# SUPPLEMENTARY MATERIAL

# Tracking Pollutants in a Municipal Sewage Network Impairing the Operation of a Wastewater Treatment Plant

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# MATERIALS AND METHODS

## 2.3. Determination of physicochemical parameters

### Text SM-1

*Biodegradability assays*

A 28-days Zahn-Wellens biodegradability test (OECD protocol (Organization of Economic Cooperation and Development (OECD) 1992)) was modified and applied to the collected water samples. Control and blank experiments were prepared using glucose (highly biodegradable carbon source) and demineralized water, respectively, instead of sample. To better represent the real conditions, for campaign 1, the biodegradability assays used biological sludge coming from another WWTP (with no reported problems) and a "real control" was made using wastewater collected at the entrance of the WWTP. Sample volume was defined considering the daily load of urban wastewater discharge at each sampling site and at the Ave WWTP (Tables SM-1 and SM-2).

**Table SM-1.** Flow rates recorded between 11th and 19th of March 2019, at the sampling points of Vila do Conde.

| Days | PS-F ($m^3/d$) | CP-VC ($m^3/d$) | PS-VC ($m^3/d$) | PS-T ($m^3/d$) | WWTP ($m^3/d$) |
|---|---|---|---|---|---|
| 03/11 | 5 006 | 7 146 | 6 750 | 154 | 12 879 |
| 03/12 | 6 063 | 4 947 | 2 340 | 211 | 11 764 |
| 03/13 | 3 427 | 7 123 | 2 823 | 150 | 11 235 |
| 03/14 | 7 050 | 6 151 | 4 704 | 190 | 13 832 |
| 03/15 | 5 486 | 6 462 | 2 462 | 129 | 12 505 |
| 03/16 | 4 727 | 7 301 | 4 001 | 167 | 12 666 |
| 03/17 | 4 234 | 5 864 | 4 383 | 173 | 10 739 |
| 03/18 | 6 801 | 6 536 | 3 749 | 157 | 14 016 |
| 03/19 | 5 268 | 6 126 | 2 856 | 163 | 12 111 |
| Average | 5 340 | 6 406 | 3 785 | 166 | 12 416 |

**Table SM-2.** Dilution factors, sample volumes and influent applied in Zahn-Wellens biodegradability tests, for Campaign 1 samples.

| Sample | Dilution Factor (%) | $V_{sample}$ (mL) | $V_{influent}$ (mL) |
|---|---|---|---|
| PVZ-1 | 0.3 | 1 | 239 |
| PVZ-2 | 0.2 | 0.5 | 240 |
| PS-F | 43.0 | 103 | 137 |
| PS-T | 1.3 | 3.1 | 237 |
| PS-VC | 30.5 | 73 | 167 |
| CP-VC | 52.0 | 125 | 115 |
| $WWTP_{INF.}$ | - | 240 | - |

For campaign 2, a fixed dilution factor of 8.3% was established for all samples, whose corresponding volume (20 mL) was replenished weekly to the respective bioassay.
The percentage of biodegradation ($D_t$) was calculated through Equation SM-1:

$$D_t = \left[1 - \frac{C_T - C_B}{C_A - C_{BA}}\right] \times 100 \qquad \text{(SM-1)}$$

where $C_T$ and $C_B$ are the sample and blank DOC concentrations (in mg/L) determined at the sampling time *t*, respectively, and $C_A$ and $C_{BA}$ are the corresponding sample and blank DOC concentrations, respectively, measured 3 h after beginning the test. Samples are considered biodegradable when $D_t$ exceeds 70% after 28 days (Organization of Economic Cooperation and Development (OECD) 1992).

## 2.4. 3D-EEM and PARAFAC analysis

### Text SM-2

For UV spectra, the excitation wavelengths were increased from 200 to 800 nm at 4 nm increment with an integration time of 0.2 sec. For EEM fluorescence, the excitation wavelengths were increased from 200 to 450 nm at 5 nm steps, while the emission was detected at longer wavelengths from 245 to 827 nm at 1.16 nm steps. The integration time was maintained at 1 sec. Total fluorescence (TF) and regional integration methods were employed in this study as reported elsewhere (Chen et al. 2003). Light scattering, as Rayleigh and Tyndall scatterings, were eliminated using ultrapure water by three-dimensional interpolation. 3D-EEM fluorescence spectra was divided into five regions, namely: Region I – tyrosine-like aromatic proteins (Ex <250 nm, Em <330 nm); Region II – tryptophan-like aromatic proteins (Ex <250 nm, 330 nm <Em <380 nm); Region III

– fulvic acid-like materials (Ex <250 nm, Em >380 nm); Region IV – soluble microbial product (SMP)-like materials (Ex >250 nm, Em <380 nm); and Region V – humic acid-like materials (Ex >250 nm, Em >380 nm).

For PARAFAC analysis, the toolbox codes were run using MATLAB (R2018a, version 9.4.0.813654). Before PARAFAC analysis, the data points below 240 nm of excitation wavelength were excised. A series of PARAFAC models from three to seven components were run with the non-negative constraints and subsequently validated using a split-half analysis with $S_3C_3T_3$ (Splits: 3, Combinations: 3, tests: 3).

## 2.5. LC-HRMS screening

### Text SM-3

*Sample preparation*

Before solid-phase extraction (SPE), water samples were vacuum filtered through 1.2 µm glass microfiber filters (Whatman International, Kent, UK) followed by 0.45 µm nylon microfiber filters (Whatman International). 200 mL of water samples were loaded onto the Oasis HLB 12 cc (500 mg) cartridges, previously conditioned with 10 mL of methanol and 10 mL of ultrapure water. After loading, sorbents were washed with 10 mL of ultrapure water. With each set of samples, a procedural blank was made with 200 mL of ultrapure water submitted to the same protocol, including filtration. Cartridges were dried under a nitrogen stream for ca. 30 min, wrapped in aluminum foil and shipped frozen to Santiago de Compostela (Spain) where they were eluted and analyzed. Within 24 h upon receipt, cartridges were defrosted at room temperature and further dried under nitrogen stream for 10 min prior to elution process. Analytes were eluted with 20 mL of methanol by gravity, and eluates were evaporated to dryness under nitrogen (99.999%) using a Turbo-Vap II (Zymark, Hopkinton, MA USA) and a Mini-Vap concentrator (Sigma-Aldrich). Then, dried extracts were redissolved in 500 µL of methanol. An aliquot of 100 µL of each extract was filtered with a GHP® 13 mm 0.2 µm Syringe filter membrane (Pall Corporation, Port Washington, NY, USA) and transferred to a micro glass insert for instrumental analysis.

*Liquid chromatographic-mass spectrometry conditions*

Sample extracts and procedural blanks (2 µL) were injected into an Agilent Technologies (Wilmington, DE) 1290 Infinity II LC system. Chromatographic separation was carried out with a ZORBAX Extend-C18 1.8 µm (2.1 x 50 mm) column supplied by Agilent Technologies and connected to a Supelco ColumnSaver 0.5 µm Precolumn Filter (Supelco, Bellefonte, PA). As mobile phases, Milli-Q water (0.1% formic acid) (A) and methanol (0.1% formic acid) (B) were used at a flow rate of 0.4 mL/min. The temperature of the column was fixed at 35 °C. The gradient elution started with 98% A, increasing to 100% B in 22 min, held for 4 min. Subsequently, it returned to initial conditions (98% A), held for 4 min for column back-conditioning (Castro et al. 2021, Wilson et al. 2021).

A quadrupole-time-of-flight (QTOF) mass spectrometer model Agilent 6550 iFunnel Q-TOF LC/MS system was coupled to the UHPLC. The QTOF was furnished with a Dual Agilent Jet Stream electrospray (ESI) ion source. The ESI interface was operated either in positive or negative modes (in different injections) and the needle voltage of the ESI was fixed at 3500 V. Nitrogen was used as nebulizing (30 psi) and drying gas (200 °C, 12 L/min) in the ESI source, and also as collision gas in the MS/MS experiments.

Instrument control and data acquisition were performed with Agilent MassHunter Workstation software B.10.00. Different data acquisition modes were employed in the screening studies: Auto MS/MS, All Ions pseudo-MS/MS and targeted MS/MS, as detailed below.

### *Auto MS/MS screening of contaminants of emerging concern*

A suspect screening of CECs was performed in Auto MS/MS (data dependent acquisition) mode, using two consecutive injections per sample and ionization polarity, where the precursors selected for MS/MS fragmentation in the previous injections are automatically excluded by the software in the second injection (iterative acquisition (Castro et al. 2021, Wilson et al. 2021)). Iterative MS/MS parameters in the acquisition method were set at 20 ppm of mass error tolerance and 0.2 min of retention time exclusion tolerance. Three collision energies (10, 20 and 40 V) were collected for each precursor ion, with a maximum of 2 precursor ions per cycle. Precursors previously selected for fragmentation were excluded after 3 spectra and released after 0.5 min. The acquisition frequency in the single-MS and the MS/MS were 3 and 6 spectra per second, and the scan range was 60-1100 m/z in single-stage MS and 30-1000 m/z in MS/MS, respectively.

The identification workflow was carried out using the Agilent MassHunter Qualitative Analysis B.10.00 software. Auto MS/MS data files were processed using the search algorithm Find by Auto MSMS, which automatically extracts the MS and MS/MS information, aligns the spectra sorts and display them as a single compound. Then, the peaks encountered are compared towards an accurate mass MS/MS spectral library of 3225 chemicals that contains pharmaceuticals, pesticides, plasticizers, and other emerging pollutants (Table SM-3), as detailed in (Castro et al. 2021, Wilson et al. 2021). Analytes were considered as tentatively identified when at least two product ions with a coherent precursor ion matched the MS/MS spectra library and when a score >80% and a mass error lower than 5 ppm was achieved in the MS level.

**Table SM-3.** - List of chemicals included in the UPLC-HRMS mass spectral library (presented in the separate Excel file).

*Suspect screening of surfactants*

A suspect screening of surfactant chemicals was firstly carried out by injecting the samples in All Ions pseudo-MS/MS mode (data-independent acquisition). This method consisted into one injection per sample and polarity, with two alternating collision energies of 0, where little fragmentation is expected, and 20 V where fragments are expected, but without selection of precursor ion. Hence, in this mode, assignations of potential MS/MS fragments are based on the coelution of such potential fragments in the 20 V channel with the precursor ion in the 0 V channel.

The EAWAG Surfactants Suspect List containing 410 substances was retrieved from the NORMAN Suspect List Exchange website (Schymanski 2014, updated 21/11/2019, Schymanski et al. 2014). Then, All ions data files were processed using the search algorithm Find by Formula, where the software was set to search peaks corresponding to the $[M+H]^+$, $[M+NH_4]^+$ and/or $[M+Na]^+$ ions in positive mode and $[M+H]^-$ in the negative mode in the low energy channel. All surfactant candidates detected in All Ions mode (i.e., score >80% and a mass error lower than 5 ppm in the low energy channel) were then reinjected in targeted MS/MS in order to gain identification confidence, unless their spectra was already available in the AutoMS/MS experiments previously performed in CECs screening (see above).

Target MS/MS experiments were performed using three collision energies: 10, 20 and 40 V. The acquisition frequency in the single-MS and the MS/MS were 2 and 3 spectra per second, and the scan range was 60-1100 m/z in single-stage MS and 60-700 m/z in

MS/MS, respectively. Targeted MS/MS data files were processed extracting the individual MS/MS spectra acquired at different collision energies. For MS/MS spectral comparison and identification, the accurate mass MS/MS spectra of the studied surfactants were not available in the in-house library. Thus, such spectra were compared to open databases, such as MassBank, mzCloud and METLIN. In the case of that no spectra were available, the MS/MS spectra were investigated by interpreting the fragmentation pattern and exact masses of the product ions detected.

## RESULTS AND DISCUSSION

### 3.1 Preliminary remarks

**Figure SM-1.** Foam detected in the treated wastewater discharged by the WWTP.

## 3.2 Physicochemical parameters and biodegradability

**Table SM-4.** Physicochemical characterization for the sampling campaign 1.

| **Parameter** | **Unit** | **PVZ-1** | **PVZ-2** | **PS-F** | **PS-T** | **PS-VC** | **CP-VC** | **WWTP$_{INF.}$** | **WWTP$_{EFF.}$** |
|---|---|---|---|---|---|---|---|---|---|
| Color | | Dark yellow | Dark yellow | Brown | Brown | Brown | Dark brown | Dark brown | Light yellow |
| Odor | | Bleach | Rubber | Sewage | Sewage | Sewage | Sewage | Sewage | n.d. |
| pH | Sorënsen scale | 7.1 | 7.8 | 8.3 | 7.2 | 7.7 | 7.4 | 7.5 | 7.6 |
| Conductivity | µS/cm | 686 | 1473 | 1466 | 1074 | 2602 | 2024 | 1475 | 1358 |
| Turbidity | NTU | 90 | 21 | 220 | 100 | 190 | 300 | 230 | 11 |
| DOC | mg/L | 129.8 | 73.9 | 77.0 | 64.5 | 75.3 | 127.6 | 84.4 | 25.0 |
| DIC | mg/L | 26.9 | 65.5 | 77.7 | 67.3 | 61.4 | 69-9 | 109.8 | 68.4 |
| Alkalinity$^a$ | mg CaCO$_3$/L | 95 | 264 | 323 | 245 | 245 | 267 | 427 | 270 |
| COD | mg O$_2$/L | 456 | 200 | 675 | 508 | 348 | 946 | 681 | 46 |
| BOD$_5$ | mg O$_2$/L | 310 | 110 | 380 | 260 | 220 | 660 | 420 | 12 |
| TSS | mg/L | 79 | 50 | 260 | 52 | 178 | 109 | 63 | 24 |
| Oil and grease | mg/L | 14 | <10 | 56 | <10 | 21 | 139 | 83 | <10 |
| Anionic surfactants | mg/L | 5.8 | 2.6 | 8.7 | 7.0 | 9.0 | 19.8 | 8.8 | 0.2 |
| Cationic surfactants | mg/L | 2.1 | 1.6 | 2.0 | 3.4 | 3.6 | 4.4 | 3.0 | 1.4 |
| Non-ionic surfactants | mg/L | 8.3 | 2.1 | 4.7 | 6.8 | 2.5 | 11.9 | 6.1 | <0.1 |
| Total surfactants | mg/L | 16.2 | 6.3 | 15.4 | 17.2 | 15.1 | 36.1 | 17.9 | 1.6 |
| Absorbance UV$_{254}$ | cm$^{-1}$ | 0.55 | 0.32 | 0.54 | 0.45 | 0.39 | 1.06 | 0.64 | 0.23 |
| SUVA | L/mg m | 0.4 | 0.4 | 0.7 | 0.7 | 0.5 | 0.8 | 0.8 | 0.9 |
| Free chlorine | mg Cl$_2$/L | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.8 | 0.2 | 0.1 |
| Total nitrogen | mg N/L | 52 | 63 | 90 | 66 | 66 | 89 | 54 | 51 |
| Total phosphorus | mg P/L | 10 | 10 | 22 | 9 | 12 | 16 | 12 | 8 |
| Fluoride | mg/L | <0.2$^b$ | <0.2$^b$ | <0.2$^b$ | <0.2$^b$ | <0.2$^b$ | <0.2$^b$ | <0.2$^b$ | <0.2$^b$ |
| Bromide | mg/L | <0.3$^b$ | <0.3$^b$ | <0.3$^b$ | <0.3$^b$ | <0.3$^b$ | <0.3$^b$ | <0.3$^b$ | <0.3$^b$ |
| Nitrite | mg/L | <1.1$^c$ | <1.1$^c$ | <1.1$^c$ | <1.1$^c$ | <1.1$^c$ | <1.1$^c$ | <1.1$^c$ | <1.1$^c$ |
| Nitrate | mg/L | 5 | 7 | 8 | 6 | 11 | 6 | 8 | 7 |
| Sulfate | mg/L | 31 | 44 | 44 | 35 | 111 | 57 | 40 | 54 |
| Sulfite | mg/L | <1.0$^b$ | <1.0$^b$ | <1.0$^b$ | <1.0$^b$ | <1.0$^b$ | <1.0$^b$ | <1.0$^b$ | <1.0$^b$ |
| Chloride | mg/L | 44 | 165 | 102 | 68 | 362 | 230 | 104 | 128 |
| Phosphate | mg/L | 21 | 19 | 25 | 21 | 29 | 22 | 16 | 24 |

[a] Alkalinity values considering that at pH <11 the inorganic carbon was in the form of carbonates and bicarbonates. [b] Limit of detection (LOD) value. [c] Limit of quantification (LOQ) value.

**Table SM-5.** Physicochemical characterization for the sampling campaign 2.

| **Parameter** | **Unit** | **PVZ-1** | **PVZ-2** | **PS-F** | **PS-T** | **PS-VC** | **CP-VC** | **$\mathrm{WWTP_{INF.}}$** | **$\mathrm{WWTP_{EFF.}}$** |
|---|---|---|---|---|---|---|---|---|---|
| Color | | Light brown | Light brown | Brown | Brown | Brown | Dark brown | Dark brown | n.d. |
| Odor | | Sewage | Sewage/rubber | Sewage | Sewage | Sewage/bleach | Sewage | Sewage | n.d. |
| pH | Sorënsen scale | 7.3 | 7.9 | 8.2 | 7.4 | 7.5 | 7.6 | 7.6 | 7.5 |
| Conductivity | µS/cm | 619 | 1124 | 1321 | 1162 | 1683 | 1750 | 1532 | 1519 |
| Turbidity | NTU | 175 | 87.5 | 275 | 120 | 210 | 330 | 360 | 9 |
| DOC | mg/L | 156.1 | 60.3 | 80.0 | 82.4 | 114.2 | 129.9 | 75.4 | 27.1 |
| DIC | mg/L | 36.1 | 58.4 | 68.3 | 56.3 | 58.0 | 67.4 | 77.9 | 73.9 |
| Alkalinity | mg $CaCO_3$/L | 135 | 237 | 283 | 215 | 226 | 266 | 307 | 287 |
| COD | mg $O_2$/L | 691 | 316 | 584 | 338 | 514 | 780 | 860 | 78 |
| $BOD_5$ | mg $O_2$/L | 480 | 160 | 380 | 230 | 350 | 540 | 480 | 22 |
| TSS | mg/L | 239 | 140 | 328 | 109 | 155 | 338 | 569 | 28 |
| Oil and grease | mg/L | 49 | 32 | 86 | 47 | 44 | 39 | 22 | 20 |
| Anionic surfactants | mg/L | 19.0 | 4.3 | 13.0 | 13.5 | 15.3 | 38.5 | 11.5 | 0.2 |
| Cationic surfactants | mg/L | 3.5 | 3.3 | 3.4 | 2.6 | 0.2 | 3.3 | 3.1 | 1.8 |
| Non-ionic surfactants | mg/L | 12.4 | 11.8 | 7.1 | 4.3 | 6.3 | 9.7 | 4.6 | <0.1 |
| Total surfactants | mg/L | 34.9 | 19.4 | 23.5 | 20.4 | 21.8 | 51.5 | 19.2 | 2.0 |
| Absorbance $UV_{254}$ | $cm^{-1}$ | 0.71 | 0.36 | 0.56 | 0.44 | 0.47 | 0.62 | 0.47 | 0.28 |
| SUVA | L/mg m | 0.5 | 0.6 | 0.7 | 0.5 | 0.4 | 0.5 | 0.6 | 1.0 |
| Free chlorine | mg $Cl_2$/L | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 |
| Total nitrogen | mg N/L | 81 | 61 | 95 | 70 | 70 | 99 | 101 | 55 |
| Total phosphorus | mg P/L | 11 | 8 | 13 | 11 | 9 | 15 | 15 | 9 |
| Fluoride | mg/L | <0.2$^b$ | <0.2$^b$ | <0.2$^b$ | <0.2$^b$ | <0.2$^b$ | <0.2$^b$ | <0.2$^b$ | <0.2$^b$ |
| Bromide | mg/L | <0.3$^b$ | <0.3$^b$ | <0.3$^b$ | <0.3$^b$ | <0.3$^b$ | <0.3$^b$ | <0.3$^b$ | <0.3$^b$ |
| Nitrite | mg/L | <1.1$^c$ | <1.1$^c$ | <1.1$^c$ | <1.1$^c$ | <1.1$^c$ | <1.1$^c$ | <1.1$^c$ | <1.1$^c$ |
| Nitrate | mg/L | 0.3 | 1.0 | 3.0 | 2.0 | 1.0 | 1.0 | 1.0 | 3.0 |
| Sulfate | mg/L | 31 | 44 | 39 | 36 | 88 | 75 | 58 | 58 |
| Sulfite | mg/L | n.m. | n.m. | n.m. | n.m. | n.m. | <1.0$^b$ | n.m. | n.m. |
| Chloride | mg/L | 69 | 82 | 80 | 74 | 127 | 158 | 120 | 131 |
| Phosphate | mg/L | 8 | 11 | 15 | 17 | 13 | 16 | 26 | 26 |

[a]Alkalinity values considering that at pH <11 the inorganic carbon was in the form of carbonates and bicarbonates; [b] Limit of detection (LOD) value; [c] Limit of quantification (LOQ) value; n.m. - not measured.

**Table SM-6.** Physicochemical characterization for the sampling campaign 3.

| Parameter | Unit | PVZ-1 | PVZ-2 | PS-F | PS-T | PS-VC | CP-VC | WWTP$_{INF.}$ | WWTP$_{EFF.}$ |
|---|---|---|---|---|---|---|---|---|---|
| Color | | Dark yellow | Light brown | Light brown | Brown | Dark brown | Dark brown | Dark brown | n.d. |
| Odor | | Sewage/food | Sewage/rubber | Sewage | Sewage | Sewage | Sewage | Sewage | n.d. |
| pH | Sorënsen scale | 6.7 | 8.1 | 7.9 | 7.2 | 7.8 | 7.4 | 7.5 | 7.5 |
| Conductivity | µS/cm | 521 | 1087 | 706 | 1097 | 1639 | 1963 | 1734 | 1462 |
| Turbidity | NTU | 650 | 90 | 220 | 260 | 390 | 330 | 450 | 5 |
| DOC | mg/L | 136.0 | 56.9 | 89.4 | 80.7 | 136.0 | 147.8 | 104.8 | 29.8 |
| DIC | mg/L | 32.3 | 65.6 | 69.2 | 55.9 | 62.1 | 68.1 | 71.7 | 62.2 |
| Alkalinity | mg CaCO$_3$/L | 92 | 270 | 281 | 203 | 250 | 260 | 279 | 242 |
| COD | mg O$_2$/L | 1334 | 322 | 664 | 566 | 918 | 864 | 834 | 42 |
| BOD$_5$ | mg O$_2$/L | 900 | 210 | 400 | 390 | 420 | 600 | 560 | 10 |
| TSS | mg/L | 934 | 114 | 221 | 251 | 229 | 266 | 415 | 21 |
| Oil and grease | mg/L | 392 | 32 | 40 | 84 | 47 | 530 | 193 | 14 |
| Anionic surfactants | mg/L | 8.3 | n.m. | n.m. | n.m. | n.m. | 19.0 | 13.5 | 0.7 |
| Cationic surfactants | mg/L | 3.1 | n.m. | n.m. | n.m. | n.m. | 1.6 | 1.3 | 1.6 |
| Non-ionic surfactants | mg/L | 11.4 | n.m. | n.m. | n.m. | n.m. | 9.2 | 4.7 | 0.2 |
| Total surfactants | mg/L | 22.8 | n.m. | n.m. | n.m. | n.m. | 29.8 | 19.5 | 2.5 |
| Absorbance UV$_{254}$ | cm$^{-1}$ | 0.80 | 0.39 | 0.60 | 0.41 | 0.55 | 0.64 | 0.55 | 0.28 |
| SUVA | L/mg m | 0.6 | 0.7 | 0.7 | 0.5 | 0.4 | 0.4 | 0.5 | 0.9 |
| Free chlorine | mg Cl$_2$/L | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 |
| Total nitrogen | mg N/L | 94 | 13 | 34 | 74 | 83 | 104 | 105 | 51 |
| Total phosphorus | mg P/L | 46 | 13 | 34 | 15 | 15 | 21 | 22 | n.m. |
| Fluoride | mg/L | <0.2[b] | <0.2[b] | <0.2[b] | <0.2[b] | <0.2[b] | <0.2[b] | <0.2[b] | <0.2[b] |
| Bromide | mg/L | <0.3[b] | <0.3[b] | <0.3[b] | <0.3[b] | <0.3[b] | <0.3[b] | <0.3[b] | <0.3[b] |
| Nitrite | mg/L | <1.1[c] | <1.1[c] | <1.1[c] | <1.1[c] | <1.1[c] | <1.1[c] | <1.1[c] | 2 |
| Nitrate | mg/L | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 3 |
| Sulfate | mg/L | 16 | 40 | 43 | 31 | 89 | 70 | 55 | 55 |
| Sulfite | mg/L | n.m. | n.m. | n.m. | n.m. | n.m. | 1.6 | n.m. | n.m. |
| Chloride | mg/L | 58 | 70 | 92 | 67 | 134 | 198 | 147 | 127 |
| Phosphate | mg/L | 34 | 17 | 20 | 16 | 21 | 22 | 30 | 20 |

[a] Alkalinity values considering that at pH <11 the inorganic carbon was in the form of carbonates and bicarbonates; [b] Limit of detection (LOD) value; [c] Limit of quantification (LOQ) value. n.m. - not measured.

**Table SM-7.** Results for the concentration of several heavy metals in samples from Campaign 1.

| Parameter | Unit | PVZ-1 | PVZ-2 | PS-F | PS-T | PS-VC | CP-VC | $\text{WWTP}_{\text{INF.}}$ | $\text{ELV}_{\text{IWW}}$[a] | $\text{WWTP}_{\text{EFF.}}$ | ELV[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Al | mg/L | 0.369 | 0.075 | 0.489 | 0.231 | 0.635 | 0.354 | 0.314 | 10 | 0.067 | 10 |
| B | mg/L | 0.12 | 0.14 | 0.10 | 0.10 | 0.39 | 0.51 | 0.30 | 1.0 | 0.19 | n.a. |
| Ba | mg/L | 0.020 | 0.028 | 0.027 | 0.017 | 0.019 | 0.017 | 0.020 | n.a. | 0.011 | n.a. |
| Cu | mg/L | <0.003[c] | <0.003[c] | <0.003[c] | <0.003[c] | <0.003[c] | <0.003[c] | <0.003[c] | n.a. | <0.003[c] | 1.0 |
| Cr | mg/L | <0.004[c] | <0.004[c] | <0.004[c] | <0.004[c] | <0.004[c] | <0.004[c] | <0.004[c] | 2.0 | <0.004[c] | 2.0 |
| Fe | mg/L | 0.681 | 0.125 | 0.609 | 0.213 | 1.002 | 1.482 | 0.636 | 2.5 | 0.180 | 2.0 |
| Li | mg/L | 0.009 | 0.012 | 0.012 | 0.015 | 0.018 | 0.038 | 0.015 | n.a. | 0.014 | n.a. |
| Mn | mg/L | 0.024 | 0.020 | 0.041 | 0.056 | 0.131 | 0.066 | 0.044 | 2.0 | 0.029 | 2.0 |
| Pb | mg/L | 0.004 | 0.004 | 0.005 | <0.002[a] | 0.003 | 0.007 | 0.009 | n.a. | 0.011 | 1.0 |
| Sr | mg/L | 0.036 | 0.113 | 0.121 | 0.055 | 0.158 | 0.088 | 0.103 | n.a. | 0.086 | n.a. |
| Zn | mg/L | 0.092 | 0.084 | 0.275 | 0.079 | 0.143 | 0.086 | 0.085 | 5.0 | 0.049 | n.a. |

[a] Emission limit value (ELV) for typical parameters of industrials wastewaters (IWW), according to the Regulation for the Public Sanitation Service of Vale do Ave (2009); [b] ELV to discharge wastewater into the environment, according to Annex XVIII of the Portuguese Decree-Law no. 236/98 (1998). [c] Limit of detection (LOD) value. n.a. – not applicable.

**Table SM-8.** Percentages of biodegradation obtained in the Zahn-Wellens biodegradability test, after 14 days for the testing of samples from Campaign 1 and 2.

| **Sample** | **Biodegradability (%)** | |
|---|---|---|
| | **Campaign 1** | **Campaign 2** |
| PVZ-1 | 87 | 84 |
| PVZ-2 | 89 | 82 |
| PS-F | 86 | 79 |
| PS-T | 80 | 80 |
| PS-VC | 85 | 84 |
| CP-VC | 76 | 81 |
| WWTP$_{\text{INF.}}$ | 85 | 85 |

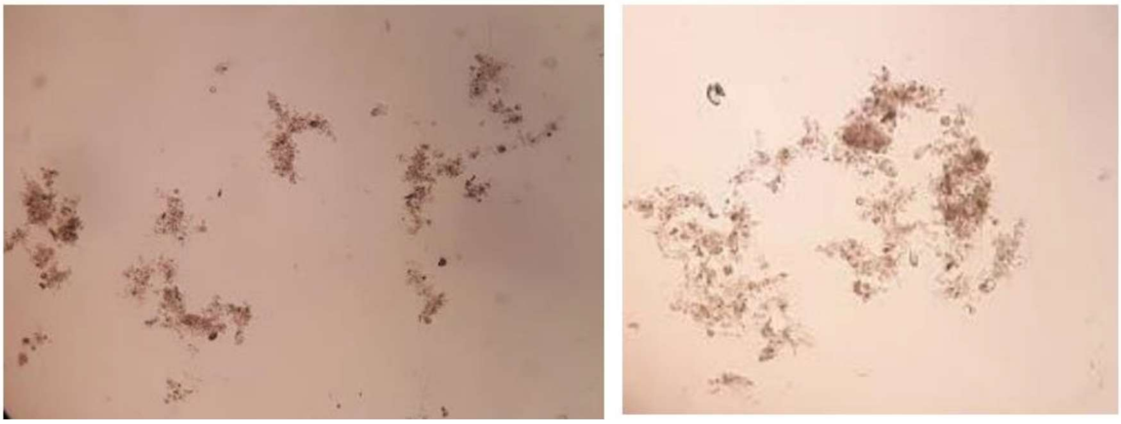

**Figure SM-2.** Observation under optical microscope of the activated sludge from the effluent at the entrance of the WWTP fed periodically with the CP-VC effluent (image on the left: 10 × 4; image on the right: 10 × 10).

## 3.3. Organic matter characterization

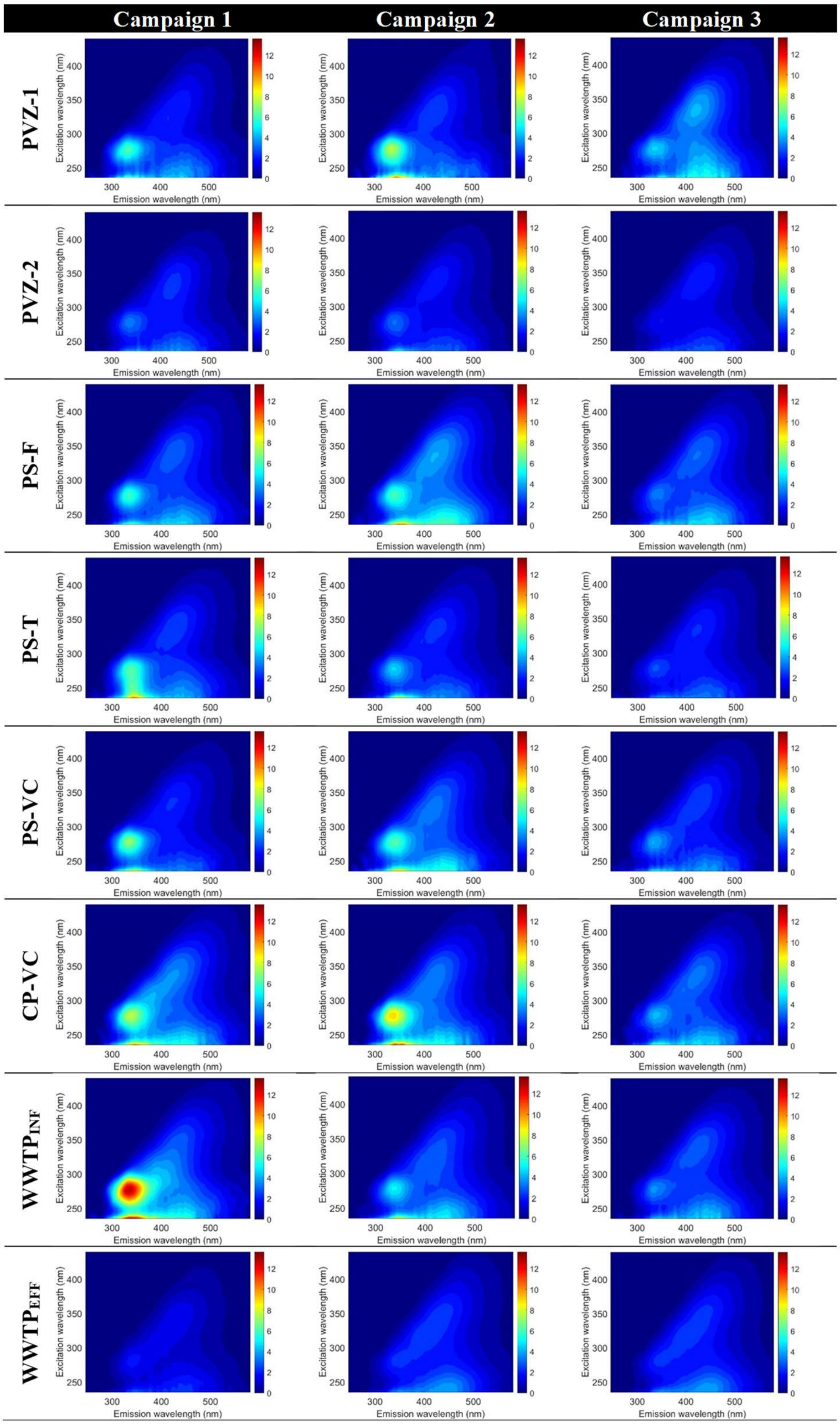

**Figure SM-3.** 3D-EEM fluorescence results for all samples and sampling campaigns (3D-EEM contour plots were normalized to the maximum fluorescence for comparison purposes).

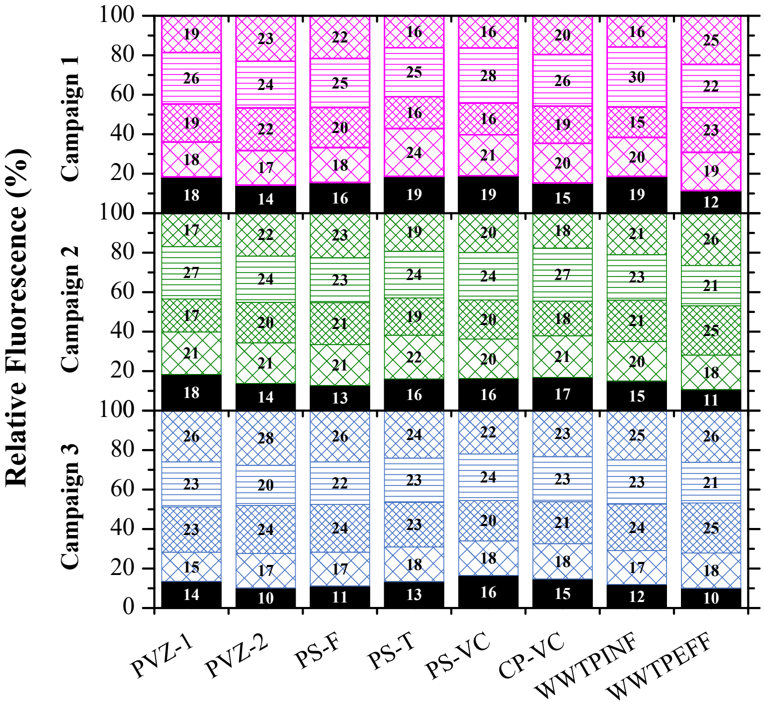

**Figure SM-4** –Relative fluorescence by region for campaigns 1, 2 and 3 (pink, green and blue colors, respectively) for the different sampling locations (■ Region I, ▨ Region II, ▩ Region III, ▤ Region IV, ▦ Region V).

## Text SM-4

PARAFAC analysis decomposed EEM into four PARAFAC components (C1 to C4 indicate the components 1 to 4, respectively, Figure 4) and the excitation and emission wavelengths at which the maximum fluorescence intensity was observed (Table SM-9). The corresponding line plots showing the split-half validations of excitation (peaks at lower wavelength at the corresponding component) and emission loadings are presented in Figure SM-5.

**Table SM-9.** $F_{max}$ values for four PARAFAC components. The unit is RU.

| **Campaign 1** | **PS-T** | **PVZ-2** | **PVZ-1** | **$\text{WWTP}_{\text{INF}}$** | **$\text{WWTP}_{\text{EFF}}$** | **PS-VC** | **PS-F** | **CP-VC** |
|---|---|---|---|---|---|---|---|---|
| C1 | 2.48560 | 2.358895 | 2.251167 | 3.072093 | 1.598050 | 2.183815 | 3.063076 | 3.269702 |
| C2 | 7.967007 | 3.444428 | 6.463634 | 14.114550 | 1.892679 | 7.583284 | 6.205210 | 7.586682 |
| C3 | 2.871577 | 2.067178 | 2.084910 | 4.396638 | 1.707450 | 2.024983 | 2.625141 | 4.373953 |
| C4 | 1.758084 | 1.153241 | 2.042728 | 2.320529 | 1.176892 | 1.366684 | 2.123019 | 2.34583 |
| **Campaign 2** | **PS-T** | **PVZ-2** | **PVZ-1** | **$\text{WWTP}_{\text{INF}}$** | **$\text{WWTP}_{\text{EFF}}$** | **PS-VC** | **PS-F** | **CP-VC** |
| C1 | 2.27986 | 1.93497 | 2.44396 | 2.91144 | 2.58926 | 3.17218 | 3.96908 | 3.20712 |
| C2 | 4.77768 | 3.29353 | 7.98579 | 5.40441 | 2.42790 | 6.41863 | 6.31971 | 9.172872 |
| C3 | 2.42901 | 2.00755 | 2.31634 | 3.32051 | 3.09318 | 3.61121 | 4.29722 | 3.793034 |
| C4 | 1.56690 | 1.28574 | 1.92042 | 2.25521 | 2.18294 | 2.11749 | 2.90216 | 2.006667 |
| **Campaign 3** | **PS-T** | **PVZ-2** | **PVZ-1** | **$\text{WWTP}_{\text{INF}}$** | **$\text{WWTP}_{\text{EFF}}$** | **PS-VC** | **PS-F** | **CP-VC** |
| C1 | 2.216988 | 2.110797 | 4.096882 | 2.977591 | 2.524549 | 2.508317 | 3.117835 | 3.134091 |
| C2 | 2.783539 | 1.664324 | 4.710185 | 3.581376 | 2.388165 | 3.987747 | 3.381051 | 4.049723 |
| C3 | 2.112009 | 1.868919 | 3.196641 | 2.812404 | 3.089993 | 2.309858 | 2.997538 | 3.127571 |
| C4 | 1.379605 | 1.208688 | 2.0979 | 1.912201 | 2.138675 | 1.619816 | 2.251808 | 1.840225 |

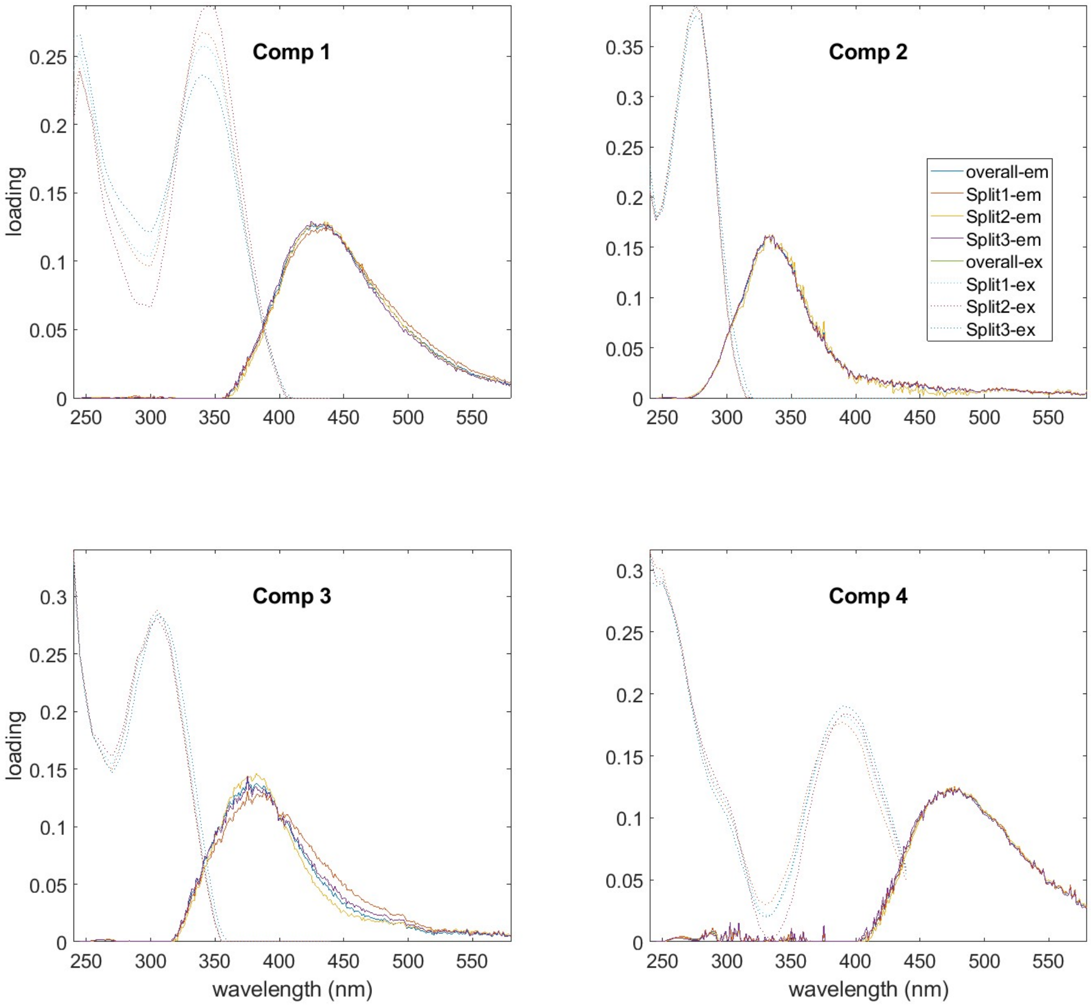

**Figure SM-5.** Split-half validations of excitation (peaks at lower wavelength at the corresponding component) and emission loadings. Split1 to 3 in the legend indicate the independent halves whereas "overall" indicates the complete dataset.

## 3.4. Identification of CECs and surfactants

**Table SM-10.** List of the 108 identified compounds in the suspect screening of CECs. Those CECs detected in all samples are highlighted in bold.

| Compound | Main use | Class | % |
|---|---|---|---|
| Methyl 4-hydroxybenzoate | Biocidal preservative | Paraben | 79 |
| Tri-iso-butyl phosphate | Flame retardant | | 96 |
| **Tris(2-butoxyethyl) phosphate** | **Flame retardant** | | **100** |
| **Tris(2-chloroisopropyl) phosphate** | **Flame retardant** | | **100** |
| Estrone hydrogen sulfate | Hormone | | 75 |
| Acetanilide | Industrial chemical | | 54 |
| Boldione | Industrial chemical | | 21 |
| 11-Nor-9-Carboxy-tetrahydrocannabinol | Metabolite | THC (cannabis) metabolite | 25 |
| **3-Hydroxycotinine** | **Metabolite** | **Nicotine metabolite** | **100** |
| 8-Hydroxyefavirenz | Metabolite | Antiviral metabolite | 96 |
| **Benzoylecgonine** | **Metabolite** | **Cocaine metabolite** | **100** |
| Carbamazepine 10,11-epoxide | Metabolite | Carbamazepine metabolite | 92 |
| **Cotinine** | **Metabolite** | **Nicotine metabolite** | **100** |
| Diacetolol | Metabolite | Acebutolol metabolite | 50 |
| Hydroxybupropion | Metabolite | Bupropion (antidepressant) metabolite | 67 |
| Iminostilbene | Metabolite | Carbamazepine metabolite | 79 |
| N-Desalkylverapamil | Metabolite | Blood pressure regulator | 46 |
| **Nordiazepam** | **Metabolite** | **Diazepam (benzodiazepine) metabolite** | **100** |
| Normeperidine | Metabolite | Phetidine (analgesic) metabolite | 4 |
| Oxcarbazepine | Metabolite | Carbamazepine metabolite | 92 |
| Paraxanthine | Metabolite | Caffeine metabolite | 92 |
| Pentoxifylline (dihydro-) | Metabolite | Pentoxyfilline metabolite | 63 |
| **Ritalinic acid** | **Metabolite** | **Metabolite of ethylphenidate (treatment of ADHD)** | **100** |
| Coumarin | Natural product | | 21 |
| E-Ferulic acid | Natural product | | 71 |
| Esculetin | Natural product | | 92 |
| o-Hydroxyhippuric acid | Natural product | | 92 |
| Piperine | Natural product | | 71 |
| **Thymotic acid** | **Natural product** | | **100** |
| Umbelliferone | Natural product | | 96 |
| Usnic acid | Natural product | | 50 |
| **Diethyltoluamide (DEET)** | **Pesticide** | **Insect repellent** | **100** |
| **Dinoterb** | **Pesticide** | **Herbicide** | **100** |
| Fipronil | Pesticide | Insecticide | 88 |
| Pirimicarb | Pesticide | Insecticide | 21 |
| Pyrimethanil | Pesticide | Antifungal | 83 |
| Terbutryn | Pesticide | Herbicide | 58 |
| 8-Chlorotheophylline | Pharmaceutical | Anti nausea | 96 |
| Abacavir | Pharmaceutical | Antiviral | 88 |
| Acebutolol | Pharmaceutical | Beta-blocker | 33 |
| Acetaminophen | Pharmaceutical | Analgesic | 29 |
| Albendazole | Pharmaceutical | Anthelmintic | 21 |
| **Amisulpride** | **Pharmaceutical** | **Antipsychotic** | **100** |
| Amitriptyline | Pharmaceutical | Antidepresant | 79 |
| Articaine | Pharmaceutical | Anhestesic | 67 |
| **Atenolol** | **Pharmaceutical** | **Beta-blocker** | **100** |
| Azithromycin | Pharmaceutical | Antibiotic | 50 |
| Baclofen | Pharmaceutical | Treatment of spastic movement disorders | 17 |
| Benzocaine | Pharmaceutical | Anhestesic | 63 |
| Benzydamine | Pharmaceutical | Non-steroidal anti-inflammatory drug (NSAID) | 54 |
| Bezafibrate | Pharmaceutical | Lipid-lowering agent | 88 |
| Bicalutamide | Pharmaceutical | Anticancer agent | 96 |
| Butylscopolaminium | Pharmaceutical | Analgesic for veterinary applications | 46 |
| **Candesartan** | **Pharmaceutical** | **Blood pressure regulator** | **100** |

| Compound | Main use | Class | % |
|---|---|---|---|
| Carbamazepine | Pharmaceutical | Anticonvulsant | 96 |
| Carbendazim | Pharmaceutical | Anthelmintic | 79 |
| Chlorothiazide | Pharmaceutical | Antihypertensive | 96 |
| Ciprofloxacin | Pharmaceutical | Antibiotic | 75 |
| **Citalopram** | **Pharmaceutical** | **Antidepresant** | **100** |
| Codeine | Pharmaceutical | Cold medication | 83 |
| Cyclobenzaprine | Pharmaceutical | Treatment of spastic movement disorders | 25 |
| Diclofenac | Pharmaceutical | Non-steroidal anti-inflammatory drug (NSAID) | 96 |
| Diltiazem | Pharmaceutical | Calcium channel blocker | 25 |
| Diphenhydramine | Pharmaceutical | Antihistamine | 38 |
| Doxylamine | Pharmaceutical | Antihistamine | 58 |
| Emtricitabine | Pharmaceutical | Antiviral | 79 |
| Enalapril | Pharmaceutical | Blood pressure regulator | 25 |
| Eprosartan | Pharmaceutical | Blood pressure regulator | 71 |
| Etodolac | Pharmaceutical | Non-steroidal anti-inflammatory drug (NSAID) | 96 |
| Flecainide | Pharmaceutical | Treatment of heart diseases | 50 |
| **Fluconazole** | **Pharmaceutical** | **Antifungal** | **100** |
| Flufenamic acid | Pharmaceutical | Non-steroidal anti-inflammatory drug (NSAID) | 75 |
| Furosemide | Pharmaceutical | Diuretic | 96 |
| Gliclazide | Pharmaceutical | Anti-diabetic | 92 |
| Ketoprofen | Pharmaceutical | Non-steroidal anti-inflammatory drug (NSAID) | 83 |
| Ketorolac | Pharmaceutical | Non-steroidal anti-inflammatory drug (NSAID) | 71 |
| Lasalocid A | Pharmaceutical | Antibiotic (veterinary uses) | 50 |
| Leflunomide metabolite A 771726 | Pharmaceutical | Leflunomide (antirheumatic) metabolite | 96 |
| Levorphanol | Pharmaceutical | Analgesic | 54 |
| **Lorazepam** | **Pharmaceutical** | **Benzodiazepine** | **100** |
| **Losartan** | **Pharmaceutical** | **Blood pressure regulator** | **100** |
| Losartan | Pharmaceutical | Blood pressure regulator | 96 |
| Metronidazole | Pharmaceutical | Antibiotic | 50 |
| Mirtazapine | Pharmaceutical | Benzodiazepine | 67 |
| Morphine | Pharmaceutical | Analgesic | 71 |
| Mycophenolic acid | Pharmaceutical | Immunosuppressant medication | 83 |
| Niflumic acid | Pharmaceutical | Non-steroidal anti-inflammatory drug (NSAID) | 58 |
| Ondansetrone | Pharmaceutical | Anti nausea | 29 |
| Pentoxifylline | Pharmaceutical | Analgesic | 88 |
| Propafenone | Pharmaceutical | Treatment of heart diseases | 50 |
| Propranolol | Pharmaceutical | Beta-blocker | 50 |
| Pyrimethamine | Pharmaceutical | Treatment of toxoplasmosis | 8 |
| Quetiapine | Pharmaceutical | Antipsychotic | 33 |
| Ranitidine | Pharmaceutical | Reduction of stomac acid | 79 |
| **Sulfapyridine** | **Pharmaceutical** | **Antibiotic** | **100** |
| Temazepam | Pharmaceutical | Benzodiazepine | 96 |
| Valsartan | Pharmaceutical | Blood pressure regulator | 96 |
| Warfarin | Pharmaceutical | Anticoagulant | 96 |
| Theophylline | Stimulant |  | 100 |
| 4-Chlorosalicylic acid | TP* | TP* or metabolite of herbicides and pharmaceuticals | 92 |
| 2,4-Dinitrophenol | Various | Industrial chemical, metabolite | 79 |
| **4-Hydroxybenzoic acid** | **Various** | **Industrial chemical, metabolite of parabens** | **100** |
| **Azelaic acid** | **Various** | **Industrial chemical, TP* of plasticizers** | **100** |
| Diphenylamine | Various | Industrial chemical, pharmaceutical, antioxidant | 54 |
| Fluorescein | Various | Industrial chemical, pharmaceutical, lab chemical… | 50 |
| Nitrophenol, 4- | Various |  | 96 |
| Pentachlorophenol | Various | Industrial chemical, metabolite, TP* | 50 |
| Salicylic acid | Various | Pharmaceutical, metabolite, natural product | 96 |

*TP – Transformation product

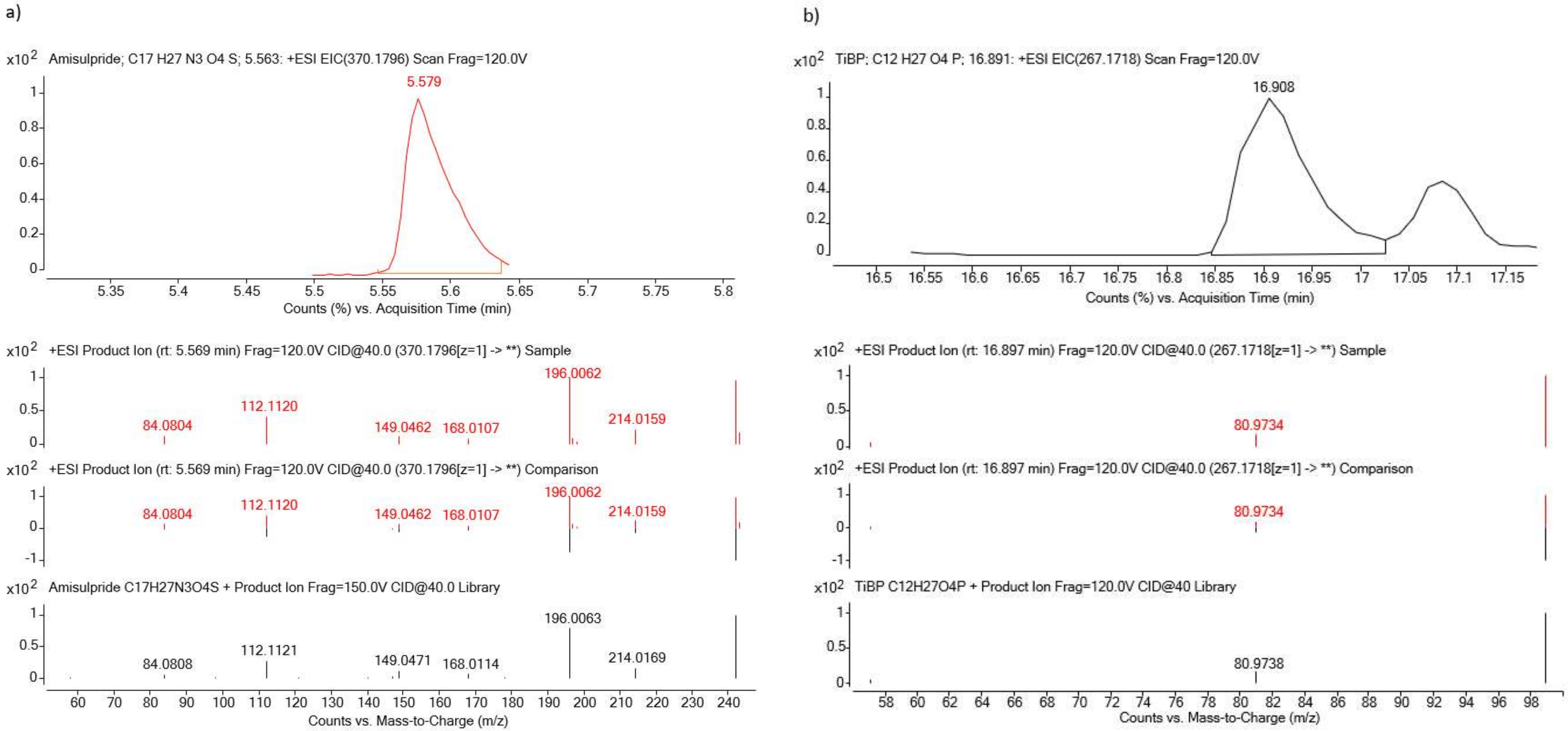

**Figure SM-6.** Examples of chromatograms and MS/MS spectra of two of the detected CECs: amisulpride (left) and Tris-*iso*-butyl phosphate (right)

## Text SM-5

*Surfactants identification by LC-QTOF*

The screening of surfactants revealed the presence of a total of 111 different surfactants, many of which correspond to the same families, differing on the size of the (poly)ethoxylated or alkyl chain, as summarized in Figure 7. In some cases, some of the chemicals with the larger number of ethoxy groups were not originally present in the database employed, but could be detected, on the basis of the single-stage MS spectra showing the characteristic profile of masses separated by a nominal difference of 44 Da (see e.g., Figure SM-7).

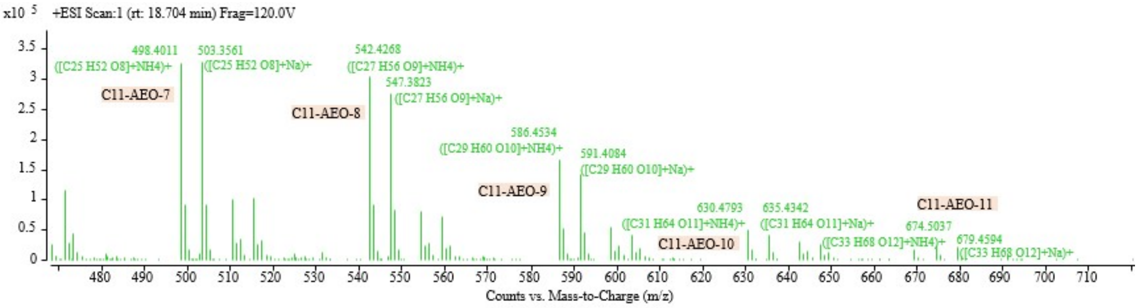

**Figure SM-7.** MS spectrum showing the $[M+NH_4]^+$ and $[M+Na]^+$ ions of C11-AEO-7, C11-AEO-8, C11-AEO-10 and C11-AEO-11 from left to right.

In the positive mode, we could identify 3 N,N-Dimethyldodecylamine N-oxides (ADAOs) with the alkyl chain varying from 8 to 12 carbon; 32 congeners of alcohol ethoxylates (AEOs), with the alcohol chain ranging from 11 to 15 carbons and the number of ethoxy groups (x) from 7 to 15 units; and 10 isomers of nonylphenol polyethoxylates (NPEOs) with the number of ethoxy units (x) ranging from 4 to 13.

ADAOs where detected as protonated molecular ions, while NPEOs and AEOs ionized as ammonium and sodium adducts (see e.g., Figure SM-7). Further identification as possible by MS/MS experiments from the protonated molecular ion (ADAOs) or ammonium adducts (ethoxylated surfactants). Thus, the MS/MS spectra of ADAOs could be compared to several Massbank records (e.g., EA278410, AU283903 and AU285202) and also tentatively identified by interpretation. For example, Figure SM-8 shows the MS/MS spectra ADAO-C10, which first fragments by losing water and then further fragments in the side chain, also yielding an ion of m/z 62 corresponding to protonated dimethyl N-oxide.

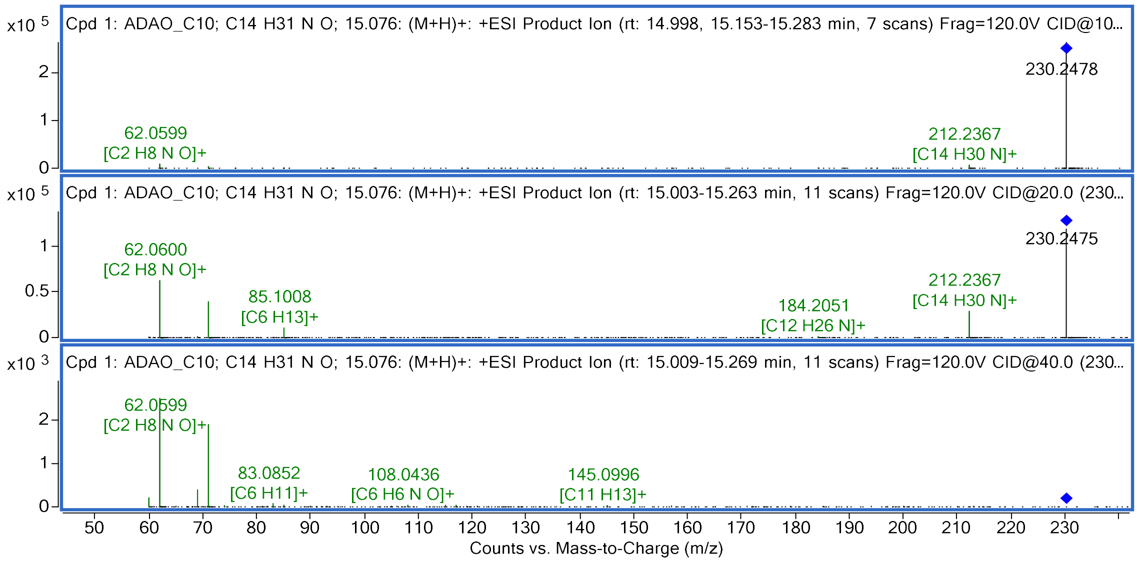

**Figure SM-8.** MS/MS spectra of ADAO-C10 at 3 different collision energies.

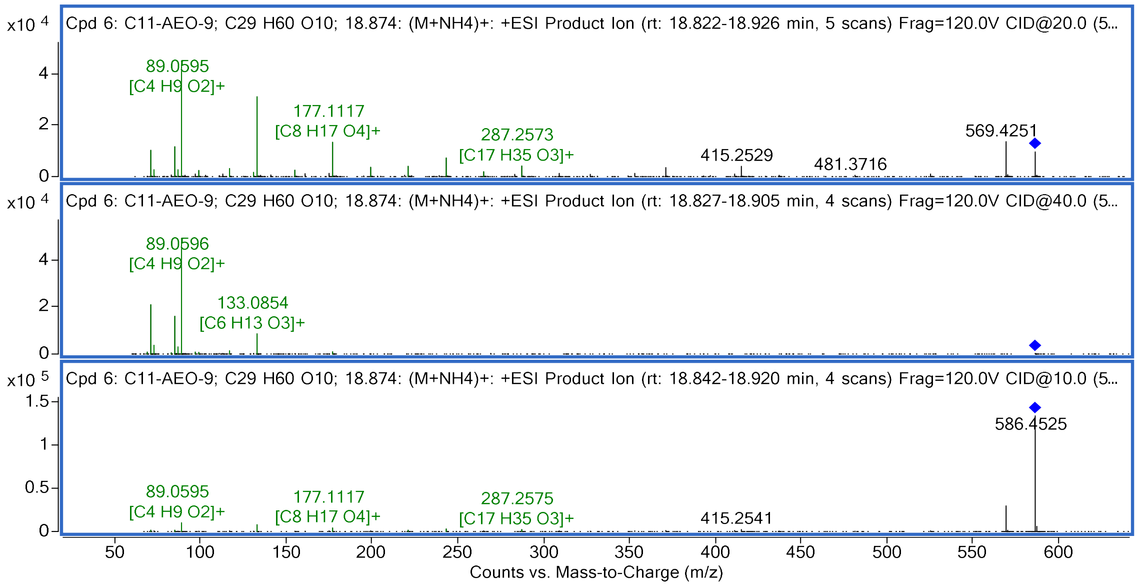

**Figure SM-9.** MS/MS spectra of C11-AEO-9 at 3 different collision energies.

NPEOs and AEOs pose also characteristic MS/MS spectra (exemplified by C11-AEO-9 in Figure SM-9) exhibiting first the loss of ammonia to produce the protonated molecular ion (m/z 569 in the case of C11-AEO-9), which fragments by further elimination of the C11 alkyl chain to produce m/z 415, and then show m/z 89, corresponding to a diethoxy dimer, as the most prominent product ion, but also presenting other product ions with a higher number of ethoxy groups (e.g. m/z 133, trimer, or m/z 177, tetramer, in Fig. SM-9).

The surfactant classes tentatively identified in the negative mode include alkyl sulphates (AS), linear alkylbenzene sulphonates (LAS), sulphophenyl carboxylic acids (SPCs), sulphophenyl dicarboxylic acids (SPA-DCs), secondary alkane sulphonates (SAS), alkyl ethoxysulfates (AES) and di-alkyl tetralin sulphonates (DATS).

The only AS detected at levels significantly above blanks was C12-AS (i.e., sodium dodecyl sulphate or SAS), which fragments to yield the monohydrogensulfate anion (m/z 99), comparable to Massbank spectra (e.g., record ETS00007). LAS spectra were characterized by the product ion at 183 m/z as being the most intense one, corresponding to the loss of a part of the alkyl chain through McLafferty rearrangements, leading to this ion (p-ethene benzenesulfonate), which is also the main ion in the Massbank spectrum of C12-LAS (record no. LIT00003). Furthermore, LAS spectra also exhibit the sulfonate anion (m/z 80) and an ion at m/z 119, corresponding to the loss of $SO_2$ from m/z 183 (see C11-LAS spectrum in Figure SM-10). This same pattern was observed for their long chain degradation products (SPCs, e.g., C10-SPC in Figure SM-11), while the shorter chain SPCs and SPA-DCs do not produce the ion at 183 m/z and their spectra is characterized by the loss of $SO_3$ or production of $SO_3^-$ (see C4-SPC spectrum in Figure SM-12). Experimental spectra of SPCs are available in Massbank (e.g., LIT00035).

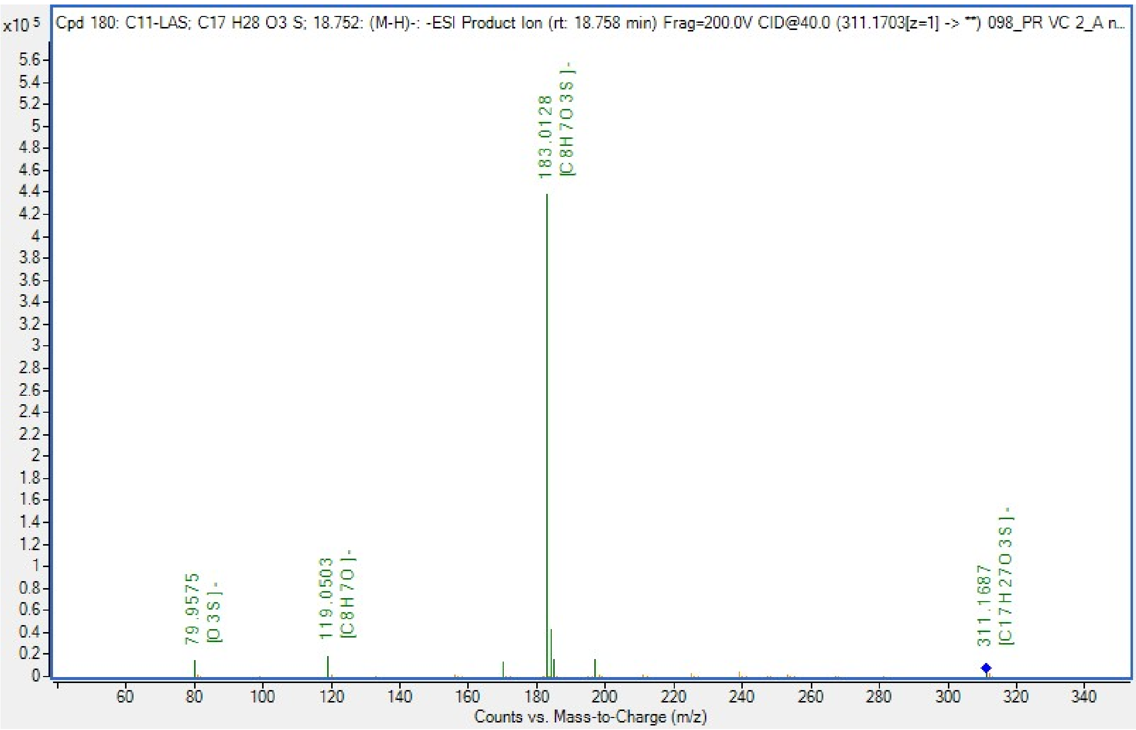

**Figure SM-10.** MS/MS spectrum of C11-LAS (CE: 40V).

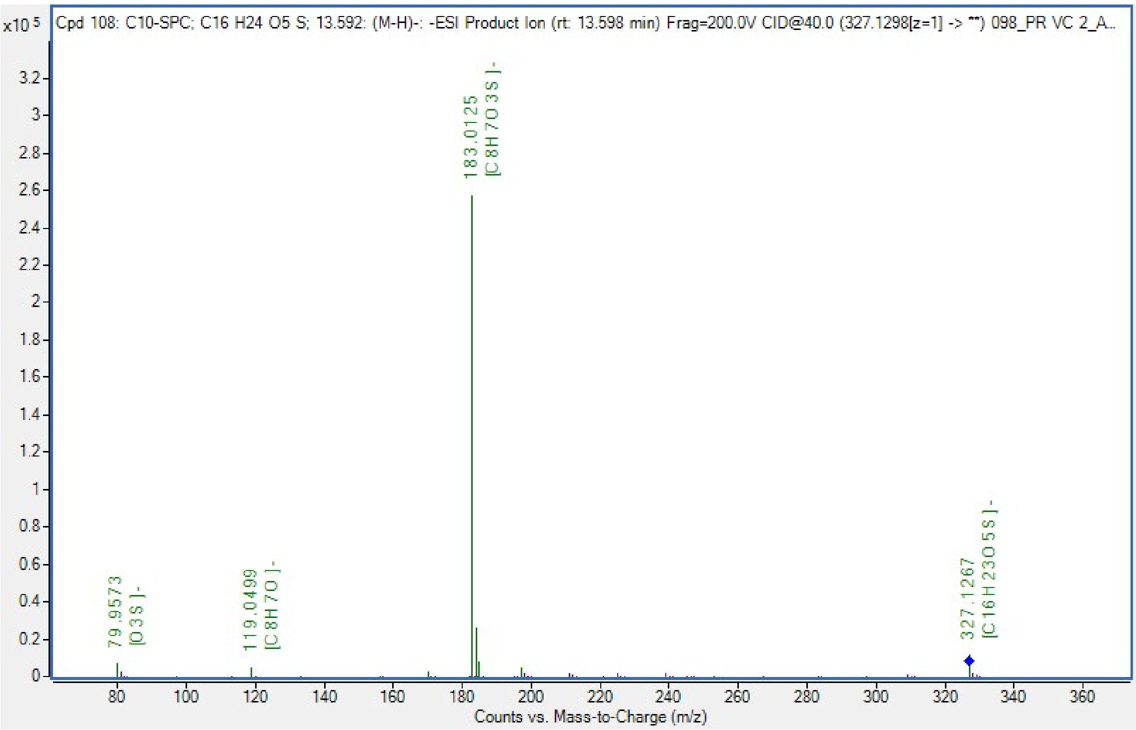

**Figure SM-11.** MS/MS spectrum of C10-SPC (CE: 40V).

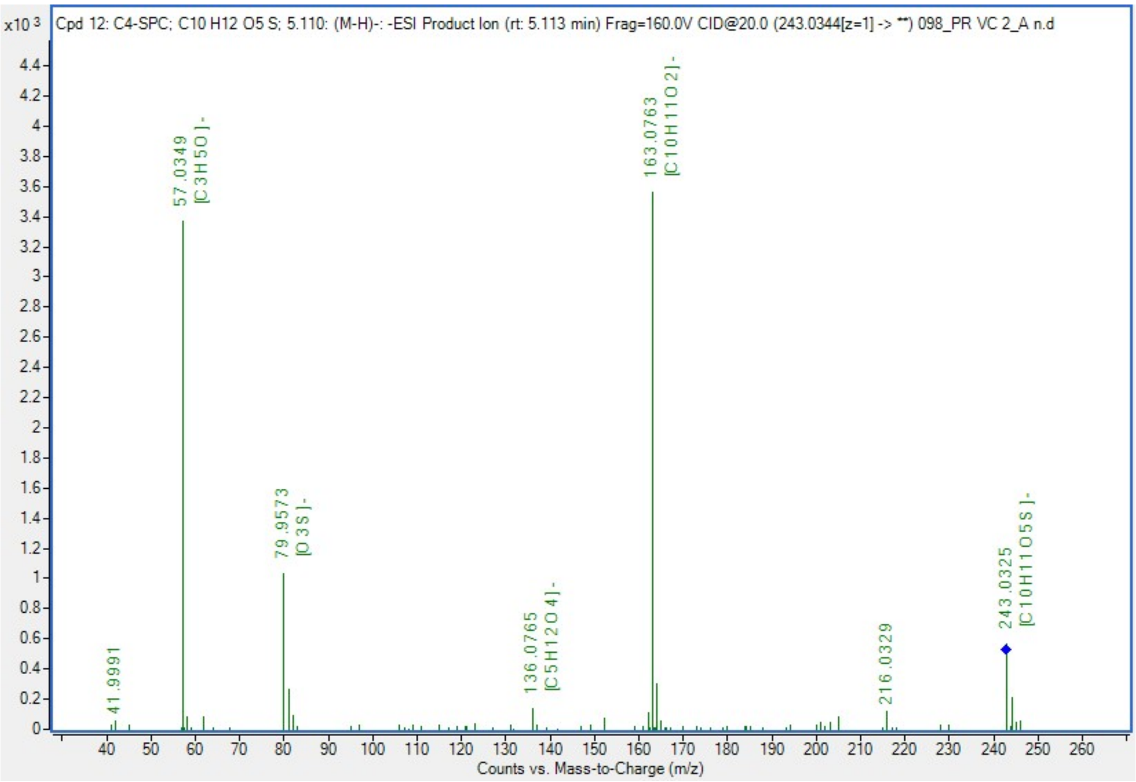

**Figure SM-12.** MS/MS spectrum of C4-SPC (CE: 40V).

SAS MS/MS fragmentation is, analogously to AS, characterized by the only production of the sulfonate anion (Figure SM-13), as described by Schymanski et al (Schymanski et al. 2014).

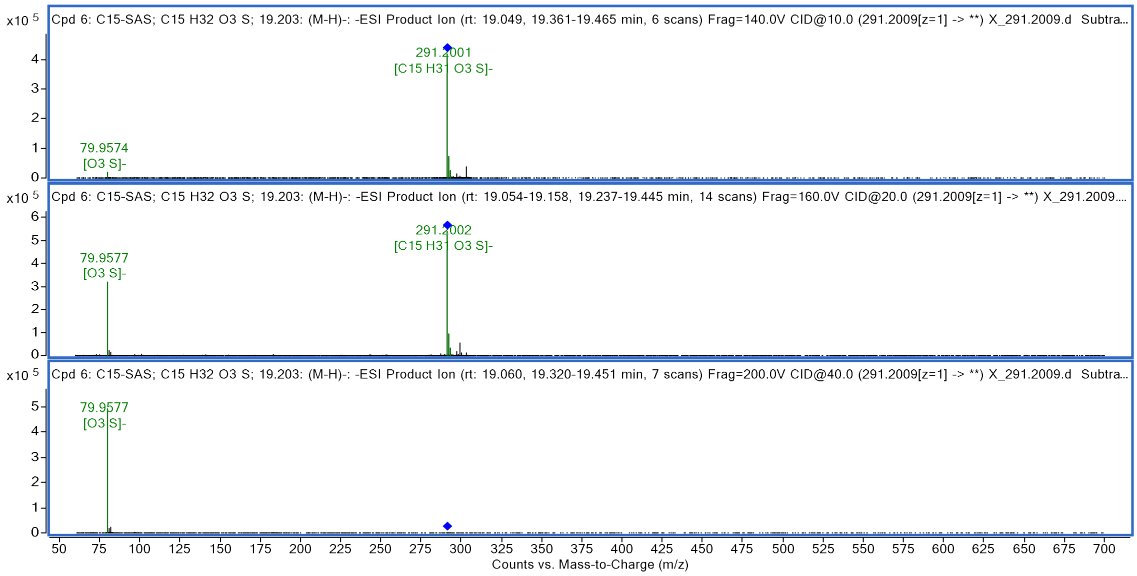

**Figure SM-13.** MS/MS spectrum of C15-SAS at three different collision energies.

Several congeners of AES were detected, all of them showing characteristic spectra (C12-AE6S spectrum is presented in Figure SM-14), that produced $HSO_4^-$ as the major product ion (m/z 97), accompanied by $SO_3^-$ (m/z 80) and monoethoxysulfate (m/z 123). These two last ions have also been reported by Schymanski et al (Schymanski et al. 2014).

The last group of surfactants tentatively identified corresponds to DATS. As an example, Figure SM-15 presents the MS/MS spectrum of C10-DATS (similar to the remaining DATS detected), which besides showing the ion at nominal mass 80 m/z (sulfonate ion) presents many product ions corresponding to fragmentation in the alkyl chains combined with the loss of sulfonic acid. A similar MS/MS spectrum is available in Massbank record LIT00001 for these same DATS.

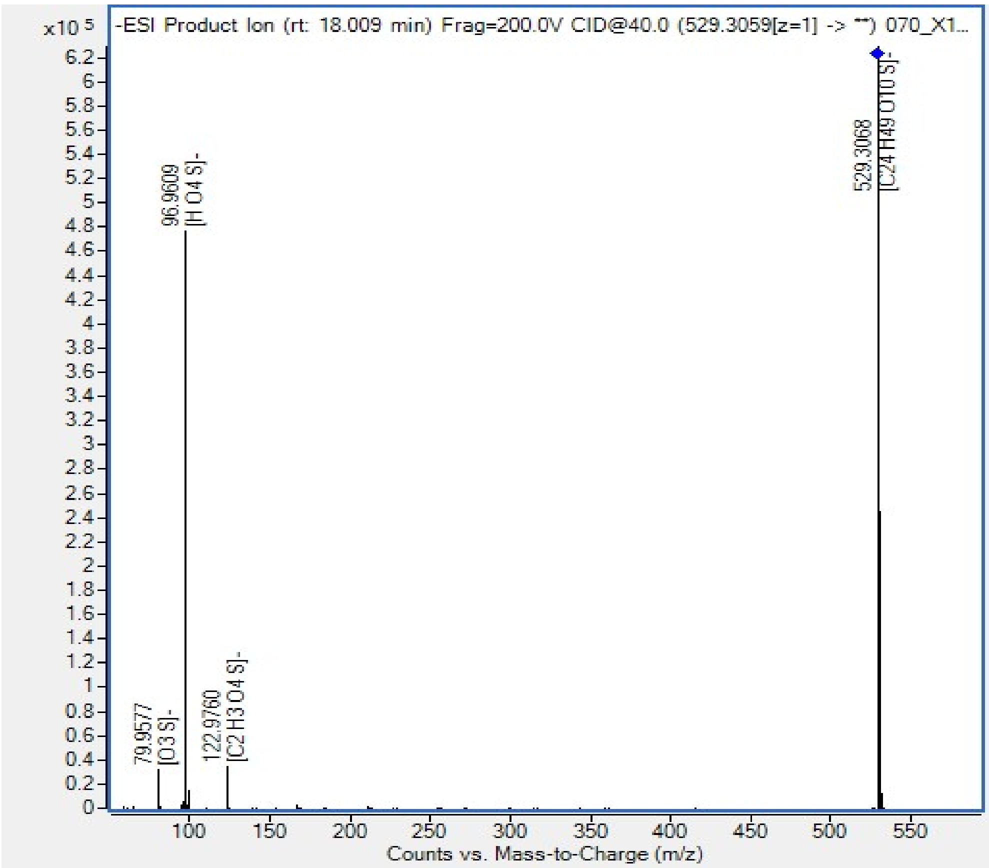

**Figure SM-14.** MS/MS spectrum of C12-AE6S (CE: 40V).

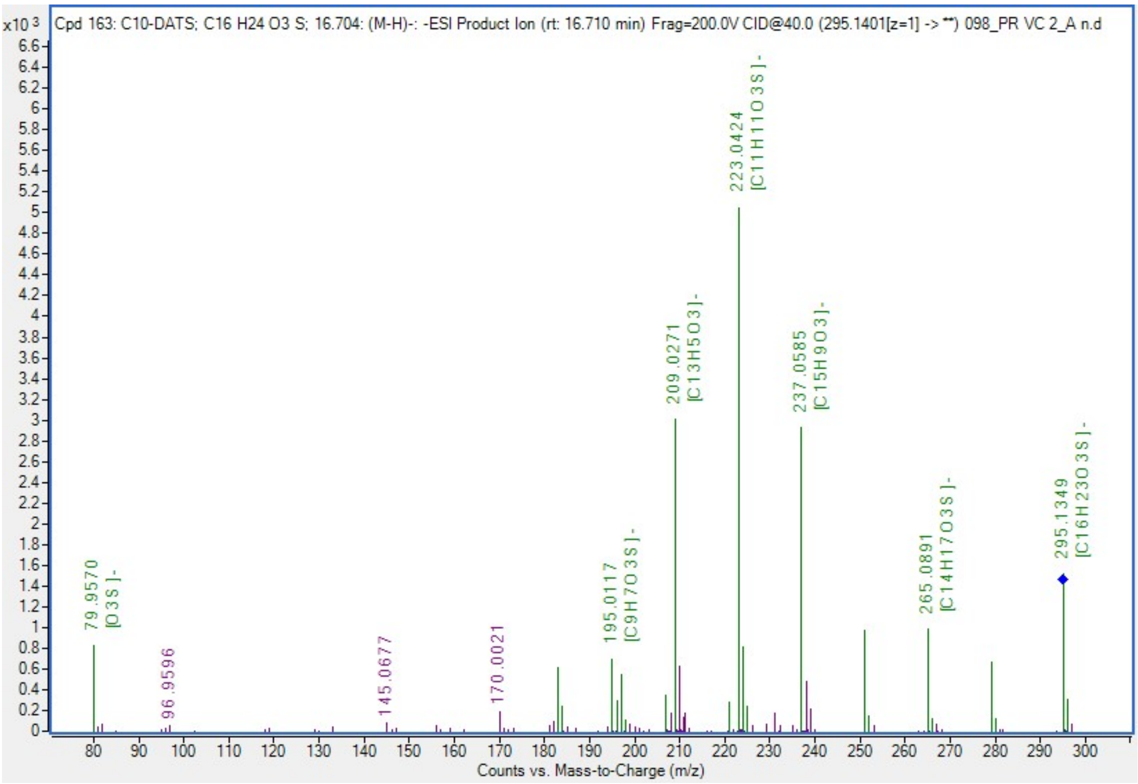

**Figure SM-15.** MS/MS spectrum of C10-DATS (CE: 40V).

## References

1998. Decree Law no. 236/98 - It establishes quality standards, criteria and objectives in order to protect the aquatic environment and improve water quality according to its main uses. M. o. t. Environment. Republic Diary no. 176/1998, Serie I-A.

2009. Regulation for the Public Service of Sanitation of Vale do Ave. Normative order nº 33/2009. S. P. a. R. D. Ministry of the Environment. Republic Diary, 2nd series.

Castro, V., Quintana, J. B., Carpinteiro, I., Cobas, J., Carro, N., Cela, R., Rodil, R., 2021. Combination of different chromatographic and sampling modes for high-resolution mass spectrometric screening of organic microcontaminants in water. Anal. Bioanal. Chem. https://doi.org/10.1007/s00216-021-03226-6

Chen, W., Westerhoff, P., Leenheer, J. A., Booksh, K., 2003. Fluorescence Excitation-Emission matrix regional integration to quantify spectra for dissolved organic matter. Environ. Sci. Technol. 37: 5701-5710.

Organization of Economic Cooperation and Development (OECD), 1992. Guideline for testing of chemicals 302 B. Inherent biodegradability: Zahn Wellens/EMPA test, Swiss Federal Laboratories for Materials testing and Research.

Schymanski, E. L., 2014, updated 21/11/2019. S7: EAWAGSURF: Eawag Surfactants Suspect List.

Schymanski, E. L., Singer, H. P., Longrée, P., Loos, M., Ruff, M., Stravs, M. A., Vidal, C. R., Hollender, J., 2014. Strategies to characterize polar organic contamination in wastewater: exploring the capability of high resolution mass spectrometry. Environ. Sci. Technol. 48(3): 1811-1818 https://doi.org/10.1021/es4044374.

Wilson, E. W., Castro, V., Chaves, R., Espinosa, M., Rodil, R., Quintana, J. B., Vieira, M. N., Santos, M. M., 2021. Using zebrafish embryo bioassays combined with high-resolution mass spectrometry screening to assess ecotoxicological water bodies quality status: a case study in Panama rivers. Chemosphere 272: 129823 https://doi.org/10.1016/j.chemosphere.2021.129823.