) 100 (max value)	≤10 or below detection limit ≤100 ≤1000 ≤10,000	A[a,e] B[b] C[c] D[d]

[a] Crop category irrigable with water quality of Class A: All food crops, including root crops consumed raw and food crops where the edible part is in direct contact with reclaimed water. All irrigation methods allowed.
[b] Crop category irrigable with water quality of Class B: Food crops consumed raw where the edible part is produced above ground and is not in direct contact with reclaimed water, processed food crops and non-food crops including crops to feed milk- or meat-producing animals. All irrigation methods allowed.
[c] Crop category irrigable with water quality of Class C: the same for the class B but only drip irrigation is admitted.
[d] Crop category irrigable with water quality of Class D: Industrial, energy, and seeded crops. All irrigation methods allowed.
[e] Performance targets for the treatment chain (log10 reduction) only required in class A: *E. coli* ≥ 5.0; Total coliphages/F-specific coliphages/somatic coliphages/coliphages ≥ 6.0; *Clostridium perfringens* spores/spore-forming sulfate-reducing bacteria ≥ 6.

Focusing on the second stage of the system, consisting of lagooning or UV treatments, the microbial mean reduction was considerably higher than those detected in CWs (Table 3). In details, after lagooning system, the highest mean reduction for the whole year (1.9 Ulog) was obtained for *E. coli*, the lowest for *C. perfringens* spores (0.2 Ulog). Overall data obtained after UV treatment indicated the highest mean reduction, extending to value of 3 Ulog for both *E. coli* and total coliform (Table 3).

3.2. Physico-chemical parameters removal efficiency

The mean values of physico-chemical parameters recorded at the different sampling points for the whole period and for the two seasons are shown in Fig. 3, whereas the mean removal efficiency is reported in Table 4. Since the mean EC value measured in the CW influent, about 1171 (μS/cm), was already suitable for crop irrigation, it was not further considered. As for microbiological parameters, no statistical differences between the CW units were observed for physico-chemical parameters, even if CWs were planted with different plant species (*Phragmites australis* and *Typha latifolia*).

In detail, TSS values decreased from a mean value of 50 mg/L to 10 mg/L, throughout all of the three CWs. The mean TSS removal efficiency was high and quite constant during the monitoring period (77 ± 10% for CW1, 80 ± 9% for CW2 and 81 ± 11% for CW3, respectively, Table 4), with standard deviation less of 11. Statistical analyses indicate significant differences in TSS values between influent and effluent of all CWs ($P < 0.05$), while no significant differences were revealed considering the seasons (Fig. 3).

The treated wastewater was characterized by a BOD_5/COD ratio > 0.6, indicating the presence of biodegradable compounds. Therefore, the mean percentage of organic compound removal was similar for both parameters (about 60%) in all the CW beds (Table 4), with a BOD_5 effluent values ranging from 2 mg/L to 23 mg/L and COD values ranging from 5 to 30 mg/L. The *t*-paired test indicated that both BOD_5 and COD values were significantly lower in the CW effluent compared to CW influent, and that no differences in the mean reduction between the two seasons were detected (Fig. 3).

Mean value of TN decreased from 21.6 mg/L to 10.2 mg/L. Although a lower TN removal was observed, compared to both organic matter and TSS (Table 4), a quite similar removal efficiency was observed for the three beds (48 ± 19% for CW1, and 44 ± 25% for CW2 and CW3). Although, an improvement in both TN and NH_4 removal during summer season was expected, any statistical differences between seasons were observed (Fig. 3). Despite the TN values in all the CW effluents met the Italian standard required for irrigation (35 mg/L), the 5% (CW2) and the 10% (CW1 and CW3) of tested the samples exceeded the European discharge limit (10 mg/L).

Regarding the TP values, they were low throughout the monitoring period, ranging from 3.8 to 9.7 mg/L, below the Italian law

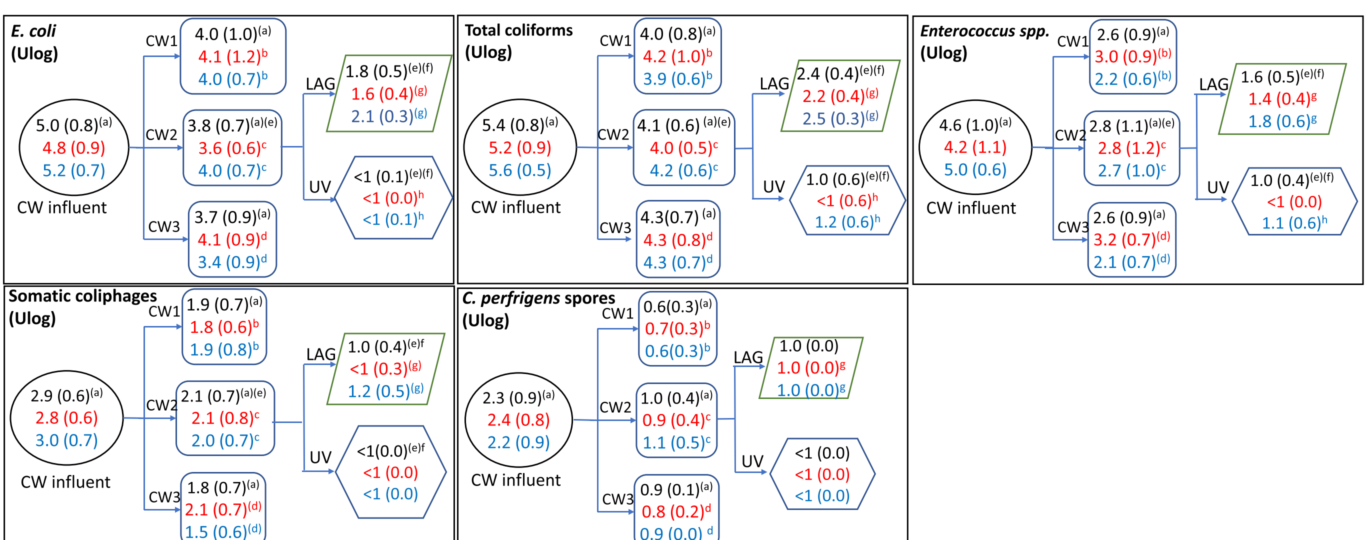

Fig. 2. Mean concentration (Ulog) of microbiological indicators at different sampling points in the whole year (black), in summer (red) and in winter (blue). *E. coli*, Total coliforms, and *Enterococcus* spp. densities are expressed as log CFU/100 mL; Somatic coliphages as PFU/mL and *C. perfringens* spores as log CFU/mL. *t*-Test was performed to compare mean concentration between: (i) inlet and outlet of each unit for the whole year; (ii) summer and winter at the effluent of each treatment unit; (iii) UV and lagooning effluents of the whole year. Mean values followed by the same letter enclosed in parenthesis are different ($P < 0.05$). Standard deviation in parenthesis. (LAG: lagooning system).

Table 3
Mean reduction of microbiological indicators (expressed in Ulog) in the whole year, in summer and in winter. Standard deviations in parentheses.

Parameters	Period	I stage			II stage (after CW2)	
		CW1	CW2	CW3	Lagooning	UV
E. coli	Whole year	1.1 (0.9)	1.3 (0.9)	1.3 (0.9)	1.9 (0.4)	3.0 (0.7)
	Summer	0.9 (0.8)	1.5 (1.0)	0.9 (0.8)	2.0 (0.3)	2.5 (0.9)
	Winter	1.3 (0.9)	1.2 (0.7)	1.7 (0.7)	1.9 (0.5)	2.8 (1.1)
Total coliforms	Whole year	1.4 (0.7)	1.4 (0.7)	1.1 (0.8)	1.8 (0.6)	3.1 (0.9)
	Summer	1.0 (0.7)	1.3 (1.0)	0.9 (0.8)	1.8 (0.6)	2.9 (1.2)
	Winter	1.7 (0.5)	1.4 (0.5)	1.3 (0.7)	1.7 (0.6)	3.0 (0.9)
Enterococcus spp.	Whole year	2.0 (1.2)	1.8 (1.3)	2.0 (1.3)	1.0 (1.3)	1.8 (1.0)
	Summer	1.0 (0.7)	1.2 (1.3)	0.9 (0.6)	0.9 (1.3)	1.9 (1.2)
	Winter	2.8 (0.9)	2.3 (1.1)	3.0 (0.9)	1.1 (1.4)	1.7 (0.8)
Somatic coliphages	Whole year	1.1 (0.9)	0.9 (1.2)	1.2 (0.9)	1.0 (0.9)	1.2 (0.7)
	Summer	1.0 (0.8)	0.7 (1.2)	0.8 (0.9)	1.1 (0.9)	1.2 (0.8)
	Winter	1.2 (1.0)	1.1 (1.2)	1.5 (0.9)	1.0 (0.8)	1.1 (0.7)
C. perfrigens spores	Whole year	1.7 (0.8)	1.3 (1.0)	1.4 (0.8)	0.2 (0.3)	0.1 (0.4)
	Summer	1.7 (0.9)	1.5 (1.0)	1.6 (0.8)	0.1 (0.2)	0.2 (0.2)
	Winter	1.6 (0.7)	1.1 (1.0)	1.3 (0.9)	0.3 (0.3)	0.2 (0.5)

standards for reuse of wastewater in agriculture (fixed as 10 mg/L). The TP concentration was significantly modified through treatment in the CW beds, from a mean value of 4.4 mg/L in CW1 and CW2 effluents, to a mean value of 5.0 mg/L in CW3 effluent (Fig. 3).

Different results were detected in the lagooning unit. Even if the HRT of the maturation pond was very high (over the recommended maximum value of 10 days, according to Mara, 2003), the efficiency in reducing the most physico-chemical parameters was quite low. The means values of TSS, BOD_5 and COD in lagooning system effluent were found significantly higher than those detected in the influent (CW2 effluent). This is also confirmed by the TSS, BOD_5 and COD values in the pond effluent, detected as significantly lower during the winter than in the summer season (Table 4), when algae bloom on the water surface was observed. Lagooning unit was also unable to further lower TN, NH_4, and TP levels, which indeed showed relative increases during the monitoring period.

3.3. Correlation between physico-chemical and microbiological parameters

In order to visualize correlations between microbial indicators and physico-chemical parameters, XLSTAT statistical software was used. Results are shown in Fig. 4. Overall, statistical data revealed a positive correlation among all considered microbial indicators and between microbial indicators and the TN and NH_4 values (Fig. 4). In particular, enterococci, together with *C. perfringens* spores, were positively correlated with all considered physico-chemical parameters, whereas a variable correlation was observed considering the other physico-chemical parameters. In addition, a negative correlation was observed between *E. coli* and TP/TSS/BOD_5/COD, while for both total coliforms and somatic coliphages negative correlation was observed towards TSS, BOD_5 and COD (Fig. 4).

4. Discussion

In this study, the removal efficiency of H-SSF CWs, combined with lagooning systems or UV treatment, in twelve consecutive months was evaluated. The performance of the whole system was assessed by comparing the main parameters of the influent with those of the effluent, at each treatment unit, considering both the Italian regulation for wastewater reuse in irrigation (Ministerial Decree No. 185/2003) and the recent European proposal regulation (European Commission, 2018).

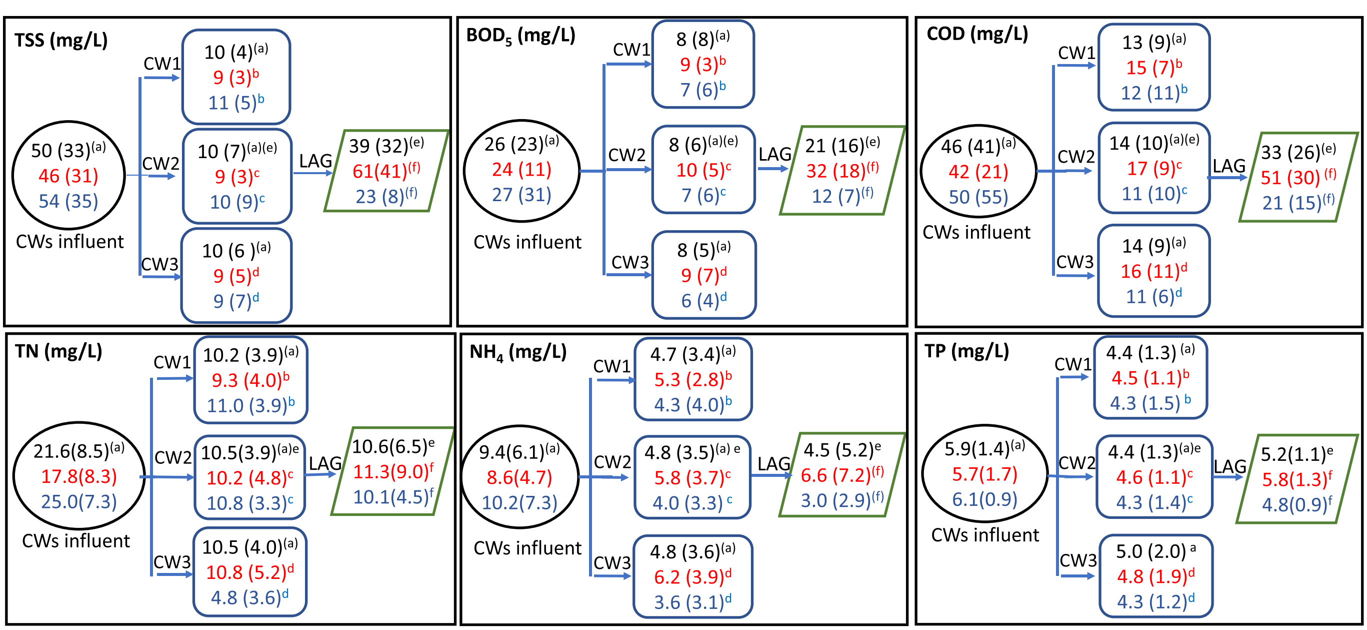

Fig. 3. Mean concentration (mg/L) of the physico-chemical parameters at the different sampling points in the whole year (black), in summer (red) and winter (blue). *t*-Test was performed to compare mean concentration between: (i) inlet and outlet of each unit for the whole year; (ii) summer and winter at the effluent of each treatment unit. Mean values followed by the same letter enclosed in parenthesis are different ($P < 0.05$). Standard deviation in parenthesis. (LAG: lagooning system).

Table 4
Mean removal efficiencies of physico-chemical parameters (expressed as percentage) for the whole year, summer and winter. Standard deviations in parentheses.

Parameters	Period	I stage			II stage (after CW2)
		CW1	CW2	CW3	Lagooning
TSS	Whole year	77 (10)	80 (9)	81 (11)	−46 (120)
	Summer	76 (11)	78 (9)	77 (11)	−525 (168)
	Winter	78 (9)	82 (9)	83 (10)	−42 (102)
BOD_5	Whole year	62 (22)	63 (18)	61 (25)	−22 (130)
	Summer	63 (13)	57 (19)	61 (27)	−276 (92)
	Winter	61 (28)	68 (16)	60 (25)	−18 (91)
COD	Whole year	63 (23)	66 (16)	59 (27)	−21 (120)
	Summer	63 (14)	60 (18)	60 (26)	−255 (110)
	Winter	62 (30)	71 (14)	59 (30)	−19 (132)
TN	Whole year	48 (19)	44 (25)	44 (24)	−2 (43)
	Summer	37 (24)	33 (29)	31 (26)	−2 (52)
	Winter	53 (18)	54 (16)	56 (16)	−1 (47)
NH_4	Whole year	42 (25)	40 (27)	39 (30)	−9 (74)
	Summer	32 (19)	25 (27)	23 (23)	−12 (62)
	Winter	51 (28)	49 (53)	53 (30)	−22 (51)
TP	Whole year	25 (20)	24 (19)	20 (18)	−17 (21)
	Summer	21 (12)	18 (13)	10 (12)	−17 (27)
	Winter	28 (25)	29 (23)	29 (19)	−19 (30)

Regarding physico-chemical parameters, the mean TSS removal efficiency was high and quite constant during the monitoring period, in accordance to previous studies (Toscano et al., 2015; Vymazal, 2002). No difference in TSS removal related to season was observed, as reported by other authors (Llorens et al., 2009; Ouellet-Plamondon et al., 2006; Steer et al., 2002). TSS removal in wetlands is mainly due to physical processes, such as sedimentation and filtration, which are not temperature dependent process (Kadlec and Wallace, 2009). TSS were always detected at levels below the threshold value (35 mg/L) propose by the European for reclaimed water quality in class B, C and D and in 95% of samples also below the Italian law limit for wastewater reuse in agriculture (10 mg/L), the same required for EU water quality in class A.

The mean percentage of organic components removal was quite the same for COD and BOD_5 parameters since municipal wastewater usually contained elevated concentration of easily degradable organic compounds (Vymazal and Kröpfelová, 2009). No differences were revealed in the mean reduction of BOD_5 and COD values between the two seasons highlighting that temperature dependence is not significant for both parameters, as reported by several authors (Akratos and Tsihrintzis, 2007; Kadlec and Knight, 1996; Kadlec and Reddy, 2001; Steinmann et al., 2003). In addition, this result could be referred to the buffer effect of both substrate and plant root systems against climatic fluctuations (Steer et al., 2002; Walaszek et al., 2018). BOD_5 and COD daily concentrations in the CWs effluents were always below the European standard for water quality in class B, C and D (25 mg/L for BOD_5) and below the Italian regulation for reuse of wastewater in irrigation (20 mg/L for BOD_5 and 100 mg/L for COD).

The observed lower mean TN removal, compared to those found for organic matter and TSS, has already been reported in HF CW systems (Aiello et al., 2016; Akratos et al., 2007) and a TN removal quite similar for all the beds has been already observed (Katsenovich et al., 2009; O'Luanaigh et al., 2010; Vymazal, 2009). Although the temperature dependence is well documented for TN and NH_4 removal (Akratos and Tsihrintzis, 2007; García et al., 2010; Garfí et al., 2012) no improvement in both TN and NH_4 removal efficiency was detected. This result could be explained by the warm climate of Mediterranean area allows year-around plant growth and a constant microbial activity (Kadlec and Wallace, 2009; Khisa and Mwakio, 2011). The microbial species involved into TN reduction sowed an optimal activity at temperature above 15 °C (Saeed and Sun, 2012; Andreo-Martínez et al., 2017). The mean air temperature during winter was 11 °C. Since the CW substrate allows maintaining the wastewater temperature higher than that of the air at least by 2–3 °C (Steer et al., 2002), it is possible to asses that the microbial activity was not affected by temperature in winter. Furthermore, this result could be also related by the higher mean rainfall during winter season, that could have a dilution effect in the CWs.

Mean value of TN at CW effluent met the Italian law requirements for wastewater irrigation (35 mg/L). No seasonal difference was observed for TP since its removal is mainly due to physical and chemical mechanisms, such as sorption and precipitation, which are not temperature dependent processes (Spieles and Mitsch, 2000; Ding et al., 2014; Thongtha et al., 2014).

Regarding the lagooning unit, different results were observed. Even if the HRT of the maturation pond was high, the average effluent values

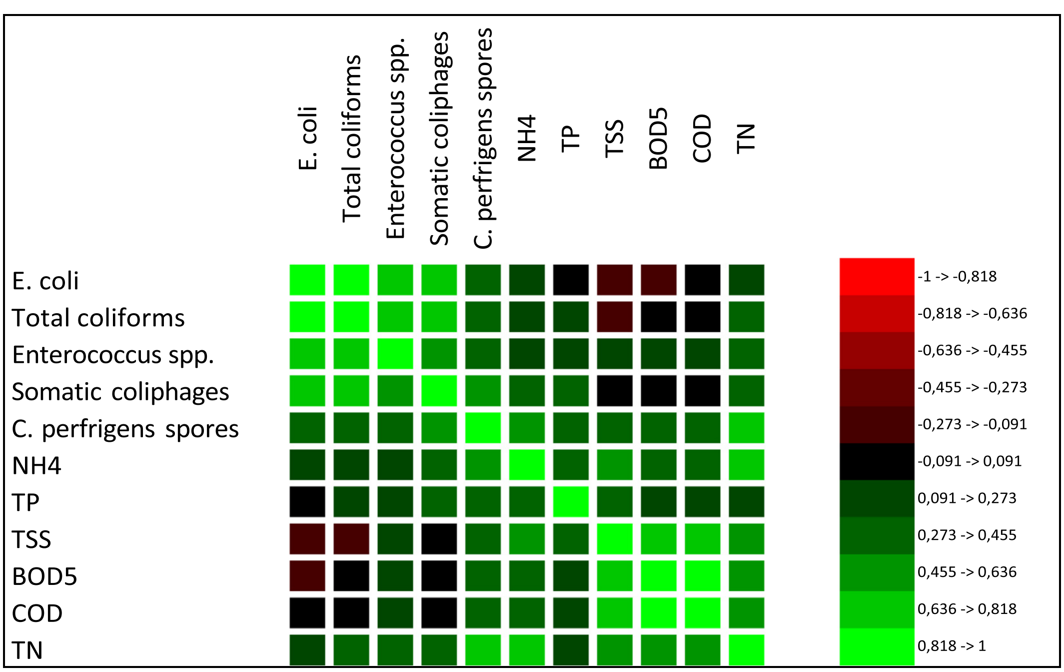

Fig. 4. Significant correlations between microbial indicators (log CFU/mL) and physico chemical parameters. The colors of the scale bar denote the nature of the correlation, with 1 indicating a perfectly positive correlation (green) and −1 indicating a perfectly negative correlation (red). Only significant correlations (False Discovery Rate < 0.05) are shown.

of TSS, BOD_5 and COD were significantly higher than the influent, highlighting a negative removal efficiency results. Similar results have been previously observed by other authors (Barbagallo et al., 2011; Dias et al., 2014; Von Sperling and Andrada, 2006), which attributed the increase of TSS and organic compounds values to algae growth and decomposition in the pond, especially during the summer period. Furthermore, the higher mean rainfall during winter season could also contribute to reduce the pollutants concertation in the pond effluent. This is confirmed by the TSS, BOD_5 and COD values found as significantly lower in the pond effluent during the winter than in the summer season.

Lagooning unit was also unable to further lower total TN, NH_4, and TP levels and this can be attributed to the low influent values of TN, NH_4, and TP (close to the background concentrations) and also to the anaerobic decomposition of algal substances.

The microbiological results showed that the densities of conventional fecal indicators (*E. coli*, total coliforms and enterococci) in the influents were consistent across the sampling period, and that the WWTP was able to achieve a mean of 1.5-log unit removal along the whole system, revealing a statistical significant improvement of water quality. These removal efficiencies were comparable to those previously reported by other authors, in different geographical locations, mainly in US, Canada, France and Belgium and Spain (Andreo-Martínez et al., 2017; George and Crop, 2002; Harwood et al., 2005; Saleem et al., 2000; 2003; Wery et al., 2008; Zhang and Farahbakhsh, 2007). In CWs the highest mean removal efficiency was observed for *C. perfringens* spores and for *Enterococcus* spp., while the lowest for *E. coli* and total coliforms, in agreement with previous reports (Wu et al., 2016). In contrast with many observations, which report a better efficacy in summer season, in the present work, the CW removal efficiency was not influenced by season. The lower efficiencies observed in summer time is in agreement with observations reported by García et al. (2008) and Tunçsiper et al. (2012) that revealed an higher incidence of both *E. coli* and total coliforms in the summer season, related to animal activity and seasonal variation in plant growth (Thurston et al., 2001). In the present work the lower removal efficiency in summer season could be related to proliferation of microorganisms in CWs, that, as already reported, is highly dependent on many factors such as temperature, water composition and solar intensity (Dixon et al., 2000). Combining CW with UV treatment a considerable improvement in microbiological water quality was obtained, with a complete removal of *E. coli*, somatic coliphages, and *C. perfringens* spores and a drastic reduction of other microbial indicators. Opposite trend was registered for enterococci, which were significantly removed by the CWs, but persist after both the lagooning system and the UV treatment, especially in winter season. This is probably due to the known genome reparation mechanisms of enterococci and to their cellular structure (with higher peptidoglycan content, presence of teichoic acids and polysaccharides) (Batch et al., 2004; Gao and Williams, 2013; Gravetz and Linden, 2005; Oguma et al., 2001). Effectiveness of UV disinfection against different organisms in wastewater systems are reported as variable, and reductions in levels of enterococci has been reported ranging from 2 to 5 orders of magnitude, depending on the treatment processes applied prior to UV exposure and on the type and intensity of the UV source (Hijnen et al., 2006; Koivunen and Heinonen-Tanski, 2005). Overall, the average concentration of *E. coli* in the UV effluent satisfied the Italian reuse standards. Furthermore, the *E. coli* reduction, from raw wastewater effluent entering the same urban wastewater treatment plant to UV effluent, reported as about 7 log CFU/100 mL by Cirelli et al. (2007), was higher than 5 log units, in compliance with the performance targets for the treatment chain reported by the last proposal law (European Commission, 2018). However, in the present study, total coliforms and enterococci, were never completely removed and remain a critical factor with linkage to human health.

From a microbiological point of view the persistence of enterococci, above all after lagooning and UV treatment remains a public concern, being WWTPs recognized as a hot spot for antibiotic resistance environmental dissemination (Michael et al., 2013; Novo et al., 2013; Oravcova et al., 2017; Patra et al., 2012). The results presented here suggest that for combined treatments it will be more appropriate to use at least two microbial indicators in order to validate the performance of the whole treatment, as already proposed by others (Byappanahalli et al., 2012; Lucena et al., 2004).

Although lagooning system produced a low mean reduction of physico-chemical parameters, a high removal efficiency, especially in summer season, against somatic coliphages, *E. coli*, total coliforms, enterococci, was observed, whereas no removal effects was observed against *C. perfringens* spores, considered as conservative surrogates for protozoa such as *Cryptosporidium parvum* and *Giardia lamblia* (oo) cysts. These results are in accordance to previous reports, which highlighted a higher resistance of spores to environmental stress and a longer persistence (several weeks) in water, depending on the temperature, physico-chemical parameters and sunlight (Ahmed et al., 2013; Araki et al., 2001; Fayer et al., 1998; Karim et al., 2004). For this reason the simultaneous use of *E. coli* and *C. perfringens* spores as indicators of recent and remote fecal contamination, respectively, has been proposed (Byamukama et al., 2005; Mayer et al., 2016). However, the effluents satisfy the WHO guidelines for unrestricted irrigation (WHO, 2006), presenting an *E. coli* mean value lower than 1000 CFU/100 mL, but fails to meet the strict reuse standard of Italian legislation (50 CFU/100 mL required for 80% of samples in the case of natural treatments).

Finally, the positive correlation between microbial indicators and the TN value is in agreement with previous reports (Wu et al., 2016), highlighting that these microorganisms might survive longer or replicate faster in presence of available nitrogen. In addition, the positive correlation between somatic coliphages and TP, could be also related to the presence of both organic and inorganic matter which represent the most important factors influencing survival of coliphages (EPA, 2012). The positive correlation between *C. perfringens* spores densities and all the considered physico-chemical parameters is in agreement with results reported by Tunçsiper et al. (2012), and could also be correlated to higher nutrient concentration. The negative correlation between *E. coli*/total coliforms/somatic coliphages and TSS/BOD_5/COD, suggests that chemical and physical parameters, such as pH, nutrient concentration, dissolved oxygen, turbidity, and conductivity, must all be within a certain range to allow bacteria survival (Wickham et al., 2006).

5. Conclusions

In conclusion, results of the present work highlight that the H-SSF CW can effectively represent a feasible solution for secondary treatment of urban wastewater, producing effluent, which, except for microbiological parameters, complies with Italian standards on wastewater reuse in agriculture. The lack of significant correlation between pollutants removal efficiency and temperature underlay that the wetland performance can be considered reasonably constant during the year-around in Mediterranean climate. Furthermore, the different plant species (*Phragmites australis* and *Typha latifolia*) seem not influence the CW performance since no statistical different was detected among the CWs.

Although the lagooning system produced a good microbiological removal efficiency, its performance was inefficient for the main physico-chemical parameters. The combination of CWs with UV treatment produced highly effective results, meeting both the strict Italian legislation and the new European proposal for agricultural reuse of reclaimed water. Although neither Italian nor European legislation provides a limit for enterococci, the risk associated with their environmental dispersal is difficult to estimate and further improvement removal efficiencies are required to obtain a better water quality with minimal detrimental health and environment impacts.

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