

Long term decentralized greywater treatment for water reuse purposes in a tourist facility by Vertical Ecosystem

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Abstract

A horizontal flow wetland (HF) was integrated, in a cascading vertical set-up (vertECO), in a Mediterranean tourist facility for decentralized treatment of real low load greywater streams. 15 different plant species were tested in LECA substrate at three different hydraulic retention times (HRTs: 1.9, 1.4 and 1.0 days) with additional aeration improving degradation processes. Beyond standard parameters, several organic micropollutants (14 pharmaceutical organic compounds and 12 endocrine disruptive compounds) were monitored over a 22 month period. The removal efficiency for standard parameters was very high, on average more than 90% for many parameters (COD, BOD₅, TSS, VSS and turbidity) and more than 80% for TOC. BOD₅ removal could be correlated with the temperature, while TN, TKN and NH₄⁺-N showed the highest removal efficiency at the lowest HRT. The effluents consistently met the standards for various reuse applications, at all three HRTs, according to Spanish Legislation (RD, 1620/2007) and EU draft. In terms of organic micropollutants, influent greywater was characterized by high concentrations and variability (for example up to $256.1 \pm 875.3 \mu\text{g L}^{-1}$ for acetaminophen, $88.7 \pm 424.5 \mu\text{g L}^{-1}$ for ibuprofen). More than 95% of removal by vertECO was achieved for most of the compounds (acetaminophen, ibuprofen, salicylic acid, caffeine, estradiol, progesterone, testosterone, triclosan, methyl-, ethyl- and propylparaben) and more than 80% for diclofenac, atenolol and trimethoprim. On the other hand,

hydrochlorothiazide, sulfamethoxazole and salbutamol could not be reduced by more than 30%. Statistically significant differences were found at different HRTs for acetaminophen, atenolol, ibuprofen, ethylparaben, TCP and TBEP ($p < 0.05$). Conversely, most of the evaluated micropollutants were consistently removed without apparent influence from temperature.

Keywords (6)

Constructed wetland, vertical ecosystem, horizontal flow, greywater, pharmaceutical active compounds, endocrine disrupting compounds

1. Introduction

Mass tourism is a global phenomenon and one of the largest and fastest growing economic sectors in the world. Tourism water consumption is normally less than 5% of domestic water consumption, but may be as high as 40% in certain countries e.g. Mauritius (Gössling et al., 2012). In fact, in the last thirty years, the frequency of droughts and the related environmental and economic damage has increased (EC, 2012). As a result, water scarcity is increasing in areas such as the Mediterranean coastal regions, one of the most important tourism destinations in the world. In tourist facilities the water consumption is usually high, 150 to 600 liters per guest per night, depending on hotel category, seasonal fluctuations during high season and weekends (Gabarda-Mallorquí et al., 2017; Gössling et al., 2012). Additionally, tourism can also have a strong impact on the water quality (Moreno-González et al., 2015).

Decentralized treatment systems could be an interesting option in the context of water reuse and therefore reduction in freshwater consumption. Possible tested solutions focus on separate water streams: black water and/ or greywater treatment. In particular, greywater has received attention, because it is less organically polluted than domestic wastewater (including toilet water), contains far less suspended solids and lower turbidity, and can be treated more efficiently for reuse purposes (Eriksson et al., 2002; Jefferson et al., 2001). The treated water might, in the near future, be reused in Europe for agricultural reuse (Alcalde-Sanz and Gawlik, 2017), but it is already a reality in specific countries (e.g. Spain) also for other industrial, urban,

environmental and recreational uses (RD, 1620/2007) with divergent water quality requirements established by national and international regulations (Alcalde-Sanz and Gawlik, 2017).

In recent decades, concerns about organic micropollutants in water and the environment, such as pharmaceutical active compounds (PhACs) and endocrine disrupting compounds (EDCs), have increased dramatically (Luo et al., 2014). Due to their low concentration and, in some cases, persistency their removal by conventional sewage treatment technologies is often incomplete (Buttiglieri and Knepper, 2008; Carballa et al., 2017). This may lead to negative consequences for the aquatic life, but also risk for the human health etc. In particular, the concentration of micropollutants in greywater was reported to be even higher than in wastewater, due to the absence of dilution with other water streams (Hernández Leal et al., 2010), but data regarding organic micropollutants in greywater are missing. Due to the lack of information, moreover, micropollutants have been rarely included in water related policies. Recently, the Watch List 2018/840 (EC, 2018) and the previous 2015/495 (EC, 2015) included for the first time, at EU level, antibiotics and hormones, like estrone and estradiol, for European monitoring (Loos et al., 2018). This demonstrates the need for further investigations regarding micropollutants presence and the development of new strategies for their removal towards potential reuse applications. Nonetheless, in most countries, no regulation is present yet regarding environmental and human health risks of micropollutants, concentration limits in water intended for different uses and removal requirements for potential reuse technologies.

Compared to other conventional technologies, constructed wetlands (CWs) are a low energy consumption alternative and economically efficient. Several studies have shown that constructed wetlands meet certain reuse standards (RD, 1620/2007) for standard parameters (Gattringer et al., 2016; Ramprasad and Philip, 2016). In particular, horizontal flow (HF) CWs are suitable for on-site treatment, due to their non-free water surface, but they require large footprints compared to other technologies. Few studies report specific CWs (e.g. GROW - Green Roof-top Water Recycling Systems) for greywater treatment on the roof top of buildings (Avery et al., 2007; Ramprasad et al., 2017). Most of these technologies (e.g. HFCW and GROW systems) were tested with lower hydraulic loading rates (HLRs) and/ or longer hydraulic retention times (HRTs) (Avery et al., 2007; Ramprasad et al., 2017;

Ramprasad and Philip, 2016). A novel vertical staggered CW (vertECO – vertical ecosystem) was recently developed, in order to face space limitations in urban areas (Gattringer et al., 2016). Finally, only very few studies are available on greywater treatment of micropollutants by means of constructed wetlands, for very few compounds and not treating real greywater (Ramprasad and Philip, 2016; Revitt et al., 2011). Also, to the best of our knowledge, no studies are available on this specific topic, with long-term real greywater treatment and application to touristic infrastructures.

In this study, a building integrated HFCW was designed to treat real greywater for water reuse purpose in a large Euro-Mediterranean resort. The simulated constructed wetland (vertECO) was monitored in terms of organic standard parameters as well as micropollutants (14 PhACs and 12 EDCs). This paper presents a long term monitoring experiment evaluating the influence of hydraulic retention time and temperature on the removal efficiencies of the selected parameters.

2. Materials and methods

2.1. Location

The experimental pilot plant is located in a Euro-Mediterranean hotel in Lloret de Mar (Spain, 41.698, 2.836), a coastal location with Mediterranean climate. The temperature ranges from 8.6 °C (average minimum last 40 years) to 20.8 °C (average maximum last 40 years) and an annual precipitation of 728 mm. This large resort offers 441 air-conditioned rooms. In order to save water, the hotel applies partial reuse of greywater for toilet flushing. Depending on the season, water consumption ranges from 25,000 to 34,000 m³ per year, i.e. an average water consumption of 100 to 135 liters per guest per night, depending on seasons and weekends. The reuse of greywater for toilet flushing is approx. 13,500 to 15,000 m³ of tap water per year, reducing water consumption per guest per night by 80% (Gabarda-Mallorquí et al., 2017).

2.2. vertECO technology

The vertECO (vertical ecosystem) was developed for the treatment of hotel greywater streams. This constructed wetland has a vertical set-up with four cascading stages combined with a horizontal subsurface water flow as shown in Figure 2.1. This vertical arrangement has the purpose of maximizing the length of water flow path through the CW and the optimal spatial utilization due to space limitation in urban areas. vertECO simulates a natural wetland ecosystem to serve as a greywater filter. The plants are cultivated in a hydroponic system with coarse expanded clay as root substrate and a permanent comparatively high saturated water level.

It has the following dimensions: 4.5 m long x 1.5 m wide and 2.5 m high (Figure 2.1.) and is semi-indoor with an encapsulation in winter. Each level has a root-substrate volume of 550 L, for a total substrate volume of 2200 L. Each level is placed 50 cm above the next level below, the containers having a height of 40 cm and an average width of 35 cm (Gattringer et al., 2016). A time-controlled pump is used to intermittently load water from an oxygenated water tank to the top floor of the vertical ecosystem. This water tank serves as a buffer tank and as a pre-treatment tank, which allows continuous load during the day and greywater homogenization. The horizontal sub surface waterflow is meandering through the rhizosphere and is forced by gravity into the next floor (each level is connected to the next level by a 1/2" hose), whereby a permanently high saturated water level is maintained. There are only minor water level fluctuations caused by the outflow of the lower level of the unit. This is in contrast to a vertical flow system, where the bed is completely drained.

As substrate, light expanded clay aggregate (LECA 8/16) was chosen to promote better biofilm development, due to its large surface area. In addition, the non-biodegradability of the substrate helps to prevent degradation and clogging. This hydroponic system contains 2 m³ expanded clay substrate. In order to promote aerobic microorganisms for efficient rhizosphere degradation processes, air is continuously pumped through perforated hoses into the bottom area of the containers using a 100 watt air compressor with a nominal capacity of 9000 L h⁻¹ at 0.045 MPa. Another advantage is the avoidance of anaerobic digestion by sulfate-reducing microorganisms and associated odors (H₂S production).

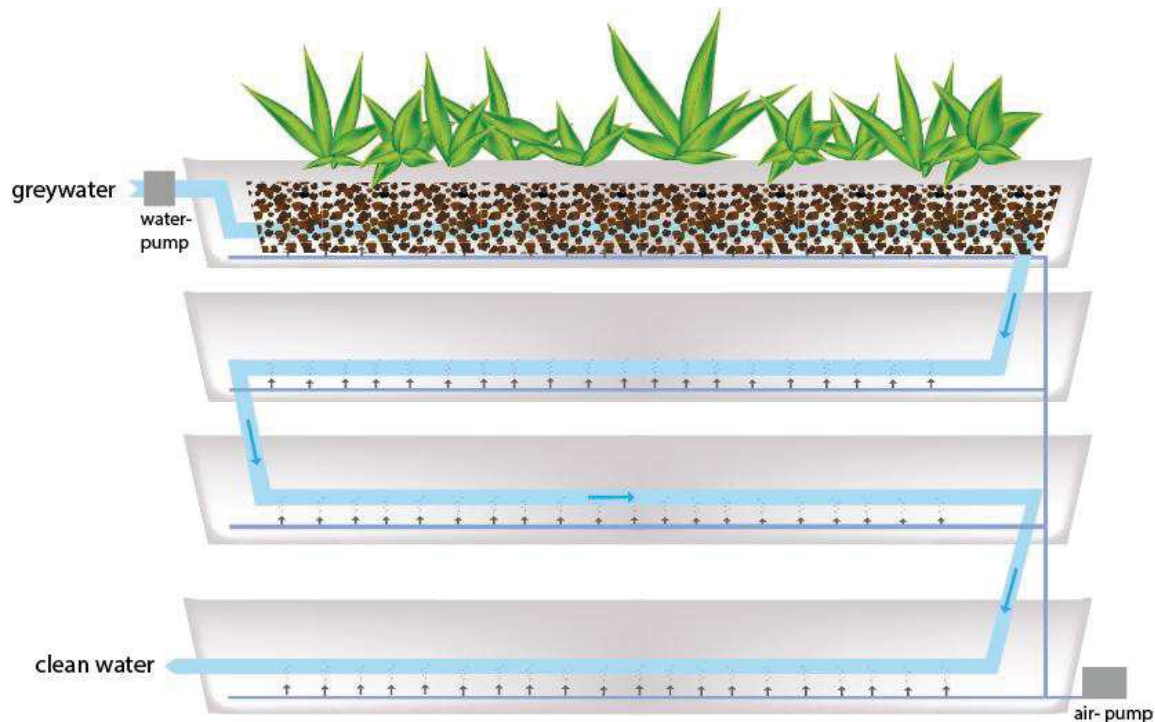


Figure 2.1. Schematic overview of the vertECO technology – all four layers are completely vegetated (planting indicated only in the top floor for a better visualization of water flow pathway and aeration)

Prior to this study, more than 150 emergent plant species were tested in various combinations and adapted to synthetic greywater on laboratory scale. Based on their special growth physiology, that guarantees their survival under difficult rhizosphere conditions (Stottmeister et al., 2003) and their performance, a combination of 15 species (helophytes, graminoids, tropical and subtropical plants, Table 2.1.) are used in the DEMO unit. All stages are fully vegetated with a planting density of approx. 28 plants per m². For successful plant establishment, a 2 month adaptation phase was allowed prior to the start of the study.

Table 2.1. Quantity of the selected plant species of the vertECO

No	Plant species	No	Plant species
25	<i>Cyperus alternifolius</i> L.	6	<i>Monstera deliciosa</i> LIEBM.
15	<i>Carex acutiformis</i> EHRH.	36	<i>Ficus pumila</i> L.
10	<i>Juncus inflexus</i> L.	9	<i>Philodendron scandens</i> K.KOCH & SELLO
10	<i>Juncus effuses</i> L.	9	<i>Philodendron erubescens</i> K.KOCH & AUGUSTIN
12	<i>Equisetum hyemale</i> L.	20	<i>Syngonium podophyllum</i> SCHOTT.
9	<i>Spathiphyllum wallisii</i> SCHOTT.	15	<i>Iris laevigata</i> FISCH. & C.A.MEY.
9	<i>Spathiphyllum wallisii</i> 'sensation' SCHOTT.	5	<i>Mentha aquatica</i> L.
10	<i>Calathea sp.</i> G.Mey.		

2.3. Experimental set-up

The vertECO was fed with real hotel low-load greywater, from the showers and washbasins of the guest rooms. Both urinals and kitchen effluents were not connected to the hotel greywater reuse system and no additional synthetic compounds were added. Influent greywater was characterized, in terms of both, standard parameters and organic micropollutants, as a function of high and low seasons (i.e. periods without encapsulation, with higher environmental temperature, and periods with encapsulation, related to colder periods).

The flow rate was 7 L min⁻¹ and the pumping interval was intermittent. During the experimental period, three different flow rates (FR), were applied, as presented in Table 2.2 with the corresponding HRTs, organic loading rates (OLR) and HLRs.

Table 2.2. Operative parameters during the three experimental periods

Mode	FR [m ³ d ⁻¹]	HLR [m ³ m ⁻² d ⁻¹]	OLR [g COD m ⁻² d ⁻¹]	HRT [d]	Temperature [°C]	Sampling [n]	Days [d]
1	0.750	0.10	15.9	1.9	15.2 ± 5.8	29	244
2	1.008	0.14	21.0	1.4	16.6 ± 5.0	13	184
3	1.400	0.19	34.0	1.0	18.9 ± 4.8	20	122

Greywater was initially fed once per hour for 4.5 min, resulting in a HLR of 0.10 m³ m⁻² d⁻¹. The inflow was then increased to 6.0 min and finally to 8.4 min per hour (corresponding to 0.14 m³ m⁻² d⁻¹ and 0.19 m³ m⁻² d⁻¹, respectively).

Inflow and outflow samples of the vertECO were collected and analyzed (for standard parameters and selected micropollutants, section 2.4) towards water reuse according to Spanish Legislation, (RD 1620/2007) and/ or draft European legislation for water reuse (Alcalde-Sanz and Gawlik, 2017). In the present study, only inlet

(after pre-treatment tank) and outlet (after the technology) were considered to represent the performance of the whole unit itself. The sampling frequency for both standard parameters and micropollutants, divided by period, is presented in Tab.2.2. In the present study, an observation time of 22 months is given.

2.4. Analytical methods, sample collection and pre-treatment

Standard chemical parameters and nutrients were analyzed with catalytic oxidation and ion chromatography. Total organic carbon and biochemical oxygen demand were analyzed with catalytic oxidation and respirometry respectively (APHA, 1998). The samples were collected in plastic bottles, with a sample volume of 500 mL for the inlet and 1000 mL for the outlet and transported in refrigerated containers. Standard parameters were analyzed on arrival at the research facilities (or frozen at -20 °C, except for the BOD₅ parameter, which was always immediately analyzed). EDCs and PhACs were selected based on micropollutants presence in greywater (Gulyas et al., 2011; Hernández Leal et al., 2010) and based on preliminary sampling campaign (data not shown). A total of 14 PhACs, including analgesics/anti-inflammatories (6), antibiotics (2), beta blockers (2), contrast media (1), diuretic (1), psychiatric drug (1), receptor antagonist (1) and 12 EDCs and related compounds were analyzed. 125 mL and 250 mL were sampled for inlet and outlet in amber PET bottles. For the analyses of micropollutants, the samples were filtered with 0.45 µm PVDF membrane filters (Merck Millipore, Germany) and immediately frozen at -20 °C, until analysis.

2.4.1 Micropollutants analyses

All compounds were purchased from Sigma-Aldrich and isotopically labelled compounds were used as internal standards from Toronto Research Chemicals and Cluzeau Info Labo. HPLC-grade methanol, acetonitrile, water (LiChrosolv), ammonium acetate, formic acid and Na₂EDTA were purchased from Merck (Germany). Nitrogen for drying 99.995% of purity was from Abelló Linde (Spain).

Analysis of pharmaceuticals

In each sample for pharmaceutical analyses, a suitable volume of 0.1 M Na₂EDTA solution was added to achieve a final concentration of 0.1% (g solute/ g solution). Samples were pre-concentrated using Oasis HLB cartridges (60 mg, 3 mL) (Waters Corp., Milford, MA, USA), which were conditioned with 5 mL of methanol followed by 5 mL of HPLC grade water. 25 mL of the sample was loaded to the cartridge at 1 mL

min⁻¹. After sample pre-concentration, cartridges were rinsed with 6 mL of HPLC grade water and air-dried for 5 min to remove all water. Analytes were eluted with 6 mL of pure methanol. Extracts were evaporated to dryness under a gentle nitrogen stream and reconstituted with 1 mL of methanol. 10 µL extract was diluted in 1 mL of methanol/ water. Finally, 10 µL of an internal standard mixture (at 1 mg L⁻¹) were added to the extract. Samples were further analyzed using Ultra-Performance™ liquid chromatograph system (Waters Corp. Milford, MA, USA) coupled to a quadrupole-linear hybrid ion trap mass spectrometer (5500 QTRAP, Applied Biosystems, Foster City, CA, USA) with a turbo Ion Spray source. Chromatographic separation was carried out using Acquity HSS T3 column (for positive electrospray ionization) and Acquity BEH C18 column (for negative electrospray ionization) (Waters Corp. Milford, MA, USA). The limits of detection were between 0.1 and 5.0 ng L⁻¹. The detailed procedure of the analysis was as described by Gros et al. (2012). Recoveries, limits of detection (LOD) and limits of quantification (LOQ) are presented in Table S1.

Analysis of endocrine disruptor and related compounds

Samples were extracted using Strata-X cartridges (200 mg, 6 mL) (Phenomenex), conditioned with 5 mL of methanol followed by 5 mL of HPLC grade water. Samples (50 mL) were loaded at 1 mL min⁻¹. Finally, the cartridges were dried under vacuum conditions in order to carry out the elution with a mixture of dichloromethane:methanol (50:50). The extract was reconstituted with 1 mL of methanol:water and 50 µL of internal standard was added. Samples were analyzed by an Ultra Performance Liquid Chromatography (Thermo Fisher Scientific) system coupled to a triple quadrupole mass spectrometer (TSQ Vantage, Thermo Fisher Scientific). Chromatographic separation was carried out using a LUNA OMEGA C18 1.6 µm (100 × 2.1 mm) (Phenomenex) column. The limits of detection were between 0.12 and 14 ng L⁻¹. The detailed procedure of the analysis was as described by Gorga et al. (2013). Recoveries, LOD and LOQ are presented in Table S1.

2.5. Data analysis

Statistical data evaluation was performed using IBM SPSS 21. The normality and homogeneity of the data were tested with Shapiro-Wilk and Levene's test. When normal distribution and homogeneity of variances of the data were confirmed, a one-

way ANOVA was performed to compare the removal efficiencies with different water flows. Tukey test was performed as post hoc test for multiple comparison of means. Where it was not possible to use ANOVA, Kruskal-Wallis test was performed. Linear Regression was used to investigate interactions between removal efficiency and temperature. Differences were considered as significant when $p < 0.05$.

3. Results and discussion

3.1. Standard parameters

3.1.1. Greywater standard parameters characterization

The average influent greywater composition is presented in Table 3.1. It generally displays results in terms of average COD ($158 \pm 112 \text{ mgO}_2 \text{ L}^{-1}$) and BOD₅ ($116 \pm 67 \text{ mgO}_2 \text{ L}^{-1}$), slightly lower compared to the literature (Jefferson et al., 2004), apparently due to higher observed per capita water consumption, and the corresponding dilution, in touristic infrastructures (Avery et al., 2007; Ramprasad et al., 2017). The calculated COD:BOD₅ ratio of 1.41 ± 0.91 was quite low, indicating an overall good biodegradability, while the COD:TN ratio of 6.85 ± 19.95 was appropriate (Eriksson et al., 2002), most probably related to the aeration in the pre-treatment tank, forcing nitrification. Compared to other greywater studies (Ramprasad et al., 2017) the concentration of nitrates (NO_3^- -N), nitrites (NO_2^- -N) and phosphates (PO_4^- -P) are low (0.02 ± 0.04 , 0.06 ± 0.39 and $0.34 \pm 0.76 \text{ mg L}^{-1}$, respectively), due to the fact that urinals, kitchen and laundry effluents were not connected to the greywater stream. The extremely low phosphorus content, on the other hand, can be related to the fact that most washing detergents no longer contain phosphates, because of their toxicity, nutrient pollution and consequently algae blooms. Suspended solids to turbidity ratio of 1.40 ± 2.73 was low, but in the literature range (Jefferson et al., 2004). Generally, higher concentrations for total suspended solids are related to washing machines and kitchen basins, not included in the hotel greywater of the present study. It is highlighted that the inlet of the technology was sampled after the pretreatment aerated influent tank and, consequently, some degradation and solids sedimentation can be expected (Dixon et al., 1999).

Finally, electrical conductivity (EC) was more than $700 \mu\text{S cm}^{-1}$, the main sources being the salts included in soaps, detergents, washing powders and, mainly, the hotel tap water itself with average EC of $600 \mu\text{S cm}^{-1}$.

Table 3.1. Greywater characterization of influent, effluent (mean values (AV) and STDEV) and removal efficiencies (%) under different HRTs

Parameter	AV influent	AV effluent	Removal Efficiencies [%]				
			AV	1.9 [d]	1.4 [d]	1.0 [d]	
COD ($\text{mgO}_2 \text{L}^{-1}$)	158 ± 112	6 ± 12	94 ± 14	92 ± 16	96 ± 11	93 ± 12	*
BOD ($\text{mgO}_2 \text{L}^{-1}$)	116 ± 67	3 ± 3	96 ± 3	95 ± 4	96 ± 2	99 ± 1	*, ‡
TOC (mgC L^{-1})	39.0 ± 25.6	5.5 ± 2.3	82 ± 11	78 ± 11	82 ± 12	87 ± 8	*
TSS (mg L^{-1})	63 ± 114	3 ± 6	91 ± 16	88 ± 17	90 ± 20	96 ± 3	*
VSS (mg L^{-1})	53 ± 82	2 ± 3	93 ± 9	91 ± 10	92 ± 11	98 ± 3	*
Turbidity (NTU)	68.4 ± 39.8	1.8 ± 1.5	96 ± 6	91 ± 8	97 ± 3	96 ± 7	*
EC ($\mu\text{S cm}^{-1}$)	767 ± 108	783 ± 52	-	-	-	-	
pH	7.08 ± 0.31	7.36 ± 0.29	-	-	-	-	*
TN (mg L^{-1})	10.4 ± 9.3	4.6 ± 2.6	43 ± 40	37 ± 47	33 ± 24	60 ± 34	*, †
TKN (mg L^{-1})	10.3 ± 9.0	2.4 ± 2.3	73 ± 31	69 ± 36	63 ± 28	87 ± 14	*, †
NO ₂ ⁻ -N (mg L^{-1})	0.06 ± 0.39	0.10 ± 0.43	-	-	-	-	
NO ₃ ⁻ -N (mg L^{-1})	0.02 ± 0.04	2.31 ± 1.80	-	-	-	-	
NH ₄ ⁺ -N (mg L^{-1})	4.88 ± 2.92	1.55 ± 1.37	56 ± 50	52 ± 29	37.9 ± 45	67 ± 53	*, †
PO ₄ ⁻ -P (mg L^{-1})	0.34 ± 0.76	0.54 ± 1.80	-	-	-	-	

total sample size: n = 58, BOD₅: n = 50; turbidity: n = 30

- effluent mean values > influent mean values

* effluent significantly lower than influent ($p < 0.05$)

† significant differences between the different HRTs ($p < 0.05$)

‡ significant differences between removal efficiency and temperature ($p < 0.05$)

3.1.2. Overall standard parameters removal efficiency

Organics, suspended solids, ECs and turbidity

vertECO achieved total average removal efficiencies higher than 90% for COD, BOD₅, TSS, VSS and turbidity and more than 80% for TOC (Table 3.1.). Similar results were reported with GROW systems and HF wetlands by Ramprasad et al. (2017) and Avery et al. (2007), but the latter with less polluted greywater. Compared to other HF systems, vertECO has higher removal efficiencies for BOD₅, due to the fact that oxygen is not the limiting factor, since air is actively pumped into the root zone by an air compressor. Additional oxygen release from the roots into the rhizosphere, increasing microbial activity, is also expected, as discussed in other studies (Stottmeister et al., 2003). In contrast to other studies, the high variability and the increase of the organic content in the influent during the monitoring period had no influence on the outlet concentration (Ramprasad et al., 2017). The OLR reached

34 g COD m⁻² d⁻¹ (depended on the number and behavior of the hotel guests) and it may be assumed that the maximum capacity of the vertECO has not been reached in the present study. Organic removal is mostly related to the rhizosphere associated microbial degradation and adsorption, but sedimentation and filtration may also contribute (Vymazal, 2002). Suspended solids removal, more than 90%, was higher than reported values for Mediterranean areas (72-84%, Masi and Martinuzzi, 2007) and related mainly to the observed constant climatic conditions and the applied high planting density, resulting in larger sedimentation, root structure filtration and biodegradation processes in the rhizosphere (Vymazal, 2005).

Effluent EC was, as expected, slightly higher than in the influent, due to water loss (evapotranspiration), plant uptake and release (Albalawneh et al., 2016) or salt release from body care products and other products.

Nutrients

TKN (73%) and TN (43%) removal was slightly lower compared to the literature (Langergraber, 2005; Ramprasad et al., 2017). Plants provide optimal rhizosphere conditions for aerobic degradation and nitrification, as well as partial uptake of nitrate and ammonia into their tissues. Moreover, in vertECO the root zone is actively ventilated and consequently nitrification was expected, but not denitrification.

In terms of phosphate (P), no statistically significant difference between influent and effluent concentrations was observed. This can be attributed to the fact that phosphate concentration in the influent was low and possibly even at deficient concentrations for the plants. On the other hand, the plant uptake and adsorption to LECA might have built up a P reservoir. Consequently, occasional findings of higher phosphate concentrations in the effluent than in the influent can be related to some P release. Although the removal efficiency for nitrate, nitrite and phosphate was limited, it can be noted that the effluent was unproblematic for water reuse and regulatory compliance (RD, 1620/2007) for many purposes.

3.1.3. Standard parameters removal under different conditions

Temperature and HRT are two recognized key factors for constructed wetlands operation. Higher temperatures are related to an increase of biodegradation rates and longer HRTs to higher removal efficiencies (Lee et al., 2009).

During the research period, the water flow was gradually increasing and the corresponding removal efficiencies were compared. As shown in Tab. 3.1., in most cases a higher removal efficiencies of the pollutants were observed by reduced HRT (not statistically significant), in controversy to other studies (Ramprasad et al., 2017). Compared to other HF CW technologies (e.g. Ramprasad et al., 2017), vertECO has a 6 times higher planting density. During the experiment, the root biomass and the corresponding root surface developed well, providing better conditions for rhizosphere associated microorganisms and most likely leading to improved pollutants biodegradation. Similar conclusions can be drawn regarding the removal of suspended solids under different conditions. The reduction of HRT did not affect the removal, the difference with the other periods being not statistically significant. Suspended solids are highly dependent on physical/ mechanical removal, e.g. filter mechanism of denser plant roots, increasing during the experimental period and compensating the increase of HRT itself.

On the other hand, statistical differences were observed for TKN, TN and $\text{NH}_4^+\text{-N}$ removals at different HRTs, with the highest removal observed at the lowest HRT of 1.0 d. The overall performance was good and with higher applied flow rates compared to other studies (e.g. Ramprasad et al., 2017; 62, 82, 100 and 120 L d⁻¹). To be highlighted that the lowest HRT was tested at the end of the experiment, with well-developed plants with much higher biomass than 2 years earlier. A better environment for microorganisms, the planting density and the higher demand for N-nutrients by the plants can be claimed to explain these results.

Regarding the possible influence of temperature, only the BOD₅ removal was higher ($p < 0.05$) and to be related to higher microbial activity during summer season (Akratos and Tsihrintzis, 2007), although the temperature differences among seasons were not extreme. In general, constructed wetlands in the Mediterranean, with average values not lower than 15 °C, show a better performance compared to the ones applied in temperate climates (Masi and Martinuzzi, 2007).

To be remarked that, also evaluating separately the periods at different HRTs and temperature, all effluent standard parameters successfully complied with the standards of RD, 1620/2007 (Spanish legislation for water reuse) and EU draft for water reuse (Alcalde-Sanz and Gawlik, 2017).

With regard to pathogens and microbiological contamination, greywater is usually less contaminated compared to wastewater. Previous experiments with vertECO showed a reduction of log2 to log3 in populations of *Escherichia coli* and *Intestinal enterococci* (Gattringer et al., 2016). These preliminary results indicate possible reuse scenarios for non-direct body contact water. However, further experiments are required to confirm this assumption.

3.2. Micropollutants

The results in terms of the presence of micropollutants in greywater and removal by the vertECO are shown in Table 3.2. Since greywater literature is largely missing, studies on wastewater treatment have been considered in some cases for comparison and discussion. Greywater with concentrations of micropollutants higher than in wastewater was previously reported and related to the absence of dilution with storm water and toilet water by a factor 2-4 (Hernández Leal, 2010). It is also worth remarking that the treated greywater in the present study was low-load, without mixing and possible dilution with laundry water or kitchen effluent.

3.2.1. Micropollutants greywater characterization

The content of organic micropollutants in the influent greywater was characterized subdividing the results based on the tourist season, (high: summer period, many young visitors; or low: retirees and older people make a much larger proportion of the visitors) and the corresponding fluctuations.

Extremely high concentrations and high variability were observed in hotel greywater for some PhACs like acetaminophen ($256.1 \pm 875.3 \mu\text{g L}^{-1}$), salicylic acid ($58.5 \pm 135.2 \mu\text{g L}^{-1}$), ibuprofen ($88.7 \pm 424.5 \mu\text{g L}^{-1}$) and diclofenac ($38.4 \pm 147.8 \mu\text{g L}^{-1}$). Results on micropollutants content in greywater are largely lacking and not easily comparable. The here reported high concentrations are in contrast with some previous studies and are only similar to spiked greywater (Ávila et al., 2010). Much lower salicylic acid and acetaminophen concentrations ($0.6 \mu\text{g L}^{-1}$ and $1.5 \mu\text{g L}^{-1}$, respectively), for example, are usually reported in greywater (Eriksson et al., 2002). Such levels are extremely high even compared to wastewater levels (e.g. Carranza-Diaz et al., 2014; Hijosa-Valsero et al., 2010).

Very few studies are also available on EDCs with most of them referring only to caffeine and bisphenol A. The concentration of Bisphenol A ($0.3 \pm 0.5 \mu\text{g L}^{-1}$) was comparable to other studies (Hernández Leal et al., 2010) and similar to the plasticizers tris(2-chloroisopropyl)phosphate (TCPP) and tris(2-butoxyethyl)phosphate (TBEP). The average caffeine concentration ($16.7 \pm 20.8 \mu\text{g L}^{-1}$) was slightly higher but comparable to other studies ($13.2 \mu\text{g L}^{-1}$, Carranza-Diaz et al., 2014). The concentrations of triclosan and the preservatives methyl-, ethyl- and propylparaben ($0.2 \pm 0.5 \mu\text{g L}^{-1}$, $6.9 \pm 8.9 \mu\text{g L}^{-1}$, $3.4 \pm 9.4 \mu\text{g L}^{-1}$ and $2.2 \pm 2.6 \mu\text{g L}^{-1}$, respectively) were also in the literature order of magnitude (Andersen et al., 2007; Eriksson et al., 2002). Finally, hormones were detected, but at limited concentrations (lower than $0.02 \mu\text{g L}^{-1}$).

For some PhACs, a statistically significant ($p < 0.05$) seasonal variability was observed in the influent greywater (Fig.3.2.1.). During the low touristic season, some PhACs (acetaminophen, diclofenac and ibuprofen) were in fact higher detected than in the high touristic season. This can be related to the variability in the hotel clients composition, changing over the year in terms of age and necessities of disease treatment, and the cold season itself with lower temperature (Kozak and Rimmington, 2000). A reverse trend, possibly also associated to tourist composition variability, was qualitatively observed for caffeine, methyl-, ethyl- and propylparaben, at higher concentrations in the high touristic season (but not statistically different, $p > 0.05$).

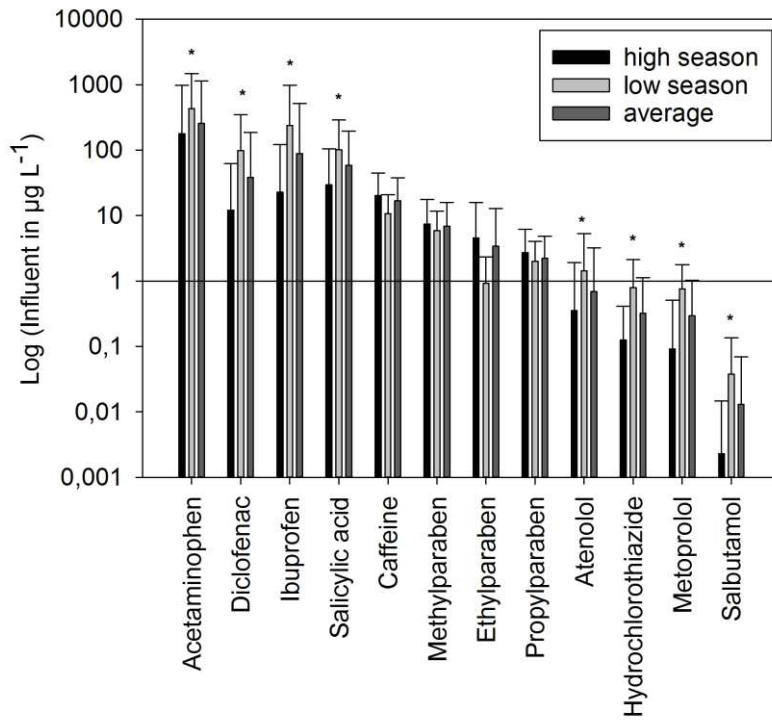


Figure 3.2.1. Micropollutants logarithmic influent concentration (in $\mu\text{g L}^{-1}$) during high season, low season and average. Significant differences ($p < 0.05$) are marked with an asterisk (*)

Table 3.2. Micropollutants characterization of influent greywater, vertECO effluent and vertECO removal efficiencies (as average and under different HRTs). AV (mean values).

Parameter	AV influent [$\mu\text{g L}^{-1}$]	AV effluent [$\mu\text{g L}^{-1}$]	Removal Efficiency [%]				
			AV	1.9 [d]	1.4 [d]	1.0 [d]	
Analgesic anti-inflammatory drugs							
Acetaminophen	256.1 ± 875.3	0.09 ± 0.11	98.2 ± 2.8	96.9 ± 3.6	98.7 ± 1.5	99.8 ± 0.6	*, †
Diclofenac	38.4 ± 147.8	0.61 ± 0.97	88.0 ± 20.6	93.6 ± 14.6	81.1 ± 18.4	83.1 ± 27.4	*
Ibuprofen	88.7 ± 424.5	0.30 ± 1.19	98.3 ± 3.0	97.9 ± 3.3	96.6 ± 3.4	99.8 ± 0.5	*, †
Ketoprofen	0.92 ± 3.54	0.32 ± 0.62	66.3 ± 58.4	53.3 ± 68.2	41.8 ± 57.9	77.8 ± 66.7	
Naproxen	0.23 ± 0.56	0.14 ± 0.30	60.7 ± 44.8	49.8 ± 54.8	77.8 ± 29.9	17.3 ± 0.0	
Salicylic acid	58.5 ± 135.2	0.09 ± 0.08	99.2 ± 1.2	99.2 ± 1.0	-	99.3 ± 1.5	*
Antiepileptic drug							
Carbamazepine	1.18 ± 7.57	0.07 ± 0.15	56.6 ± 63.8	28.6 ± 100.2	40.7 ± 55.2	83.4 ± 12.6	
Antibiotics							
Sulfamethoxazole	0.06 ± 0.39	0.05 ± 0.25	21.7 ± 53.4	31.1 ± 85.5	- 19.5 ± 54.3	29.1 ± 83.1	
Trimethoprim	0.06 ± 0.03	0.02 ± 0.19	91.1 ± 21.2	96.1 ± 15.2	99.9 ± 0.0	81.7 ± 28.1	*
Beta blockers							
Atenolol	0.69 ± 2.53	0.01 ± 0.02	93.2 ± 8.3	88.5 ± 9.8	89.9 ± 6.6	98.9 ± 3.2	*, †
Metoprolol	0.29 ± 0.72	0.08 ± 0.26	65.3 ± 36.9	56.8 ± 46.4	70.6 ± 10.7	70.6 ± 34.9	*
Contrast media							
Iopromide	0.003 ± 0.006	0.00 ± 0.00	75.0 ± 68.3	99.9 ± 0.0	99.9 ± 0.0	33.3 ± 103.3	*
Diuretics							
Hydrochlorothiazide	0.32 ± 0.81	0.10 ± 0.18	28.3 ± 60.4	13.2 ± 58.6	26.1 ± 57.3	51.38 ± 61.7	*, ‡
Receptor antagonist							
Salbutamol	0.01 ± 0.06	0.02 ± 0.18	- 7.6 ± 96.3	- 5.6 ± 100.5	64.7 ± 80.7	- 51.6 ± 83.9	
Stimulant drugs							
Caffeine	16.7 ± 20.8	0.73 ± 2.19	96.2 ± 7.7	93.9 ± 11.4	97.0 ± 2.7	98.2 ± 2.6	*
Hormones							
Estrone	0.02 ± 0.07	0.004 ± 0.031	98.9 ± 0.0	98.5 ± 3.6	n.d.	99.9 ± 0.0	*
Estradiol	0.001 ± 0.000	0.00 ± 0.00	99.9 ± 0.0	99.9 ± 0.0	n.d.	n.d.	
Progesterone	0.003 ± 0.010	0.00 ± 0.00	99.9 ± 0.0	n.d.	99.9 ± 0.0	n.d.	
Testosterone	0.02 ± 0.09	0.00 ± 0.00	99.9 ± 0.0	99.9 ± 0.0	n.d.	n.d.	
Plasticizer							
Bisphenol A	0.27 ± 0.45	0.18 ± 0.40	58.0 ± 43.7	56.6 ± 42.9	56.7 ± 54.0	58.9 ± 37.8	*
TCP	0.37 ± 0.89	0.19 ± 0.14	33.9 ± 32.9	24.0 ± 32.6	56.5 ± 28.0	19.3 ± 20.6	†
TBEP	0.19 ± 0.25	0.08 ± 0.14	54.2 ± 31.2	45.5 ± 37.3	40.4 ± 29.3	67.7 ± 21.4	*, †
Preservatives							
Methylparaben	6.88 ± 8.91	0.01 ± 0.02	99.7 ± 0.5	98.5 ± 0.5	99.7 ± 0.5	99.8 ± 0.5	*, ‡
Ethylparaben	3.35 ± 9.43	0.00 ± 0.01	99.4 ± 1.1	98.8 ± 0.5	99.4 ± 0.9	99.8 ± 1.5	*, †
Propylparaben	2.22 ± 2.60	0.00 ± 0.01	99.0 ± 4.7	97.8 ± 8.0	99.9 ± 0.4	99.9 ± 0.8	*
Disinfectant							
Triclosan	0.20 ± 0.47	0.02 ± 0.04	98.9 ± 2.5	99.9 ± 0.0	n.d.	98.4 ± 2.9	*

total sample size: n = 62; naproxen, salicylic acid: n = 47, triclosan: n = 31; n.d. not detected; - no measurement;

* effluent significantly lower than influent ($p < 0.05$)

† significant differences between the different HRTs ($p < 0.05$)

‡ significant differences between removal efficiency and temperature ($p < 0.05$)

3.2.2. Overall micropollutants removal efficiency

The removal of micropollutants by vertECO as a function of HRTs is presented in table 3.2. As previously mentioned, there is very limited literature on the removal of many micropollutants, in particular on EDCs, in greywater matrix. Moreover, greywater treatment by means of wetlands are rare and, in most cases, only comparison with wastewater treatment was possible. No difference in removal efficiency was found as a function of high or low touristic season for the studied PhACs and EDCs ($p > 0.05$).

Extremely high removal efficiencies ($> 98\%$) were generally observed for acetaminophen, ibuprofen and salicylic acid (Fig.3.2.2.). Similar results were also obtained by HF wetlands treating grey- and wastewater (Ávila et al., 2013; Gattringer et al., 2016; Ranieri et al., 2011). Removal of acetaminophen and ibuprofen is mostly accomplished by biodegradation processes under aerobic conditions (Matamoros et al., 2012; Ranieri et al., 2011), as it is the case of vertECO. Salicylic acid is found to be easily degraded in all surface and subsurface flow systems in high redox potential environments (Hijosa-Valsero et al., 2010). Macrophytes and/ or their root surface associated microorganisms appear to have an impact in the removal of acetaminophen, ibuprofen and salicylic acid, as it has been demonstrated in several studies of planted and unplanted reactors (Ávila et al., 2010; Matamoros and Bayona, 2006; Ranieri et al., 2011; Ternes et al., 2004). In addition, due to its alkaline nature, LECA is a good sorbent for acidic pharmaceutical compounds (such as salicylic acid) through electrostatic interactions (Dordio et al., 2009).

High removal efficiencies could be achieved for atenolol (93%), diclofenac (88%) and trimethoprim (91%). In the case of atenolol an alkaline, positive charged compound, strong sorption can occur through ion exchange with LECA (Dordio et al., 2009). Diclofenac removal in the literature is often marked by inconsistent and contrasting results but mostly associated to biodegradation (Ávila et al., 2013). According to the literature, HF wetlands showed diclofenac degradation up to 52%, while higher removal efficiencies (65-96%) were only reported in hybrid systems (Hijosa-Valsero et al., 2010). In vertECO the higher removal of diclofenac could be related to the high oxidation status of the wetland (Hijosa-Valsero et al., 2010), probably due to the additional aeration and oxygen release in the rhizosphere by the plant roots of certain species (Stottmeister et al., 2003). Trimethoprim, finally, seems to be easily eliminated in HFCWs by microbial degradation (Hijosa-Valsero et al., 2011).

Lower removal efficiencies, in the range 50-70% were observed for ketoprofen, metoprolol, naproxen, iopromide and carbamazepine, while hydrochlorothiazide was only 28% removed. Ketoprofen has a similar structure to ibuprofen, but is more recalcitrant to degradation due to the keto group and the extended aromatic rings (Camacho-Muñoz et al., 2012; Matamoros et al., 2009). Concerning the chemical structure and the functional groups, similar conclusions can be drawn for naproxen, hydrochlorothiazide and metoprolol. Carbamazepine is usually a very persistent compound and recalcitrant to biodegradation (Matamoros et al., 2009). Here, however an average removal of 56% was observed, probably due to extreme variability in the influent concentration and/ or sorption capacity of the substrate (Tixier et al., 2003). Finally, negative removal values were obtained only for salbutamol and in some cases for sulfamethoxazole. This could be attributed to release phenomena during the treatment (Anjos et al., 2018), low observed concentrations and high influent variability, or not time adjusted sampling according to the HRT (Roberts and Thomas, 2006).

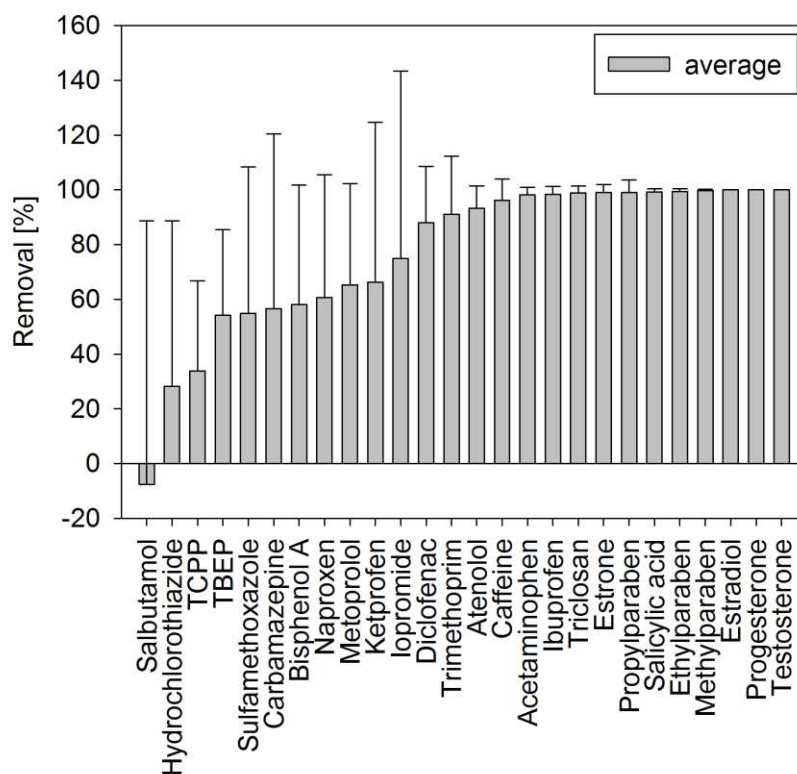


Figure 3.2.2. Total Average (AV) Removal (%) of micropollutants during long term monitoring

Regarding the EDCs and related compounds, extremely high removal (> 99%) were observed for estradiol, progesterone, testosterone, methyl-, ethyl- and propylparaben and high removal (> 96%) for estrone, caffeine and triclosan. Estrogens and progesterone are reported to be degradable in both, aerobic and anaerobic environments, as well as removed by adsorption (Froehner et al., 2011; Vymazal et al., 2015). A recent study investigated estrone and estradiol under different conditions and observed the benefit of aerobic conditions to degrade these compounds (Dai et al., 2016), which is the case of vertECO due to the artificial aeration. Only little literature is generally available on the removal of estrogen, progesterone and testosterone by means of constructed wetlands. The three preservatives (methyl-, ethyl- and propylparaben) also showed an extremely high removal rate (> 99%), mostly related to biodegradation processes. Caffeine is commonly an easy biodegradable substance and it is therefore often chosen as a reference compound (Matamoros and Bayona, 2006). Triclosan, mostly used as antibacterial agent for hygiene and personal care products, can be removed by both substrate and plant sorption as well as biodegradation, as previously reported in HF wetlands (Carranza-Diaz et al., 2014; Hijosa-Valsero et al., 2010). Plasticizers like bisphenol A, TCPP and TBEP were characterized by smaller removal (58%, 34% and 54%, respectively). According to the literature, plasticizers higher in the outflow than in the inflow are often reported, but the reason is sometimes still obscure (Ávila et al., 2010). In the present study, however, the lower removal rate of these compounds may be related to some release from plastic parts, valves, pipes and other technical devices.

In terms of water reuse, to be remarked that no legislation is available at European levels regarding the here studied organic micropollutants, although some of them (including estrone and estradiol) are now included in the EU Watch List 2018/840 (EC, 2018). This European Decision states that the listed contaminants have to be monitored across Europe to obtain high quality data on their occurrence, to inform further legislative action, and the present paper contributes in this direction.

3.2.3. Micropollutants removal under different condition

Influence of hydraulic retention time (HRT)

As previously mentioned, higher removal efficiencies are usually reported at higher HRTs (Matamoros et al., 2008). Conversely, in the present study, higher removal

efficiencies were observed for acetaminophen, atenolol and ibuprofen at the lowest HRT ($p < 0.05$) (Fig.3.2.3.), even though with average removal rates always higher than 88% at all tested HRTs. This could be related to the fact that decreasing HRTs were tested during the experimental periods. Denser root systems and more retained solids in the system were more likely present at lower HRTs. It can be consequently speculated that the high planting density (28 plants per m^2), the growth of the plants and the increase in root density in the rhizosphere, with consequently extended contact surface between pollutants and plant roots, compensated the decrease in HRT. HRTs had no significant influence on the efficiency of PhACs removal. A similar pattern was observed for EDCs, with no influence of HRT on the removal efficiencies ($p > 0.05$) for most compounds except for ethylparaben, TCP and TBEP (higher removal at lower HRT).

To be remarked that no data are available in the literature on the correlation between micropollutants removal and HRT in terms of greywater treatment by means of constructed wetlands. Moreover, wetlands for wastewater treatment usually apply higher HRTs (e.g. 3.5 - 5.5 days; Ávila et al., 2010; Carranza-Diaz et al., 2014; Hijosa-Valsero et al., 2010). This confirms the relevance of the results presented here regarding the removal of organic micropollutants with short HRTs (1.0 - 1.9 days, table 2.2), and the resulting reduced needed footprint.

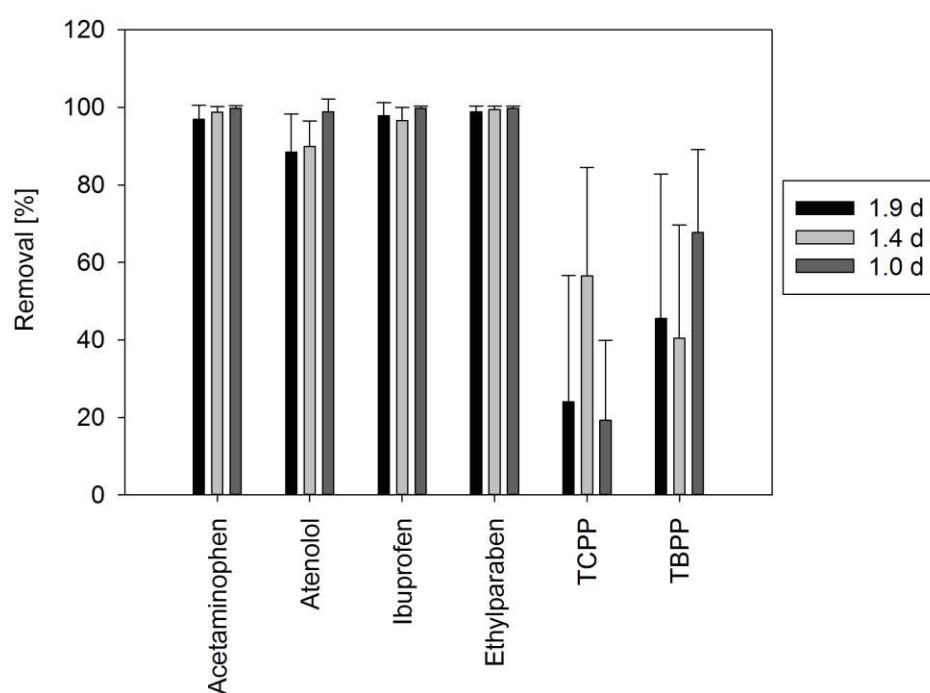


Figure 3.2.3. Micropollutants Average (AV) removal (%) and STDEV according to the three tested HRTs

Influence of temperature

With the exception of hydrochlorothiazide for PhACs and methylparaben for EDCs ($p < 0.05$), no significant influence of temperature on the removal of micropollutants was observed ($p > 0.05$).

According to the literature, temperature is reported to influence the removal of micropollutants (e.g. caffeine, naproxen and salicylic acid, Hijosa-Valsero et al., 2010). The optimal activity of microorganisms in CWs is favored by warm temperatures of 15 – 25 °C, which was the case in our study for most of the experimental time. vertECO, in fact, was semi indoor, also due to some tropical plant species. Consequently, the temperature difference among the seasons was limited and the average never below 15°C (Table 2.2.), resulting in an overall good and constant performance. Moreover, it is reported that the effect of lower temperature on the removal efficiency is higher in superficial flow than in sub-superficial flow CW (Verlicchi and Zambello, 2014).

4. Conclusions

vertECO was confirmed as an effective nature-based option for decentralized greywater treatment and reuse, meeting the requirements of the Spanish legislation and European draft for water reuse for many purposes.

Extremely high concentrations and variability were observed in the influent greywater in terms of organic micropollutants, most likely due to the low dilution. vertECO confirmed to be highly effective in removing many PhACs and EDCs. Other compounds (ketoprofen, naproxen, carbamazepine, metoprolol, sulfamethoxazole and hydrochlorothiazide) show more persistency and, in the case of bisphenol A, TCPP and TBEP, likely related to the release of plastic valves and technical devices. Higher removal efficiency could be established at lower HRT for TN, TKN, $\text{NH}_4^+\text{-N}$, acetaminophen, atenolol, ibuprofen, TCPP and TBEP. The increase in root density in the subsurface might have compensated the HRT variance.

Temperature had a limited influence and only correlated with BOD_5 , hydrochlorothiazide and methylparaben removal; most likely because vertECO was

semi-indoor and sub-superficial flow.

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