USE OF GC×GC-(HR)MS AND NON-TARGET ENVIRONMETRICS TO ASSESS SEWAGE TREATMENT PLANT REMOVAL EFFICIENCIES AND IDENTIFY POORLY REMOVED EMERGING POLLUTANTS

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Introduction

In the modern society, large quantities of chemical substances are used, and more than 30 000 compounds are estimated to be in daily use in Europe, of which many will reach the municipal sewage treatment plants (STPs). STPs can therefore be considered as significant secondary sources for hazardous substances such as carcinogenic, mutagenic and reprotoxic (CMR) chemicals, persistent organic pollutants (POPs), pesticides, toxic metals, etc. to surface water. However, STPs are primarily designed to remove nutrients, not hazardous compounds. It is therefore essential to identify compounds poorly removed, characterize their physico-chemical properties, and use this information to improve the treatment process. This is a challenging task that only can be solved by combining powerful analytical techniques and systematic data interpretation.

Materials and methods

The sewage influent and effluent samples were collected from the Umeå STP, which is located in northern Sweden. The treatment process at the STP includes mechanical (screening and removal of sand and fat), chemical (flocculation of phosphorus using ferrous sulfate, FeSO4), and biological (degradation of organic material by microorganisms and removal of the remaining phosphorus) treatment steps. The Umeå STP processes mixed sewage from residential and commercial areas, a large hospital, surface runoff and a few industrial sites. Flow proportional water samples were collected using an automatic sampler over three consecutive days (influent samples 1 L per day, effluent samples 3.5 L per day) in May 2009 when the weather was normal for the season (average day-temperature 12℃) and the plant was functioning normally. Sampling was carried out mid-week to minimize the influence of different (often lower) industrial activity during the weekend, as well the effect of other activities that are largely performed during the weekend, e.g., domestic cleaning and pesticide application. The samples were transferred to dark pre-cleaned bottles and put in cold storage (4℃) until analysis.

The water samples from the three sampling days were combined and then divided into 5 replicates, and filtered through glass microfiber and membrane filters. The particle filters were wrapped in pre-cleaned aluminum foil and stored in a refrigerator until all water had evaporated. To serve as controls, laboratory blanks (Milli-Q water) were run in parallel to the samples according to the same protocols to ensure that any contamination introduced during pre-treatment, extraction, clean-up, and instrumental analysis did not significantly influence the results. The water samples (aqueous phase, <0.45 µm) were liquid-liquid extracted (half the volume at a time in the case of the effluent water samples) using a separator funnel, by vigorous shaking with 50, 20, and 15 mL of DCM (5 min each). The organic phases and emulsions formed between the water and organic phases were combined separately. 20 mL of DCM and 6 mL NaCl were added to the combined emulsions, the mixture was gently shaken, and the DCM was drained and added to the combined DCM phase. The solid samples (filters) were packed in 33 mL Dionex accelerated solvent extractor (ASE) cells fitted with glass microfiber filters. The cells were then filled with Celite® 545. The filters were pressurized liquid extracted (Dionex, ASE 200) with DCM by using the following settings: temperature, 100℃; pressure, 15 MPa; heat time, 5 min; static time, 5 min; flush volume, 60%; purge time, 90 s; number of cycles, 3.

After extraction the extracts were rotary evaporated to 3 mL. Isotopically labelled internal standards (IS) $^{13}C_{12}$-PCBs (28, 52, 105, 114, 118, 123, 156, 157, 167, 187; 20 ng each) were added and gel permeation chromatography (GPC) was used for non-specific clean-up, i.e., to remove macromolecules. The target fraction was collected, fortified with 12 ng of recovery standards (RS) $^{13}C_{12}$-PCBs (97 and 188) and evaporated into 500 µL

toluene. An aliquot (50 µL) from each influent replicate (both aqueous and solid samples) was combined and then subjected to the same instrumental analysis as the samples.

Analysis of the samples was performed using a Pegasus® 4D comprehensive two-dimensional gas chromatograph connected to a time-of-flight mass spectrometer (GC×GC-TOFMS) (LECO Corporation, St. Joseph, MI, USA) equipped with a 6890N GC (Agilent Technologies, Palo Alto, CA, USA), a secondary GC-oven, and a cryogenic modulator. A 30 m DB-XLB column (0.25 mm i.d. x 0.25 µm film thickness, J&W Scientific) was used for the first dimension (^{1}D) separation and a 1.5 m BPX-50 column (0.10 mm i.d. x 0.10 µm film thickness, SGE) was employed for the second dimension (^{2}D) separation. Helium was used as the GC carrier gas (constant flow, 1.4 mL min^{-1}). The injection volume was 1 µL and injections were performed in split-less mode, using an autosampler and an injector temperature of 250°C. The primary GC-oven temperature program started at 80°C (1 min) followed by an increase of 4°C min^{-1} to 340°C (5 min). The secondary GC-oven and the modulator temperature were operated in the same manner as the primary oven but with a +20°C and +35°C offset, respectively. The modulation period was 2 s with 0.6 s hot pulse duration and 0.4 s cooling time between stages. The transfer line and ion source temperatures were set at 350°C and 250°C, respectively. Electron ionization at 70 eV was used and ions were recorded in full-scan mode over the mass range 50-900 amu, using a sampling rate of 100 Hz. ChromaTOF® -GC software (LECO Corporation, St. Joseph, MI, USA) was used for data acquisition and processing.

The combined influent water sample (described in the previous section) was used to create a template for the GC×GC peak detection and data evaluation. The automatic baseline, peak find, peak and spectra deconvolution, and library search functions were applied, and the resulting peak table was used as a reference sample. The remaining samples were automatically processed to detect and integrate peaks associated with the components present in the template (reference sample), and individual peak tables were copied to separate worksheets in a Microsoft Excel workbook. All peak area columns were then extracted and compiled in a single worksheet. The resulting large data set was pre-treated to remove background and low intensity components, and avoid false positives. The following data were excluded: compounds with low detection frequencies (i.e., present in less than 3 out of 5 replicates); compounds having elevated blank values (i.e., >20% of the median value for the respective sample matrix); and peaks due to column and septa bleed or partially resolved isomers that were difficult to automatically integrate. The remaining 1128 compounds were then semi-quantified using one of the internal standards (IS 13C12-PCB 118) and the isotope-dilution methodology. The total area of all apexing peaks (corresponding to ions that maximized at the same ^{1}D and ^{2}D retention times) were used in the calculations, and an equal response to the IS was assumed.

Principal component analysis (PCA) was used (SIMCA-P+11, Umetrics, Sweden) to generate an overview of the final complex data set and to detect suspected outliers. The data were mean-centered and scaled to unit variance to make all parameters equally important. To assess the STP treatment efficiency, calculation of the total removal efficiency of all compounds was performed, as well as the removal efficiency of aqueous- and solid-phase contaminants. In cases where the average concentrations in the effluent were higher than those in the influent, which may occur because of e.g. transformation/biodegradation reactions lead to the formation of a compound present in the influent, the removal efficiencies were set to zero.

An attempt was made to identify as many as possible of the compounds that were poorly removed by the STP processes. All components that had a removal efficiency less than 65% were evaluated using the NIST library. Compounds that passed the spectra quality cutoffs and a manual inspection (all major spectral features present and absence of abundant ions above the molecular weight of the library candidate) were assigned as tentatively identified. The validity of these assignments were tested by reanalyzing selected samples using a prototype GC×GC high-resolution TOFMS (LECO Corporation, St. Joseph, MI, USA). It was operated using very similar chromatographic conditions as the Pegaus 4D system, but at a higher resolution (> 25,000).

Results and discussion

The results of the PCA revealed distinct groupings of replicates of different sample types, which were clearly separated from each other. No suspected outliers could be detected. The first principal component (PC1) reflected differences in concentration, whereas the second principal component (PC2) reflected contaminant pattern differences between aqueous and solid samples. Blank samples contain low concentrations and have a profile that matches neither that of aqueous- nor particle-phase samples; hence the samples were not significantly affected by laboratory or instrument background.

The main objective of this study was to characterize compounds that were not efficiently removed using the current STP treatment technology. Thus, all compounds with a removal efficiency better than 65% were excluded from further analysis, and the remaining data were examined manually to eliminate any data that could be due to instrument background. In general, the compounds that were most retained (most polar or polarizable) in ^{2}D were less efficiently removed. A group of poly(ethylene/propylene)glycols was however found to behave differently to the other polar contaminants. They eluted early in ^{2}D and exhibited low removal efficiencies (large STP breakthrough). Thus, despite the high polarity of these compounds and their tendency to pass through the STP unaffected, they did not interact with the semi-polar stationary phase (50%-phenyl polysilphenylene-siloxane) in the ^{2}D column. These compounds are all H-bond acceptors, and therefore, unable to interact strongly with the (polarizable) phenyl groups in the stationary phase. In future studies, it would be interesting to use another (complementary) secondary column with the ability to form H-bonds, e.g., a polyethylene glycol (WAX) column, which should result in a higher retention times for these compounds.

An attempt was made to identify as many as possible of the compounds that were poorly removed by the STP processes. Of the 188 components that had a removal efficiency less than 65%, we were able to tentatively identify 68 compounds (Table 1). NIST library searches produced matches that were generally good with a median similarity of 87% and a median probability value of 7,200 (out of 10,000). Generally, the library matches were concentration dependent, and compounds with a high concentration gave the best fit. Only compounds that passed a manual inspection were considered.

Table 1. List of compounds poorly removed (< 65%) by the STP process (in concentration order top-down, starting with left column).

2,4,7,9-Tetramethyl-5-decyn-4,7-diol	5-Hydroxy-4-nitroguaiacol	Indano[2,1-d]1,3-dioxane
Benzenesulfonamide, N-butyl-	4-Amino-acetophenone	5-Benzothiazolamine, 2-methyl-
Tris(butoxyethyl) phosphate	Dimethyl benzyl carbinol	2-Propanone, 1-phenoxy-
Benzothiazole, 2-(methylthio)-	Phthalic acid, mono(-2-ethxyethyl) ester	1-Ethyl-2-pyrrolidinone
Tricyclo[5.2.1.0(2,6)]dec-3-en-10-one	1H-Inden-1-one, 2,3-dihydro-	3,5-Dichlorophenyl isocyanate
Tris(3-chloropropyl)phosphate (3 isomers)	4-Hydroxy-2-methylacetophenone	2(3H)-Furanone, 5-acetyldihydro-
Benzophenone	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	1H-Inden-1-one, -3,3-dimethyl-
Ethyl citrate	1(2H)-Naphthalenone, 3,4-dihydro-	Ethyl acetoacetate ethylene acetal
Caffeine	2,4(1H,3H)-Pyrimidinedione, dihydro-3-methyl-	9H-Fluoren-9-one
Tris(2-chloroethyl)phosphate	1(3H)-Isobenzofuranone, 3,3-dimethyl-	9,10-Anthracenedione
2,2,2-Trichloro-1-phenylethanol	1-[4-(1-hydroxy-1-methylethyl)phenyl]-ethanone	Benzo[f]quinoline
Oxybenzone	Benzenesulfonamide, N-ethyl-4-methyl-	Naphthalene
4-*tert*-butyl-cyclohexanone	Triphenyl phosphate (TPP)	2-Propanone, 1,3-diphenyl-
Ethosuximide	1H-Benzotriazole, 4-methyl-	2(5H)-Furanone, 5-methyl
Tris(1,3-dichloroisopropyl)phosphate	1H-Benzotriazole, 5-methyl	Bayer 28,589
Isoquinoline	Disulfide, methyl (methylthio)methyl	Ethanone, 1-[4-(1-methylethyl)phenyl]-
4-*tert*-octyl-phenol	1-Phenoxypropan-2-ol	2,4,6-Cycloheptatrien-1-one
Hexadecenoic acid, Z-11-	Phenol, 2,5-bis(1-methylethyl)-	Coumarin
Diethyltoluamide (DEET)	1'-Acetonaphthone	1H-Indole-3-carboxaldehyde*
Benzenesulfonamide, N-ethyl-2-methyl-	2,5-Pyrrolidinedione, 1-ethyl-	Triacetin
2,3,6,7-Tetramethylquinoxaline	Carbamazepine	Indan-1,2,3-trione

Effluent concentrations of the tentatively identified compounds varied between 0.2 and 12 000 ng L 1, of which 87% were found to be predominantly (>90%) dissolved in the aqueous phase (median, 100%; average, 94%). A lower fraction of the pollutants (59%) were found in the aqueous phase of the influent (median, 81%; average, 82%). This was also reflected in the removal efficiencies; compounds that are more water-soluble were less retained. One of the compounds, 1H-Indole-3-carboxaldehyde (the only aldehyde), appeared to be attached to particles in both influent and effluent (marked with an asterisk in Table 1) but nevertheless was not efficiently

removed. This may indicate that this compound is present in the influent and also formed (e.g. from 1H-Indole) during the STP process.

The chemicals that were filtered out using the non-target environmetrics approach and tentatively identified using the NIST library shared some common structural features. Many of the compounds were aromatic (68%), (hetero)cyclic (16%) or both (19%), and a few were saturated compounds. Among the remaining compounds that did not belong to these classes, seven were (halogenated) organophosphate esters (10%). These structural features make the contaminants more stable and less prone to degradation. It is also worth noting that all the identified compounds have polar functional groups or moieties, most commonly keto/ester-groups (53%), followed by S,N,O-heterocyclic groups (31%), phosphate esters (10%), S/O-ethers (10%), amides (7%), nitro-groups (3%), acids (3%) and, finally one amine (1.5%). These functional groups and moieties increase the water solubility of the compounds, and thus they are more likely to follow an aqueous route through the STP.

Complementary analyses using GC×GC high-resolution TOFMS were found to be very valuable. The accurate mass determinations were found to be in excellent agreement with the expected theoretical values (Table 2). With few exceptions the NIST EI-MS library similarity was better than 80% and the deviation from the expected value < 1 ppm. The combined use of library and accurate mass matching greatly reduces the risk of false positive identification. Naturally, comparisons to pure reference compounds will be required for final verification. It was also possible to tentatively identify additional poorly removed compound (among those that did not have unambiguous spectra), for example, a hydroxy metabolite of 2-(methylthio) benzothiazole (MBT).

Table 2. Mass accuracies of the 10 most abundant poorly removed contaminants.

Compound	Similarity %	Nominal mas	Ion	Formula	Expected mass	Detected mass	Deviation, ppm
2,4,7,9-Tetramethyl-5-decyn-4,7-diol	81	169	$[M-C_4H_9]^+$	$C_{10}H_{17}O_2$	169.1223	169.1221	1.12
		151	$[M-C_4H_{11}O]^+$	$C_{10}H_{15}O$	151.1117	151.1116	0.87
Benzenesulfonamide, N-butyl-	87	213	M^+	$C_{10}H_{15}NO_2S$	213.0818	213.0818	0.14
		170	$[M-C_3H_7]^+$	$C_7H_8NO_2S$	170.027	170.0269	0.48
Tris(butoxyethyl) phosphate	87	299	$[M-C_6H_{11}O]^+$	$C_{12}H_{28}O_6P$	299.1618	299.1615	0.97
Benzothiazole, 2-(methylthio)- (MBT)	90	181	M^+	$C_8H_7NS_2$	181.0014	181.0014	0.01
Tricyclo[5.2.1.0(2,6)]dec-3-en-10-one	88	148	M^+	$C_{10}H_{12}O$	148.0883	148.0885	1.66
Tris(3-chloropropyl) phosphate	81	277	$[M-CH_2Cl]^+$	$C_8H_{16}Cl_2O_4P$	277.0158	277.0156	0.64
Benzophenone	87	182	M^+	$C_{13}H_{10}O$	182.0726	182.0726	0.16
Ethyl citrate	77	203	$[M-C_3H_5O_2]^+$	$C_9H_{15}O_5$	203.0948	203.0918	1.89
		157	$[M-C_5H_{11}O_3]^+$	$C_7H_9O_4$	157.0495	157.0498	1.95
Caffeine	86	194	M^+	$C_8H_{10}N_4O_2$	194.0798	194.0797	0.37
Tris(2-chloroethyl) phosphate	81	249	$[M-Cl]^+$	$C_6H_{12}Cl_2O_4P$	248.9844	248.9843	0.74
		205	$[M-C_2H_4OCl]^+$	$C_4H_8Cl_2O_3P$	204.9582	204.9585	0.95

In conclusion, this study demonstrates new tools for assessing which compounds are poorly removed during sewage treatment. The systematic approach was shown to work satisfactorily for GC amenable compounds and enabled the tentative identification of several well-known water-soluble contaminants as well as many new and emerging pollutants. The approach offers better chromatographic (GC×GC) and mass spectrometric (peak and spectra deconvolution) resolution than traditional GC-MS methodologies, allows comprehensive comparisons of influent and effluent concentrations, systematic prioritization of contaminants according to (lack of) removal efficiency, and provides pure spectra, which facilitate library identification. It also provides information on the physicochemical properties of the contaminants via the first and second dimension retention times, which can be used to support or reject tentatively proposed contaminant structures. Furthermore, it allows for the first time, systematic analysis of which compound classes are not efficiently removed using the current sewage treatment technology and could thus aid the development of future STP technologies. Finally, the prototype GC×GC high-resolution TOFMS system proved to be very useful for accurate mass determination and structure verification. If it reaches the market, it will make the process of identification faster, easier and more reliable.

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