On the performance of a pilot hybrid constructed wetland for stormwater recovery in Mediterranean climate

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ABSTRACT

The overall efficiency of a pilot-scale hybrid constructed wetland (H-CW), located on a retail store's parking area in Eastern Sicily, for alternative treatment of stormwater runoff and of sequential batch reactor (SBR) effluent was evaluated. Experimental activities were focused on system performances, including wastewater (WW) quality and hydraulic monitoring. System design, macrophyte growth and seasonal factors influenced the pilot plant performance. Very high removal efficiency for microbial indicators were reported within the subsurface horizontal flow unit (HF), playing a strategic role for *Clostridium perfringens*. The algal growth occurred in the free water surface (FWS) unit and inhibited removal efficiencies of total suspended solids (TSS), biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD), impairing water quality. The whole H-CW showed good efficiency in trace metals removal, especially for Pb, Zn, and Cu. Preliminary results suggested the reliability of the H-CW technology in decentralised water treatment facilities for enhancing water recovery and reuse.

Key words | alternate feeding, decentralised treatment, hybrid constructed wetland, hydraulic conductivity, stormwater

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INTRODUCTION

Constructed wetlands (CWs) are green treatment technologies simulating natural wetlands. They have been traditionally used to treat conventional wastewater (WW), but during last two decades their application also included industrial and agricultural wastewaters, landfill leachate, as well as stormwater runoff (Vymazal 2011). In particular, in recent years, CWs have become more common for urban stormwater treatment in order to remove contaminants that would be potentially detrimental to the receiving water ecosystem. In many countries (USA, Australia, Malaysia, etc.) the CWs were suggested among the best management practices (BMPs) for stormwater treatment. The European Union (EU) has clearly implemented water protection in its environmental policy through various relevant directives (2000/60/EC, 2008/105/EC), but concerning the stormwater regulation, only a quantitative

approach was adopted (EU Floods Directive 2007/60/EC), while the quality issue is still lacking. Italian normative (LD 152/2006, art. 74, 113) has a general content, which does not effectively deal with stormwater runoff treatment and management, relying mainly on regional 'case-by-case' evaluations. Sicily, in particular, is among those regions without a consistent regulation. Moreover, in semiarid regions, the unconventional water source reuse would represent a key factor for local-scale integrated water management plans, in the light of the recent EU proposal for water reuse (European Commission 2018), whose adoption at national levels could eventually conform the strict and uneven normative barriers in the Mediterranean area (Barbagallo *et al.* 2012; Aiello *et al.* 2013; Salgot *et al.* 2016; Ventura *et al.* 2019). A large number of authors have described the CWs performances for the urban runoff treatment (Adyel *et al.* 2016) and removal efficiencies of major pollutants (total suspended solids (TSS), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD)) are generally high (58–99% for TSS mainly in the subsurface flow systems, and 31–98% for COD). The removals of TSS

and organic matter are strongly correlated because most of the COD and BOD$_5$ is in particulate form while only a few portions are in soluble form. The average efficiency of nutrients widely varies from 1% to 90% for total nitrogen (TN) and from 10% to 90% for total phosphorus (TP) due to the large fluctuations of hydraulic loading rates. In general, when hydraulic rates exceed the system's assimilative capacity, threat of nutrient release may occur. The nutrient's removal rates are not usually influenced by CW configuration and input concentrations, suggesting retention time is a critical factor in nutrient removal efficiencies in any system. Finally, performances on micropollutants, like trace metals, vary according to author and substance. The explanation lies in the different physical forms they can assume. When attached on TSS, trace metals are efficiently eliminated by sedimentation and filtration. When mainly present in dissolved form, most trace metals are not degraded. However, the main CWs removal processes for metals are: binding with the granular medium; precipitation as insoluble salts and uptake by plants and bacteria (García *et al.* 2010), even if temperature, conductivity and pH variations promote metal release in water (Walaszek *et al.* 2017). The knowledge on CWs hydrocarbons removal mechanisms still need to be extended, though the biological and microbiological processes are by now considered the preferential degradation paths. In fact, bacterial consortia and plant-bacteria mutual associations in the plant rhizosphere and endosphere can promote the microbial gene expression of catabolic enzymes linked to hydrocarbons degradation (Hashmat *et al.* 2018). Also, in formed microbial mats, the characteristic communities apt to oil-derivatives metabolization can vary depending on oil and nutrients levels (e.g. ammonia), and also pH, temperature, dissolved oxygen (DO) and sulfate concentration (Abed *et al.* 2014). Also,

bioaugmentation strategy has been proposed for oil-contaminated soils treatment (Tao *et al.* 2019). Overall, physicochemical processes (e.g. photoreactions, chemical precipitation, sedimentation and filtration) alone or combined allow us to foster the biological hydrocarbon removal and nowadays are widely studied as the base of novel technologies, particularly for the degradation of the more persistent polycyclic aromatic hydrocarbons (PAHs) (Li *et al.* 2019; Wang *et al.* 2019). The latter can be biologically degraded, but a lack of data makes conclusions on the CWs efficiency not reliable. Besides the general focus, based on the development of effective decentralised wastewater treatment-water recovery system, the specific aim of this study was to evaluate the removal efficiency of an experimental hybrid CW (H-CW), alternatively treating stormwater of a parking area and the effluent of a sequential batch reactor (SBR) installed in a retail store (toilets, kitchens and bar). This would avoid feeding shortage and system block during warmer seasons and provide system robustness in Mediterranean climate changing conditions (heavy rain episodes and dry weather periods), besides offering a social amenity for the local community. The hydraulic behaviour of the subsurface horizontal CW (HF) unit, after less than two years of operation, allowed us to assess the clogging's effects on the wetland hydraulic properties (i.e. hydraulic conductivity at saturation, K_s). Finally, the possibility of reclaimed water use was evaluated.

METHODS

The pilot-scale H-CW consists of a retention pond, followed by two identical parallel lines, each one including a subsurface HF and a free water surface CW (FWS) unit in series (Figures 1 and 2). It is located within the parking area of
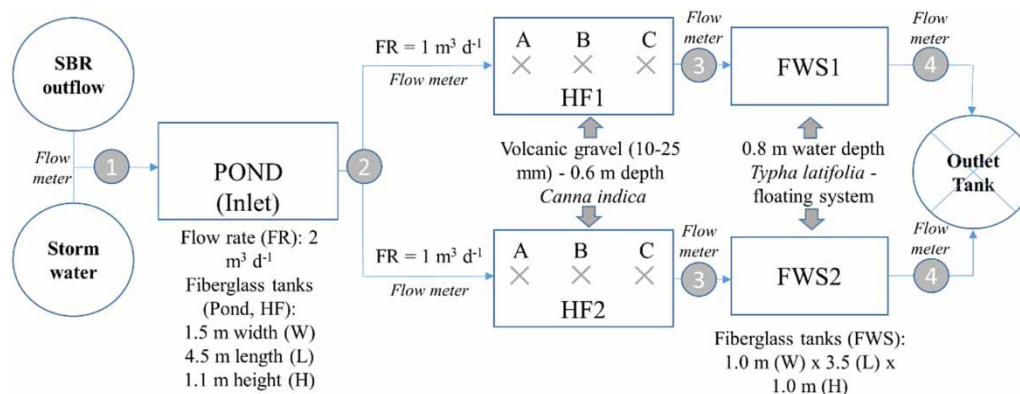
Figure 1 | Pilot-scale hybrid constructed wetland (H-CW) layout and monitoring design (grey circles and crosses, respectively, for water quality and hydraulic surveys). Sequential batch reactor (SBR); retention pond (POND) and free water surface CW (FWS).
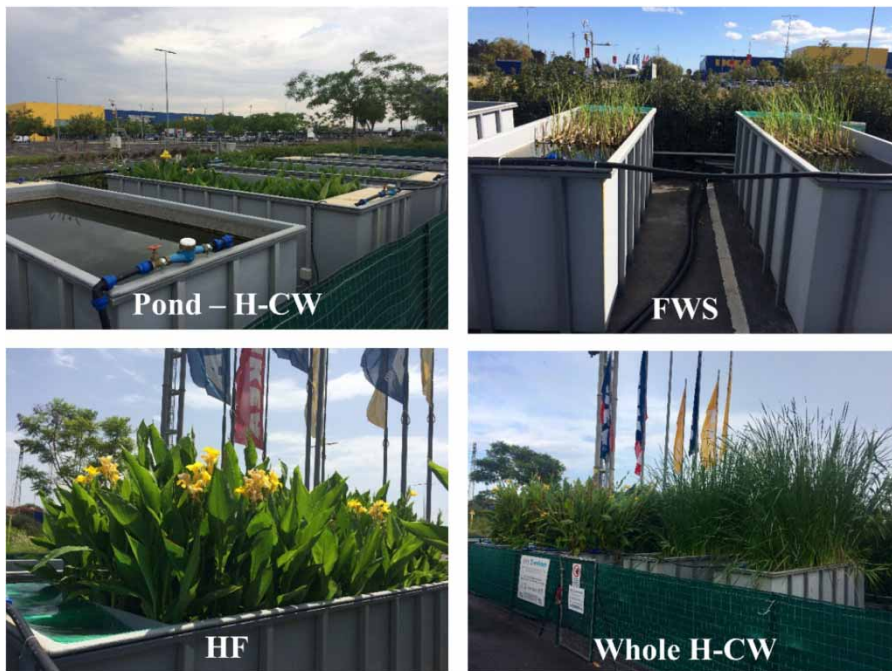
Figure 2 | View of the pilot-scale hybrid constructed wetland (H-CW): single stages (FWS and HF) and overall view (with a detail of the system inlet retention tank, named as 'Pond').

the retail store Ikea®, in the industrial district of Catania, Eastern Sicily (Italy) (37°26′54.2″N 15°02′05.2″E).

The system has been operated since the end of 2016 with a nominal hydraulic retention time (HRT) of 96 h per line. Water quality monitoring was divided into three periods (number of sampling per period, $n = 15$). I period ranged from May to September 2017, with SBR effluent treatment (feeding frequency of four cycles/day), while the II and III periods, respectively, from November 2017 to February 2018 and from March to May 2018, with storm-water runoff for feeding. During stormwater collection, sampling campaigns were carried out, trying to match moments immediately after significant rainfall events (>5 mm). Water quality and removal efficiency (R.E., %) were analysed for TSS, COD, BOD_5, TN, TP (Marzo *et al.* 2018), trace metals (As, Cd, Cr, Fe, Ni, Pb, Cu, Zn) and PAHs as the total sum of benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, indeno(1.2.3-cd)pyrene. Inductively coupled plasma mass spectrometry (Perkin Elmer NexION 350X) and gas chromatography (GC-Perkin Elmer Clarus 500) were performed, respectively, for trace metals and PAHs. Monitoring points are (circled numbers) reported in Figure 1 as: 1 (In Pond), 2 (Out Pond), 3 (Out HF) and 4 (Out FWS). Statistical analysis was performed using the Kruskal-Wallis test (R Core Team 2014) to evaluate the two treatment lines differences. Significance was shaped as $p < 0.05$. On February and June

2018, the first two hydraulic surveys were conducted on HF units (HF1 and HF2) in order to evaluate the hydraulic conductivity in saturated conditions (K_s) of the filtering volcanic gravel beds. The traditional *in situ* falling head test was therefore applied: sampling points are reported in Figure 1 as grey crosses (A, B, C) in the HF units. The falling head permeameter, based on Lefranc's test, has been largely used to measure K_s of CW (Nivala *et al.* 2012), applying different schemes and equations suggested in literature (NAVFAC 1986). Among the different schemes and equations, the standpipe method was chosen (Licciardello *et al.* 2019). Compared to other methods, a standpipe test does not require a borehole to be drilled as the pipe is merely inserted directly into the sediment, which can save much time and money. K_s was calculated by using the following equation (Equation (1)):

$$K_s = \frac{2\pi R + 11L}{11t} \ln\left(\frac{H_1}{H_2}\right) \tag{1}$$

where R is the radius of the tube (m), L is the submerged length of the tube (m), t is time (s) and H_1 and H_2 are the water levels (meters, m) in the permeameter cell corresponding to time t_1 and t_2 (s), respectively. In order to obtain the best fit between modelled H_2 and the measured H_1, the squared difference between the theoretical curve and that obtained in the field was minimized to estimate

the value of K_s by means of an iterative, non linear, procedure that can be done easily using Excel solver (Frontline Systems, Incline Village, NV, USA):

$$\sum_{t=0}^{n} = (H_{obs}(t) - H_{est}(t))^2 \qquad (2)$$

where H_{obs} is the height of the water table level measured inside the tube at time t during the test (m); H_{est} is the corresponding modelled data, by Equation (2).

In order to fill the permeameter with water in a pulse mode, as required, a permeameter unit was assembled. In particular, an impervious steel tube (internal diameter of 0.10 m and length of 1.5 m) for the insertion into the medium was connected to a plastic water reservoir (6.6 L volume) by means of a bulb valve. The permeameter was inserted by using a mallet into the medium to a depth of about 0.32 m, after the creation of a small hole in the granular medium to reach the water table. The decrease of water height inside the tubes was monitored until the water level reached the water table by using a pressure probe (STS–Sensor Technik Sirnach, AG), connected to a laptop by means of a CR200-R (Campbell Scientific) data logger, positioned inside the steel tube. Pressure data were then converted into water heights. For each test, the experimental monitoring time was fixed in 60 seconds and the pressure probe was configured to collect four water height data per second.

RESULTS AND DISCUSSION

Effective data monitoring and collection were the most challenging issues faced when stormwater fed the experimental

system. In fact, the Sicilian region was clearly affected by heavy rainfall deficit (>30–40%) in 2017, with respect to the period 2003–2016 (SIAS), and very few significant events were recorded (Figure 3).

Mean values of main physical parameters for each period are reported in Table 1. Identical treatment units were further averaged (HF1-HF2: monitoring point, 3; FWS1-FWS2: monitoring point 4), since non-parametric data analysis did not show differences (Kruskal-Wallis test p-values > 0.05). P-values are respectively embedded in Table 1 (see caption [a]) and Table 2 for physical and chemical parameters. EC values varied between I and II–III periods, and DO along the treatment units. The latter has been described as a key indicator of wetland metabolism, and its variability can describe the fluctuating balance between autotrophic and heterotrophic processes. All these features, influenced by seasonal changes (climate and plant phenology), together with multi-compartment CW design and diversified flow conditions (and also different HRT along the treatment units) become very challenging when managing these types of systems (Adyel *et al.* 2016). The alternation of lentic and lotic phases strongly characterizes many wetlands in Mediterranean environments, clearly impacting on DO dynamics. In this case, the only H-CW unit exhibiting higher hydrodynamic regime was the retention pond placed at the inlet system, probably because of the incoming influent flushing, while the remaining stages mainly presented lentic characteristics. A proof of that could be linked to higher levels of DO and pH recorded in the retention pond (Table 1, point 2), while temperature seemed less influent. Also, during the warmer periods, when the exposure to solar radiation was high, the accumulation of thick algal mats as floating mass in the FWS units (Table 1, point 4) could have limited both photosynthetic
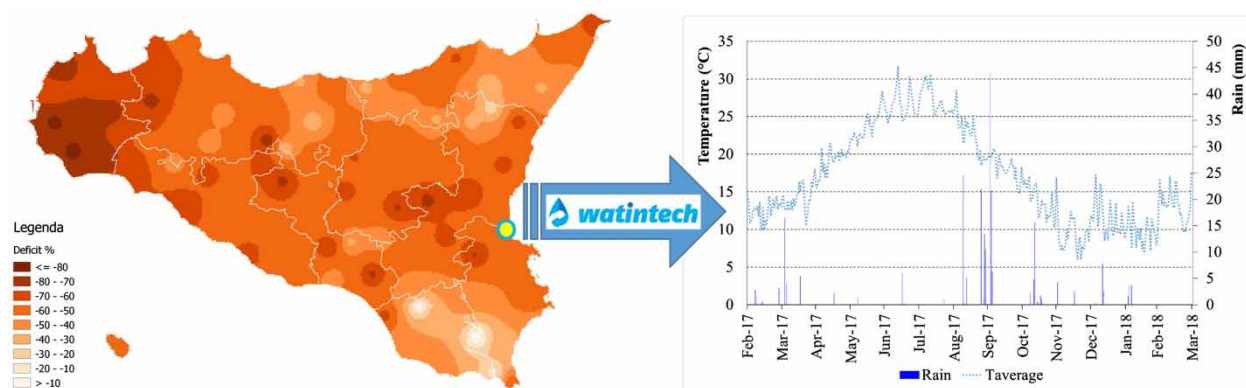
Figure 3 │ Rainfall deficit recorded in Sicily in 2017, in comparison to the period 2003–2016 (data SIAS) and *in situ* pilot H-CW meteorological data: total rain (mm/day) mean air temperature (°C) (February 2017–March 2018).

Table 1 │ Average values and standard deviation (SD, ±) of physical parameters recorded at each sampling point during the three monitoring periods; dissolved oxygen (DO), electrical conductivity (EC, at 20 °C), pH and T (°C)

Point	I period				II period				III period			
	1	2	3	4	1	2	3	4	1	2	3	4
DO (mg L^{-1})	6.7 (1.8)	8.6 (0.9)	4.0 (2.1) [a]0.63	5.6 (2.5) [a]1	4.9 (1.5)	9.8 (1.8)	1.6 (0.6) [a]0.91	8.4 (2.3) [a]0.08	3.1 (0.7)	8.9 (0.9)	2.5 (0.1) [a]0.6	3.3 (0.1) [a]0.59
EC (µS cm^{-1})	1,910 (388)	2,129 (236)	2,130 (224) [a]0.26	2,851 (1,443) [a]0.87	114 (14)	161 (55)	319 (161) [a]0.25	471 (291) [a]0.35	160 (73)	174 (68)	211 (32) [a]0.35	218 (30) [a]0.07
pH	7.5 (0.3)	8.4 (0.3)	7.8 (0.5) [a]0.69	7.2 (0.2) [a]0.57	6.7 (0.9)	7.9 (1)	7.1 (0.8) [a]1	7.5 (0.8) [a]0.92	6.7 (0.7)	8.6 (0.4)	7.1 (0.01) [a]0.67	7.4 (0.01) [a]0.91
T (°C)	28.9 (3.1)	26 (2.1)	24.7 (2.2) [a]0.57	26.5 (3) [a]0.87	15.2 (1.9)	15.1 (2.1)	14.8 (1.3) [a]0.35	15.21 (1.4) [a]0.75	23.9 (6.1)	22.7 (6)	20.4 (0.1) [a]0.92	21.7 (0.2) [a]0.75

[a]Kruskal-Wallis test *p*-values calculated between identical treatment units (HF1-HF2: monitoring point 3; FWS1-FWS2: monitoring point 4).

activity and atmospheric DO exchange. Only during the II period, ranging from November to February, with lower temperature and solar radiation but higher wind velocity (data not shown), DO in FWS units increased.
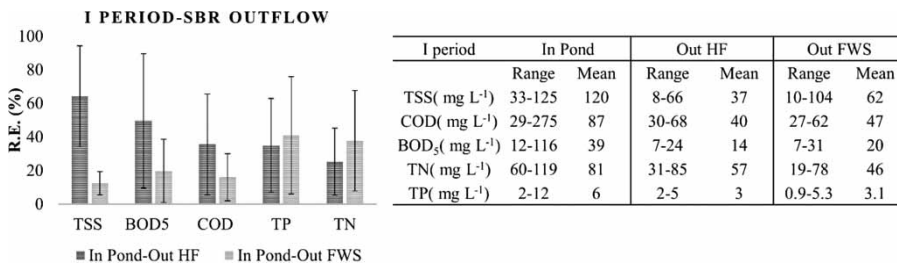
Figure 4 reports concentrations and removal efficiency (R.E., %) of the main chemical parameters during I monitoring period. Higher R.E. took place at HF outlet for TSS and organic matter, while the opposite trend was observed for nutrients. The H-CW presented undeveloped *Typha latifolia* root systems, that were introduced into the FWS on floating support, to act as further filter for retention. The algal blooms occurred in FWS tanks and were determined by increased temperature, caused water quality worsening at the H-CW outlet (TSS, COD and TN values were higher than national standard limits for reuse, Table 3) suggesting the system was still at initial and unstable conditions. On the other hand, as already described by others (Vymazal 2013; Àvila *et al.* 2017), FWS confirmed to play a positive role in nutrients removal. Nevertheless, as reported by Àvila *et al.* (2017) the effluent recirculation could be an effective solution for TN removal in hybrid CW systems, when unfavourable conditions occur.

In Figure 5, trace metals detected during both runoff treatment periods (II and III) are reported. Overall trace metals removal efficiencies (%), for Cr, Fe, Ni, Pb, Cu, Zn, were, respectively, 12 (±9), 9 (±1), 6 (±5), 53 (±30), 30 (±28), 61 (±5) in the II period and 63 (±40), 33 (±32), 39 (±27), 90 (±12), 74 (±9), 91 (±7) in the III. Removal efficiencies mostly enhanced during the III period, possibly more appreciable because of higher concentrations in the case of Cr, Ni and Pb, while Cu and Zn showed very high removal efficiencies even present at lower concentrations. According to Schmitt *et al.* (2015), metals in urban runoffs are mainly particle-bound but Cu and Zn are also present in the dissolved fraction (retained both, in the pond and in the filter; the latter in fact constitutes further retention for dissolved fraction), but the biological uptake of plants and bacteria should be assessed as well. However, the overall concentrations were always below the Italian standard limits for discharge in water bodies (Table 3).

The indicator microorganisms (Table 4) analysed, among those recommended in the last European proposal for water reuse (European Commission 2018) were *Escherichia coli* and *Clostridium perfringens,* respectively, the most appropriate for pathogenic bacteria and protozoa assessment. Both indicators were efficiently removed during the treatment process. Microbial inlet concentrations were below Italian standard limits (Table 3) but a peculiar path was observed in the case of *Clostridium perfringens*, for which removal (2 u-log) was always reached at the HF

Table 2 │ Kruskal-Wallis test *p*-values calculated for chemical parameters between identical treatment units (HF1-HF2: monitoring point 3; FWS1-FWS2: monitoring point 4)

Period	Point	TSS	COD	BOD$_5$	TN	TP	Cr tot	Fe tot	Ni tot	Pb tot	Cu tot	Zn tot
I	3	0.26	0.33	0.87	0.87	0.87	–	–	–	–	–	–
	4	0.52	1	0.87	0.23	0.63	–	–	–	–	–	–
II	3	0.06	0.88	0.25	0.07	0.39	1	0.6	0.83	1	0.46	0.37
	4	0.25	1	0.15	0.66	0.56	0.7	0.12	0.6	0.75	0.83	0.5
III	3	0.88	0.77	0.66	0.05	0.77	0.41	0.75	0.4	0.91	0.17	0.4
	4	0.75	0.15	0.08	0.44	0.09	0.88	0.17	0.6	0.74	0.14	0.92

I period	In Pond		Out HF		Out FWS	
	Range	Mean	Range	Mean	Range	Mean
TSS(mg L^{-1})	33-125	120	8-66	37	10-104	62
COD(mg L^{-1})	29-275	87	30-68	40	27-62	47
BOD$_5$(mg L^{-1})	12-116	39	7-24	14	7-31	20
TN(mg L^{-1})	60-119	81	31-85	57	19-78	46
TP(mg L^{-1})	2-12	6	2-5	3	0.9-5.3	3.1

Figure 4 │ I monitoring period (SBR effluent treatment). Maximum–minimum and mean concentrations of conventional water quality parameters: TSS, COD, BOD$_5$, TN, TP at the system inlet (In Pond), the horizontal unit outlet (HF, monitoring point 3) and system outlet (free-water unit, FWS, monitoring point: 4). Removal efficiencies (R.E., %) calculated between the inlet of the system and the intermediate treatment stage (Pond + HF unit) and the overall (Pond + HF unit + FWS unit).

Table 3 │ Physical-chemical (mgL^{-1}), trace metals (mgL^{-1}), and bacteriological (CFU 100 mL^{-1}) Italian standard limits for wastewater discharge and reuse

Italian standard limits	TSS	BOD$_5$	COD	TN	TP	Tot Cr	Fe	Ni	Pb	Cu	Zn	*E. coli*
WW discharge[a]	80	40	160	≈35[c]	10	2	2	2	0.2	0.1	0.5	5.0×10^{3}[d]
WW reuse[b]	10	20	100	15	2	0.1	2	0.2	0.1	1	0.5	100[e]

[a]Legislative Decree 152 (2006).
[b]Ministerial Decree 185 (2003).
[c]Limit for discharge into surface water bodies (as the rough sum of NH$_4$, N-NO$_2$ and N-NO$_3$).
[d]Recommended value for P.E. >2,000.
[e]Maximum value in 80% of samples.
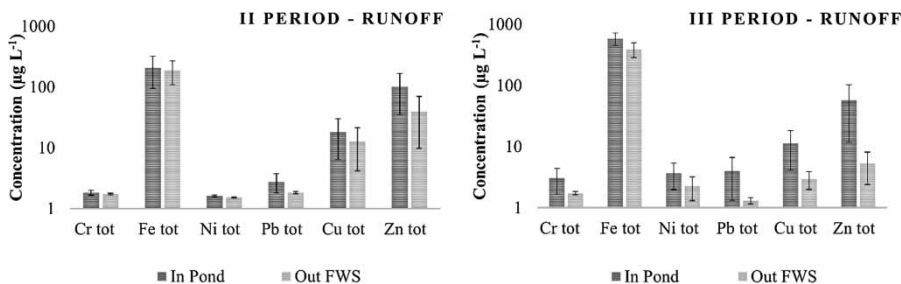
Figure 5 │ Log-scale average concentrations (µg L^{-1}) and SD for trace metals at the hybrid CW inlet (In Pond) and outlet (Out FWS), during stormwater runoff treatment (II–III monitoring periods).

outlet. Further investigation might be useful to clarify the possible influence of DO and suitable substrates presence for anaerobic respiration along the length of the H-CW.

TSS and organic matter (BOD$_5$, COD) average concentrations (mg L^{-1}) at the hybrid CW inlet and outlet recorded when the system was fed with stormwater were,

Table 4 │ Average concentrations (CFU 100 ml^{-1}) and removal efficiencies (R.E., u-log) of indicator microorganisms in hybrid CW inlet (In Pond) and outlet (Out FWS) during II monitoring period

| II monitoring period	In Pond (CFU 100 ml^{-1})	Out FWS (CFU 100 ml^{-1})	R.E. (u log)
Escherichia coli	106 (±83)	3 (±1)[a] 0.12	1.5(±0.4)
Clostridium perf.	464 (±623)	6(±3)[a] 0.05	2 (±0.4)

Standard deviation (±SD) are in brackets.
[a]Kruskal-Wallis test *p*-values calculated between identical treatment units (FWS1-FWS2: monitoring point 4).

respectively, 7 (±0.05), 12 (±5), 21 (±7) and 11 (±0.6), 9 (±2), 26 (±9). Values were below the Italian standard limits (Table 3). Similarly, TN and TP average concentrations (mg L^{-1}) at the hybrid CW inlet and outlet were below the national standard limits: inlet and outlet TN and TP mean values between II and III monitoring periods were, respectively: 3 (±1.3), 2.6 (0.3) and 0.1 (±0.01) and 3.5 (±2.4). A slight TP increase at the outlet was observed, probably influenced by the II period, as also shown by reference data (mean HF and FWS outlet concentrations were, respectively (mg L^{-1}), 5.5 (±2.7) and 5 (±3.6)). In the Mediterranean climate, the ranging period from November to February corresponds to macrophyte seasonal senescence, which in this case could have reasonably contributed to internal nutrient release along the system (Adyel *et al.* 2016). PAHs monitored during II and III periods were in very low concentrations (0.006 μg L^{-1} ±0.003) with a reduction of 40% (±23) already at the pond outlet. As previously described, the interaction between plants and microorganisms is a preferential path in hydrocarbons degradation. Despite the absence of macrophytes in the stabilization pond, the slight removal trend observed at PAHs low concentrations suggest that microbial activities and physicochemical processes could have already occurred. In that regard, Schmitt *et al.* (2015) described sedimentation as a relevant mechanism of PAHs retention in CWs, but the present study did not investigate specific removal paths.

Figure 6 reports Ks mean values (number of sampling per point $n = 3$) measured in both pilot beds at different distances from the inlet in February and June 2018 (after 1–1.5 years' operation). K$_s$ values measured in June were very similar to those measured in February for all measuring points in both beds. Just in the middle point of HF1 there was a significant reduction between Ks values measured in February and in June equal to 2,243 m/d. K$_s$ values measured during the operation period were also compared with K$_s$ value measured for the clean gravel, equal to 12,135 m/d (SD = 1,591). K$_s$ values after the operation periods decreased just in the area close to the inlet in both beds and in the HF2 bed also in the area close to the outlet of about 17%. These reductions were higher than the maximum SD of measurement repetitions in the same place and time and equal to about 1,500 m/d. Mean K$_s$ values measured in the middle areas and close to the outlet, were sometimes even higher than the values observed for the clean gravel (i.e. point HF1-A and HF2-C when IMP permeameter was used and HF1-C when P permeameter was used). This behaviour could be due to the high variability of Ks values, being those differences in the range of the measured variability of the parameter.

CONCLUSIONS

Preliminary experimental results on a pilot-scale H-CW are promising and suggest good reliability in the perspective to combine this technology in decentralised water treatment facilities (DWTFs) for enhancing the water recovery and reuse (e.g. green area irrigation and toilet flushing) in Mediterranean climate conditions. Particularly in Sicily, DWTFs by natural system can play a strategic role for promoting the wastewater reuse, as suggested by several authors (Barbagallo *et al.* 2012; Ventura *et al.* 2019). However, predictions on the main critical issue became heavier than expected when considering the effect of such a strong rainfall scarcity. The feeding modalities of H-CW, particularly
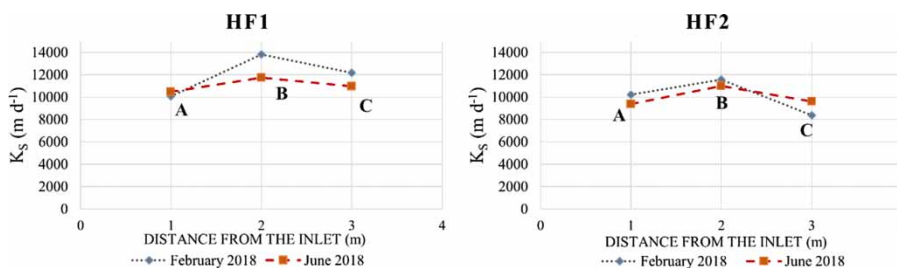
Figure 6 │ Ks values obtained during hydraulic monitoring campaigns of February and June 2018, through *Falling head test* method on both subsurface horizontal CW: HF1 and HF2.

in a climate change scenario, have highlighted the following main critical points:

- The need to properly assess the interaction among nutrient-rich or poor effluent/feeding frequency/plant phenology. In fact, as reported above, the feeding switching from nutrient-rich SBR effluent to poor runoff at the end of the I period corresponded with the macrophyte senescence starting phase, presumably causing the TP increase from the inlet to the outlet of the system. Consequently, different feeding wastewater affects the H-CW treatment efficiency.
- The need for recirculation phase could be required in order to improve a multi-compartment CW treatment efficiency and cope with wastewater variability, addressing the most feasible option between reuse or discharge.
- The difficulty to conduct a robust monitoring activity and data collection.

However, general conclusions pointed out the following:

- The overall trace metals and PAHs removal efficiencies were good in spite of the low inlet concentrations detected, which were actually much lower than expected; hydrocarbons removal paths and processes occurring in hybrid CWs would be further investigated.
- The hydraulic monitoring of a novel H-CW from earlier operation phases allows to provide a long-term complete screening of the hydraulic conductivity at saturation, starting from a zero point survey.

Finally, further research is required to increase knowledge on hybrid CWs 'metabolic pathways' in highly variable climate regions.

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