Science of the Total Environment 817 (2022) 152518

Contents lists available at ScienceDirect

Science of the Total Environment

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

Tracking pollutants in a municipal sewage network impairing the operation of a wastewater treatment plant

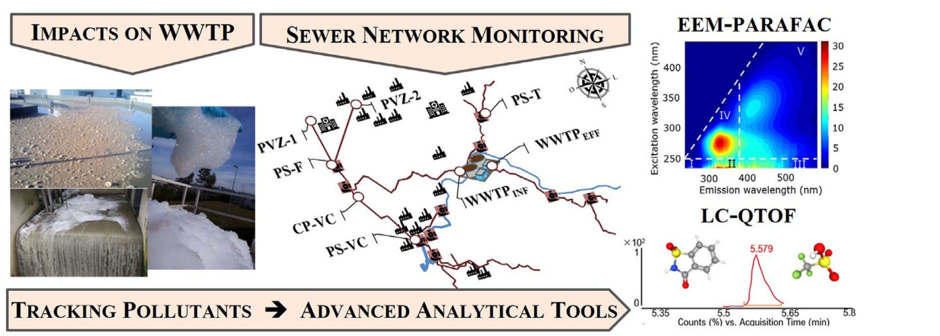
Mariana F.T. Sá [a,1], Verónica Castro [b,1], Ana I. Gomes [a,*], Daniela F.S. Morais [a], Rui V.P.S. Silva Braga [c], Isabel Saraiva [c], Bianca M. Souza-Chaves [d,e], Minkyu Park [d], Victoria Fernández-Fernández [b], Rosario Rodil [b], Rosa Montes [b], José Benito Quintana [b,*], Vítor J.P. Vilar [a,*]

[a] Laboratory of Separation and Reaction Engineering-Laboratory of Catalysis and Materials (LSRE-LCM), Departamento de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[b] Department of Analytical Chemistry, Nutrition and Food Sciences, Institute of Research on Chemical and Biological Analysis (IAQBUS), Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain
[c] Efacec Engenharia e Sistemas S.A. (Unidade de Negócios Ambiente), Rua Eng. Frederico Ulrich – Guardeiras, Apartado 3003, 4474-907 Moreira da Maia, Portugal.
[d] Department of Chemical & Environmental Engineering, University of Arizona, 1133 E James E Rogers Way, Harshbarger 108, Tucson, AZ 85721-0011, USA
[e] CNPq - National Council for Scientific and Technological Development, Brazil

HIGHLIGHTS

- Sewer network monitoring identified several organic pollutants.
- 111 surfactants from 10 families tentatively identified by LC-HRMS in the sewer network.
- Foam/biomass changes are associated with the presence of LAS/NPEOs in the $WWTP_{INF}$.
- WWTP performance impairment was tentatively associated to a food industrial area.
- Several CECs and HMW DOM components detected in the $WWTP_{EFF}$.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 October 2021
Received in revised form 26 November 2021
Accepted 14 December 2021
Available online 4 January 2022

Editor: Paola Verlicchi

Keywords:
Contaminants of emerging concern (CEC)
EEM-PARAFAC analysis
Industrial pollution hotspots
Suspect screening
surfactans
LC-QTOF

ABSTRACT

This work provides a screening of organic contaminants and characterization of the dissolved organic matter in the sewer network until the municipal wastewater treatment plant (WWTP), identifying the network areas with a higher degree of contamination and their impact on the WWTP performance, particularly in the activated sludge reactor. Three monitoring campaigns were carried out at six selected locations of the sewage system (PVZ-1, PVZ-2, PS-F, PS-VC, CP-VC, and PS-T), influent ($WWTP_{INF}$) and effluent ($WWTP_{EFF}$) of the WWTP. Advanced analytical techniques were employed, namely excitation/emission matrix fluorescence-parallel factor analysis (EEM-PARAFAC), size exclusion chromatography with organic carbon detector (SEC-OCD), and liquid chromatography with high-resolution-mass spectrometric detection (LC-HRMS). EEM-PARAFAC showed higher fluorescence intensity for the protein-like component (C2), particularly at CP-VC (near seafood industries) associated with the presence of surfactants (~50 mg/L). SEC-OCD highlighted the WWTP efficiency in removing low molecular weight acids and neutrals. LC-HRMS tentatively identified 108 compounds of emerging concern (CEC) and similar detection patterns were obtained for all wastewater samples, except for PVZ-2 (lower detection), many of which occurred in the effluent. Eight CECs included on relevant Watch-Lists were detected in all $WWTP_{EFF}$ samples. Furthermore, 111 surfactants were detected, the classes more frequently found being alcohol ethoxylates (AEOs), nonylphenol polyethoxylates (NPEOs) and linear alkylbenzene

* Corresponding authors.
E-mail addresses: ana.isabelgomes@fe.up.pt (A.I. Gomes), jb.quintana@usc.es (J.B. Quintana), vilar@fe.up.pt (V.J.P. Vilar).
[1] The first two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.scitotenv.2021.152518

sulphonates (LAS). The continuous presence of LAS and NPEOs allied to surfactants concentrations in the $WWTP_{INF}$ of 15–20 mg/L, with CP-VC location (linked with food industries) as an important contributor, explain the morphological changes in the activated sludge and high LAS content in the dewatered sludge, which may have impacted WWTP performance.

1. Introduction

Industrial effluents are known to contain a wide variety of pollutants, such as persistent organic pollutants (POP) and many other contaminants of emerging concern (CECs), culminating in a highly complex matrix (Saghafi et al., 2019; Yu et al., 2019). Even when legally regulated, the discharge of industrial wastewater into the municipal sanitation network may cause unexpected adverse effects on the performance of municipal wastewater treatment plants (WWTPs) (Purschke et al., 2020). WWTPs performance is routinely verified by assessing wastewater influents and effluents in terms of chemical and biochemical oxygen demand (COD and BOD, respectively), total nitrogen (TN), and phosphorous (TP) contents, among other current physicochemical parameters. These general wastewater quality parameters are normally required to overview legal compliance and are used as the basis for the design of WWTPs. Nonetheless, those parameters are indicative and do not provide detailed information on the properties of specific components of dissolved organic matter (DOM), nor the presence or identification of contaminants that reach the WWTP and that may impact the treatment performance, especially the biological treatment stage (Ignatev and Tuhkanen, 2019). Thus, to ensure the proper operation of WWTPs, it is essential to check the presence of (organic) microcontaminants and characterize DOM throughout the sewage network, allowing the detection of specific hotspots and the implementation of preventive measures at their source.

In this context, in recent years, advanced analytical tools have evolved to analyze the composition and structure of DOM, generally based on its molecular weight (MW) distribution, hydrophobicity, and optical properties (Wang and Chen, 2018). Three-dimensional fluorescence excitation-emission matrix (3D-EEM) spectroscopy is a relatively inexpensive and high sensitivity tool to characterize DOM constituents found in water systems. 3D-EEM has been widely implemented to detect the fluorescent properties of DOM in water samples and to differentiate its origins (e.g., humic acid-like, fulvic acid-like, soluble microbial products-like, etc.) (Baghoth et al., 2011; Goldman et al., 2012; Ishii and Boyer, 2012; Park and Snyder, 2018). When coupled with parallel factor (PARAFAC) analysis, a statistical method based on a multi-way spectral deconvolution algorithm, the complex fluorescence spectrum is decomposed into individual fluorescent components for both quantitative and qualitative analysis (Baghoth et al., 2011; Stedmon and Bro, 2008). Size exclusion chromatography in combination with organic carbon detection (SEC-OCD), broadly used to characterize apparent MW of DOM, has also been applied as an effective tool in following changes in the DOM distribution through wastewater treatment trains (Baghoth et al., 2011). Together, these advanced analytical tools can provide insight into the distribution and sources of organic contamination throughout the entire sewage system.

WWTPs are pointed among the main sources for CECs spread into the environment (Kroon et al., 2020; Rizzo et al., 2019), although generally there is a lack of regulation on the release of CECs via WWTPs (except in Switzerland, which demands 80% removal for 5 out of 12 selected CECs (Water Protection Ordinance, 1998). CECs include a wide range of chemicals (pharmaceuticals, personal care products, surfactants, plasticizers, industrial additives, and others) and most WWTPs were not designed to remove low levels of these contaminants, being only partially effective in their removal or degradation (Krzeminski et al., 2019). Beyond that, many of these contaminants, namely surfactants, can disrupt biological treatment systems, jeopardizing the WWTP performance and potentially increasing the release of these compounds to the environment. In this sense, it is recognized that new requirements for the Urban Wastewater Treatment Directive (Council Directive 91/271/EEC (1991)) should be adopted, namely the need to introduce specific measures to address CECs and/or their effects on wastewater systems (European Comission, 2019). Notwithstanding, so far, the monitorization of these contaminants in public sewage systems seems to have been overlooked. Given the fact that the list of (potential) CECs is already too large (and keeps on growing) to develop and implement dedicated quantitative analytical methods, (non-target) screening analysis has been proven to be a great alternative, particularly in complex matrices like wastewater, thus allowing a more comprehensive view of the existing contaminants. Currently, liquid chromatography (LC) in combination with high-resolution mass spectrometry (HRMS) instruments plays an important role in environmental analysis and quality assessment (Bader et al., 2016; Wilson et al., 2021). HRMS instruments, such as quadrupole-orbitrap and quadrupole-time-of-flight (QTOF) mass spectrometers, have shown high-performance detection capabilities for the screening of a wide range of targeted and untargeted analytes in wastewater samples from WWTPs (Assress et al., 2019; Cardoso et al., 2020; Wang et al., 2020).

The current work aims to make an integrated and comprehensive analytical characterization of DOM and identification of organic microcontaminants in the sewage network affecting the performance of a WWTP. An exhaustive physicochemical characterization of 24 wastewater samples was performed (three monitoring campaigns, each with eight sampling points: six throughout the sewer network, plus the WWTP influent and effluent). Advanced analytical tools such as 3D-EEM, SEC-OCD, and LC-HRMS were further employed to characterize DOM properties and origins and identify organic microcontaminants (particularly, surfactants) potentially related to the problems detected at the WWTP.

2. Materials and methods

2.1. Description of the sewer network

The studied area is located in the municipalities of Vila do Conde and Póvoa de Varzim, northern Portugal, and comprises a municipal WWTP and the respective sewer network (Fig. 1). Designed to serve up to 257,557 inhabitants (42,935 m^3 of wastewater per day, with an estimated 13% coming from industries), this WWTP currently serves a population of around 150,000 inhabitants (receiving ~17% of effluent produced by local industry). The WWTP scheme includes: (a) pumping station (Archimedes screws), to ensure a continuous and stable flow of wastewater for treatment; (b) pre-treatment (2-channel filter drum sieves and 3 rectangular degreasers), to remove large solids, sands, oils, and greases; (c) primary treatment (lamellar settlers); (d) secondary treatment (3 parallel independent lines), by activated sludge reactors and secondary settlers (with partial recirculation of the biological sludge); and (e) tertiary treatment, with a microtamisation filtration step (to ensure TSS $\leq$20 mg/L) followed by UV polishing step. The final effluent is partially reused for irrigating green spaces and washing floors and equipment. The produced sludge (primary and biological) is treated by gravitational and mechanical thickening, anaerobic digestion (with the recovery of biogas in a cogeneration unit), stabilization, and dewatering.

Sampling locations were strategically selected from the drainage sewer network that serves both municipalities, including the influent ($WWTP_{INF}$) and effluent ($WWTP_{EFF}$) of the WWTP. Wastewater samples were collected from the sewage pump stations of Touguinho (PS-T), Vila do Conde (PS-VC), and Forte (PS-F), and specific points at Vila do Conde (CP-VC) and Póvoa de Varzim (PVZ-1, PVZ-2) counties. PS-T is located near a hospital, while the urban surroundings of PS-VC and CP-VC sampling points include several food industries (mainly fish/cannery/frozen industries), some textile and metalworking. CP-VC is also a route to another pump station

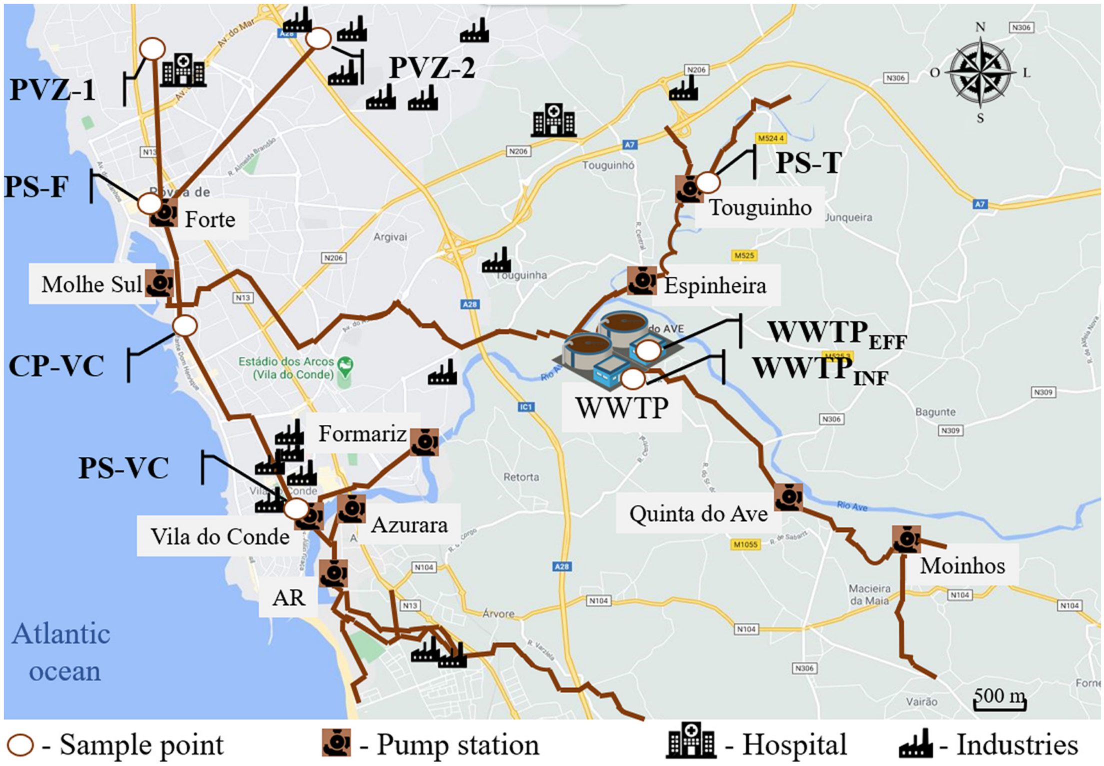


Fig. 1. Map representing the sewage network and sampling locations.

(Molhe Sul) that receives all the effluent from the city of Vila do Conde that reaches the WWTP. The sampling site PVZ-1 is in an urban area also close to a hospital, and PVZ-2 is located within an industrial network (mainly automobile and metalworking).

2.2. Sampling campaigns and collection

Three monitoring campaigns were carried out at 8 different sampling locations (Fig. 1), in consecutive weeks in the spring of 2019, comprising a total of 24 samples. All samples were collected using automatic samplers, allowing the collection of composite samples (hourly sampling over 24 h). Sampling campaigns 1 and 2 occurred under similar climatic conditions, with relatively dry and hot weather, while campaign 3 took place in a period of intense precipitation.

2.3. Determination of physicochemical parameters

All the analytical procedures used for the determination of diverse physicochemical parameters to characterize the wastewater samples were completed according to recognized international standards. The Zahn-Wellens biodegradability test (OECD protocol (EMPA, 1992)) was also applied to the collected water samples (further details can be consulted at Text SM-1, Tables SM-1 and SM-2). Each sample/parameter was analyzed in duplicate, and all reagents used were of analytical grade.

2.4. 3D-EEM, PARAFAC, and SEC-OCD analysis

UV spectra and excitation-emission matrix (EEM) fluorescence analysis were conducted using a fluorescence spectrophotometer (Aqualog, Horiba, USA). All EEM spectra were normalized by the Raman peak of ultrapure water to convert fluorescence data in Raman units (R.U.). A maximum intensity of 13.5 R.U. nm^2 was defined for comparative purposes. PARAFAC was conducted using the drEEM toolbox (downloaded at http://www.models.life.ku.dk/algorithms) and a four-component model was validated by the split-half analysis (Murphy et al., 2013; Sanchez et al., 2013). Additional information about EEM technique and PARAFAC analysis can be found in the Supplementary Material (Text SM-2).

Size distribution of DOM was measured using SEC separation applying a hydroxylated methacrylic polymer column (TOYOPEARL® HW-50S, Tosoh Bioscience LLC; 21 mm × 250 mm). High-performance liquid chromatography (HPLC) (Agilent 1290) hyphenated with an organic carbon detector (Suez GE Sievers M9 TOC analyzer) was employed to measure the organic size distribution. The eluent was prepared with 4 mM phosphate buffer (pH 6.8) and sodium sulfate with 96 mM ionic strength. 100 μL of injection volumes were employed for all analyzed samples.

2.5. LC-HRMS screening

All samples were solid-phase extracted in the University of Porto. The dried cartridges were shipped frozen to the University of Santiago de Compostela (Spain), and further desorbed and analyzed by LC-QTOF to perform a suspect screening of CECs (Castro et al., 2021; Wilson et al., 2021) and a selection of surfactants (Schymanski et al., 2014) combining data-dependent and data-independent acquisition. All positive detections were confirmed by a duplicate analysis and accounting for blanks. Further details are provided in the Supplementary Material (Text SM-3).

3. Results and discussion

3.1. Preliminary remarks

The municipal WWTP in this study came into operation in August 2010, under a wastewater disposal license and respective applicable legislation (Decree Law no. 236/98, 1998; Decree-Law n° 152/97, 1997), concerning emission limit values (ELV) and monitoring details). Until October 2015, no procedural problems were affecting the removal of organic matter (final discharge with BOD_5 < 25 mg/L and COD <125 mg/L), as well as compliance with the TSS parameter (<35 mg/L). Occasionally, however, episodes of inhibition of the nitrification stage, which impaired the nitrogen removal up to the desired values (15 mg N/L), were detected and reported. On these occasions, the monitoring data of the WWTP influent allowed the detection of a significant occurrence of saline intrusion phenomena, with conductivity values greater than 15 mS/cm. These conductivity spikes probably led to nitrifying bacteria inhibition. Another problem detected within that period was that, from time-to-time, a very significant amount of foam was formed, requiring the addition of anti-foam to minimize the impact on the receiving medium. This event, which was not related to high salinity, suggested the existence of another type of interference, possibly from an industrial source, that reached the WWTP through discharges into the drainage network. From October 2017, these episodes became increasingly frequent and acute, negatively affecting the performance of the treatment system in terms of BOD_5, COD, and TSS parameters. Sudden changes in the microbial community of the biological reactor were detected, such as the disappearance of the microfauna (i.e., protozoa), as well as the filamentous bacteria species that were endemic in the biomass of the WWTP (*Microthrix parvicella* and type 0092). Only the constant and significant impact of compounds with direct or indirect bactericidal/bacteriostatic effects can explain such a drastic occurrence. Also, foaming has become almost permanent during treatment, requiring the constant addition of anti-foam. Beyond that, the foam has been detected in the treated wastewater discharged by the WWTP (Fig. SM-1), although this was not observed during the period of this study. The presence of foam (white and clear, and with no indication of sludge contamination) indicated the action of surfactants (Collivignarelli et al., 2020), implying the need for a thorough analysis of this type of compounds.

3.2. Physicochemical parameters and biodegradability

During the monitoring period, the WWTP effluent ($WWTP_{EFF}$) complied with the legal requirements for discharge as regards COD, BOD_5, and TSS (<125, <25, and <35 mg/L, respectively, Tables SM-4 to SM-6). However, the total nitrogen values (~50 mg N/L) remained above the ELV, indicating the occurrence of constraints in the biological treatment. To evaluate the possible industrial pollution hotspots, several physicochemical parameters were analyzed in the wastewater collected throughout the sewer network that flows to the WWTP. The effluents from industrial (or services) facilities that are discharged into this sewer system must comply with the provisions imposed by the Regulation for the Public Service of Sanitation (2009) - ELV_{WW} (ELV for wastewater discharge into the sewerage). This regulation also establishes reference values for several parameters for an effluent to be classified as comparable to municipal wastewater (MWW). Taking this, Table 1 summarizes the main results for all the sewage samples.

The measured pH values (between 6.7 and 8.1) are considered typical of municipal wastewaters (MWW), contrary to the conductivity (from 1074 up to 2602 μS/cm) and TSS values (particularly in the $WWTP_{INF}$ (campaigns 2 and 3) and in the PVZ-1 site (campaign 3)) that exceeded the reference limits for MWW. For the parameters related to organic matter, the PVZ-1 site (nearby a hospital) also exceeded both COD and BOD_5 reference values at campaign 3. High BOD_5 values were also measured for CP-VC (near seafood industries) and $WWTP_{INF}$ samples in all campaigns. PVZ-1 and CP-VC had also high values for the oil and grease parameter, with greater relevance in campaign 3. Similar features were detected for the parameters of total nitrogen and total phosphorus, with values above typical MWW in locations PVZ-1, CP-VC, and PS-F. These events may be due to the existence of a hospital, restaurants/canteens, and food industries connected to those sampling locations. Sulfate concentrations above typical MWW values were measured in PS-VC (all campaigns) and CP-VC (campaigns 2 and 3). As for chlorides, high levels at all sampling points in the three campaigns, namely in PS-VC and CP-VC, were detected. The source of sulfates and chlorides can be associated with the saltwater intrusion phenomenon and/or some canning industries whose effluent is drained into the public sanitation network. High amounts of surfactants (particularly anionic, followed by non-ionic) were detected in almost all sampling locations (up to ~50 mg/L for CP-VC, at campaign 2 - Fig. 2), including in the $WWTP_{INF}$ (up to 6.1 and 13.5 mg/L, for non-ionic and anionic surfactants, respectively). In turn, low surfactant concentrations were observed at $WWTP_{EFF}$, suggesting that their loads were reduced during the treatment.

Table 1
Physicochemical parameters analyzed in the wastewater samples from the sewer network to the WWTP.

Parameter	Unit	ELV_{WW}[a]	MWW[a]	Compliance with MWW
pH		5.5–9.5	5.5–8.5	All samples complied.
Conductivity	μS/cm	3000	1000	All samples exceeded typical MWW values, except PVZ-1[b,c,d] and PS-F[d].
COD	mg O_2/L	1000	1000	PVZ-1 (1334[d])
BOD_5	mg O_2/L	500	400	CP-VC (660[b]; 540[c]; 600[d]) PVZ-1 (480[c]; 900[d]) $WWTP_{INF}$ (420[b]; 480[c]; 560[d])
TSS	mg/L	1000	350	PVZ-1 (934[d]) $WWTP_{INF}$ (569[c]; 415[d])
Oil and grease	mg/L	100	100	CP-VC (139[b]; 530[d]) PVZ-1 (392[d]) $WWTP_{INF}$ (193[d])
Total nitrogen	mg N/L	90	85	PS-F (95[c]) PVZ-1 (94[d]) CP-VC (99[c]; 104[d]) $WWTP_{INF}$ (101[c]; 105[d])
Total phosphorus	mg P/L	20	15	PS-F (22[b]; 34[d]) PVZ-1 (46[d]) CP-VC (21[d]) $WWTP_{INF}$ (22[d])
Sulfate	mg/L	1000	50	PS-VC (111[b]; 88[c]; 89[d]) CP-VC (75[c]; 70[d]) $WWTP_{INF}$ (58[c]; 55[d])
Chloride	mg/L	1000	100	PVZ-2 (165[b]) PS-F (102[b]) PS-VC (362[b]; 127[c]; 134[d]) CP-VC (230[b]; 158[c]; 198[d]) $WWTP_{INF}$ (104[b]; 120[c]; 147[d]).

[a] Emission limit values (ELV_{WW}) admissible for wastewater to be discharged into the sewer system and typical municipal wastewater (MWW) values for an effluent to be considered comparable to municipal wastewater (2009).
[b] Concentration values obtained for campaign 1.
[c] Concentration values obtained for campaign 2.
[d] Concentration values obtained for campaign 3.

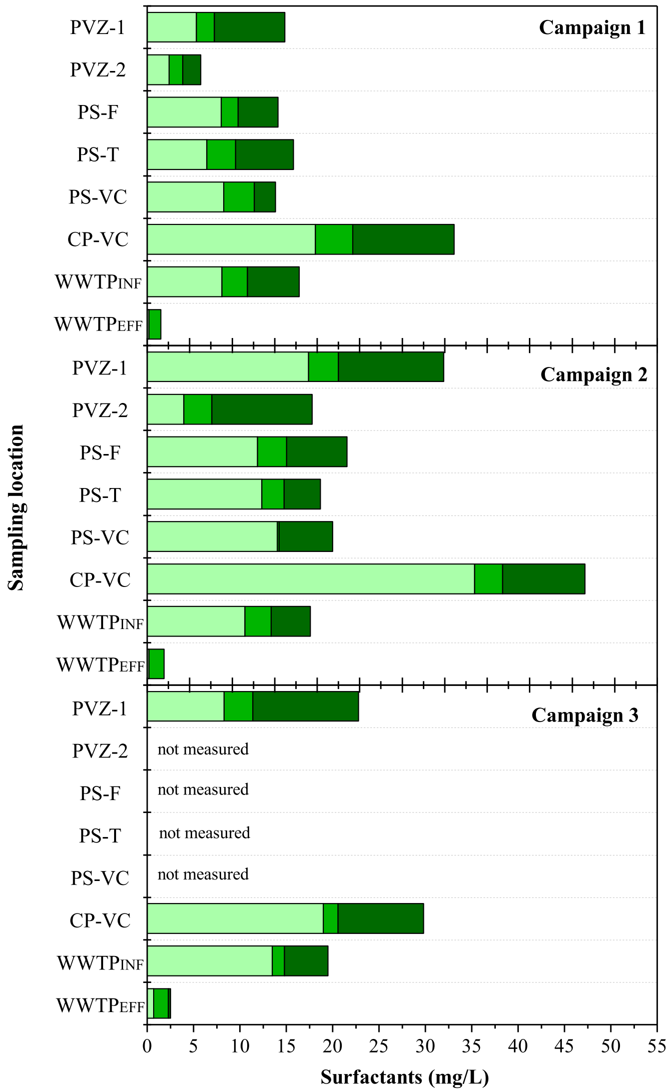


Fig. 2. Concentration values for surfactants (anionic, cationic and non-anionic) measured for all collected samples.

It is also noteworthy that in the sludge control analyses of this WWTP, concentrations between 4.7 and 9.7 mg LAS/g dry sludge were measured, reflecting a significant occurrence of LAS adsorption mechanisms in the activated sludge. Considering the various heavy metals (Al, B, Ba, Cu, Cr, Fe, Li, Mn, Pb, Sr, Zn) measured in campaign 1 (Table SM-7), Cu and Cr were below the detection limit for all samples. For the remaining elements, low concentration values were obtained for most samples and the $WWTP_{EFF}$ presented legal compliance for all analyzed parameters (Table SM-7).

The biodegradability of the wastewater samples was also evaluated through Zahn-Wellens 28-days biodegradability tests (Table SM-8). Although all sewer samples have been classified as biodegradable, the continuous addition of a pollutant load from CP-VC (which presented the highest surfactants levels) led to a modification in the sludge characteristics. From microscopic analysis (Fig. SM-2), filamentous and flagellate organisms were observed, as well as the disaggregation of biological flocs, which are clear indicators of increased difficulties on sludge settling and decreased efficiency for organic matter removal.

3.3. Organic matter characterization

3.3.1. 3D-EEM fluorescence analysis

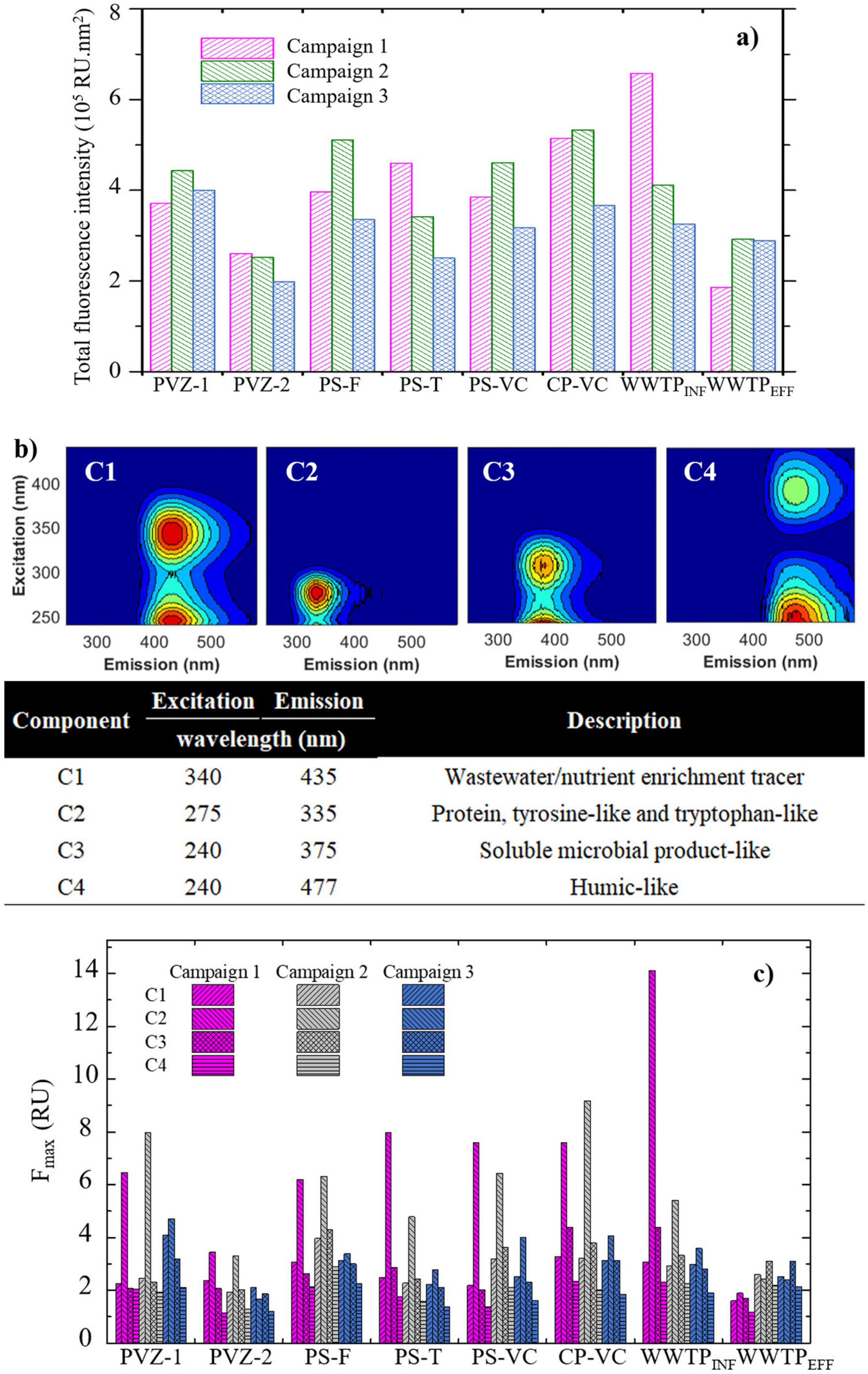
The total fluorescence (TF) values of the sewage network samples from campaigns 1 and 2 were higher than in campaign 3, which is likely associated to the rainy period (Fig. 3a). The EEM fluorescence values ranged from 1.9×10^5 R.U. nm^2 (PVZ-2, campaign 3) to 5.3×10^5 R.U. nm^2 (CP-VC, campaign 2). Compared to other sewer sampling locations, PVZ-2 presented the lowest fluorescence intensity, which agrees with the lower DOC and total surfactant concentration measured for this sampling point. This suggests that the wastewater discharge from the industrial complex surrounding PVZ-2 region may be complying with regulations in terms of organics (Table 1) and have a lower contribution to the organic content reaching the WWTP. In turn, the higher fluorescence intensity of the samples collected at CP-VC is likely associated to the high concentration of industrial organic contaminants due to the proximity to tinned fish and/or textile dyeing industries. The samples collected at sewage pump stations (centralized wastewater-discharge locations) also presented relatively high TF values. From all wastewater samples, the highest TF intensity was measured for the $WWTP_{INF}$ in campaign 1 (6.6×10^5 R.U. nm^2), which is likely due to the accumulation of the high organic content from the sewer network, and may also be related to the high surfactant concentrations in campaigns 1 and 2 since surfactants can interact with protein-like components and increase fluorescence trends (Maqbool and Hur, 2016).

The samples collected from the sewage network and hydraulically connected with the WWTP presented similar EEM fluorescence contours (Fig. SM-3). Also, the plume at the entrance of the WWTP was very similar to the fluorescence profile at most sampling locations. Wastewater sources were also examined based on the regionally integrated fluorescence intensities under the five specific excitation-emission regions (Fig. SM-4). In general, all wastewater sources presented a similar distribution of relative fluorescence intensities for each region and stayed consistent during all three campaigns, suggesting similar physicochemical characteristics of fluorophores. Protein-like (tyrosine-like (10–19%) and tryptophan-like (15–24%)) and SMP-like (20–31%)) were found to be the main fluorophores, whose sum of the integrated regional volume accounted for more than ~60% (average) of the DOM, while fulvic-like and humic-like components represented 19% and 20%, respectively, on average.

3.3.2. PARAFAC components and fluorescence maximum intensity (Fmax) values

PARAFAC analysis was performed to decompose EEM spectra into four dissimilar fluorescent components (Fig. 3b), named C1-C4. The component C1 is typically found in wastewater and nutrient-rich environments (Murphy et al., 2011), and is similar to the fulvic-acid component (Murphy et al., 2011; Yu et al., 2016). Component C2 is related to the protein-like peak, which was previously ascribed to tyrosine-like and tryptophan-like fluorophores (Cory and McKnight, 2005; Kulkarni et al., 2017; Yamashita and Tanoue, 2003). Components with emission <380 nm, such as C2, are usually associated to domestic/industrial waste. These fluorophores can be related to phenols, DNA, polyaromatic carbons, indoles, amino acids, lignin, and other compounds derived from chemical, pharmaceutical, textile, automotive, or petrochemical industries, for example (Carstea et al., 2016). Component C3, included in this emission range (<380 nm), is related to SMP-like substances (polysaccharides, proteins, organic acids, exocellular enzymes, structural components of cells and products of microorganism metabolism (Abdelrady et al., 2018, Barker and Stuckey, 1999)), and occurs universally in agriculturally and industrially/urban impacted rivers, eutrophic lakes, and wastewaters. Component C4 represents humic-like fluorophores characterized by high MW (>1000 Da) and may have a terrestrial or anthropogenic origin (Baghoth et al., 2011).

After validation of the four-component model, the fate of the components across all sampling campaigns was tracked using their maximum fluorescence intensities (F_{max}). For most of the studied water samples, F_{max} in campaigns 1 and 2 was considerably higher for component C2 than for components C1, C3, and C4 (Fig. 3c and Table SM-9). This component is related to tyrosine-like and tryptophan-like proteins, confirming that they are the predominant components in the sewer network. Recent studies have demonstrated that proteins can interact with surfactants via available binding sites of proteins (Tomaszewski et al., 2011). Thus, the high



Component	Excitation	Emission	Description
	wavelength (nm)		
C1	340	435	Wastewater/nutrient enrichment tracer
C2	275	335	Protein, tyrosine-like and tryptophan-like
C3	240	375	Soluble microbial product-like
C4	240	477	Humic-like

Fig. 3. a) Total fluorescence (TF) intensity for campaigns 1, 2 and 3 (pink, green and blue colors, respectively) for the different sampling locations; b) excitation-emission matrices (EEMs) of the four PARAFAC components with indication of excitation/emission wavelengths at which the maximum fluorescence was observed for each PARAFAC component; and (c) maximum fluorescence intensity (F_{max}) of PARAFAC components for campaigns 1, 2 and 3 at different sampling locations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentration of surfactants in CP-VC may have contributed to an increase in C2 fluorescence intensity due to interactions between the tyrosine-like and tryptophan-like components with alkyl chains (Maqbool and Hur, 2016).

3.3.3. SEC-OCD fingerprints and fraction characteristics

SEC-OCD is an established method to segregate the pool of natural organic matter into major fractions of different MW, contributing to a better understanding of DOM species in municipal wastewater. The SEC-OCD chromatograms (Fig. 4) support the 3D-EEM fluorescence results. Hence, in general, the DOM size distribution profiles were similar for all sampling points, but lower OC-signal responses were observed in campaign 3 (aforementioned rainy period), except for PVZ-1, where an increase in the signal response was observed compared to previous campaigns. This event may be associated with the existence of a hospital connected to this sampling location, with an increased discharge of CECs during campaign 3, which is consistent with the highest 3D-EEM fluorescence and TF intensity observed (Figs. SM-3 and 3a), and probably caused by an increase in the discharge of components C1 and C3 (Fig. 3b).

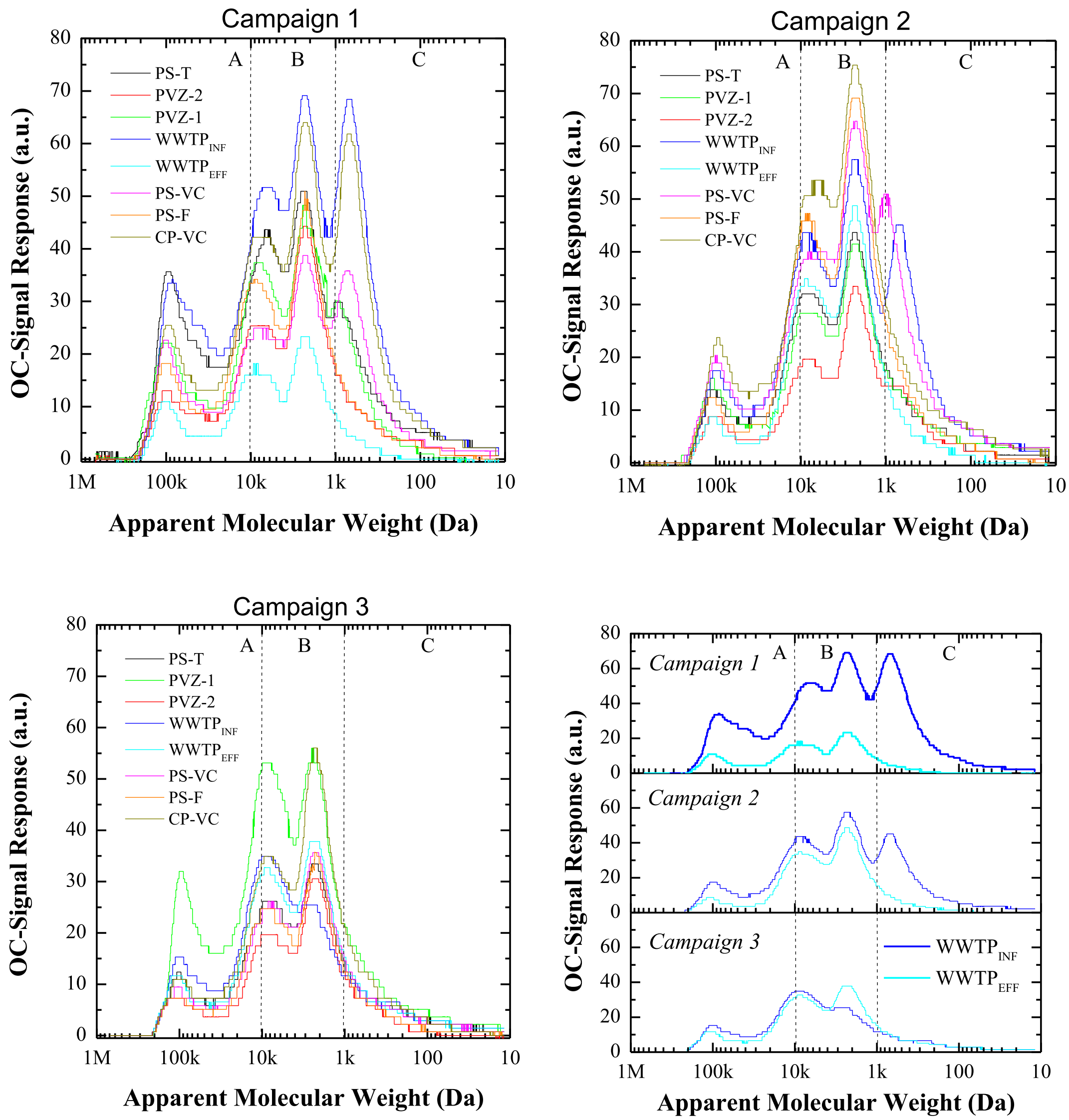


Fig. 4. SEC-OCD fingerprint for campaigns 1 to 3. Fraction A: proteinaceous biopolymers; Fraction B: Humic substances and building blocks of humic substances; Fraction C: carbohydrates, amino acids, aliphatic, low molecular weight acids and neutrals.

In Fig. 4, fraction A corresponds to the MW of 10 kDa or higher and refers to the more hydrophilic substances with a high molecular weight (HMW). This fraction is usually related to HMW humic-like or fulvic-like substances. Fraction B points to the presence of polysaccharides with a possible contribution from nitrogen-containing material such as proteins or amino sugars. Polysaccharides are considered to be the dominating material of polymeric extracellular substances (PES) (Flemming et al., 2007). The contribution of SMP-like and protein-like substances (fraction B) was the most pronounced peak for all effluents in all three campaigns, and the contribution of LMW acids and neutrals fraction (fraction C) to the organic carbon content is low for all effluents. These results are consistent with the absorbance UVA_{254}, fluorescence, and DOC results (Tables SM-4 to SM-6).

Furthermore, in campaign 1, $WWTP_{INF}$ and CP-VC exhibited the highest concentrations of all fractions compared with the other water matrices (Fig. 4), likely because $WWTP_{INF}$ receives all the sewage from the city and contains a high load of organics. On the other hand, CP-VC is located close to food industries and a pump station and showed the highest surfactant level (Fig. 2), which may explain the higher signal response in comparison with the other sampling points. $WWTP_{EFF}$ demonstrated a lower signal response, and the fractions A and B were more prominent in campaign 1, suggesting that the treatment train in the WWTP is efficient in reducing LMW acids and neutrals (fraction C) since this peak was completely removed. This peak was not detected in $WWTP_{INF}$ campaign 3, likely influenced by rain-diluted wastewater. Although particle size distribution may vary for different wastewater types and treatment trains, the removal of fractions A-C was found to be in accordance with literature (Gursoy-Haksevenler and Arslan-Alaton, 2020; Sophonsiri and Morgenroth, 2004; Wu and He, 2009). According to Levine et al. (1985) and Gursoy-Haksevenler and Arslan-Alaton (2020), a faster biological degradation is expected for HMW substances (fraction A) compared to colloidal and LMW organic substances (fractions B and C) in the activated sludge system. Then, the water quality of the final effluent ($WWTP_{EFF}$) will depend on the tertiary treatment and its efficacy in removing the residual refractory compounds from the biological process.

3.4. Identification of organic microcontaminants

3.4.1. Screening of CECs

A total of 108 different compounds were identified (see details in Table SM-10), of which 56% were pharmaceuticals (e.g., amisulpride (Fig. SM-6a) and diclofenac) and 15% metabolites (in most cases from pharmaceuticals). To a lesser extent, pesticides (e.g., terbutryn and fipronil), flame retardants (e.g., tri-isobutyl phosphate (TiBP) (Fig. SM-6b) and tris (2-butoxyethyl) phosphate), natural products and other industrial

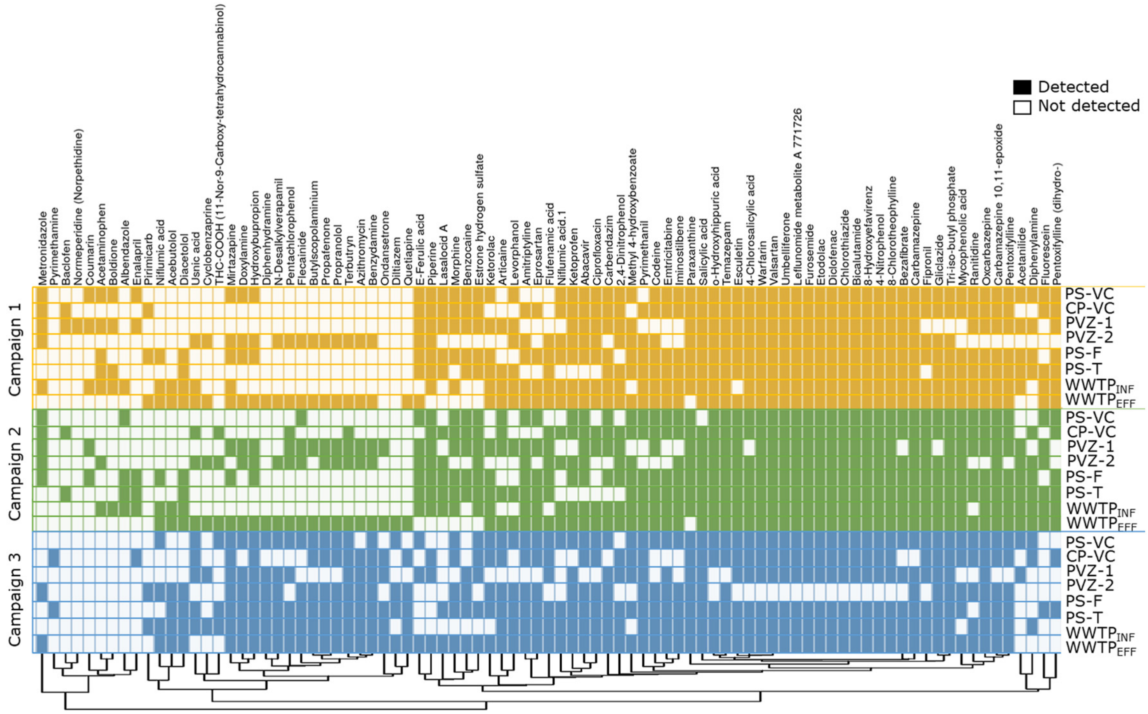


Fig. 5. Graphical summary of the presence or absence of CECs in the samples. Created with https://biit.cs.ut.ee/clutvis/. CECs clustered by Manhatan distance to cluster averages.

chemicals have also been detected. Among these CECs, up to 21 substances could be detected in all samples and 90 were present in >50% of them (Table SM-10). The detection rate is also summarized in Fig. 5 where CECs were also clustered. Among the clusters, the left two represent CECs which are, either seldomly detected (first one), or systematically detected in WWTP$_{EFF}$ samples but inconsistently in the raw wastewaters (e.g., enalapril or flecaidine). The fact that they are more frequently detected in effluents is likely because matrix effects during LC-QTOF analysis are much more prominent in raw than treated wastewater (Kloepfer et al., 2005), preventing their detection in raw sewage, not meaning that they are produced during treatment, but anyway showing that they are incompletely removed. On the other hand, the cluster in the right part of Fig. 5 represents CECs which are present in almost all samples, including effluents. In general terms, all untreated wastewater samples showed a similar profile, except the samples from PVZ-2, where some of the pharmaceuticals could not be detected, likely because of the type of industries (automobile and metalworking) that are predominant in this area.

Among those CECs, 8 compounds included in the Swiss Watch List (1998) or the different versions of the European Union Water Framework Directive Watch List (2020) were detected in all WWTP$_{EFF}$ samples viz.:

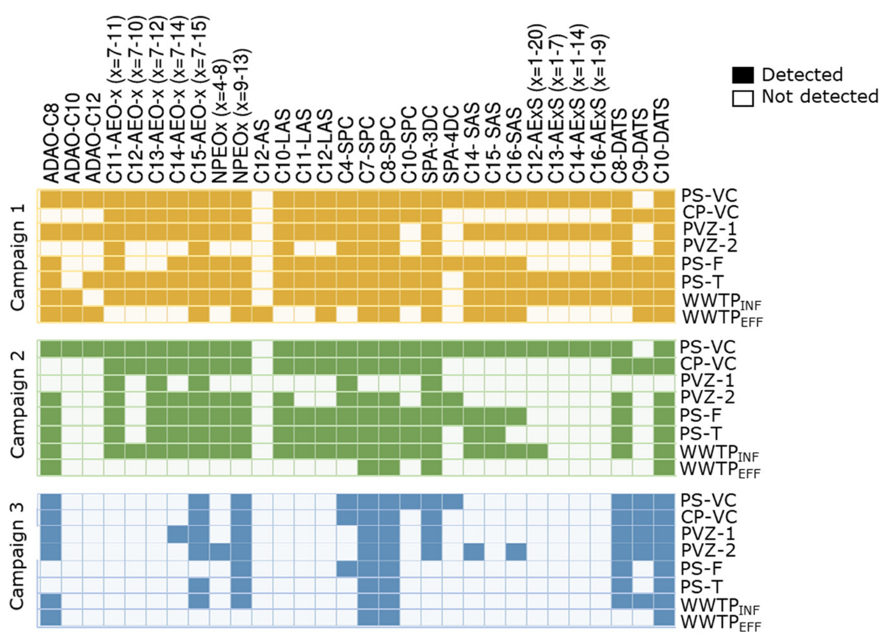


Fig. 6. Graphical summary of the presence or absence of the surfactants screened for in the samples. Created with https://biit.cs.ut.ee/clutvis/.

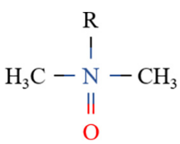
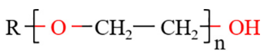
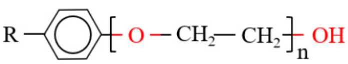
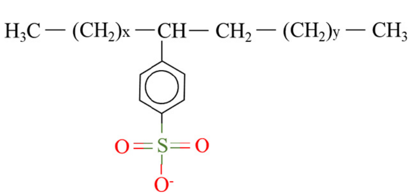
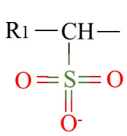
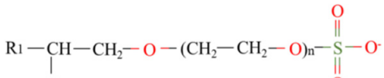
amisulpride, azithromycin, candesartan, carbamazepine, ciprofloxacin, citalopram, fluconazole, and diclofenac. Furthermore, 3 metabolites of carbamazepine were detected and several other sartan and azolic drugs besides candesartan and fluconazole were also detectable. Most of these compounds are typically detected into effluents of WWTPs equipped with conventional activated sludge treatment (O'Flynn et al., 2021; Patel et al., 2019; Rodil et al., 2012). Altogether, this reflects the inability of conventional WWTPs to remove CECs, which could also be further impacted in this case by the operational problems.

3.4.2. Suspect screening of surfactants

The screening of surfactants revealed the presence of a total of 111 different surfactants (Fig. 6), corresponding to 10 different families (details on the identification are provided in Text SM-3). This includes the zwitterionic surfactants: *N*,*N*-Dimethyldodecylamine N-oxides (ADAOs); non-ionic surfactants: alcohol ethoxylates (AEOs) and nonylphenol polyethoxylates (NPEOs); and seven different types of anionic surfactants: alkyl sulfates (AS), linear alkylbenzene sulphonates (LAS), sulfophenyl carboxylic acids (SPCs), sulfophenyl dicarboxylic acids (SPA-DCs), secondary alkane sulphonates (SAS), alkyl ethoxysulfates (AES) and di-alkyl tetralin sulfonates (DATS), SPCs and SPA-DCs being known as microbial degradation products of LAS (Schymanski et al., 2014). These findings are consistent with the total surfactant concentrations presented in Fig. 2, since anionic and then non-ionic surfactants represent the most relevant classes, in that order.

Fig. 6 clearly shows a higher prevalence of surfactants in the first two campaigns and that the detection rate in $WWTP_{EFF}$ samples was much lower than in the raw wastewater, although more surfactants were detectable in the first campaign. Thus, given the above-mentioned fact that matrix effects are less pronounced in effluents, this would indicate a good removal of the surfactants detected, which is in line with most literature (Capodici et al., 2015; Jardak et al., 2015; Palmer and Hatley, 2018). As regards the

Table 2
List of the main groups/classes of surfactants detected, respective applications, biodegradability data and degradation pathways.

Surfactant group	Application/biodegradation/degradation pathways
Alkyldimethylamine oxides (ADAO)  R = C_4–C_{14}	Regularly employed in cleaning and personal care products as foam stabilizers, thickeners, emollients, emulsifying and conditioning agents. Upon discharge, a pipe loss >90% is expected during transport in the sewers, after which ~98% removal by conventional activated sludge treatment (McDonough et al., 2018). The application of several biodegradability tests (OECD guidelines 301 C, D, and F and 302 B) demonstrated that the ADAO C12-14 compounds are readily biodegradable (McDonough et al., 2018), and a constant biodegradation rate of 1 h^{-1} can be assumed for WWTPs. The metabolic pathway of N,N-dimethyldodecylamine generally occurs via fission of the C_{alkyl}-N bond with the formation of dimethylamine and decanal. The toxicity of the parent substance is strongly reduced by this metabolization.
Alcohols ethoxylates (AEO)  R = C8–C18 (typical) n = no. of repeating ethoxylate units	Non-ionic surfactant used in multiple applications, including paper and textile processing and hard surface cleaners and degreasers. AEO homologues with linear hydrocarbon chain lengths from C4 to C15 are considered readily biodegradable in water compartments and efficiently removed (>99%) in conventional WWTP (Morrall et al., 2006). The biodegradation of alcohol polyglycol ethers occurs simultaneously under two main degradation pathways: intramolecular scission of the surfactant and ω- and β-oxidation of the alkyl chain.
4-Nonylphenol polyethoxylates (NPEO) 	Synthetic chemicals widely used in both the textile and leather industry, whose occurrence has been related with discharges coming mainly from industrial activities (Camacho-Muñoz et al., 2014). According to a 28-day biodegradation study (OECD Guideline 301 C), with a DOC removal of 81% at the end of the test, nonylphenol ethoxylate is considered readily biodegradable in nature . Notwithstanding, surfactants of the nonylphenol ethoxylates group (Tergitol NP-7, Tergitol NP-9 and Trigotol 15-S-9) have presented inhibitory effects on activated sludge oxygen uptake ratio (OUR) and nitrification (Othman et al., 2010). Moreover, some studies have reported that alkylphenol ethoxylates degradation metabolites were more toxic than the parent surfactant and demonstrated endocrine disrupting characteristics (Dereszewska et al., 2015, Scott and Jones, 2000). The compounds, nonylphenol monoethoxylate (NP1EO) and nonylphenol diethoxylate (NP2EO) are formed during activated sludge treatment from 4-nonylphenol polyethoxylates. These metabolites, which are not readily biodegradable, are relatively lipophilic and accumulate in the sludge and may also be discharged with the treated sewage effluent.
Linear alkylbenzene sulfonates (LAS)  (x + y) = 6–9	Widespread use in households and industries being applied in washing and cleaning products, adhesives and sealants, plasters, polishes and waxes, cosmetics and personal care products. The breakdown mechanism of LAS involves first the degradation of the straight alkyl chain, the sulfonate group and finally the benzene ring whose degradation cannot be through oxidation by microorganisms rather it must be through loss of carbon atoms one at a time (Scott and Jones, 2000). LAS occurs in sludge in large concentrations meaning that these compounds are not fully degraded during secondary biological treatment and there is significant accumulation in digested sludge. OUR tests carried out in the presence of 10 to 100 mg/L of C12-LAS showed inhibitory effects which increased from 27.6% to 75.5%, respectively (Othman et al., 2010). Strong inhibitory effects of LAS were reported for different strains of autotrophic ammonia-oxidizing bacteria, with 50% effective concentration (EC_{50}) ranging between 6 and 38 mg LAS/L for metabolic activity, and 3 to 14 mg LAS/L for microbial growth rate and viability (Brandt et al., 2001). LAS also affects the morphology of activated sludge, presenting a predicted no effect concentration (PNEC) of 1.35 mg/L in WWTPs and for concentrations c.a. 50 mg/L LAS is able to depolarize the bacterial cell wall and destroy structure and function, ultimately causing fragmentation of flocs and lysis of protozoa cells (Othman et al., 2010, Palmer and Hatley, 2018). The exposures of activated sludge to C12-LAS have shown negative effects on its settling properties and led to increased effluent suspended solids concentration (Yulian et al., 2014).
Secondary alkane sulfonates (SAS)  Linear alkyl chain (R_1 + R_2) = C11 to C17 SO_3^- group placed randomly along the alkyl chain.	Widely employed in household cleaning applications, especially dishwashing and laundry detergents. SAS is removed readily in WWTP mostly by biodegradation (~83%) - presenting faster biodegradation rates for the shorter-chain homologs than for longer-chain - and by sorption to sewage sludge (~16%) - in this case a larger fraction of the more hydrophobic longer-chain SAS homologs compared to the shorten-chain are transferred to sludge (Field et al., 1995). The aerobic biodegradation of SAS proceeds by α-hydroxylation with the insertion of a hydroxyl group alpha to the carbon atom bonded to the sulfonic acid group to form the bisulfite and the corresponding alcohol intermediate (Field et al., 1995).
Alkyl ethoxy sulfates (AES)  R_1 = C10–C14; R_2 = H; n = 1–4	Used in various consumer products (shampoos, hand dishwashing liquids, and laundry detergents), as well as in industrial processes (cleaning, aids in emulsion polymerization, and as additives in the plastics and paint production). AES primary biodegradation rates were shown to be 1.9 to 3.7 h^{-1} (half-lives = 0.16 to 0.21) and the dominant biodegradation pathway is central scission followed by rapid oxidation of the resulting fatty alcohol and slower oxidation of the corresponding sulphated ethoxylate (Menzies et al., 2017).

distribution of surfactants among the different samples, the higher concentrations of surfactants, particularly anionic, detected in CP-VC campaigns 1 and 2 (Fig. 2) are likely associated to LAS and its degradation products, SPC and DPA-3 DC (Fig. 6). In general, these compounds, as well as AEOs and NPEOs, were the surfactant classes more frequently detected (Table 2).

The removal of several surfactants detected in this study, whether non-anionic (AEOs and NPEOs) or anionic (LAS, SAS, and AES), in WWTPs has been reported by several researchers (Camacho-Muñoz et al., 2014; Field et al., 1995; Morrall et al., 2006), in many cases partially due to sorption by the activated sludge (Table 2). Moreover, for some surfactants (ADAOs, AES, AS, and LAS), meaningful removals along the sewer system may occur (McDonough et al., 2018), particularly due to the activity of biofilms growing in the sewage pipes (Menzies et al., 2017). Notwithstanding, when surfactants concentrations of 15–20 mg/L reach WWTP, as in this study, they can negatively impact the treatment causing (Capodici et al., 2015; Collivignarelli et al., 2020; Dereszewska et al., 2015; Jardak et al., 2015; Liwarska-Bizukojc and Bizukojc, 2006; Mitru et al., 2020; Othman et al., 2010; Palmer and Hatley, 2018): (i) foam formation, (ii) constrains on oxygen diffusion (surface-active compounds affect the rheology of fluid interfaces causing variable viscosity, elasticity and surface tension gradients), (iii) increased solubility of pesticides and other chemical agents with potential toxic effects, (iv) inhibition of the oxygen uptake rate (OUR) and nitrification of the activated sludge (possibly due to the decrease of dehydrogenase enzyme activity). The presence of anionic surfactants has also been shown to cause morphological changes on the activated sludge flocs (smaller size and more circular) diminishing the settling ability (Brandt et al., 2001; Palmer and Hatley, 2018; Yulian et al., 2014) (and verified by the biodegradability studies and microscopic analysis presented in Section 3.2). Beyond that, changes in the characteristics of activated sludge can negatively affect several parameters related to the WWTP sludge treatment line, such as the time required for clarification and thickening, and the efficiency of the dewatering step (Dereszewska et al., 2015). Some studies have reported that NPEOs (mainly related to industrial activities discharges and systematically detected in this study) degradation metabolites were not only persistent but more toxic than the parent surfactant and demonstrated endocrine-disrupting characteristics (Nasu et al., 2001; Scott and Jones, 2000).

4. Conclusions

In this work, several innovative tools were integrated aiming to characterize the sewerage network in terms of DOM and organic micropollutants, to trace down the potential sources of WWTP performance impairment. In this context, 3D-EEM coupled with PARAFAC, and supported by SEC-OCD analysis and physicochemical parameters concluded that protein-like (tryptophan-like and tyrosine-like) and soluble microbial product (SMP)-like components, usually predominant in urban and food industry wastewaters, are the dominant contributors to DOM in the sewer network samples. Yet, higher fluorescence values in PS-VC and CP-VC were measured, which was linked to a higher presence of surfactants (with concentrations up to ~50 mg/L).

The LC-HRMS screening of the samples detected several CECs along the sewage network, many of which occurred in treated wastewater, which may be partly attributed to the inefficient operation of the plant. Overall, the WWTP could maintain its capacity to remove high and low molecular weight organic substances, but variations in the WWTP feed water may influence the removal of these constituents.

The screening for surfactants concluded that LAS, AEOs and NPEOs are the surfactant classes more frequently detected in the sewage samples. Their constant presence and measured concentrations in the $WWTP_{INF}$ (15–20 mg/L), particularly at CP-VC and, to a minor extent PS-VC, are believed to be responsible for the changes in the characteristics of the activated sludge and LAS accumulation in the sludge. These locations are associated with several food industries pointing to intermittent uncontrolled discharges of the above-mentioned surfactants (during cleaning operations from some of those food industries) as the major sources of WWTP performance problems, including modification of activated sludge communities. Further closer monitoring is advised in those areas in order to detect the ultimate responsible of surfactant discharges and enforcement measures being put into practice.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

LC-HRMS data will be available at https://doi.org/10.5281/zenodo.5830725. All other relevant data generated or analyzed during this study are included in this article.

CRediT authorship contribution statement

The statement to specify the contribution of each co-author is as follows:

- **Conceived and designed the experimental procedure:** José Benito Quintana**, Vítor J.P. Vilar.**
- **Performed the sampling:** V.P.S. Silva Braga, Isabel Saraiva.
- **Performed the analytical procedures:** Mariana F.T. Sá, Verónica Castro, Daniela F.S. Morais, Victoria Fernández-Fernández, Bianca *M. Souza*-Chaves, Minkyu Park.
- **Analyzed the data:** Ana I. Gomes, Bianca *M. Souza*-Chaves, José Benito Quintana, Rosario Rodil, Rosa Montes.
- **Contributed reagents/materials/funding:** José Benito Quintana, Vítor J. P. Vilar.
- **Drafted or revised the manuscript:** Mariana F.T. Sá, Verónica Castro, Ana I. Gomes, Rui V.P.S. Silva Braga, Isabel Saraiva, Bianca *M. Souza*-Chaves, Minkyu Park, Victoria Fernández-Fernández, Rosario Rodil, Rosa Montes, José Benito Quintana, Vítor J.P. Vilar.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was financially supported by: i) Base-UIDB/50020/2020 and Programmatic-UIDP/50020/2020 Funding of LSRE-LCM, funded by national funds through FCT/MCTES (PIDDAC); ii) European Regional Development Fund through the Interreg V-A Spain-Portugal Programme (POCTEP) 2014–2020 (ref. 0725_NOR_WATER_1_P); iii) Xunta de Galicia (Verónica Castro predoctoral contract: ED481A-2017/156, and ED431C2017/36), the Spanish Agencia Estatal de Investigación – MCIN/AEI/ 10.13039/501100011033 (ref. PID2020-117686RB-C32); iv) NORTE-01-0145-FEDER-000069 (Healthy Waters) co-funded by European Regional Development Fund (ERDF), through North Portugal Regional Operational Programme (NORTE2020), under the PORTUGAL 2020 Partnership Agreement. The authors also acknowledge Águas do Norte, S.A. for supporting the development of this work. Daniela F.S. Morais acknowledges her Ph.D. scholarship supported by FCT (SFRH/BD/146476/2019). Bianca M. Souza Chaves gratefully acknowledges her postdoctoral scholarship supported by CNPq through the Science Without Borders Program (Process No. 201989/2014-0). Vítor J.P. Vilar acknowledges the FCT Individual Call to Scientific Employment Stimulus 2017 (CEECIND/01317/2017). It must be stated that this work only reflects the author's view; thus Program authorities are not liable for any use that may be made of the information contained therein.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.152518.

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