Removal of organic carbon, nitrogen, emerging contaminants and fluorescing organic matter in different constructed wetland configurations

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ABSTRACT

The elimination of organic carbon, nitrogen, five emerging organic contaminants (EOCs) and fluorescence signature was evaluated in two treatment lines comprising different constructed wetland (CW) configurations: (i) partially saturated vertical subsurface flow (SVF) wetland (treatment line 1) and (ii) unsaturated vertical subsurface flow (UVF), horizontal subsurface flow (HF) and free water surface (FWS) wetlands in series (treatment line 2). Results showed important differences between the different CW configurations. The highest removal of BOD_5 (81%), COD (67%), TOC (72%) and fluorescing organic matter were observed in the UVF wetland, whereas the HF and FWS wetlands were the most efficient units for total nitrogen removal (60 and 69%, respectively). The SVF wetland showed a greater performance in the reduction of total nitrogen than the UVF bed (52 vs 35%). In addition, the SVF wetland exhibited a higher removal of the EOCs caffeine (95 vs 90%), trimethoprim (99 vs 87%) and sulfamethoxazole (64 vs 4%), as opposed to DEET (34 vs 63%), whose removal was superior in the UVF unit. Sucralose was negligibly removed in all the CWs. PARAFAC analysis of fluorescence measurements revealed that the proteinaceous tryptophan-like fluorescent component was the most highly removed one in all the investigated CWs (>28%) and, particularly, in the UVF wetland (66%), whereas humic and fulvic-like components resulted recalcitrant to decomposition. Increases of fluorescence intensities were often observed for fulvic-like substances in CWs operating with saturation of the bed, and these were particularly relevant in the SVF unit. Finally, important correlations ($r>0.7$) between the tryptophan-like fluorescent component and the water quality parameters COD and BOD_5 suggest fluorescence spectroscopy as an useful monitoring tool for water treatment efficiency in CW systems.

Keywords: pharmaceutical and personal care products, vertical subsurface flow, partial saturation, fluorescence spectroscopy, PARAFAC, real-time monitoring.

1. Introduction

Constructed wetlands (CWs) are engineered systems that emphasizes the physical-chemical and microbiological processes occurring in natural wetlands in a controlled manner to treat wastewater [1]. The use of this technology has developed rapidly over the last decades for the treatment of wastewater in decentralized areas both in industrialized and low-income countries due to their various advantages over conventional wastewater treatment systems. These include low to no energy consumption, ease of maintenance and operation, and good integration into the landscape and promotion of biodiversity, among others [2,3].

CWs have exhibited a great capacity to degrade contaminants from many different origins and also fecal microbial indicators, oftentimes complying with the requirements for potential reuse applications [4]. The removal of emerging organic contaminants (EOCs), including pharmaceuticals and personal care products (PPCPs), and other priority substances has also been lately explored, displaying a remarkable degradation capacity, mainly owed to the complex microbial interactions occurring within the bed media and the rhizosphere promoted by a large range of redox conditions [5–8]. However, more studies are still needed for a complete and thorough understanding of the behavior of these micropollutants in CW systems, which should also shed some light into the transformation pathways of these contaminants in treatment systems and the environment.

Despite CWs have been traditionally considered as ‘black boxes’, the research and technical development in the technology over the last decades has shown that the system’s design and operation parameters influence the dominating environmental conditions inside the wetland, which, in turn affects degradation processes [9,10]. While dissolved oxygen concentration is one of the main limiting factors for biodegradation processes in traditional horizontal subsurface flow CW (HF) operating mostly under anoxic/anaerobic conditions due to the permanent saturation of the wetland

bed, unsaturated vertical flow CW (VF) units were developed to increase the oxygen transfer capacity by specific design and operational conditions, such as intermittent feeding and resting periods [1,11]. Various design and operational alternatives have been proposed to improve the performance of CWs, comprising from the use of hybrid systems (VF and HF wetlands operated in series) to other strategies or intensifications that involve the use of induced energy through active aeration, increased pumping or recirculation [10]. Recently, the use of a saturated zone at the bottom of a classic VF wetland aims at creating anaerobic/anoxic conditions in the lower part of the bed, and aerobic conditions in the top part, so as to increase the microbial diversity in the wetland and promote various contaminant removal pathways. This strategy is especially targeted to enhance the removal of total nitrogen through the promotion of the nitrification-denitrification (NDN) processes [12]. Although presumably no studies have yet evaluated the removal of PPCPs in saturated VF wetlands, their transformation might also be benefited by the strategy and their behavior should be addressed.

On the other hand, the monitoring of wastewater treatment plants, including CWs, and its compliance with regulatory standards is generally assessed using physical, chemical and microbiological tests. Among these techniques, reliance is often placed on biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC) [13]. However, these parameters depend on time-consuming methods, offering only snapshots of moment in time, which makes them unsuitable for online monitoring [14]. An alternative promising approach is the use of fluorescence spectroscopy, which could be used for wastewater quality assessment as a tool for discharge detection in natural systems and for a continuous process control during wastewater treatments [15–17]. Fluorescence spectroscopy is a rapid, cost-effective, reagentless technique that re-

quires little or no sample preparation prior to analysis. The acquisition of 3-dimensional excitation−emission matrices (EEMs) provides a 'map' of contributions of different component classes comprising dissolved organic matter (DOM). This has resulted in the development of fluorescence indexes that have been shown to be useful indicators of water treatment efficacy [15–18], or surrogate parameters useful for monitoring the fate of EOCs during conventional and advanced wastewater treatments [19–23]. However, to the best of our knowledge, there are no studies dwelling on the use of fluorescence measurements as indicator of treatment performance and EOC surrogate in CW systems.

In the current study, two CW treatment lines operating in parallel were evaluated for a period of 4 months. One of the lines consisted of a partially saturated vertical subsurface flow wetland (SVF). The other line consisted of a typically unsaturated VF wetland (UVF), which was followed by a HF and a free water surface (FWS) wetland in series. The objectives of this study were: (i) to investigate the removal of conventional water quality parameters, EOCs and fluorescing organic matter in the two treatment lines, with special emphasis on the comparison of the two VF wetlands; (ii) to examine possible relationships between the removal of fluorescence indices, conventional water quality parameters and EOCs in the wetlands and (iii) to evaluate the suitability of fluorescence measurements to produce indices that are effective indicators of water treatment efficiency and/or EOCs surrogate parameters for real time monitoring in CW systems.

2. Materials and methods

2.1. Chemicals and reagents

All purchased solvents, standards, and reagents were of high purity. The details concerning these materials are reported in the Supplementary material section (Text S1). The selection of PPCPs

analyzed in this study (i.e., caffeine, trimethoprim; sulfamethoxazole, N,N-diethyl-meta-toluamide –DEET-, sucralose) was based on data presented in previous literature that rely on chemical-physical properties, occurrence data, detection frequency, availability of robust analytical methods and removal during wastewater treatments [24,25]. Detailed information about all target analytes used in this study is indicated in Table S1.

2.2. Wastewater treatment plant

The experimental treatment plant was set outdoors at the facilities of the GEMMA group (Department of Civil and Environmental Engineering of the Universitat Politècnica de Catalunya-BarcelonaTech, Spain) in a Mediterranean climate. It was commissioned in 2010 and since then it has been continuously operated and monitored as a hybrid system applying increasing hydraulic loads to assess its treatment capacity [25–27]. During the period of this study (April to July 2016) the configuration of the treatment plant was slightly modified, including two treatment lines operating in parallel (Fig. 1). Firstly, the urban wastewater was collected from a nearby sewer and conveyed to a continuously stirred storage tank with negligible effects on water quality (1.2 m^3). Subsequently, the wastewater was conducted to an Imhoff tank (0.2 m^3; hydraulic retention time = 12 h), from where it was pumped to the two treatment trains. The two lines contained a VF wetland as first stage of the secondary treatment (1.5 m^2). However, while Treatment Line 1 included a VF wetland whose filter bed was partially saturated (SVF) (0.35 m saturated out of 0.8 m) with the purpose of testing possible contaminant removal improvement, Treatment Line 2 consisted of a typically unsaturated VF wetland (UVF), which was followed by a HF (2 m^2) and a FWS (2 m^2) wetland in series. The treatment system was very mature at the time of this study and had been working under this configuration for 3 months before sampling took place. The flow applied to each treatment train was of 200 L d^{-1}, implying on the VF beds a mean organic loading rate (OLR)

of 40 g COD m^{-2} d^{-1} and hydraulic loading rate (HLR) of 133 mm d^{-1}. Feeding of the VF beds was done intermittently and simultaneously by means of pumps and distribution pipes on the top that provided about 8 pulses a day. Both wetlands were operated in a continuous mode -as opposed to previous operational periods where the two units alternated feed-rest cycles of 3.5 d [25]. An electromagnetic flow meter (SITRANS F M MAGFLO®) was installed before each wetland unit so as to assist on the follow up of the flow values entering the treatment system. All CWs were constructed on polypropylene and were planted with *Phragmites australis,* which was very well developed at all wetland units at the time of this study. Further CW features are detailed in Table S2.

2.3. Sampling strategy

The treatment plant was monitored weekly at the same time (about 10 AM) for a period of 4 months from Apr to Jul 2016 (n=12). After measurement of on-site water quality parameters (i.e. temperature, pH, dissolved oxygen, electrical conductivity and redox potential), samples were collected from the raw influent and from the effluent of the Imhoff tank and the different CW units (Fig. 1). The collected samples were taken to the adjacent laboratory for the immediate analysis of conventional water quality parameters, whereas samples for the determination of PPCPs were stored in 500 mL amber glass bottles at 4°C after filtration at 0.7 μm (Whatman glass microfiber filter, Clifton, NJ). Solid phase extraction (SPE) for PPCPs quantification was carried out within 24h. Aliquots of filtered samples were also used for spectroscopic measurements, which were performed within few days.

2.4. Analytical methods

Onsite measurements of water temperature, pH, dissolved oxygen (DO) and electrical conductivity (EC) were taken by using a Checktemp-1 Hanna thermometer, a Crison pH-meter, Eutech Ecoscan

DO6 oxymeter and an EH CLM 381 conductivity meter. Redox potential (E_H) was also measured onsite by using a Thermo Orion 3 Star redox meter and values were corrected for the potential of the hydrogen electrode. The determination of chemical oxygen demand (COD), total suspended solids (TSS) and ammonium nitrogen (NH_4-N) was done following the standard methods [28]. Biochemical oxygen demand (BOD_5) was measured by using a WTW® OxiTop® BOD Measuring System. Nitrate and nitrite nitrogen (NO_x-N), orthophosphate phosphorus (PO_4-P) and sulfate (SO_4^{2-}) were analyzed using a DIONEX ICS-1000 chromatography system, whereas total nitrogen (TN) and total organic carbon (TOC) were determined using a Multi N/C (2100 S) analyzer.

Analysis of the target PPCPs was performed by an Agilent 6410 Triple Quadrupole LC/MS-MS at the University of Catania (Catania, Italy) according to the procedure reported by Sgroi et al. [22] and here described in Supplementary Material (Text S2, Table S3, Table S4, Table S5).

Absorbance measurements were performed with a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). Fluorescence data acquired by a Shimadzu RF-5301PC fluorescence spectrophotometer (Kyoto, Japan) were corrected as described in Sgroi et al. [22] and also reported in the Supplementary Material section (Text S3). Fluorescence intensities were produced in Raman unit (RU).

2.5. PARAFAC modeling

Parallel Factor (PARAFAC) analysis was carried out using the drEEM toolbox [29]. Non-negative constraints were applied for excitation and emission loadings. In accordance with Murphy et al. [29], several criteria were applied to a dataset of 71 EEMs to ensure the soundness of the PARAFAC modeling and to identify the number of fluorescence components: (i) examination of the core consistency, (ii) evaluation of the shape of the spectral loading, (iii) leverage analysis regarding the influence of a specific sample or certain excitation and emission wavelengths, (iv) residuals

analysis and (v) the split half analysis. Only one outlying sample emerged during the PARAFAC modeling, and it was needed to exclude part of the EEMs with excitation wavelength <240 nm that exerted disproportionate leverage on the model and impeded a correct model validation. A PARAFAC model with 5 components was validated. The fluorescence intensity at the maximum of each PARAFAC component was used as a specific fluorescence index.

2.6. Statistical data analysis

The T-test, which follows a Student's t distribution, was used to identify whether there were significant differences on removal for the investigated contaminants and water quality parameters between the SVF and UVF wetlands.

3. Results and discussion

3.1. General wastewater treatment performance

Average values as well as the removal efficiency of water quality parameters after each CW unit of the wastewater treatment system are shown in Table 1. Pollutant removal processes varied between the CWs, owing to the variations in the redox potential (E_H) provided by the design and operational parameters of each wetland unit (Table 1 and Fig. S1). In general, high E_H (from +250 to +700 mV) can offer oxidized conditions to promote aerobic nitrification, while lower redox potential furnishes reduced environments (+250 to -400 mV) to promote anaerobic methanogenesis and sulphates reduction [30]. Although the bulk water at all wetland outlets exhibited relatively low E_H values that resembled reduced environments, aerobic and anoxic zones always tend to coexist in the filter bed and rhizosphere of these systems, which can foster the occurrence of different routes of contaminant removal [31,32]. Particularly, the partial saturation of the SVF bed

lead to consistently lower E_H and DO values in the effluent compared to the UVF unit (Table 1), owing to the reduced oxygen transfer capacity. The pH remained close to neutrality in all CW units (6.9-7.5), displaying slight fluctuations.

The Imhoff tank removed 79% of influent TSS despite the large concentrations (356 ± 169 mg TSS L^{-1}). The further entrapment of solids was slightly higher in the UVF (76%) than in the SVF (67%), and the HF unit in series with the UVF diminished concentrations down to 5 ± 4 mg TSS L^{-1}. Moreover, the UVF outperformed the SVF in regards to organic matter removal, including BOD_5 (81% vs. 54%), COD (67% vs. 53%) and TOC (72% vs. 48%). The superior performance of this VF wetland configuration can be associated with the relatively higher redox conditions and oxygen availability occurring within its bed media. To this respect, anaerobic routes of organic matter removal are generally slower when compared to aerobic pathways [33]. In treatment Line 2, the HF wetland showed high removal of easily biodegradable organic matter (79% BOD_5). Neverthless, the FWS wetland showed in general a poor performance similarly to its prior operational period, presumably related to the senescence of the plant biomass in this unit, which caused the release of solids and organic compounds from the rhizosphere to the water column [25]. Overall removal efficiencies for Treatment lines 1 and 2 were of 93 and 97% for TSS, 64 and 95% for BOD_5. 56 and 70% for COD, respectively.

The transformation of the different nitrogen species varied within the different CW units of the treatment system (Fig. 2). The saturation of the bottom part of the SVF wetland resulted in a lower removal of the sum of organic and ammonia nitrogen, which is in agreement with Saeed and Sun [33]. However, the negligible amount of NO_x-N in its effluent suggests that the denitrification of all previously nitrified ammonia was entirely taking place within this wetland unit. Overall, the partial saturation allowed significantly higher removal of TN in respect to the UVF wetland (52%

vs. 35%), demonstrating the enhanced capacity to remove this nutrient, while involving no additional energy or land requirements. Despite this, the high denitrification capacity exhibited by the HF and FWS wetlands (about 60% and 69% TN removal in HF and FWS) resulted in an overall removal efficiency of 93% for TN in Line 2. The high TN removal of the FWS during this study period was most likely promoted by the adverse conditions taking place in the unit, which decreased redox values in the water column, thus promoting the denitrification process (Table 1).

No orthophosphate reduction was observed in any of the CW treatment lines (Table 1). Instead, its concentrations increased in all treatment units, which could be majorly attributed to desorption and hydrolysis mechanisms [34]. The increment was particularly significant within the SVF wetland, which could be explained by the higher contact time of the water and the filter media, as observed by Dong and Sun [35]. Sulphate (SO_4^-) removal occurred only in the SVF wetland (51%), favoured by the occurrence of anaerobic/anoxic conditions and organic carbon in the bed media of this unit [33].

The T-Test, performed with a level of significance $\alpha = 0.05$, confirmed statistically significant differences on the removal of COD, BOD_5, TOC, TN and SO_4^- between the SVF and UVF wetlands (Table S6).

3.2. Behavior of pharmaceuticals and personal care products

Fig. 3 shows average PPCPs concentrations normalized by the influent values along the two treatment lines for the whole period of study. The concentration of the target compounds in ng L^{-1} for every collection day along the different treatment units can be observed in Table S7. The highly biodegradable compounds caffeine and trimethoprim were significantly removed in both VF wet-

lands, observing a slightly higher reduction in the SVF than in the UVF unit, especially for trimethoprim (99 vs. 87% in the SVF and UVF, respectively). The high removal of the stimulant caffeine in the VF wetlands (≥ 90%) is also in accordance with the high degradation capacity exhibited for this compound [8]. While trimethoprim, which occurred at very low concentrations in the UVF effluent, was not detected after the HF bed, the concentration of caffeine was reduced by 82% in this unit. Sulfamethoxazole was negligibly removed in all CW units (≤30%) of treatment line 2, which agrees with the poor degradation of this substance reported in the literature [8,25,27]. However, the SVF reactor showed an enhanced capacity for the removal of this antibiotic, achieving moderate removal efficiencies (64 ± 32%), as opposed to Treatment Line 2 (Fig. 3). A different trend was observed for the insect repellant DEET, which revealed superior elimination rate in the unsaturated UVF (63 ± 21%) than in the SVF (34 ± 35%). The low removal efficiency of this compound in the HF wetland (25 ± 45%) suggests that fully aerobic conditions might be important for its degradation, in agreement with previous studies [8,25]. The sweetener sucralose, which has been shown to be highly recalcitrant during biological treatments [22,36,37], was not degraded in any of the CWs. Finally, the FWS wetland exhibited limited removal of all the investigated PPCPs with the highest one observed for caffeine and sulfamethoxazole (about 25%), being in agreement with earlier sampling campaigns [27]. The poor performance of this wetland unit in respect not only of PPCPs but also conventional water quality parameters indicates that a higher retention time would be required for improved contaminant removal.

While the removal efficiencies of EOCs observed in the VF and HF wetlands are similar to those reported in previous studies with a generally higher performance in typically-unsaturated VF than in saturated HF wetlands, due to higher microbiological degradation under aerobic conditions [7,8,25,38], very surprising is the enhanced removal of some PPCPs (i.e., caffeine, trimethoprim,

sulfamethoxazole) in the partially saturated VF wetland. The occurrence of a larger redox range under conditions of partial saturation may enable complex microbial interactions under aerobic and anaerobic microenvironments or promote the presence of certain microbial communities that can influence the removal of some contaminants. For example, the removal of sulfamethoxazole has been shown to be influenced by the occurring nitrification and denitrification conditions, which influence the microbial population in the bed reactor [39,40]. In addition, the microbial community metabolic function has been demonstrated to be significantly different between different CW designs when treating domestic wastewater [41]. However, this is the first study investigating the removal of EOCs in a partially saturated VF wetland and further investigations are needed for a better understanding of the phenomenon.

The T-test confirmed statistically significant differences for the removal of caffeine, trimethoprim, sulfamethoxazole and DEET between the SVF and UVF wetlands, when performed with a level of significance $\alpha = 0.1$ (Table S6).

3.3. Fluorescent organic matter removal in constructed wetlands

Analysis of fluorescence EEMs can provide a qualitative estimate of the treatment performance of the different CW configurations as displayed in Fig. 4, where EEM fluorescence spectra of the CW effluents collected during a same sampling event are reported. A strong decolorizing effect can be observed in the EEM of the UVF wetland in agreement with the high removal of TOC, COD and BOD_5 observed in this unit. On the other hand, the EEMs of all the other CW effluents exhibited a reduction of the intensity of some fluorescence peaks, mainly in the region of the protein-like fluorescence, and an increase of other fluorescence peaks, such as fluorescence intensities related to fulvic-like and microbial product substances.

A useful tool for EEMs interpretation is PARAFAC analysis. This method, which is a technique of multivariate data analysis, enables the deconvolution of complex EEMs into independent components that represent groups of similarly behaving fluorophores. Such deconvolution helps discriminate and ascertain contributions of different DOM types, and by providing the fluorescence maxima intensity for the identified components it gives a basis for quantitative analysis of change in the composition of fluorescent organic matter during water treatment [18,29,42]. In this study, PARAFAC analysis identified 5 independently varying fluorescing components as reported in Table S8. The excitation and emission loadings of these fluorescent components, denoted henceforth as C1 – C5, are shown in Fig. S2. Fig. S3 shows the corresponding spectral fingerprints. Based on the position of the excitation and emission peaks, components C1 ($\lambda_{ex}/\lambda_{em}$= <240/415 and 310/415), C2 ($\lambda_{ex}/\lambda_{em}$= 245/440 and 350/440), and C3 ($\lambda_{ex}/\lambda_{em}$= 250/470 and 380/470) were identified as humic and fulvic-like fluorescence (Table S8). Specifically, component C1 represents the contribution of a microbial humic-like component, and component C3 that of terrestrial fulvic/humic-like fluorescence, as has been established in prior PARAFAC studies of surface water and wastewater [18,22,42–45]. Component C2 corresponds to a group of humic-like fluorescing species found in prior studies in high nutrient and wastewater impacted environments [18,42,43,46]. Component C4 ($\lambda_{ex}/\lambda_{em}$= <240/340 and 295/340) was relatively rarely reported in published literature and it was mainly associated with amino acids and biologically labile matter produced in aquatic environments [42,44,47]. Finally, protein and tryptophan-like fluorescing compounds observed in prior studies are associated with components C5 ($\lambda_{ex}/\lambda_{em}$= <240/330 and 275/330) [18,42–44,48].

In Fig. 5, changes in the fluorescent DOM along the different CW units were characterized by using the maxima intensities of fluorescent PARAFAC components normalized by the influent

values. The tryptophan-like fluorescence component C5 was the most highly removed component in all the investigated CW units, except for the FWS wetland, where this component exhibited an increase of 27 ± 47%. In this wetland unit, production of fluorescence intensities were observed for all the identified PARAFAC components, except for component C2, and it is in agreement with the increase in concentration of typical water quality parameters, such as COD and BOD_5, TOC, TSS, and probably related to plant senescence stage, which caused release of biodegradable organic matter in this unit. The highest removal (66±14%) for component C5 was obtained in the UVF wetland, which is the CW with the highest aerobic biodegradation potential, whereas moderate removal (around 30%) was observed in the SVF sat and HF wetlands (Fig. 5). The humic-like component C2 had moderate removal in both VF wetlands, which were the two wetlands with reaeration of the reactor bed, whereas negligible removals occurred in the HF and FWS units. This suggests a higher biodegradation capacity for this components under aerobic conditions as it was observed in a study conducted within a conventional wastewater treatment plant (WWTP) [49]. All the PARAFAC components related to soluble microbial products and/or fulvic-like substances (i.e., C1, C3, C4) resulted resistant to biodegradation processes and were not removed in all the investigated CW configurations unless excluding the moderate removals observed for component C1 and C4 in the UVF wetland (20-30%). Particularly, increases of fluorescence intensities were often observed for these three components in wetlands operating with saturation of the bed, and these increases were particularly relevant for component C3 in the saturated VF bed (Fig. 5).

Studies on the removal of fluorescent organic matter in CW systems are very limited and related to investigations of DOM removal in five CW beds in series and alternating biological ponds and plant gravel beds, and in a pilot-scale UVF unit [44,50]. Similarly to the results found in this work, protein and tryptophan-like substances, which represent the most biodegradable fraction of DOM,

revealed the highest removal, whereas lower elimination rates were observed for fulvic and humic-like fluorescence components [44,50]. Studies on DOM removal by fluorescence spectroscopy in conventional WWTPs have also reported the highest removal (40-99%) for fluorescing substances in the region of EEM with emission < 380 nm (i.e., protein, tryptophan-like and tyrosine-like fluorescence) and the observed removal rates were similar under aerobic or anoxic/anaerobic conditions [16,22,49,51–53]. On the contrary, lower removals (10-30%) were reported for fulvic and humic-like components and sometimes production of fluorescence intensities were observed for components sensitive to microbial activity under both aerobic and anoxic/anaerobic conditions [16,22,49,51–53]. It has been suggested that microbial product and fulvic-like fluorescence components are either potentially produced by microbial activity during the process or are recalcitrant to decomposition [14,53,54].

In this study, fluorescence components were removed or produced in different extent in different CW configurations. These results are in agreement with the hypothesis reported in literature of significant differences in microbial community structure and metabolic function between different CW designs (Barbieri et al., 2012; Button et al., 2015; Kassotaki et al., 2016; Pelissari et al., 2017a) and may justify the differences in removal capacity observed for conventional water quality parameters and PPCPs in the investigated wetlands.

The T-Test, performed with a level of significance $\alpha = 0.05$, confirmed statistically significant differences on the removal of the PARAFAC component C1, C3 and C5 between the SVF and UVF wetlands (Table S6).

3.4. Correlation analysis between the removals of fluorescence, PPCPs and conventional wastewater quality parameters in constructed wetlands

In order to assess the capability of fluorescence spectroscopy to act as monitoring tool of water treatment processes, it is important to consider the correlations between fluorescence signals and BOD_5, COD and TOC, commonly used indicators of organic matter concentration in natural water and wastewater. While studies on wastewater quality monitoring using fluorescence spectroscopy in CW systems are inexistent, researches carried out in conventional WWTPs have shown the existence of correlations between fluorescence indexes in the region of tryptophan-like and humic-like fluorescence and the water quality parameters BOD_5, COD, TOC [15,16,55]. However, in these studies stronger correlations were observed when using protein and tryptophan-like fluorescence indexes, as opposed to humic-like fluorescence [15,16,55].

In the present study, good correlations ($r > 0.7$) were observed between tryptophan-like fluorescence index (component C5) and the water quality parameters COD and BOD_5 (Fig. 6) confirming for CWs and these water quality parameters the same relationships observed in conventional WWTPs. On the contrary, very weak relationships were observed when correlations were investigated between fluorescence PARAFAC components and PPCPs. The correlation with the highest Pearson correlation coefficient value among the selected PPCPs was observed for DEET, which exhibited moderate removal during biological processes, and the PARAFAC component C4 (Fig. 6). No correlations were observed using UV absorbance at 254 nm.

4. Conclusions

The removal of conventional water quality parameters, selected pharmaceutical and personal care products and fluorescent organic matter was evaluated in two treatment lines comprising different CW configurations. After settling in an Imhoff tank, urban wastewater was treated by a partially saturated VF wetland in the first treatment line, whereas it was pumped to unsaturated VF, HF and

FWS wetlands in series in the second treatment line. Results of twelve periodic monitoring campaigns conducted for four months showed important differences in the removals of the investigated substances between the different CW designs. Particularly:

- The water quality parameters COD, BOD_5 and TOC showed a higher removal in the UVF than in the partially saturated VF wetland due to the occurrence of a higher volume of oxygenated media. However, the SVF wetland exhibited a greater performance in the reduction of total nitrogen, producing an effluent with negligible concentration of oxidized nitrogen species (NO_x).
- The HF wetland, which operated under anoxic conditions, revealed moderate and low removal of TOC and COD, respectively, but it was highly efficient for TN elimination. While relevant concentration of NO_x were still present in the effluent after the HF wetland, the FWS wetland was very efficient on their conversion into nitrogen gas through denitrification.
- The highly biodegradable PPCPs caffeine and trimethoprim were almost completely removed in both VF wetlands, exhibiting slightly higher relimination in the SVF than in the UVF wetland, as opposed to DEET that showed superior removal in the unsaturated unit. Sucralose and sulfamethoxazole were negligibly removed in all the CW wetlands, except for the enhanced removal capacity of the antibiotic in the SVF bed.
- PARAFAC analysis was performed to investigate changes in the composition of fluorescent organic matter during wastewater treatment in CW systems. The identified fluorescence components showed a different removal behaviour depending on the CW configuration and the highest removal was always observed in the UVF wetland. Particularly, the proteinaceous tryptophan-like fluorescent was the component exhibiting the highest

removal, whereas humic and fulvic-like components resulted recalcitrant to decomposition. Furthermore, increases in fluorescence intensities were observed for fulvic-like substances in wetlands operating with saturation of the bed, and these increases were particularly relevant in the SVF wetland.

- Significant correlations ($r > 0.7$) between the proteinaceous tryptophan-like fluorescent component and the water quality parameters COD and BOD_5 suggest the possibility to use fluorescence spectroscopy as monitoring tool for water treatment efficacy in CW systems.

Supplementary data

Texts S1 – S3, Table S1 – S8 and Figures S1 – S3. This material is available free of charge.

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Tables

Table 1. Conventional water quality parameters (average ± s.d.) at the different sampling points of treatment lines 1 and 2 (n=12). Average removal efficiencies (%) are shown in parentheses.

Parameter	Influent	Imhoff tank	SVF wetland	UVF wetland	HF Wetland	FWS Wetland
T (°C)	20.4 ± 3.4	20.3 ± 3.2	20.8 ± 3.7	19.8 ± 3.7	19.9 ± 3.6	19.8 ± 3.2
EC (mS/cm)	2.0 ± 0.3	2.1 ± 0.4	2.6 ± 0.7	2.3 ± 0.4	2.7 ± 0.7	2.7 ± 0.5
DO (mg/L)	0.2 ± 0.3	0.4 ± 0.4	0.5 ± 0.3	2.1 ± 0.7	2.2 ± 1.3	0.8 ± 0.6
E_H (mV)	+33 ± 84	-43 ± 48	-16 ± 32	+107 ± 18	+131 ± 36	+69 ± 64
pH	7.7 ± 0.1	7.3 ± 0.2	6.9 ± 0.2	7.5 ± 0.2	7.2 ± 0.4	7.4 ± 0.1
TSS (mg/L)	356 ± 170	75 ± 28 (79%)	25 ± 12 (67%)	18 ± 11 (76%)	5 ± 4 (72%)	11 ± 10 (neg)
BOD_5 (mg/L)	368 ± 71	290 ± 75 (21%)	133 ± 24 (54%)	56 ± 27 (81%)	12 ± 3 (79%)	18 ± 3 (neg)
COD (mg/L)	300 ± 117	277 ± 74 (8%)	131 ± 49 (53%)	91 ± 36 (67%)	85 ± 40 (7%)	90 ± 56 (neg)
TOC (mg/L)	194 ± 81	88 ± 33 (55%)	46 ± 17 (48%)	25 ± 6 (72%)	17 ± 4 (32%)	31 ± 13 (neg)
TN (mg/L)	67 ± 9	62 ± 13 (7%)	30 ± 5 (52%)	40 ± 6 (35%)	16 ± 2 (60%)	5 ± 2 (69%)
NH_4-N (mg/L)	18.5 ± 4.0	21.2 ± 4.4 (neg)	5.0 ± 1.9 (76%)	4.9 ± 2.2 (77%)	1.1 ± 1.0 (78%)	1.6 ± 3.2 (neg)
NO_x-N (mg/L)	<LOD	<LOD	0.9 ± 0.8	28.5 ± 8.0	12.5 ± 5.1	2.4 ± 1.7
SO_4^{2-} (mg/L)	102 ± 11	91 ± 24 (11%)	45 ± 40 (51%)	113 ± 13 (neg)	125 ± 25 (neg)	118 ± 15 (6%)
P-PO_4^{3-} (mg/L)	4.3 ± 0.6	5.7 ± 1.0 (neg)	8.3 ± 2.3 (neg)	6.2 ± 1.2 (neg)	6.6 ± 1.8 (neg)	7.3 ± 1.6 (neg)

LOD = limit of detection
neg = negative removal

Figures

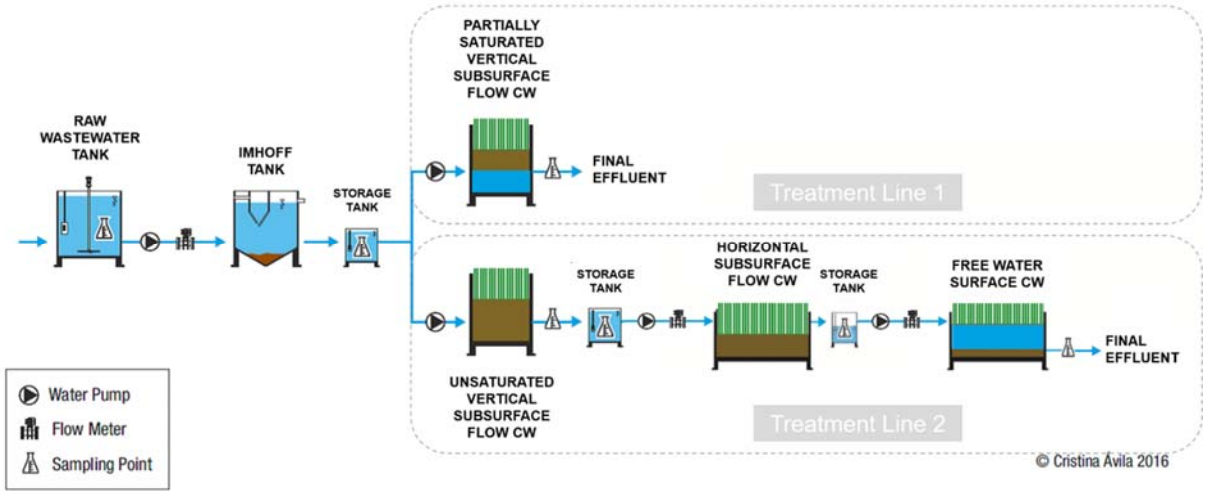

Figure 1. Diagram of the experimental wastewater treatment plant.

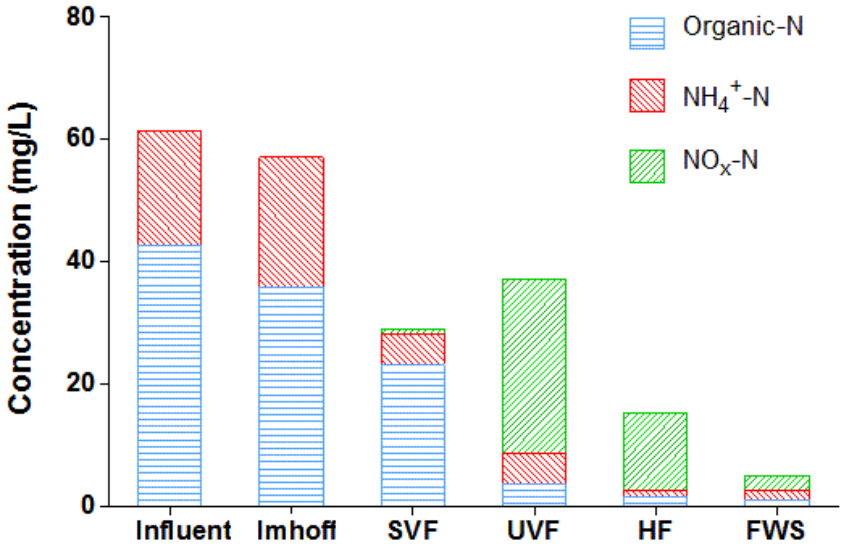

Figure 2. Average concentration of the different nitrogen species along the treatment trains of the constructed wetland system (n=12).

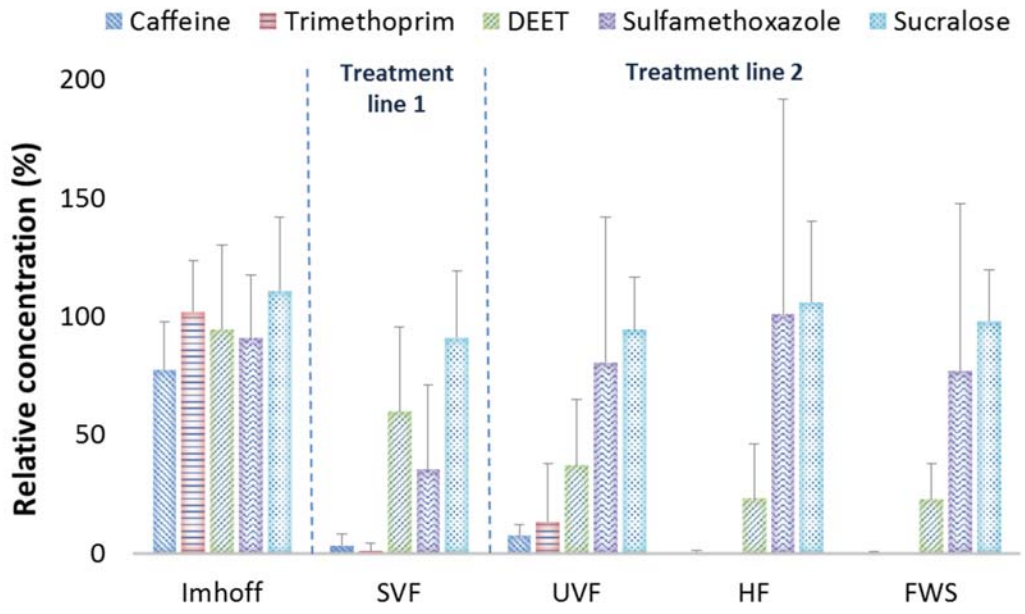

Figure 3. Average (±sd) concentration of target emerging organic contaminants normalized by influent values in the different units of treatment lines 1 and 2 (n=12).

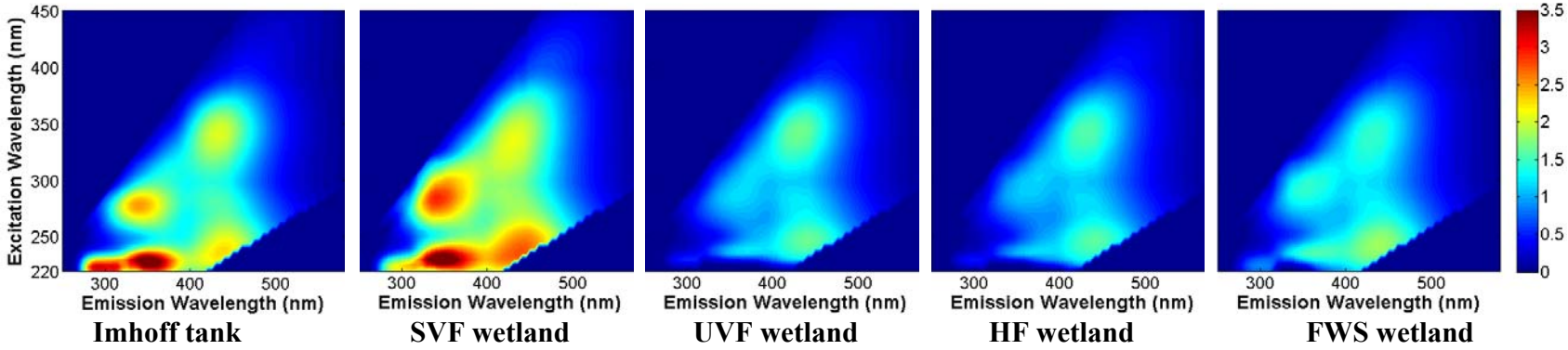

Figure 4. Fluorescence EEMs (RU) in the Imhoff tank and the different units of the treatment system for samples collected on May 30th 2016.

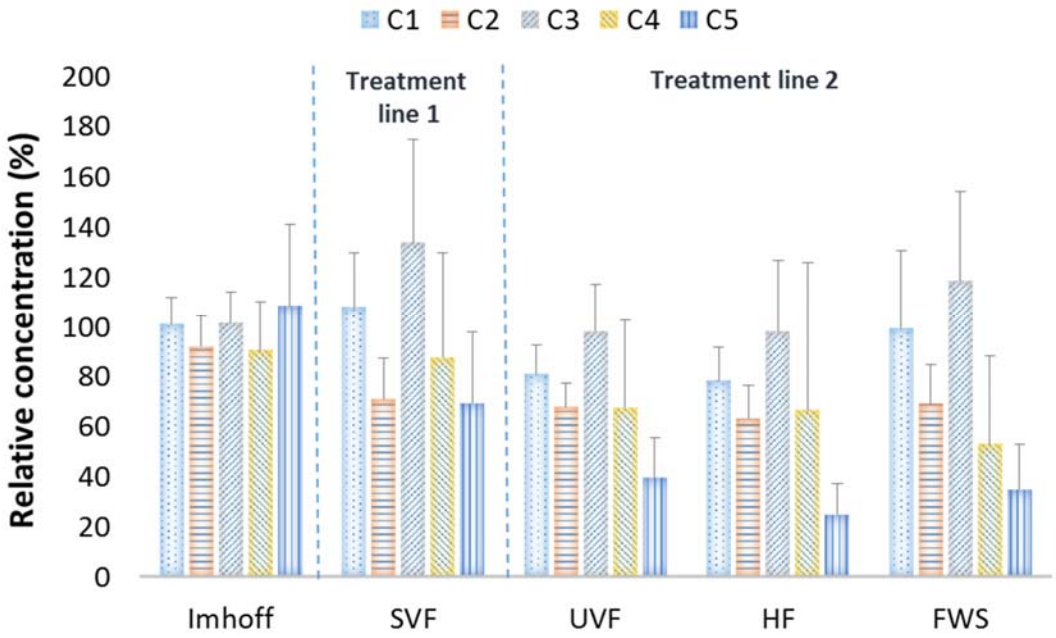

Figure 5. Average (±sd) of PARAFAC intensities normalized by influent values in the Imhoff tank and the different units of treatment lines 1 and 2 (n=12).

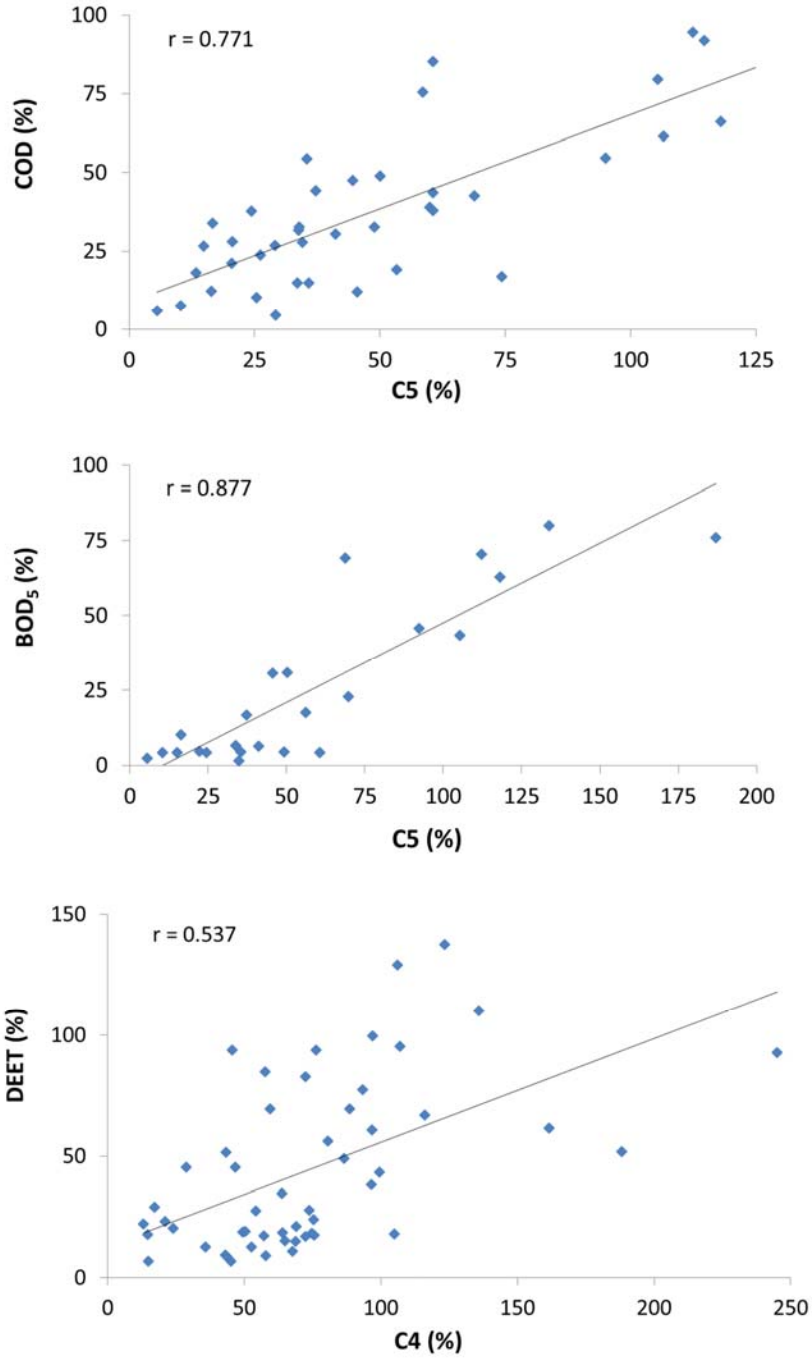

Figure 6. Examples of regression analysis between PARAFAC components and COD, BOD_5 and DEET (n=29-53). Relative concentrations normalized by influent values were used to produce correlation models.

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