In the present work we fractioned the effluent water from an urban sewage treatment plant (USTP) of Ribarroja (Valencia, Spain) using the conventional protocol based on DAX8 and XAD4 resins. The fractions were analyzed by elemental analysis, FT-IR, 1H-NMR, COSY-NMR, HSQC-NMR, FAB-MS and also by derivatization with bis(trimethylsilyl)trifluoroacetamide with 10% of trimethylchlorosilane. The four fractions obtained have common spectroscopic features and individual compounds indicating that the fractioning procedure is inefficient at separating different families of compounds. Gas chromatography/mass spectrometry (GC-MS) analysis of the derivatized fractions allowed identification of many individual compounds. The main classes of organic compounds present in the effluent are saccharides, amino acids, fatty acids, hydroxyacids, aromatic compounds and steroids. Also we were able to identify in the effluent the emerging pollutants paracetamol and ketoprofen – two best-selling anti-inflammatory drugs used in humans.

Keywords: urban wastewater effluent; dissolved organic matter analysis; NMR spectroscopy; mass spectroscopy; emergent pollutants

Introduction

Fresh water is becoming a precious natural resource and is at the base of economic development and social well-being [1]. Considering the negative impact on the environment of urban wastewater (UWW) [2] and also the need to reuse the effluents [3–7], developed countries have made a considerable effort to adequately treat UWW, decreasing the concentration of dissolved organic matter (DOM) and removing suspended particles. The most common UWW treatment consists of biological degradation of the organic matter using activated sludges [8]. In this way, urban sewage treatment plants (USTP) are effectively reducing the total organic carbon (TOC) of the wastewater. Nevertheless, biological treatment does not completely remove all the organic chemicals present in UWW [8,9], some of these compounds being notably reluctant to undergo biological degradation. Therefore it is of interest to undertake an analytical study to gain as much understanding as possible of the chemical composition of treated UWW [10]. These studies on the composition of USTP effluents are particularly relevant when treated UWW is going to be reused for agricultural purposes or if it is going to be combined with fresh water resources to supply water for human consumption [11]. While there are some studies aimed at detecting the presence of certain chemicals [12], particularly antibiotics and other drugs [13,14], studies that try to gain a general view of the UWW effluent composition are scarce [10,15].

In the present manuscript we report our results on the fractioning and spectroscopic and chromatographic analysis of a UWW effluent from a USTP. As will be shown, we have been able to characterize a large number of carboxylic acids together with saccharides and steroids. Also we have detected paracetamol and some other drugs of therapeutic use.

Materials and methods

Materials

Supelite DAX8 (an acrylic ester-based polymer) and Amberlite XAD4 (a styrene-divinylbenzene-based copolymer) resins were supplied by Supelco (Madrid, Spain). Amberlite IRN-78 (hydroxide-form) was supplied by Sigma-Aldrich (Madrid, Spain). The resins were washed before their use in order to remove organic impurities (see Supplementary Material, available online) [16].

Paracetamol, ketoprophen, chloresterol, stigmasterol, β-sitosterol, oleic acid, citric acid, nitrobenzene, fumaric acid, glyceric acid, benzoic acid, tyrosol (2-(4-
hydroxyphenyl)ethanol), cholesterol, 4-hydroxybenzoic acid, inositol, lauric acid, myristic acid, stearic acid, palmitic acid, oxalic acid, propanedioic acid, maltotriose and 21-L amino acids kit, were supplied by Sigma-Aldrich (Madrid, Spain) and were used to identify the presence of these compounds in the UWW.

All the solvents used were of HPLC analytical grade. N,O-bis(trimethylsilyl)trifluoroacetamide with 10% of trimethylchlorosilane (BSTFA+10% TMCS) was supplied by Sigma-Aldrich (Madrid, Spain).

**Sampling and fractioning procedure**

The treated UWW correspond to the effluent from a public USTP, located at Ribarroja del Turia (Valencia, Spain), belonging to the public network for water treatment plants (EPSAR). This USTP serves the towns of the ‘Comarca del Turia’ (Eliana, Ribarroja del Turia and Villamarchante) zone with a population about 70,000 equivalent inhabitants, and the incoming water (16,296 m$^3$ day$^{-1}$), is almost exclusively domestic wastewater with minor inlets from industrial or agricultural origin. The main treatment process of the plant is prolonged aeration using activated sludges operating with a biological oxygen demand (BOD) of 0.15 kg per kg of mixed liquor suspended solids (MLSS). The effluent and sludge retention times on the USTP were 24 h and nine days, respectively. The DOC values at the entrance and at the exit of the plant were 96 and 10 mg L$^{-1}$, respectively. The free pH of the effluent was 7.2. Other quality parameter data are summarized in Table S1 in the Supplementary Material.

The sample that was analyzed was obtained by combining seven samples (each 25 L). Each of the seven samples was obtained by collecting 1 L at different hours using an automatic collector. They were collected from September to December 2008. Each of the seven samples was fractioned using DAX8 and XAD4 resins following a standard protocol previously reported in the literature [16–19]. The fractioning procedure was done immediately after collection in order to prevent any modification to the composition upon storage. The different organic fractions (HPO-A, HPO-N, TPI-A and TPI-N) were stored at −20 °C and combined when the last sample was fractioned. Therefore, the four fractions of the seven samples represent an integrated average of the effluent, minimizing daily variations in a four-month period.

The HPO-N and TPI-N fractions, initially dissolved in methanol, were concentrated under reduced pressure at 40 °C and the residue dissolved in Milli-Q water. All the fractions, once in aqueous solution, were acidified at pH 2 with HCl (0.1 M) and were contacted with the anionic IRN-78 exchange resin in order to remove anions (mainly HCO$_3^-$, SO$_4^{2-}$, Cl$^-$, Br$^-$ and NO$_3^-$) that will interfere in the subsequent characterization of the organic compounds. After removal of part of the inorganic salts, the IRN-78 resin was filtered, and the aqueous solution concentrated under reduced pressure at 40 °C. Finally the organic residue was recovered with methanol, accounting for 85% of the initial DOC.

Table 1 summarizes the operational fractioning procedure followed for each of the seven samples immediately after collection and indicates the percentage of each fraction with respect to the DOC of the effluent. The final four fractions correspond to the combination of the seven individual fractions.

| Fraction Column | Elution | Initial DOC percentage (%) | N:C | S:C |
|-----------------|---------|-----------------------------|-----|-----|
| Hydrophobic acid (HPO-A) | Retained in DAX8 | First adsorbed, then eluted with NaOH (0.1 M) | 42 | 0.045 | 0.019 |
| Hydrophobic neutral (HPO-N) | Retained in DAX8 | First adsorbed Second not eluted with NaOH Third eluted with MeOH | 21 | 0.050 | 0.051 |
| Transphilic acid (TPI-A) | Not retained in DAX8 Retained in XAD4 | First not adsorbed in DAX8 Second adsorbed in XAD4 Third eluted with NaOH (0.1 M) | 16 | 0.027 | <0.005 |
| Transphilic neutral (TPI-N) | Not retained in DAX8 Retained in XAD4 | First not adsorbed in DAX8 Second adsorbed in XAD4 Third not eluted with NaOH Fourth eluted with MeOH | 7 | 0.016 | <0.005 |

$^a$Each of these four fractions correspond to the combination of seven individual fractions from the seven samples. Fractionation was performed immediately after collection.
**Spectroscopic techniques**

By means of proton nuclear magnetic resonance ($^1$H-NMR), the type of protons and their relative percentage present in each fraction can be determined. Heteronuclear single quantum coherence NMR (HSQC-NMR) and correlation spectroscopy NMR (COSY-NMR) are two bidimensional NMR techniques that provide information about the connectivity of the hydrogen with carbon or with neighbouring hydrogen. In HSQC-NMR the hydrogen is correlated with the carbon that is bonded. The combination of the chemical shift of the hydrogen and the corresponding carbon can serve to disregard or support structural assignments. For instance, in our case, the signals appearing between 3.3 and 3.9 ppm can be attributed either to saccharides or peptides, but the fact that we observe in HSQC-NMR a signal at 50–55 ppm indicates that some of the hydrogen corresponds to peptides. Correlation spectroscopy NMR is, in contrast, a homonuclear technique that determines which protons are coupled. When complex signals are recorded, as is the case here, the COSY-NMR spectra indicate the series of protons that are neighbours and, therefore, magnetically coupled. Fourier transformed infrared (FT–IR) spectroscopy on the other hand will provide evidence of the functional groups present in the fractions. For volatile trimethylsilyl derivatives, gas chromatography/mass spectroscopy (GC–MS), as the analytical tool, would indicate the relative quantity, the molecular weight and the fragmentation pattern and will give some hints about the structure and the presence of subunits. Fast atom bombardment (FAB) mass spectrometry can detect, without fragmenting, all the components in a mixture, giving the mass of the individual components.

**NMR techniques**

The $^1$H-NMR, COSY-NMR and HSQC-NMR spectra of the sample solutions were recorded in perdeuterated methanol on a Brucker spectrometer, DRX-600 model, using CH$_3$OH as reference. In all the cases the NMR spectra were recorded using the water suppression mode.

**IR spectroscopy**

The FT–IR spectra were recorded for ambient equilibrated samples using a Jasco FT–IR-460 PLUS spectrophotometer. Solid residues corresponding to each fraction were mixed with anhydrous KBr powder and pressed to 10 ton for 5 min to obtain a wafer. Prior to recording the FT–IR spectra, the wafers were dried at 40 °C for 24 h.

**Derivatization and GC–MS analysis**

The four fractions obtained were silylated as previously reported [20]. Briefly, a weighed amount of each fraction was suspended in BSTFA containing 10% TMCS and the solution stirred at 80 °C for 8 h. The resulting silylated mixture was dissolved in anhydrous acetonitrile, filtered through a 0.45 μm membrane and injected in a GC–MS (Hewlett Packard HP6890 chromatograph and quadrupolar mass detector Agilent 5973). The capillary column (30 m) contained crosslinked (5%) phenylmethylsilicone (HP-5MS) as the stationary phase. Helium was used as a carrier gas (1.2 mL min$^{-1}$). The injection volume was 1 μL. The injection and detector temperatures were 250 and 280 °C, respectively. The oven temperature was initially 50 °C for 3 min, then the temperature was increased at a rate of 8 °C min$^{-1}$ up to 90 °C, which was maintained for 2 min, and then it was increased again at a rate of 15 °C min$^{-1}$ up to 280 °C. Finally, the temperature was maintained at 280 °C for 10 min. The derivatization procedure was repeated in full twice in order to confirm the quantification data. Percentages of the individual products in the two runs differed by less than 5%.

In some cases, product identification was done based on the mass spectra of the silylated derivative present in the reaction mixture by comparing them with NIST98 the library. For these cases, the spectra are shown in Table S2 of the Supplementary Material, indicating the matching quality number. In other cases, when possible, the compounds present in the fractions have been compared with commercially available samples of the proposed compound. For these cases, retention time and mass spectra were the parameters used to confirm the proposed compound.

Product quantification was made using nitrobenzene as external standard except for the cases of paracetamol, tyrosol, ketoprophen, cholesterol, stigmasterol, β-sitosterol, 4-hydroxybenzoic acid, lauric acid, stearic acid and palmitic acid, for which authentic samples were used to quantify the concentration of each of these compounds in the UWW effluent after silylating known amounts of the individual components and determining the ratio between with the area of the chromatographic peak after injection in the GC–MS instrument and the mass of the compound. It is worth commenting that no decomposition of the previous standards was observed in the silylation process.

**FAB$^+$-MS**

The FAB$^+$-MS spectra were recorded in a VG Autospec Trio 1000 (Fisons). The samples (10 mg) were prepared using nitrobenzyl alcohol as a matrix. The resulting mixture was placed in the sample holder and introduced...
in to the spectrometer. Caesium ion was used for the bombardment.

**Dissolved organic carbon (DOC)**

Dissolved organic carbon was analyzed using a High-TOC Elementar II analyzer. Before the analysis the sample was filtered through a nylon 0.45 µm filter. Measurements were based on the Pt-catalysed total combustion of organic matter in water at 950 °C and IR detection and quantification of evolved CO₂.

**Combustion chemical analysis of C, N and S content of the fractions**

Elemental analyses of the dry powders obtained after exhaustive solvent removal (40 °C, under vacuum) from the fractions were carried out with a Perkin Elmer CHNOS analyzer. Calibration was made using benzene-sulphonylamide as standard.

**Results and discussion**

**Fractioning of the UWW effluent**

The sample that was analyzed corresponds to a daily average of the USTP effluent over a period of four months (see Experimental). This sampling minimizes daily variations and provides a balanced composition of the effluent. The amount of each fraction, as a percentage of the initial DOC of the effluent, and the nitrogen and sulphur analyses of each fraction are indicated in Table 1. As can be seen in this table, the hydrophobic fractions (HPO-A and HPO-N) are the most important in terms of DOC. Although DOM fractions vary depending on the kind of wastewater and type of treatment process [10], similar results to our work have been reported for activated sludges, indicating that, in these effluents, hydrophobic fractions prevail over hydrophilic ones [21,22]. All the fractions contain nitrogen although the higher percentages correspond to the two HPO fractions. These two fractions are also the ones in which significant amounts of sulphur are detected. In particular, it is noteworthy that HPO-N is the fraction with the highest nitrogen and sulphur content. It should be noted that the total amount of the fractions correspond to 86% of the initial DOC. This mean that a remaining 14% is not retained in the columns and is lost during the fractionating procedure.

**Spectroscopic study**

Each of the four fractions was analyzed by FT–IR, ¹H-NMR, COSY-NMR and HSQC-NMR spectroscopy. Figure 1 shows the FT–IR spectra of each of the four fractions. In this figure, the wavenumbers of the most characteristic peaks have been indicated together with their corresponding value. The HPO-A fraction is characterized by having a band corresponding to –OH groups (3123 cm⁻¹) as well as an intense –C = O stretching vibration (1730 cm⁻¹), typical of ester groups, accompanied by C–O stretching around 1100 cm⁻¹ [23]. Thus, these FT–IR spectral features are in general agreement with the relative hydrophobicity of the HPO-A fraction [24]. In contrast to HPO-A, the FT–IR spectrum of the HPO-N fraction exhibits a much more intense –OH band in the 3700–2700 cm⁻¹ region as well as two carbonyl peaks at 1730 and 1657 cm⁻¹. Since fractioning does not lead to pure compounds [24] and considering the operational procedure, it is not unreasonable to assume that some of the compounds responsible for the 1730 cm⁻¹ –C = O band in the first HPO-A fraction will also be present in the following HPO-N fraction. However the nature of the compounds forming part of the fractions should gradually change and for this reason, in addition to the 1730 cm⁻¹ band there is also a new C = O peak at 1657 cm⁻¹. This low-frequency carbonyl peak is likely to be due to amide-forming oligopeptides or N-acetyl-glucosamines [25]. We notice that, according to the combustion chemical analysis, HPO-N is the fraction with a higher N and S content, a fact that is compatible with the intensity of the 1657 cm⁻¹ peak. The TPI-A fraction exhibits a much more intense –OH band, with absence of intense carbonyl groups, and single –C–O bonds. The last fraction (TPI-N) also exhibits a broad –OH band together with two carbonyl peaks, one with a low intensity at 1730 cm⁻¹ and a more intense second one at 1657 cm⁻¹. Another characteristic peak present in TPI-N is the C–O stretching vibration at 1133 cm⁻¹.
Overall, the FT–IR spectra of the fractions shown in Figure 1 indicate the presence and the relatively intensity of –OH, –C = O and –C–O groups, but does not give information about the complexity of the mixture and individual compounds.

To complement FT–IR spectroscopy, we performed 1H-NMR spectra of the fractions and recorded also their COSY-NMR and HSQC-NMR spectra. Figures S1–S17 (in Supplementary Material) present the most relevant data obtained from these spectroscopic techniques.

Table 2 lists the peaks recorded by 1H-NMR spectroscopy grouped according to their chemical shift region, as is normally reported in the literature [26,27], with an indication of the corresponding integration area.

Table 2. Proportion and assignment of non-exchangeable hydrogen as determined form the 1H-NMR spectra of the fractions.

| δ (ppm) | Assignment | Relative percentage (%) |
|---------|------------|-------------------------|
|         |            | HPO-A | TPI-A | HPO-N | TPI-N |
| 0.5–1.9 | Aliphatic  | 36.5  | 22.4  | 46.5  | 42.4  |
| 1.9–3.1 | Neighbour to unsaturated groups | 22.3 | 17.7 | 16.7 | 13.6 |
| 3.1–4.6 | O–CH₂ | 26.3 | 50.3 | 27.9 | 32.6 |
| 4.7–6.0 | O–CH–O | 4.0 | 3.6 | 1.9 | 2.1 |
| 6.0–9.0 | Aromatic | 10.9 | 6.0 | 7.0 | 9.3 |

Table 3. Characteristic signals of the different compound types in NMR spectroscopy data.

| Type of compounds            | Chemical shift assignment                                      | Chemical shift NMR references |
|------------------------------|----------------------------------------------------------------|-------------------------------|
| Fatty acids, lipids or related compounds | CH₃–C                                                                 | [23,26,33–35]                |
|                              | ¹H-NMR: 0.9 ppm                                                  |                               |
|                              | ¹³C-NMR: 25 ppm                                                  |                               |
|                              | C–CH₃–C (polymethylene chains)                                   |                               |
|                              | ¹H-NMR: 1.3 ppm                                                  | [23,26,33–35]                |
|                              | ¹³C-NMR: 30 ppm                                                  |                               |
|                              | C–CH=CH–C                                                       | [23]                         |
|                              | ¹H-NMR: 5.5 ppm                                                  |                               |
|                              | ¹³C-NMR: 130 ppm                                                 |                               |
| Mono- and polysaccharides    | ¹H-NMR 3.3–3.9 ppm                                               | [23,32,33,35]                |
|                              | ¹³C-NMR (O-alkyl): 63–90 ppm                                     |                               |
|                              | ¹³C-NMR (Acetal group): 90–110 ppm                               |                               |
| Proteins, peptides or amino acids | ¹H-NMR: 3.3–3.9 ppm (α-carbon)                                   | [23,31,35]                   |
|                              | ¹H-NMR: 2.0–3.3 ppm (aliphatic chain)                            |                               |
|                              | ¹³C-NMR: 30–55 ppm (α-carbon)                                    |                               |
|                              | ¹³C-NMR: 20–74 ppm (aliphatic chain)                             |                               |
| Aromatic compounds           | Phenolic compounds                                               | [23,35,36]                   |
|                              | ¹H-NMR: 6.6–7.0 ppm                                              |                               |
|                              | ¹³C-NMR: 120–150 ppm                                             |                               |
|                              | Benzoic-like compounds                                           | [23,34,36]                   |
|                              | ¹H-NMR: 8.1–7.8 ppm                                              |                               |
|                              | ¹³C-NMR: 120–140 ppm                                             |                               |
that the $^1$H-NMR peaks in the 3.3–3.9 ppm region cross with carbons appearing from 60 to 75 ppm, there is an important contribution of amino acids and peptides as clearly evidenced by the presence of important cross-section peaks between the 3.3–3.9 ppm peak in $^1$H-NMR and the signals at 50–55 ppm in $^{13}$C-NMR (see Figure 2). It has to be mentioned that, whereas 50–55 ppm is a very common value for the $\alpha$-carbons of $\alpha$-amino acids [23,31], none of the saccharide carbons appear below 63 ppm [23,30].

On the other hand, HSQC-NMR spectroscopy also shows that most of the 0.8–1.2 ppm signals in $^1$H-NMR are due to aliphatic carbons, appearing in 2–30 ppm in $^{13}$C-NMR [23,33–35], thus indicating the presence of alkyl chains.

Mass spectrometric study

The previous two spectroscopic techniques provide an overall view of the general characteristics of the four fractions obtained from the USTP effluent. However, they do not provide information about the complexity of the mixture and the structure of individual compounds. This information can be obtained by mass spectrometric techniques.

In order to advance further in the characterization of the USTP effluent, we proceeded to the silylation of the mixtures by derivatization of the fractions with BSTFA+10%TMCS [20]. This protocol allows the analysis by gas chromatography of complex mixtures of non-volatile compounds by increasing their volatility through silylation. It has to be mentioned, however, that this technique could provide a biased view since it can happen that some compounds present in the mixture are not derivatized into any volatile derivatives, and therefore they will go undetected. To minimize this limitation we added an external standard (nitrobenzene) that allows the quantification of the proportion of volatile compounds with respect to the total amount of the fraction. In addition, FAB$^+$-MS (see Figure S18) of the fractions established that the maximum molecular weight of the compounds of the mixture is below 1000 Da, the majority of the components of the mixture being of molecular mass below 450 Da. Similar molecular weight distributions have been observed by others authors working with domestic sewage wastewaters and using activated sludges as biological treatment [10]. The relatively low molecular mass of the organic compounds present in the USTP effluent makes it more likely that the volatile compounds can be analyzed by GC after derivatization. The results obtained are summarized in Table 4.

As can be seen in Table 4, there are common features in the four fractions. Thus, monosaccharides with five, six or seven carbons have been characterized in all fractions (except in HPO-N fraction), but particularly in fractions HPO-A (49.3 $\mu$g mg$^{-1}$) and TPI-A (137.9 $\mu$g mg$^{-1}$). It has been reported that the saccharide fraction is typically retained in the XAD4 resin [37], and this agrees with our results in which we observed that the band around 1133 cm$^{-1}$, attributable to carbohydrates in the FT–IR spectrum of the TPI-A fraction, is much more intense than for the HPO-A fraction (see Figure 1). Although the mixture of monosaccharides is complex, the most abundant type of monosaccharides in the TPI-A fraction is aldopentose stereoisomers (92.2 $\mu$g mg$^{-1}$).

Apart from monosaccharides, the other common trait is the presence of carboxylic acids, mostly aliphatic. These carboxylic acids can have one or two hydroxyl groups, but the most prevalent are fatty acids. This is not surprising considering that fatty acids form part of the composition of soaps and domestic detergents [38].

The third feature is the presence of steroid compounds. These are characterized in the HPO-N fraction. The detection of cholesterol, even though in minimum quantities, is noteworthy as it indicates anthropogenic contamination of the water [39].

Also of interest is the detection of two therapeutic drugs in the USTP effluent: paracetamol (28.7 $\mu$g L$^{-1}$) and ketoprofen (1.8 $\mu$g L$^{-1}$), two best-selling non-steroidal anti-inflammatory drugs. These concentrations agree with reported values for paracetamol (from 6 to 65 $\mu$g L$^{-1}$) [14,40,41] and ketoprofen (0.84 $\mu$g L$^{-1}$) [42] in some wastewater effluents.

Overall the individual compounds shown in Table 4 and their presence in several of the different fractions show once again that the standard procedure commonly
Table 4. Individual compounds detected by GC–MS upon silylation of the HPO-A, HPO-N, TPI-A and TPI-N fractions. The number represents the estimated quantity (µg mg\(^{-1}\) sample) in the mixture.

| Compounds                        | HPO-A (Total identified 137.9 µg mg\(^{-1}\)) | HPO-N (Total identified 51.9 µg mg\(^{-1}\)) |
|----------------------------------|-----------------------------------------------|---------------------------------------------|
| Glycine (3.8)                    | 1.8                                           | 0.7                                         |
| Threonine (1.3)                  | 2.1                                           | 0.6                                         |
| Tyrosol (2.0)                    | 1.4                                           | 1.4                                         |
| Dehydroabietic acid (9.7)        | 3.8                                           | 0.9                                         |
| Mixture of stereoisomers (13.8)  | 7.2                                           | 2.1                                         |
| Mixture of stereoisomers (6.2)   | 4.1                                           | 3.2                                         |
| Oleic acid (3.2)                 | 5.3                                           | 3.0                                         |
| Cholesterol (2.1)                | 1.5                                           | 1.8                                         |
| Stigmasterol (1.3)               | 12.1                                          | 1.2                                         |
| β-Sitosterol (3.1)               | 17.0                                          | 4.3                                         |

(Continued)
used for water fractionation, consisting of the adsorption/desorption of organic compounds in two resins, is rather inefficient at producing a sharp separation of the components. The product distribution shown in Table 4 provides evidence that the resins used for fractionation are not able to specifically retain any type of compound that is spread in similar concentrations across the four fractions. Recently, the same conclusions about the inefficiency of the use of resins for fractionation have been reached using different hydrophilic and hydrophobic model compounds which were not fully retained in the corresponding resin [43].

A final comment is that, although it may seem that the data in Table 4 correspond to a low percentage of the total mass of each fraction (HPO-A, 137.9 μg mg⁻¹; HPO-N, 51.9 μg mg⁻¹; TPI-A, 292.0 μg mg⁻¹; TPI-N 193.7 μg mg⁻¹), i.e. between 5.2% and 29.2% of the total mass being determined, similar types of compounds could account for additional percentages of the mixture, but some silyl derivatives are not sufficiently volatile to be analyzed by GC. In other words, it could be possible that, even though the derivatization protocol is able to almost quantitatively transform hydrogen bonded to heteroatoms (–OH, –NH₂, –
COOH) into their silyl derivatives, the resulting silylated compounds may not be sufficiently volatile because of their high mass, particularly when there are multiple trimethylsilyl groups. To support this hypothesis, we submitted maltotriose (a trisaccharide having three glucose units and 504 Da molecular mass) to derivatization, and, upon GC–MS analysis, we were able to observe around 1% of monosaccharide isomers, probably derived from acid hydrolysis of maltotriose under the derivatization conditions. Thus, for a given mass of maltotriose, only 1% is detected as glucose. It can be assumed that a similar proportion can apply to other oligosaccharides. In summary, although a large proportion of the total mass is undetected, it is not unreasonable to speculate that a large percentage of the undetected fraction will be similar to the detected material and that the characterized compounds are representative for a larger percentage of organic compounds than those indicated in Table 4.

The effluent under analysis corresponds to an urban wastewater treated by prolonged aeration using activated sludge, and our analytical data agree with related studies with respect to the diminution of the DOC content [44], the distribution of the molecular weights of the compounds present in the different fractions [10], the prevalence of hydrophobic fractions over the hydrophilic ones [21,22], and also the presence of carbohydrates in the transphilic fraction [37]. Also, recent studies have shown that treatment by activated sludge is inefficient to eliminate drug and other compounds used by consumers and that these compounds can be detected in the effluents of treatment plants [42].

A large number of the studies derived from wastewater effluents have focused on the quality of the effluent with respect to the by-products formed in the disinfection process (i.e. chlorination or chloramination) and their implications for water reuse [21,45]. Some other works have been interested in the speciation of dissolved metals (i.e. copper) in the wastewater effluents, because of the impact of the metals on the effluent toxicity and because of the biological availability of the metals [46]. However, most of the studies have not made much effort to determine the chemical composition of the effluent, and are concerned with the behaviour of the bulk water. This approach cannot provide a deep understanding of the interactions of the effluent with disinfectants and/or with dissolved metals. In this sense, our work has established the presence of a large number of individual compounds in the different fractions and confirms some general similarities of our effluent with other studies, thus suggesting a general trend. It is well known that some of the identified

Table 5. Environmental impact of the individual compounds detected by GC–MS after derivatization with BSTFA+10% TMCS.

| Group               | Compound name                  | Environmental impact                                      | Reference b |
|---------------------|--------------------------------|-----------------------------------------------------------|-------------|
| Therapeutic drugs   | Paracetamol                    | Undesirable environmental compounds                       | [49–51]     |
|                     | Ketoprofen                     |                                                           |             |
| Sterols             | Stigmasterol                   | Often faecal indicators                                   | [39]        |
|                     | Beta-sitosterol                |                                                           |             |
|                     | Cholesterol                    |                                                           |             |
| Saccharides         | Aldopentoses                   | DBP-precursors†                                          | [52–54]     |
|                     | Aldohexoses                    |                                                           |             |
|                     | Aldoheptoses                   |                                                           |             |
| Amino acids         | Glycine                        | DBP-precursors†                                          | [55,56]     |
|                     | Threonine                      |                                                           |             |
| Carboxylic acids    | Citric acid                    | DBP-precursor                                            | [57]        |
|                     | 1,3 propanedioic acid          | DBP-precursors†                                          | [58]        |
|                     | 3-hydroxy-butyric acid         |                                                           |             |
|                     | 4-oxo-pentanoic acid           |                                                           |             |
| Fatty acids         | Lauric acid                    | All behaving similarly.                                   | [38,59]     |
|                     | Myristic acid                  | Influence on water retention capacity of soils, the mobility of heavy metals or the mineralization processes. |             |
|                     | Palmitic acid                  |                                                           |             |
|                     | Stearic acid                   |                                                           |             |
|                     | Oleic acid                     |                                                           |             |
| Aromatic compounds  | Dehydroabietic acid            | Aquatic and microbial toxic                               | [60,61]     |
|                     | Benzoic acid                   | DBP-precursor †                                           | [62,63]     |
|                     | Tyrosol                        | Inhibitor of biodegradation                               | [64,65]     |
|                     | p-hydroxyphenol                | DBP-precursor †                                           | [62,63]     |

*DBP-precursor. Disinfection by-product precursor is assigned when the compound develops, upon chlorination, important quantities of halogenated compounds, e.g. trihalomethanes, haloacetic acids and halonitriles, and has been identified to cause high total organic halogen (TOX).

bThe reference describes the direct effect of the compound in the environment or if it has been reported as a DBP-precursor.
compounds interact with disinfectants to form disinfection by-products (DBPs) (see Table 5), and they can also complex some metal ions (i.e. carbohydrates or citric acid) [47,48].

**Impact of the detected compounds for the aquatic environment**

Finally we would like to make some general observations about the influence of the detected compounds in the quality of the resulting UWW effluent. In the present work we have characterized a large quantity of compounds from an integrated UWW effluent, which are summarized in Table 5. In this table we indicate the expected impact of these compounds for the environment, based on reported data.

The detected compounds can be distinguished as undesirable when present in natural aquatic resources or if they are precursors of DBPs. The first group includes paracetamol and ketoprofen as undesirable compounds [66]. Another important identified product is dehydroabietic acid, which occurs naturally in softwood cellulose and sometimes is present (in µg L\(^{-1}\)) in the pulp industry effluent even after biological treatment [67–69]. The dehydroabietic acid is recognized in aquatic resources to be detrimental to fish and other forms of aquatic life [70,71].

A second group of compounds is those that have a recognized impact on environmental resources when they interact with chlorine as disinfectant. It has to be mentioned that the most extensive final disinfection procedure in the USTP is chlorination [72]. This group of compounds are formed by saccharides that have been previously reported as trihalomethane (THM) precursors [53]. Also, amino acids are considered to be responsible for THMs and haloacetonitriles [56,73]. With respect to the composition of carboxylic acids detected in the USWW effluent, it is worth commenting the presence of citric acid that is one of the best-studied compounds as a precursor of THMs and haloacetic acids (HAAs) [57,58]. Finally, some detected phenolic compounds are also known to react fast with chlorine to produce considerable amounts of THMs and HAAs [74–76].

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