Biofiltration for Advanced Treatment of Wastewater

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The Urban Water Security Research Alliance (UWSRA) is a $50 million partnership over five years between the Queensland Government, CSIRO's Water for a Healthy Country Flagship, Griffith University and The University of Queensland. The Alliance has been formed to address South East Queensland's emerging urban water issues with a focus on water security and recycling. The program will bring new research capacity to South East Queensland tailored to tackling existing and anticipated future issues to inform the implementation of the Water Strategy.

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Cover Photograph:

Description: pilot scale biofilters at South Caboolture Water Reclamation Plant
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FOREWORD

Water is fundamental to our quality of life, to economic growth and to the environment. With its booming economy and growing population, Australia's South East Queensland (SEQ) region faces increasing pressure on its water resources. These pressures are compounded by the impact of climate variability and accelerating climate change.

The Urban Water Security Research Alliance, through targeted, multidisciplinary research initiatives, has been formed to address the region's emerging urban water issues.

As the largest regionally focused urban water research program in Australia, the Alliance is focused on water security and recycling, but will align research where appropriate with other water research programs such as those of other SEQ water agencies, CSIRO's Water for a Healthy Country National Research Flagship, Water Quality Research Australia, eWater CRC and the Water Services Association of Australia (WSAA).

The Alliance is a partnership between the Queensland Government, CSIRO's Water for a Healthy Country National Research Flagship, The University of Queensland and Griffith University. It brings new research capacity to SEQ, tailored to tackling existing and anticipated future risks, assumptions and uncertainties facing water supply strategy. It is a $50 million partnership over five years.

Alliance research is examining fundamental issues necessary to deliver the region's water needs, including:

- ensuring the reliability and safety of recycled water systems.
- advising on infrastructure and technology for the recycling of wastewater and stormwater.
- building scientific knowledge into the management of health and safety risks in the water supply system.
- increasing community confidence in the future of water supply.

This report is part of a series summarising the output from the Urban Water Security Research Alliance. All reports and additional information about the Alliance can be found at http://www.urbanwateralliance.org.au/about.html.

Chris Davis
Chair, Urban Water Security Research Alliance

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EXECUTIVE SUMMARY

Wastewater treatment plants (WWTPs) are designed to remove nutrients from wastewater in order to limit the impact of effluent discharge on the environment. Nowadays, they achieve high removal of organic matter, nitrogen and phosphorus. However, it has been observed that even highly treated effluents still have an impact on aquatic wildlife. This impact has been linked to the presence of residual organic matter and, in particular, trace levels of compounds from anthropogenic origins. Effluent organic matter (EfOM) contains thousands of these organic micropollutants (OMPs) such as pharmaceuticals and their human metabolites, personal care products, pesticides, and industrial chemicals as well as their biodegradation products. Moreover, humans can be exposed to OMPs where wastewater impacted sources are used for drinking water production. Conventional drinking water treatment processes are indeed ineffective for the removal of these chemicals. Pharmaceuticals raise particular concerns because they have been designed to be bioactive and the long term effects of low level, lifetime exposure are unknown. EfOM also contains disinfection by-products (DBP) precursors which could increase their formation during the disinfection of drinking water. Exposure to DBPs has been associated with some forms of cancer. It is therefore important to remove EfOM and OMPs from the treated effluents before their discharge in order to further reduce their impact on the environment and protect drinking water sources from contamination.

Several water treatment technologies such as adsorption, oxidation and high pressure membrane filtration have proved effective for the removal of EfOM and, in particular, OMPs but they are expensive. On the contrary, biological filtration systems are typically simple and robust. Natural systems such as riverbank filtration and managed aquifer recharge can provide significant removal of EfOM and OMPs. However, engineered biofiltration systems such as sand and biological activated carbon (BAC) have not yet been extensively studied despite their potential. In this context, the objectives of this study were to:

- determine the efficacy of biofiltration as a single step for the advanced treatment of wastewater.
- compare biofiltration alone to the conventional combination of ozonation and biofiltration.
- assess the impact of filtering media (sand or activated carbon) and empty bed contact time (EBCT) on the biofiltration performance.
- determine whether operating conditions impact the microbial community composition in the filters.

Pilot scale biofiltration columns were set up at South Caboolture Water Reclamation Plant and the water quality and treatment efficiency were assessed by various analyses:

- Dissolved organic carbon (DOC) concentration was used as an indicator of EfOM content.
- A wide range of pharmaceuticals and personal care products (PPCPs) were quantified.
- Bioanalytical tools were used to determine non-specific and specific toxicity via five toxic modes of action.
- The removal of disinfection by-product precursors was estimated with formation potential tests for nitrosamines, trihalomethanes and haloacetic acids.
- The microbial communities' composition was determined by DNA extraction follow by 16S rRNA pyrotag sequencing.

The first sampling campaign was performed with an EBCT of 2 hours in the pilot scale filters and samples were also collected from the full scale BAC filter which has an EBCT of 18 minutes. BAC filtration alone (i.e. without prior ozonation) was capable of removing up to 60% of the residual DOC from the WWTP effluent. BAC filtration also removed a wide range of PPCPs by more than 90%, generally down to levels below 0.1 ng L^{-1}. BAC filtration alone reduced non-specific toxicity by more than 60% and five specific toxic modes of action by 50 to more than 90%. BAC filtration alone also removed DBP precursors, it reduced the formation potential of *N*-nitrosodimethylamine (NDMA), total trihalomethanes and five haloacetic acids by 81, 40 and 47% respectively. When compared to other filters, BAC alone performed similarly to the combination of ozonation and BAC filtration with

the same EBCT and better than ozonation followed by BAC filtration with a shorter EBCT. Sand filtration alone led to limited improvement of the water quality.

The EBCT was varied from 30 to 120 minutes in both BAC filters (i.e. with and without prior ozonation). Generally, the performance of the filters did not vary much when the EBCT varied. However, the number of compounds detected in the effluent of the filters increased and the removal of gabapentin decreased with decreasing EBCT suggesting that this parameter is nonetheless important. Non-specific toxicity reduction also decreased with decreasing EBCT in the BAC filter without prior ozonation. The dissolved oxygen concentration in the effluent of the filters was low, suggesting that it could be a factor limiting the performance of the filters.

Two additional sampling campaigns were performed approximately 1 and 2 years after the first one to check the performance of the filter on the long term. In particular, the aim was to make sure the high removal of DOC and PPCPs observed in the BAC filters in the first sampling campaign was not due to adsorption. The results showed that the efficacy of the BAC filters for DOC and PPCPs removal, as well as non-specific toxicity reduction, was stable over two years, suggesting the main mechanism is biodegradation and not adsorption. In the meantime, the sand filter was placed after the ozonation but it did not perform significantly better than without ozonation. The dissolved oxygen concentration in the filters influents was increased using 90% oxygen to avoid any limitation. This did not lead to a significant increase of efficacy in the BAC filters.

The analysis of the microbial communities' composition at different depths in the various filters revealed that the number of species present tended to decrease with increasing depth. This could be due to a change in conditions such as lower nutrient availability and/or lower dissolved oxygen concentration. The comparison of the filters also revealed that ozonation impacts the type of bacteria present, particularly for deeper samples. Differences were also observed between the BAC and the sand filter. However, it is not possible to draw conclusions owing to the limited number of samples; this warrants further research in the area.

BAC filtration without prior ozonation is capable of significantly improving the quality of the WWTP effluent. BAC filtration proved more effective than sand filtration and ozonation before BAC filtration did not significantly improve the performance. BAC filtration is therefore suggested as a simple and cheap option for the upgrade of WWTPs with advanced treatment in order to improve effluent quality before discharge. Further research is required to better understand the parameters influencing the performance of BAC filters and to provide information for the design of full scale units.

1. INTRODUCTION

Traditionally, wastewater is treated to remove pathogens, organic matter and nutrients to limit the environmental impact of its discharge in surface water and avoid the contamination of drinking water sources. Nowadays, state-of-the-art wastewater treatment plants (WWTPs) achieve high levels of nutrient removal and disinfection. Therefore, in recent years, attention has turned to the residual organic matter present in treated effluents.

1.1. Effluent Organic Matter Composition and Impact on Water Quality

Effluent organic matter (EfOM) consists of natural organic matter originating from the drinking water and anthropogenic pollutants brought by human activities (Shon *et al.*, 2006). EfOM contains thousands of organic micropollutants such as pharmaceuticals and their human metabolites, personal care products, pesticides, industrials chemicals as well as their biodegradation products. Although they are typically present at sub µg L^{-1} concentrations, pharmaceuticals and personal products (PPCPs) raise concerns regarding the adverse effects they could have on human health for a lifetime exposure to low doses. Pharmaceuticals received particular attention because they have been designed to be bioactive. While there is still no evidence of their potential impact on human health, it has been demonstrated that WWTP effluent discharge can affect river wildlife. For instance, feminisation of male fishes due to the presence of estrogenic compounds at ng L^{-1} levels in effluents have been observed (Sumpter, 2005). Therefore, following the precautionary principle, these compounds should be removed from the treated wastewater to protect the environment and prevent the contamination of drinking water sources.

Drinking water is systematically disinfected with chlorine or chloramine to provide a disinfectant residual in the distribution network, preventing undesired bacterial growth. The reaction of the disinfectant with organic matter leads to the formation of disinfection by-products (DBPs), which is an unintended consequence of this process. Bladder and colorectal cancers have been associated with exposure to chlorination by-products in drinking water (Villanueva *et al.*, 2007). EfOM contains DBP precursors and, in particular, dissolved organic nitrogen (Krasner *et al.*, 2008). Drinking water sources impacted by wastewater could therefore lead to an increased formation of DBPs, in particular nitrogenous DBPs such as *N*-nitrosodimethylamine (NDMA). DBP precursors should therefore be removed from reclaimed water to reduce DBP formation during the disinfection of wastewater impacted sources.

1.2. Effluent Organic Matter Removal in Treated Wastewater

Technologies that can be used to remove EfOM include flocculation, adsorption, (bio)filtration, ion exchange, (advanced) oxidation processes and membrane filtration (Shon *et al.*, 2006). However, only some of these technologies are effective for the removal of PPCPs: activated carbon adsorption (Ternes *et al.*, 2002; Westerhoff *et al.*, 2005; Nowotny *et al.*, 2007; Snyder *et al.*, 2007; Yu *et al.*, 2008), ozonation and advanced oxidation processes (Zwiener and Frimmel, 2000; Huber *et al.*, 2003; Ternes *et al.*, 2003; Huber *et al.*, 2005; Esplugas *et al.*, 2007; Nakada *et al.*, 2007; Kim *et al.*, 2008; Hollender *et al.*, 2009; Reungoat *et al.*, 2010; Reungoat *et al.*, 2012) and high pressure membrane filtration (Kimura *et al.*, 2004; Snyder *et al.*, 2007; Yoon *et al.*, 2007). Activated carbon adsorption and ozonation are considered to be economically feasible for advanced treatment of WWTPs effluents (Joss *et al.*, 2008). Their combination has proven to be very effective in removing organic micropollutants and decrease non-specific and specific toxicity in treated wastewater (Reungoat *et al.*, 2012). However, ozonation is known to lead to the formation of by-products largely not identified to date, which raises concerns regarding their potential impact on the environment and human health (Benner and Ternes, 2009; Radjenovic *et al.*, 2009; Dodd *et al.*, 2010; Stalter *et al.*, 2010). While activated carbon does not generate by-products, it has to be renewed regularly and disposed of or regenerated, generally off site.

1.3. Biofiltration

Biofiltration systems are typically robust, simple to construct and have low energy requirements (Pipe-Martin *et al.*, 2010). The most common technologies are sand filtration, biological activated carbon (BAC) filtration, riverbank filtration and managed aquifer recharge. Whereas the removal of organic matter and PPCPs from drinking water sources and treated wastewater has been investigated in river bank filtration and managed aquifer recharge systems (Petrovic *et al.*, 2009; Baumgarten *et al.*, 2010; Rauch-Williams *et al.*, 2010), the treatment of WWTP effluents with engineered BAC filters has not received much attention so far. Recently, Reungoat *et al.* (2012) observed significant removal of some PPCPs in BAC filters after ozonation.

A BAC filter consists of a fixed bed of granular activated carbon (GAC) supporting the growth of bacteria attached to the GAC surface. This technology has been used for many years for drinking water treatment, usually after ozonation, and has proven to be able to significantly remove natural organic matter, ozonation by-products, DBP precursors as well as odour and taste compounds (e.g. geosmin and 2-methylisoborneol) (Simpson, 2008). A Swiss study estimated the cost of several options to upgrade WWTPs for PPCP removal: sand filtration and ozonation were in the same range, 5.9 to 32.2 and 4.8 to 36.7 CHF EP^{-1} a^{-1} respectively (depending on the plant size) whereas activated carbon adsorption cost was higher, between 21.5 and 95 CHF EP^{-1} a^{-1} (Moser, 2008). BAC filtration costs can be expected to lie in the same range as sand filtration and therefore it potentially represents an interesting alternative technology for the removal of EfOM and PPCPs to improve the quality of WWTP effluents.

1.4. Objectives

In that context, the objectives of the enhanced treatment project were to:

- determine the efficacy of biofiltration as a single step for the advanced treatment of wastewater.
- compare biofiltration alone to the combination of ozonation and biofiltration.
- assess the impact of filtering media (sand or activated carbon) and empty bed contact time (EBCT) on the biofiltration performance.
- determine whether operating conditions impact microbial community compositions in the filters.

Pilot scale biofiltration columns were set up at South Caboolture Water Reclamation Plant and the water quality and treatment efficiency was assessed with various analyses:

- Dissolved organic carbon (DOC) concentration was used as an indicator of EfOM content.
- A wide range of PPCPs were quantified.
- Bioanalytical tools were used to determine non-specific and specific toxicity via five toxic modes of action.
- The removal of DBP precursors was estimated with formation potential tests for nitrosamines, trihalomethanes and haloacetic acids.
- The microbial communities' composition was determined by DNA extraction follow by 16S rRNA pyrotag sequencing.

2. MATERIAL AND METHODS

2.1. Pilot Scale Biofilters

Three pilot scale biofilters (Figure 1 and front cover) were set up in December 2006 at the South Caboolture Water Reclamation Plant (van Leeuwen *et al.*, 2003; Reungoat *et al.*, 2012). The reclamation plant receives water from a 40,000 equivalent people WWTP using a sequencing batch reactor process which achieves partial nutrient removal. The columns are 3 m high and 22.5 cm internal diameter; they consist of 80±1 cm filtering bed supported by a 20 cm layer of gravel at the bottom, the top of the columns are filled with water. One column uses sand as filtering medium and the other two are filled with “Acticarb BAC GA1000N” granular activated carbon (Activated Carbon Technologies Pty Ltd, Australia). Details on the filtering media can be found Table 1.

The filters were fed with water from the main stream of the reclamation plant. The sand filter was originally fed with non-ozonated water (referred to as SAND 1) and later with ozonated water (referred to as SAND 2). The activated carbon filters BAC 1 and BAC 2 were continuously fed with non-ozonated and ozonated water respectively. Non-ozonated water refers here, and in the rest of the manuscript, to the effluent before the main ozonation stage but after the denitrification, the pre-ozonation and the dissolved air flotation and filtration. A prior study showed that the ozone dose added in the pre-ozonation is very low relatively to the DOC concentration at this stage (0.1 mg_{O3} mg_{DOC}^{-1}) and does not lead to any significant removal of DOC or PPCPs (Reungoat *et al.*, 2012).

Compressed air was bubbled in the water above the filtering bed to ensure a high level of dissolved oxygen to support biological activity; this was later switched to 90% oxygen. The empty bed contact time (EBCT) was controlled by adjusting the effluent flow rate at the bottom of the columns. The top layer of each filtering bed (sand and BAC filters) was stirred weekly to avoid clogging of the columns and water was withdrawn from above the filter at the same time. This operation removed some of the biomass from the top of the filter; however no backwash of the entire filter was performed. A previous study showed that biological activity had developed on the filtering media and dissolved organic removal had reached a steady state by June 2007 (Pipe-Martin *et al.*, 2010).

Table 1: Characteristics of the sand and granular activated carbon used in the pilot-scale and full scale filters (n/a = not applicable).

Filter	Sand	Acticarb BAC GA1000N
Bulk density (kg m^{-3})	1,417	564
Mean 2D equivalent diameter (mm)	2.25	1.60
Particle effective size (mm)		0.6 (0.9 for full scale)
Uniformity coefficient		< 1.8
Iodine number (mg g^{-1})	n/a	1,050
BET surface area (m^2 g^{-1})	n/a	1,146
Total porous volume (cm^3 g^{-1})	n/a	0.476
Microporous volume (cm^3 g^{-1})	n/a	0.360
Mesoporous volume (cm^3 g^{-1})	n/a	0.116

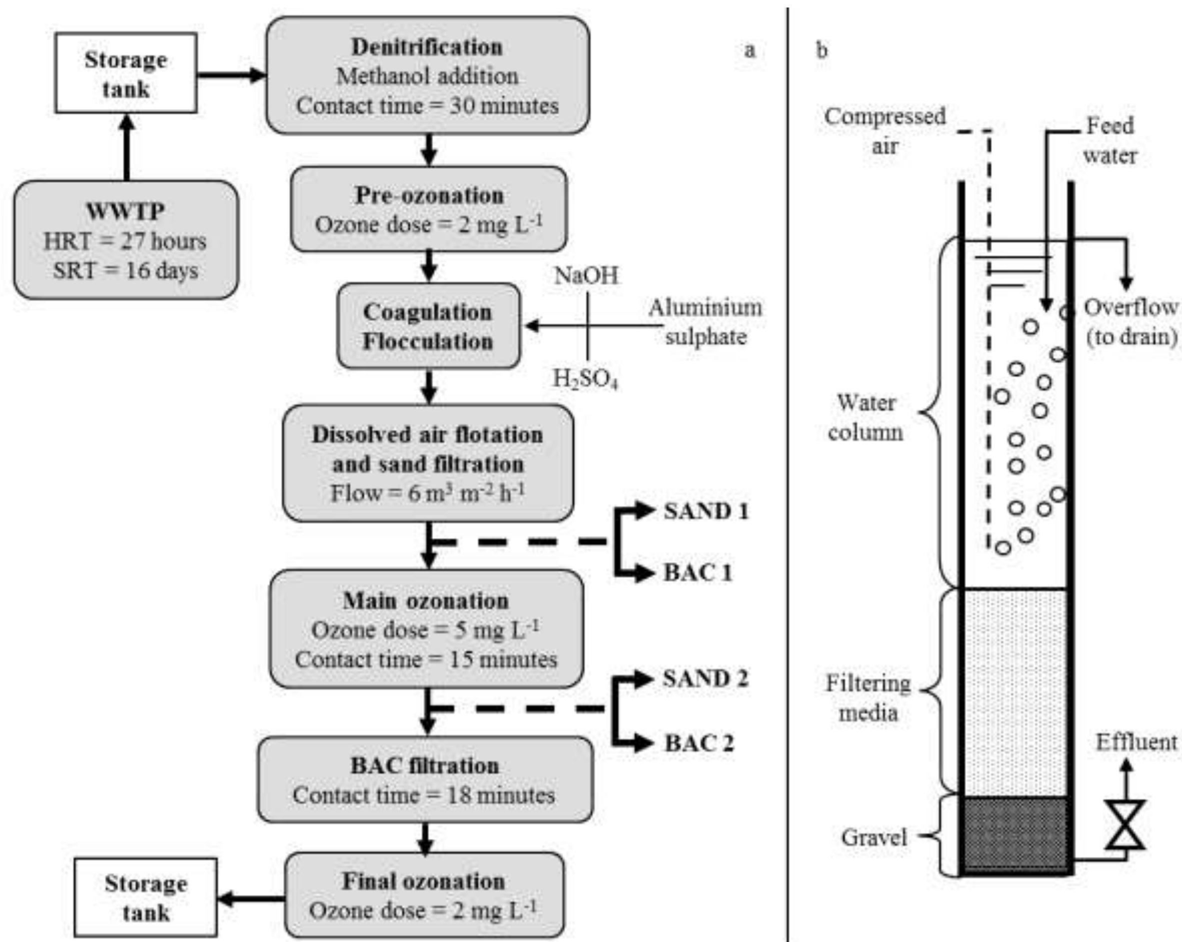

Figure 1: South Caboolture Water Reclamation Plant (a) and pilot-scale biofilters (b). HRT= hydraulic residence time, SRT = sludge residence time.

2.2. Full Scale Biological Activated Carbon Filter

The BAC filter of the reclamation plant (BAC FS) has an empty bed contact time of 18 minutes. The filtering media is of the same type of granular activated carbon as the one used in the pilot-scale columns with a slightly higher particle diameter (Table 1). The granular activated carbon was replaced in March 2008, 4 months prior to the first sampling campaign.

2.3. Sample Collection

Samples were collected during 4 campaigns from the influent and effluent streams of the filters. For the first campaign (July and August 2008), four sets of 24-hours composite samples were collected. As the flow rate in the reclamation plant (due to the presence of storage tanks) and in the pilot scale filters was constant at the time of sampling, representative samples were collected using continuous pumping at 7 ml min^{-1}. The pilot-scale filters were then operating with an empty bed contact time (EBCT) of 120±10 minutes. During the second campaign (December 2008), four sets of 24-hours composite samples were collected from both BAC filters operating with various EBCTs (30±1, 60±2, 90±4 and 120±6 minutes) to investigate the influence of this parameter. A waiting period of one week was observed from the moment the EBCT was modified prior to the sample collection. Finally, a third and fourth sampling campaigns were carried out in October 2009 and July 2010 to confirm the results obtained in previous campaigns on a longer term and to investigate the performance of sand filtration after ozonation. The feed to the sand filter was changed from non-ozonated to ozonated water six months prior to the third campaign. Two sets of 24-hour composite samples and three grab samples were collected and in the third and fourth campaign respectively. Pilot scale filters were operating with

an EBCT of 1 hour. Three months prior to the fourth campaign, the air supply at the top of BAC 1 and BAC 2 was switched to 90% oxygen used in the reclamation plant's ozone generators to ensure higher dissolved oxygen levels in the influent water.

Samples were collected into glass bottles pre-washed with MilliQ water and HPLC grade methanol and rinsed with the water sampled moments before sampling commenced. The samples were protected from light and refrigerated during collection. In the fourth campaign, the grab samples were collected directly in amber glass bottles (see below). The water temperatures were 22±2°C, 27±2°C, 26±1°C, and 22±1°C during the first, second, third and fourth sampling campaigns respectively and pH was 7.0±0.5 for all sampling events.

For micropollutant analysis, 1 to 2 L of sample were transferred into methanol washed amber glass bottles and preserved with sodium thiosulfate (80 mg L^{-1}) when sent to Queensland Health Forensic and Scientific Services (QHFSS). For the bioassays, 2 L of sample were transferred into methanol washed amber glass bottles and hydrochloric acid (36%) was added to a final concentration of 5 mM for preservation. For DOC measurements, 100 mL were collected in plastic (HDPE) bottles. All bottles were rinsed with sample before filling. All samples were transported on ice and stored frozen or at 4°C prior to analysis.

2.4. Chemical Analysis

2.4.1. Dissolved Oxygen

During the second sampling campaign, dissolved oxygen (DO) concentration was measured with a YSI 6562 Dissolved Oxygen Probe connected to a YSI MDS 650 multi-parameter display system. A YSI 6560 conductivity and temperature probe connected to the same multi-parameter display system allowed to simultaneously correct the DO concentration value and display it directly as a concentration. During the third sampling campaign, DO was measured using a CyberScan PCD 650 multi-parameter instrument (Eutech Instruments) equipped with temperature, pH, DO and conductivity probes. The simultaneous measurement of DO, temperature and conductivity allowed correction of the DO concentration value and displaying it directly as a concentration.

2.4.2. Dissolved Organic Carbon

Prior to analysis, samples were filtered through a 0.45 μm PTFE membrane. The DOC was measured as non-purgeable organic carbon with an Analytik Jena multi N/C 3100 instrument. For each sample, 2-3 replicates were measured, giving a relative standard deviation of less than 3%.

2.4.3. Pharmaceuticals and Personal Care Products

For the first and second campaigns, 57 PPCPs were quantified by QHFSS according to the method described in Appendix 1. For the third and fourth sampling campaigns, 29 PPCPs were quantified at the Advanced Water Management Centre (AWMC) at the University of Queensland using a different method described in the Appendix 2. Both methods consisted of solid phase extraction, elution, concentration, and analysis by liquid chromatography coupled with tandem mass spectrometry (LC/MS-MS). A list of compounds with some of their properties is available in Appendix 3.

2.4.4. Disinfection By-Products

N-nitrosodimethylamine (NDMA) and other nitrosamines – *N*-nitrosodiethylamine (NDEA), *N*-nitrosomorpholine (NMOR), *N*-nitrosopiperidine (N-Pip), *N*-nitrosodibuthylamine (NDBA) – were quantified using method based on U.S. Environmental Protection Agency's Method 251 (Munch and Bassett, 2004). After solid phase extraction on activated carbon and elution with dichloromethane, the extracts were concentrated down by evaporation leading to a concentration factor of 1,000. Extracts were then injected in a gas chromatograph coupled with a mass spectrometer with chemical ionisation.

The limit of quantification (LOQ) for nitrosamines was 5 ng L^{-1} for NDMA, 10 ng L^{-1} for NDEA and NMOR, and 20 ng L^{-1} for N-Pip and NDBA.

The trihalomethanes (THMs) – trichloromethane (TCM), bromodichloromethane (BDCM), dibromochlorometane (DBCM) and tribromomethane (TBM) – were quantified using gas chromatography equipped with a purge and trap system coupled with a mass spectrometer. The LOQ is 1 µg L^{-1} for all THMs.

Five haloacetic acids (5HAAs) – monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), bromochloroacetic acid (BCAA) and dibromoacetic acid (DBAA) – were extracted from aqueous samples by portioning into methyl tert-butyl ether. The analysis was carried out using gas chromatography coupled with an electron capture detector. The LOQ is 10 µg L^{-1} for MCAA, DCAA and TCAA and 5 µg L^{-1} for BCAA, MBAA and DBAA.

More details on the sampling, the formation potential tests and the quantification methods can be found in (Farré *et al.*, 2011).

2.5. Bioanalytical Tools

A battery of six bioassays described in Table 2 was used. The experimental procedure for these bioassays is available elsewhere (Macova *et al.*, 2010). Water samples were extracted by solid phase extraction using Oasis HLB cartridges. Full dose response curves were determined for a serial dilution of the extract for each bioassay. Results were expressed as toxic equivalent concentrations (TEQ) except for the umuC assay. The TEQ represents the concentration of a given reference compound that would be required to produce the same effect as the mixture of compounds present in the sample. When the outlet TEQ was below the LOQ of the bioassay, removal efficiency was calculated as a minimum value using the LOQ as outlet TEQ. In the umuC assay, the response is determined as an induction ratio (IR), an IR ≥ 1.5 is considered genotoxic. For genotoxic samples, $EC_{IR1.5}$ corresponds to how many times the sample must be concentrated or diluted to elicit an IR of 1.5. Results are expressed as $1/EC_{IR1.5}$ therefore a higher number represents a higher genotoxic effect.

Table 2: Short description of the bioassays used (EqC = equivalent concentration).

Toxic Mode of Action Bioassay Expression of Result	Targeted Chemicals
Non-specific toxicity *Vibrio fischeri* bioluminescence inhibition test Baseline-TEQ_{bio} (mg L^{-1})	Non-specific bacterial toxicity test widely recognised in the field of ecotoxicology as the standard assay for acute cytotoxicity. The assay reflects the general “energy status” of the bacteria and is sensitive to a broad spectrum of compounds with different modes of action. The toxic potential of organic micropollutants is generally directly related to their hydrophobicity (Escher *et al.*, 2008).
Estrogenicity E-SCREEN Estradiol EqC (ng L^{-1})	Specifically responds to natural hormones and other compounds that can mimic the activity of the female sex hormone estradiol.
AhR response CAFLUX assay 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) EqC (ng L^{-1})	Dioxins and dioxin-like compounds such as polychlorinated biphenyls (PCBs) but can also respond to other chemicals such as polycyclic aromatic hydrocarbons (PAHs).
Genotoxicity umuC assay $1/EC_{IR1.5}$	Responds specifically to genotoxic compounds that cause DNA damages.
Neurotoxicity acetylcholinesterase inhibition assay Parathion EqC (µg L^{-1})	Organophosphate and carbamate pesticides specifically bind to this enzyme.
Phytotoxicity PSII inhibition I-PAM assay Diuron EqC (µg L^{-1})	Herbicides that directly inhibit photosynthesis.

In order to assess the fraction of the non-specific toxicity measured with the bioassay (noted baseline-TEQ_{bio}) that can be explained by the quantified PPCPs, a quantitative structure activity relationship was used. It allows estimation of the relative potency (RP) of any organic compound from its chemical structure (Escher *et al.*, 2008). The reference compound used is the same as the one used to express the baseline-TEQ_{bio}. The concentrations of the quantified PPCPs are then multiplied by their respective relative potencies and summed according to Equation 1 to derive the baseline-TEQ of the mixture of these specific PPCPs then called baseline-TEQ_{chem}. The baseline-TEQ_{chem} of the mixture can be then compared to the baseline-TEQ_{bio}. More details of this approach are given by Vermeirssen *et al.* (2010).

$$\text{baseline-TEQ}_{chem} = \sum_{i=1}^{n} \text{baseline-TEQ}_i = \sum_{i=1}^{n} RP_i \cdot C_i \qquad \text{Equation 1}$$

2.6. Disinfection By-Products Formation Potential Test

To determine the DBP formation potential, chlorine (for THMs and HAAs) or chloramines (for NDMA) were added to a buffered sample at high concentrations and kept reacting for at least seven days to achieve the maximum formation of the specific DBPs. The THMs and HAAs formation potential test was performed following Standard Methods for the Examination of Water and Wastewater (Eaton *et al.*, 2005). The NDMA formation potential test closely follows the procedure described as nitrosamine precursor test by (Mitch *et al.*, 2003). The DBPs are quantified in the original sample and at the end of the formation potential test, the difference is the formation potential.

2.7. Microbial Community Characterisation

2.7.1. Sample Collection

Filtering media samples were collected from various depths (top, middle and bottom) of the pilot scale filters in March 2010 using a coring tool. A sample was also collected from the biofilm layer that had developed on top of each of the filter beds.

2.7.2. DNA Extraction and 16S rRNA Gene Amplification and Sequencing

DNA was extracted from 500 mg of each sample using a MO BIO PowerBiofilm™ DNA Isolation Kit according to the manufacturer's instructions. The quality of the extracted DNA was assessed on a 1% agarose gel. Extracted DNA concentrations were determined using a QubitTM fluorometer with Quant-iT dsDNA BR Assay Kits (Invitrogen) and then normalised to 10 ng μL^{-1}.

Universal 16S rRNA genes were amplified by polymerase chain reaction (PCR) in 50 µL volumes containing 20 ng DNA, molecular biology grade water, 1X PCR Buffer minus Mg (Invitrogen), 50 nM of each of the dNTPs (Invitrogen), 1.5 mM $MgCl_2$ (Invitrogen), 0.3 mg BSA (New England Biolabs), 0.02 U *Taq* DNA Polymerase (Invitrogen), 8 µM each of the primers 926F and 1392R (Engelbrektson *et al.*, 2010) modified on the 5' end to contain the 454 FLX Titanium Lib L adapters B and A, respectively. The reverse primers also contained a 5-6 base barcode sequence positioned between the primer sequence and the adapter. A unique bar-code was used for each sample. Thermocycling conditions were as follows: 95°C for 3 min; then 30 cycles of 95°C for 30 s, 55°C for 45 s, 72°C for 90 s; then 72°C for 10 min. Amplifications were performed using a Veriti® 96-well thermocycler (Applied Biosystems). Amplicons were purified using a QIAquick PCR purification kit (Qiagen), quantified using a Qubit™ fluorometer with a Quant-iT dsDNA BR Assay Kit and then normalised to 25 ng μL^{-1} and pooled for 454 pyrosequencing. Sequencing was performed by Macrogen Inc. (Seoul, Korea).

2.7.3. Analysis of Sequence Data

Sequences were quality filtered and dereplicated using the QIIME script split_libraries.py with the homopolymer filter deactivated (Caporaso *et al.*, 2010) and then checked for chimeras against the GreenGenes database using uchime ver. 3.0.617 (Edgar *et al.*, 2011). Homopolymer errors were corrected using Acacia (Bragg *et al.*, 2012). The number of sequences per sample was normalised to 800 to facilitate comparisons of diversity without bias from unequal sampling effort. The normalised and quality filtered data were then subjected to the following procedures using QIIME scripts with the default settings: 1) sequences were clustered at 97% similarity; 2) cluster representatives were selected; 3) GreenGenes taxonomy was assigned to the cluster representatives using BLAST; 4) tables with the abundance of different operational taxonomic units (OTUs) (and their taxonomic assignments) in each sample were generated; 5) the cluster representatives were aligned against the GreenGenes database; and 6) the alignments were filtered and then midpoint rooted, Newick format phylogenetic trees were generated.

The number of OTUs observed at equal numbers of sequences between samples (observed richness) and Faith's Phylogenetic Diversity index (PD; (Faith, 1992)) were calculated as measures of within sample diversity. The effects of position within the reactor on observed richness and PD were investigated using GLM. The effects of position within the reactor on the composition of communities were determined using Permutational Multivariate Analysis of Variance. Differences were investigated using Hellinger transformed OTU abundances (Legendre and Gallagher, 2001) and weighted-unifrac distances (Lozupone and Knight, 2005). The key difference between these data formats is that the latter incorporates information concerning the evolutionary relatedness of 'species', whereas the former does not. All analyses were implemented using R 2.12.0 (R Development Core Team).

3. RESULTS AND DISCUSSION

3.1. Removal of Dissolved Organic Carbon

- Can biofiltration alone remove residual effluent organic matter from WWTP effluents?
- How does biofiltration alone compare with the combination of ozonation and biofiltration?
- What is the influence of the filtering media on the DOC removal efficacy?

In the first campaign, the DOC in the feed water (non-ozonated) was 11.2±0.4 mg L^{-1}. With an EBCT of 120 minutes, SAND 1 reduced the DOC by 22±3% (Figure 2). This is in agreement with what has been previously observed by other researchers in sand columns simulating riverbank filtration or managed aquifer recharge: Maeng *et al.* (2008) observed up to 20% DOC removal for an EBCT of 4 days; Rauch and Drewes (2004) obtained a removal of 25% after 18 hours of residence time. The investigators observed a faster removal at the top of the columns (corresponding to shorter EBCTs) which is consistent with the result of the present study. The effect of the full scale sand filter preceding the pilot scale filter is assumed to be negligible since the EBCT there is only 15 minutes and backwashes are performed typically every hour, preventing the establishment of a biologically active layer. It is therefore suggested that the fraction of DOC removed here corresponds to the more easily biodegradable fraction of the EfOM.

In BAC 1, the DOC influent level was reduced by 63±1%, which is much higher than what was observed in SAND 1 suggesting that the biodegradable fraction of EfOM is not totally removed in SAND 1. It is also possible that the higher removal observed is due to adsorption of EfOM onto activated carbon. However, a previous study on these filters showed that DOC removal had reached a steady state one year prior to the collection of the samples for the present study (Pipe-Martin *et al.*, 2010). This indicates that complete breakthrough of EfOM has been reached and it is therefore suggested that biodegradation is responsible for the removal observed even though adsorption might still play a role in the mechanism. Indeed, in BAC filters, the surface of the activated carbon is not totally covered by the biofilm and the free areas might still take part in adsorption/desorption processes leading to an increased flux of pollutants to the biofilm (Herzberg *et al.*, 2003).

Ozonation reduced the DOC concentration by less than 10% to 10.3±0.6 mg L^{-1} showing that oxidation did not lead to extensive mineralisation of the EfOM but rather to a transformation of the organics present. The BAC 2 reduced the DOC by 60±2% reaching the same effluent level as BAC 1. The similar DOC removal observed in BAC 2 compared to BAC 1 is surprising because ozonation is known to increase biodegradability of the organic matter. This indicates that there might be another factor limiting the degradation of EfOM (e.g. EBCT, DO).

The activated carbon filter of the full-scale plant reduced the DOC concentration by 36% only but with a much shorter EBCT of 18 minutes. The activated carbon in this filter was renewed four months before the sampling campaign took place but that is considered to be a sufficient time for the biological activity to establish (Simpson, 2008; Pipe-Martin *et al.*, 2010). Initial DOC removal efficiencies in GAC filters have been reported to be in the order of 40 to 90% and then gradually decreases as the DOC breaks through the filter and the biomass establishes. When complete breakthrough of DOC has been reached and the biomass is fully established, the DOC removal stabilises and is only due to biodegradation (Simpson, 2008). Here, it is difficult to say in which phase the filter is given the lack of data before the sample was collected. However, the lower DOC removal observed here compared to the other filters could indicate that the initial phase has already ended and the EfOM is removed mainly by biodegradation. In that case, the lower removal efficiency compared to BAC 1 and BAC 2 is most likely due to the shorter EBCT. However, with an EBCT almost seven times shorter than in the BACs, the DOC removal was reduced by a factor of 1.5 only consistently with the fact that the most easily (fastest) biodegradable fraction of EfOM would be removed first.

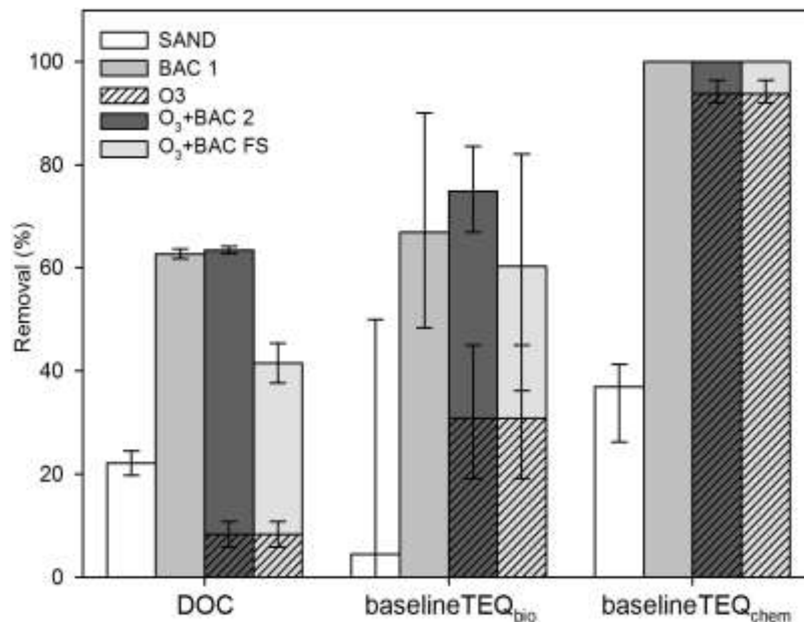

Figure 2: Median removal of DOC, baseline-TEQ$_{bio}$, baseline-TEQ$_{chem}$ observed after SAND 1, BAC 1, ozonation (O_3), O_3+BAC 2 and O_3+full-scale BAC filter (BAC FS) relatively to the feed for the first sampling campaign. EBCT =120 min for SAND 1, BAC 1, BAC 2 and EBCT = 18 min for BAC FS. Error bars represent the maximum and minimum of the four independent samples collected and therefore reflect the temporal variability.

- BAC filtration alone was capable of removing up to 60% of the residual dissolved organic carbon in the WWTP effluent.
- The removal of DOC by the BAC filter alone was similar to what was observed with the combination of ozonation and BAC filtration with the same EBCT. The BAC filter alone performed better than the combination of ozonation and BAC filtration with a shorter contact time.
- The sand filter alone lead to limited removal of DOC (20%) compared to the BAC filter.

3.2. Removal of Pharmaceuticals and Personal Care Products

3.2.1. Chemical Analysis

- Can biofiltration alone remove PPCPs from WWTP effluents?
- How does biofiltration alone compare with the combination of ozonation and biofiltration?
- What is the influence of the filtering media on the PPCP removal efficacy?

During the first sampling campaign, 37 PPCPs out of the 57 targeted had a median concentration above their LOQ in the feed water with gabapentin reaching 3.05 µg L^{-1} (Table 15, Appendix 4). The concentration of each compound remained in the same range during the whole sampling campaign as indicated by the maximum and minimum values measured. Most of the compounds quantified were reported to be poorly to moderately removed in WWTPs except caffeine, gabapentin and paracetamol (Table 14, Appendix 3). Caffeine and paracetamol are typically present in very high amounts in raw wastewaters (tens of µg L^{-1} in the present case, data not shown) and therefore can still be quantified in the treated wastewater despite the high removal rates observed. Gabapentin has been reported to be well degraded in WWTPs (> 99%) but this is based on one study only (Yu *et al.*, 2006). A preliminary investigation of the WWTP producing the effluent used in this study showed limited removal of around 30% (data not shown). Among these 37 compounds 21 had a median concentration at least 10 times higher than their LOQ; removal percentages are reported for these compounds only, in order to be able to express removals in the range of 0 to > 90% and to avoid over-interpretation of variations

for the other compounds that could be due to limitations in the chemical analysis method. These compounds still cover a wide range of classes and physico-chemical properties.

After filtration through SAND 1, 32 compounds still had a median concentration above their LOQ. The five compounds removed (atorvastatine, fluoxetine, sertraline, sulfadiazine, triclosan) had initial concentrations that were close to their LOQ before filtration. Among the 21 selected compounds, 12 were not or poorly removed (≤ 20%), eight experienced intermediate removal (22-54%) and only one compound, paracetamol, was well removed (84%) (Figure 3). Even though no direct conclusion can be drawn, the fate of PPCPs in WWTPs can be used as a qualitative indication of their biodegradability and/or their propensity to adsorb on the biomass. Indeed, the behaviour of most of the compounds is in accordance with their fate in WWTPs (Table 14, Appendix 3). It has to be stressed that the contact time in the sand filter is much shorter than typical hydraulic residence time in WWTPs and the biomass density can be assumed to be far lower than in an activated sludge process. Nevertheless, erythromycin, trimethoprim and roxythromycin experienced intermediate removals of 30, 38 and 54% respectively even though they are poorly removed in WWTPs. Göbel *et al.* (2007) also observed significant removal of these compounds in a sand filter with prior aeration (similarly to this study). This observation clearly indicates a difference in the biodegradation rates of these compounds between the activated sludge and biofiltration processes. Sulfamethoxazole's concentration consistently increased between 15 and 83%. This fact has already been observed by several researchers in WWTPs and is likely due to the de-conjugation of a sulfamethoxazole metabolite, N^4-acetyl-sulfamethoxazole (Bendz *et al.*, 2005; Clara *et al.*, 2005; Göbel *et al.*, 2005; Göbel *et al.*, 2007).

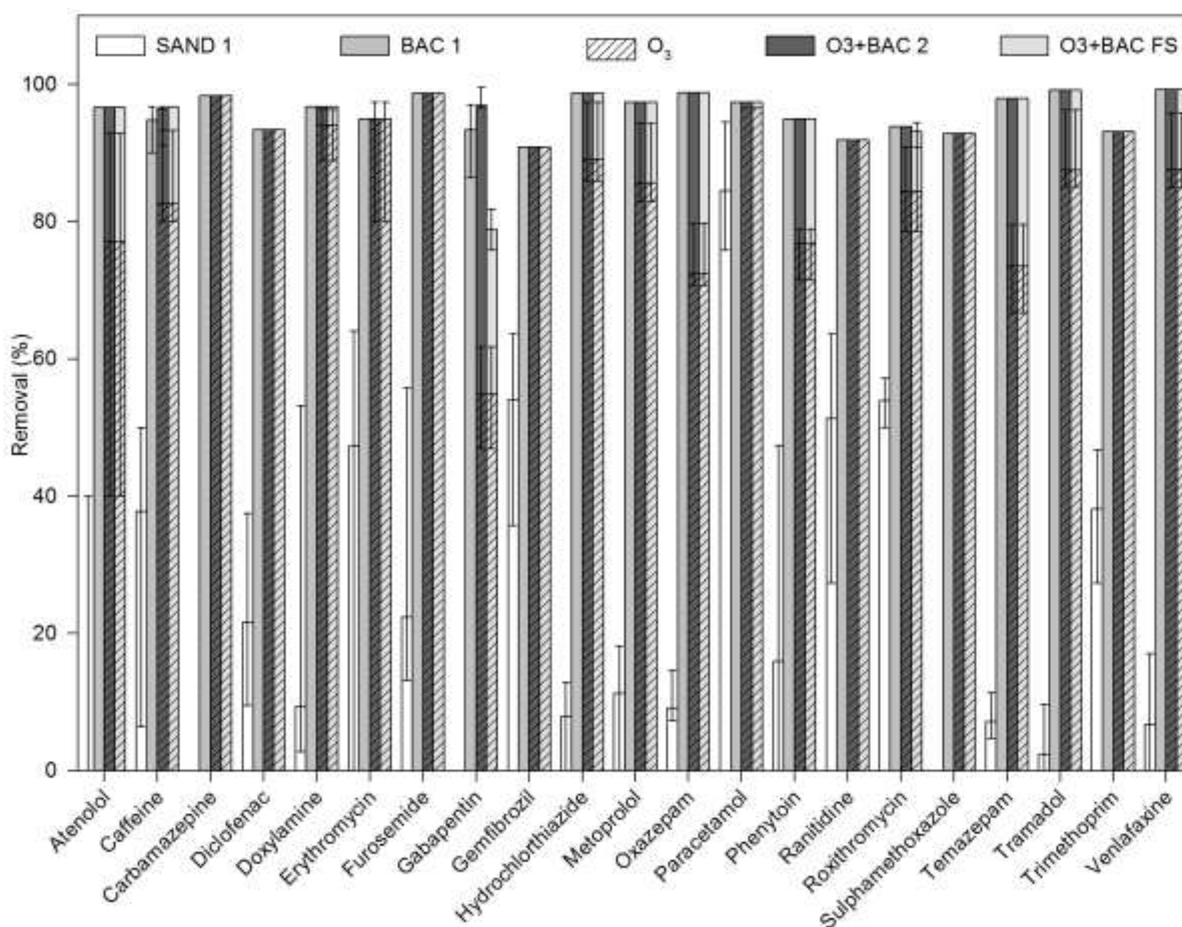

Figure 3: Median removal of selected pharmaceuticals observed after SAND 1, BAC 1, ozonation (O_3), O_3+BAC 2 and O_3+full-scale BAC filter (BAC FS) relatively to concentrations in the feed (i.e. before the main ozonation stage) for the first sampling campaign. EBCT = 120 min for SAND 1, BAC 1, BAC 2 and EBCT = 18 min for BAC FS. Error bars represent the maximum and minimum of the four independent samples collected and therefore reflect the temporal variability. No error bar means that the removal indicated is the minimum observed (i.e. compound was below LOQ after treatment).

Filtration through BAC 1 removed 35 PPCPs to levels below their LOQ. Only gabapentin and caffeine were quantified in the effluent with median concentrations of 0.20 and 0.03 µg L^{-1} respectively. Among the 21 selected compounds, seven were removed by 90 to 95% and 14 by more than 95% (Figure 3). Some of these compounds have been repeatedly reported to be poorly removed in WWTPs: carbamazepine, diclofenac, erythromycin, metoprolol, roxithromycin, sulfamethoxazole and trimethoprim. Another compound known to be poorly removed in WWTPs, iopromide, is here removed by more than 85%. The high removal efficiencies observed for these compounds could be due to adsorption onto the activated carbon surface.

Adsorption onto activated carbon is difficult to predict as the mechanism involves several types of interactions. Westerhoff *et al.* (2005) showed that removal efficiencies of PPCPs by powder activated carbon tend to increase with increasing $\log K_{ow}$ (i.e. increasing hydrophobicity) but some protonated bases and deprotonated acids did not follow this general trend. The activated carbon contained in BAC 1 has been exposed to typical concentrations of these compounds for several months before sampling. Biomass exposed to low concentration of trace organic chemicals can adapt over time and become able to significantly degrade even compounds considered to be persistent (Rauch-Williams *et al.*, 2010). However, activated carbon filters can also retain an adsorption capacity for compounds present at micrograms per litre levels even after the breakthrough has been observed for DOC (Wang *et al.*, 2007). Moreover, the breakthrough for individual OMPs will occur at different filtered volumes depending on their adsorption propensity (Snyder *et al.*, 2007). Therefore, it cannot be concluded at this point whether the PPCPs are merely adsorbed or also biodegraded.

After ozonation, only 16 compounds were quantified with a median concentration above their LOQ. Ozone is known to be able to oxidise many PPCPs but some compounds such as gabapentin and iopromide can be refractory. Ozonation has been extensively studied and was not the focus of this work; it will therefore not be discussed further here. For more information on ozonation, refer to the Urban Water Security Research Alliance Technical Report #69 (Reungoat *et al.*, 2012).

Filtration through BAC 2 removed the remaining compounds below their LOQ except for gabapentin and caffeine, which had remaining median concentrations of 0.10 and 0.01 µg L^{-1} respectively. In the ozonated water, 5 compounds had a concentration at least ten times above their LOQ: gabapentin (1.30 µg L^{-1}), oxazepam (0.20 µg L^{-1}), temazepam (0.12 µg L^{-1}), tramadol (0.14 µg L^{-1}) and venlafaxine (0.17 µg L^{-1}). These five compounds were further removed by more than 90% in BAC 2 alone, in accordance with their fate in BAC 1. Following the same reasoning as for BAC 1, both adsorption and biodegradation could be responsible for the removal observed in BAC 2. Among the 21 selected compounds, eight were removed by 90 to 95% and 13 by more than 95% by the combination of ozonation and BAC 2.

In the full-scale plant, after filtration through the BAC, two compounds were quantified with a median concentration above their LOQ: gabapentin (0.70 µg L^{-1}) and roxithromycin (0.01 µg L^{-1}). The five compounds with initial concentrations of at least ten times their LOQ were removed by more than 90%, except gabapentin, which was removed by only 52%. The activated carbon in the full-scale BAC had been replaced only four months before sampling occurred. Removal by adsorption is therefore likely to be even more relevant than for the pilot scale filters. Among the 21 selected compounds, eight were removed by 90 to 95% and 12 by more than 95% by the combination of ozonation and the full-scale BAC filter.

- BAC filtration removed a wide range of PPCPs by more than 90%, generally down to levels below the LOQ (0.1 ng L-1)
- BAC filtration alone performed as well as ozonation followed by BAC filtration with the same EBCT. BAC filtration alone performed better than ozonation followed by BAC filtration with a shorter contact time.
- The sand filter removed PPCPs by less than 50%.

3.2.2. Reduction of Non-Specific Toxicity

- Can biofiltration alone reduce non-specific toxicity of WWTP effluents?
- How does biofiltration alone compare with the combination of ozonation and biofiltration?
- What is the influence of the filtering media on the PPCPs removal efficacy?
- How does the removal of non-specific toxicity compare with the removal of DOC and PPCPs?

While the chemical analytical concentrations of many PPCPs fell below detection limit after treatment, the baseline-TEQ_{bio} of all samples (excluding the blanks) were above detection limit and significantly different from the blank in all samples (Table 3). Thus it was possible to calculate robust removal efficiencies without invoking any assumptions with respect to the detection limit.

Table 3: Minimum (Min), median (Med), and maximum (Max) DOC, baseline-TEQ_{bio}, baseline-TEQ_{chem} and percentage of baseline-TEQ_{bio} explained by baseline-TEQ_{chem} for the first sampling campaign.

	DOC (mg L^{-1})			baseline-TEQ_{bio} (µg L^{-1})			baseline-TEQ_{chem} (µg L^{-1})			TEQ_{chem}/TEQ_{bio} (%)		
	Min	Med	Max	Min	Med	Max	Min	Med	Max	Min	Med	Max
Feed	10.9	11.2	11.6	890	1300	2000	2.16	2.96	4.03	0.14	0.24	0.31
SAND 1	8.2	8.8	9.3	880	1100	1600	1.37	1.97	2.53	0.14	0.17	0.21
BAC 1	4.2	4.2	4.2	200	420	480		0			0	
Ozonation	9.7	10.3	10.9	720	900	1100	0.11	0.19	0.24	0.01	0.02	0.03
BAC 2	4.1	4.1	4.2	360	415	830		0			0	
BAC SF	5.9	6.6	7.2	160	375	430		0			0	

The SAND 1 filter showed limited decrease of baseline-TEQ_{bio} whereas BAC 1 was able to reduce it by 50 to 90% which is higher than what was achieved by ozonation alone (19 to 45%). After ozonation, BAC 2 and BAC FS further decreased the baseline-TEQ_{bio} to reach 67 to 84% and 36 to 82% reduction respectively. Figure 2 shows the higher removal observed in BAC 2 compared to BAC FS confirming that EBCT is an important operational parameter. With similar EBCT (120 min) in the BAC filters, prior ozonation allowed an overall slightly higher removal of baseline-TEQ_{bio} compared to filtration alone. However, when a shorter EBCT (18 min) was used after ozonation, the removal of baseline-TEQ_{bio} was lower compared to filtration without prior ozonation but with long EBCT (120 min).

The baseline-TEQ_{bio} were compared with the baseline-TEQ_{chem} derived for a mixture of all quantified PPCPs in order to evaluate how large the tip of the iceberg of the identified chemicals is in comparison to the overall burden of biologically active pollutants. With every treatment step, more PPCPs fell below their LOQ in the chemical analysis. This does not mean that they disappeared altogether; they might still contribute to mixture toxicity when present at concentrations below the LOQ, as even concentrations below any theoretical expected effect may contribute to mixture toxicity. This so-called "something from nothing" effect was first shown by Silva *et al.* (2002) for estrogenic chemicals but later confirmed for many other endpoints.

If the baseline-TEQ_{chem} was calculated by Equation 1, and the PPCPs that fell below their LOQ were not considered, then the percentage of baseline-TEQ_{bio} that could be explained by the baseline-TEQ_{chem} fell from 0.14 to 0.31% to less than 0.0001% during the treatment process, incrementally decreasing with every treatment step (Table 3). A fraction of 0.1 to 1% of toxicity in this bioassay explained by the detected chemicals is a typical result for wastewater effluents and surface waters as has been demonstrated by Vermeirssen *et al.* (2010). The unknown fraction accounts for PCPPs not on the analytical target list, but also for other compounds such as pesticides, industrial chemicals and natural compounds, which in addition to exhibiting a defined specific effect also add to the underlying

baseline toxicity. Furthermore, transformation products of organic micropollutants may also contribute to the observed mixture toxicity.

The variability of the fraction of toxicity explained by chemical analysis and its decrease with advanced water treatment indicates that chemical analysis is not necessarily a robust parameter for assessing overall removal efficiencies for a given process but that the toxicity sum parameter of baseline-TEQ_{bio} might be more appropriate to estimate the reduction of the mixture of organic micropollutants.

This phenomenon is also illustrated in Figure 2, where the median reductions of baseline-TEQ_{bio} and baseline-TEQ_{chem} are depicted for all filtration steps. Using baseline-TEQ_{chem}, it looks as if the removal efficiency is always >90%, but this is somewhat misleading as many PPCPs fall below their LOQ and consequently do not contribute to the calculation of baseline-TEQ_{chem} using Equation 1. The baseline-TEQ_{bio} gives a more subtle picture of the different processes, which is also consistent with the removal of DOC and individual PPCPs.

- BAC filtration alone reduced non-specific toxicity by more than 60%.
- The BAC filter alone performed similarly to the combination of ozonation and BAC filtration, regardless of the EBCT.
- The sand filter alone led to limited removal of non-specific toxicity
- The reduction of non-specific toxicity was similar to the observed removal of DOC but lower than the removal of selected PPCPs. The non-specific toxicity derived from the PPCP concentrations could explain less than 0.3% of the effect observed in the bioassays, demonstrating the potential of this bioanalytical tool in addition to chemical analysis.

3.2.3. Reduction of Specific Toxicity

- Can biofiltration alone reduce various specific toxic modes of action in WWTP effluents?
- How does biofiltration alone compare with the combination of ozonation and biofiltration?
- What is the influence of the filtering media on the reduction of specific toxic modes of action efficacy?
- How does the reduction of specific toxic modes of action compare with the removal of DOC and PPCPs?

For all bioassays, the toxicity levels in the feed water were significantly higher than the blank levels (Table 4). For a given bioassay, the toxicity remained in the same range for the four samples collected.

Table 4: Specific toxicity levels in the feed water compared to the blank levels (EqC = equivalent concentration).

Toxic Mode of Action	Results Expression	Blank	Feed		
			Min	Med	Max
Estrogenicity	Estradiol EqC, ng L^{-1}	< 0.02	7.9	10.3	10.9
AhR response	TCDD EqC, ng L^{-1}	0.08	0.77	0.84	1.02
Genotoxicity	$1/EC_{IR1.5}$	< 0.01	0.05	0.06	0.17
Neurotoxicity	Parathion EqC, µg L^{-1}	< 0.3	1.7	2.15	2.50
Phytotoxicity	Diuron EqC, µg L^{-1}	< 0.01	0.07	0.14	0.17

Generally SAND 1 had limited effect on the toxicity levels, consistent with other analysis (Figure 4). An exception was estrogenicity which was reduced by more than 90%. This shows that the compounds inducing the estrogenic effect are easily biodegradable as this is the only mechanism occurring in the sand filter. On the contrary, BAC 1 significantly reduced the level of toxicity in all bioassays from 60% for phytotoxicity to more than 99% for estrogenicity. The effect of ozonation varied greatly

depending on the bioassay. It had almost no impact on neurotoxicity but reduced estrogenicity by more than 95%. This is probably due to the specific nature of ozone reactivity with organic compounds. Ozone reacts quickly only with electron rich moieties such as aniline, pyrimidine, naphthalene, aromatic rings, double bounds and tertiary amines. Therefore, depending on the nature of the compounds inducing the toxicity, they might be degraded quickly or be refractory to oxidation by ozone. Both BAC filters placed after ozonation (BAC 2 and BAC FS) further reduced the toxicity. The improvement was significant for AhR response and neurotoxicity, less so for phytotoxicity. For estrogenicity and genotoxicity, levels fell below the LOQ of the assay and the impact of BAC filters cannot therefore be accurately evaluated. BAC 1, O_3+BAC 2 and O_3+BAC FS all reduced estrogenicity, genotoxicity and neurotoxicity to levels below the LOQ of the bioassay. For AhR response and phytotoxicity, the levels after treatment were comparable for the three options.

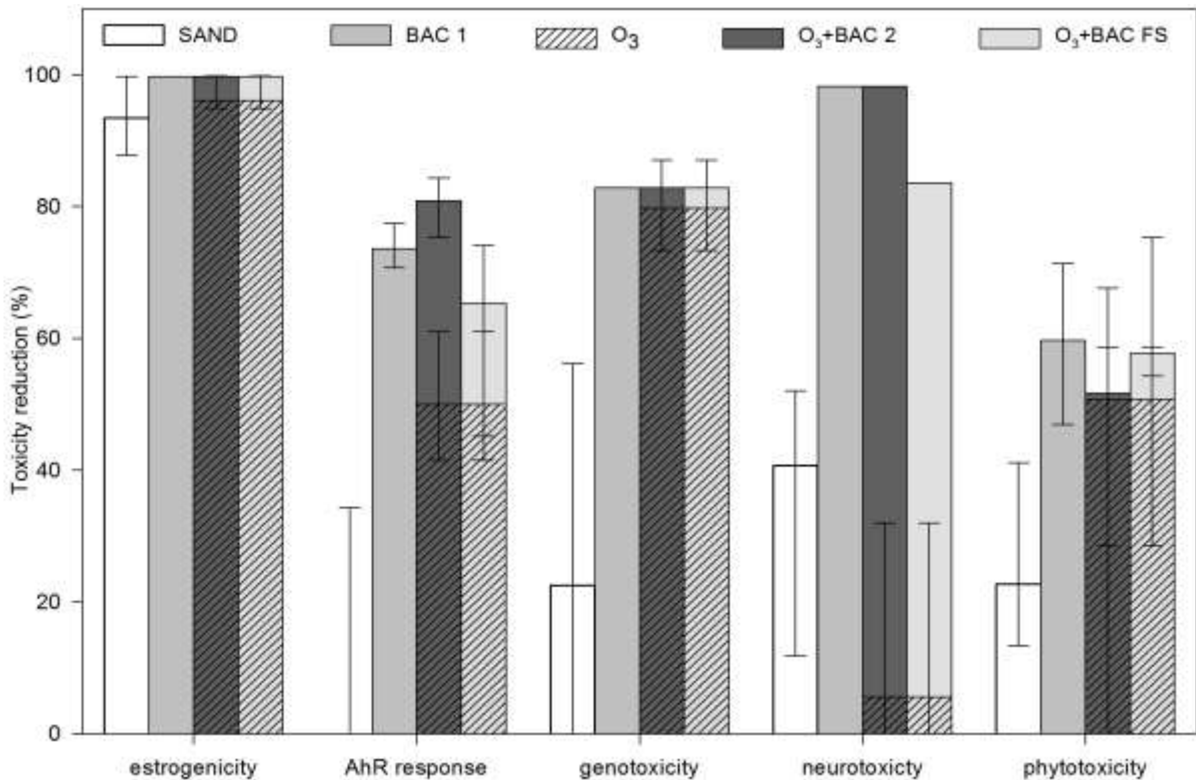

Figure 4: Median reduction of specific toxicity. Errors bars represent minimum and maximum of four samples.

- BAC filtration alone reduced all toxic modes of action by 50 to more than 99%.
- BAC filtration alone performed similarly to the combination of ozonation and BAC filtration regardless of the EBCT.
- The sand led to less than 40% reduction of toxic modes of action except for estrogenicity (90%).
- Various specific modes of action were affected differently by the treatments and also behaved differently from the removal of DOC and PPCPs. Bioanalytical tools give a more detailed picture of water quality and treatment efficacy compared to chemical analysis alone.

3.3. Influence of Empty Bed Contact Time

- How does the EBCT affect the removal of DOC, PPCPs and the reduction of non-specific toxicity in BAC filters before and after ozonation?

The comparison of BAC 2 and BAC FS in the first sampling campaign suggested that EBCT could play an important role in the removal of EfOM and particularly PPCPs. A second sampling campaign was therefore performed in December 2008 with BAC 1 and BAC 2 to investigate the influence of this parameter. Four sets of 24-hour composite samples were collected from both BAC filters operating with various EBCTs (30±1, 60±2, 90±4 and 120±6 minutes). A waiting period of one week was observed from the moment the EBCT was modified prior to the sample collection.

The DOC levels in the feed water were slightly lower than during the first campaign except for 90 minutes (Table 5). However, among the 57 target compounds, 32 were quantified with a median concentration above their LOQ (Table 15, Appendix 4). These 32 compounds were also detected in the first sampling campaign and their concentrations were in the same range. Fluoxetine, salicylic acid, sertraline and triclosan were not detected in this second campaign; their respective concentrations were close to their LOQ in the first campaign. Diclofenac could not be quantified in the second campaign due to the presence of interferences in the analytical instrument. Baseline-TEQ_{bio} levels were also similar to the first campaign and remained in the same range during the sampling campaign except for the influent of BAC 1 with EBCT=30 min (Table 6).

Table 5: Dissolved organic carbon and dissolved oxygen concentrations in the influent (IN) and effluent (OUT) of BAC 1 and BAC 2 in the second sampling campaign. The dissolved oxygen concentration in the influent of BAC 2 was higher than for BAC 1 because the ozone is generated in the plant from 90% oxygen enriched gas; therefore a high amount of oxygen is transferred into the liquid phase in the main ozonation reactor.

EBCT (minutes)	BAC 1					BAC 2				
	DOC (mg L^{-1})		Dissolved oxygen (mg L^{-1})			DOC (mg L^{-1})		Dissolved oxygen (mg L^{-1})		
	IN	OUT	IN	OUT	Consumption	IN	OUT	IN	OUT	Consumption
30	8.2	5.0	5.5±0.1	0.6±0.1	4.9±0.1	7.1	3.5	11.2±0.5	1.0±0.2	10.2±0.3
60	8.7	5.7	6.7±0.1	0.5±0.1	6.2±0.1	7.0	4.6	10.0±0.5	0.9±0.1	9.1±0.5
90	11.5	6.11	6.3±0.2	0.7±0.3	5.6±0.3	10.2	4.1	9.7±0.1	1.4±0.3	8.3±0.4
120	8.8	5.7	6.3±0.1	0.6±0.1	5.7±0.1	8.3	4.5	10.1±0.1	1.2±0.1	8.9±0.1

Table 6: Baseline-TEQ_{bio} (average of duplicate samples±standard deviation) and gabapentin concentrations of the second sampling campaign.

EBCT (minutes)	BAC 1				BAC 2			
	Baseline-TEQ_{bio} (µg L^{-1})		Gabapentin (µg L^{-1})		Baseline-TEQ_{bio} (µg L^{-1})		Gabapentin (µg L^{-1})	
	IN	OUT	IN	OUT	IN	OUT	IN	OUT
30	1989±198	1346±5	1.86	0.89	996±62	617±45	0.57	0.43
60	996±5	570±5	3.10	0.82	693±29	427±34	0.96	0.78
90	787±5	340±32	2.50	0.67	510±41	339±1	1.30	0.51
120	846±3	325±11	4.60	0.05	436±7	276±1	1.90	0.29

For 120 minutes EBCT, the removal of DOC was lower in both BACs compared to the first campaign (Figure 5). This is probably due to the fact that the feed water contained less easily biodegradable EfOM as the DOC level was lower. Indeed, BAC 1 and BAC 2 reached similar removal levels of DOC as in the first campaign for an EBCT of 90 minutes for which the level in the feed water was comparable to the first campaign (Figure 5). Contrary to what was observed in the first campaign, the removal of DOC in BAC 2 (after ozonation) was higher than in BAC 1. This is also consistent with the hypothesis that there was less easily biodegradable EfOM in the feed water. The concentrations of a majority of the targeted PPCPs were reduced to levels below their LOQ in the effluents of BAC 1 and BAC 2 consistently with the observation made for the first sampling campaign. Two compounds were quantified in the effluent of BAC 1, DEET and gabapentin, with respective concentrations of 0.02 and 0.24 µg L^{-1}. Gabapentin was also detected in the effluents of BAC 1 and BAC 2 in the first campaign with median concentrations of 0.20 and 0.10 µg L^{-1} respectively. The concentration of DEET was

below 0.01 µg L^{-1} in the effluent of BAC 1 in the first campaign but its influent concentration was also lower: 0.05 to 0.07 µg L^{-1} compared to 0.12 µg L^{-1} here. The removal of gabapentin observed here, 95% and 85% for BAC 1 and BAC 2 respectively, is consistent with the removal observed in the first campaign (i.e. 93% in both filters). These facts support the hypothesis that the removal of PPCPs is mainly due to biodegradation (or adsorption followed by biodegradation) rather than to adsorption alone because adsorption efficacy typically decreases with operating time as adsorption sites are gradually saturated. The reduction of baseline-TEQ_{bio} in BAC 1 (62%) was similar to the first campaign (68%) despite the fact that the DOC removal was lower. On the contrary, in BAC 2, the reduction of the baseline-TEQ_{bio} was lower (32%) compared to the first campaign (63%) consistently with the lower DOC removal observed.

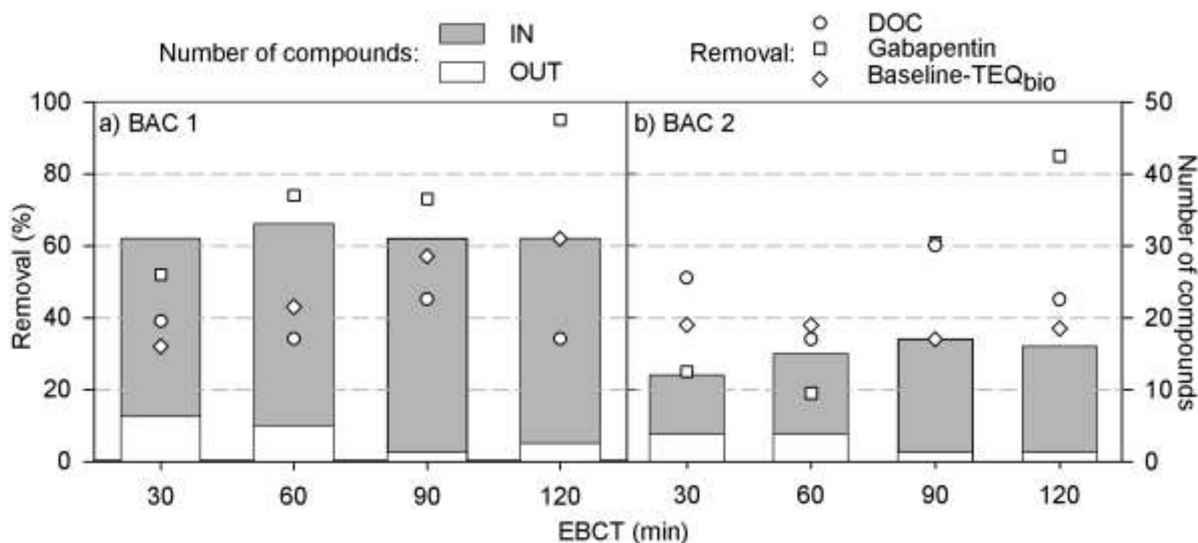

Figure 5: Influence of EBCT on number of PPCPs detected (concentration > 10 ng L^{-1}) and on DOC, Gabapentin and baseline-TEQ_{bio} removal in BAC 1 (a) and BAC 2 (b).

Decreasing the EBCT from 120 to 30 minutes did not affect the performance of BAC 1 regarding the removal of DOC, it remained in every case between 34 and 47% (Figure 5a). However, there was a consistent decrease of baseline-TEQ_{bio} removal from 62 to 32% when the EBCT was shortened from 120 to 30 minutes (Figure 5a). This shows that compounds contributing to baseline toxicity need a longer contact time to be degraded by the biomass but their concentration is probably too low to have an influence on DOC removal. Indeed, an increasing number of compounds, from two to five, were quantified at a concentration above 10 ng L^{-1} in BAC 1 effluent when the EBCT was decreased from 120 to 30 minutes respectively. The concentrations of these compounds were always close to their LOQ after filtration except for gabapentin. Gabapentin removal decreased from 95% to 52% when EBCT decreased from 120 to 30 minutes (Figure 5a). This effect cannot be attributed to an increase in the gabapentin concentration in the feed water as it actually decreased from 4.60 to 1.86 µg L^{-1} in the meantime (Table 6).

Similar observations can be made from the results obtained with BAC 2: the decrease in EBCT did not seem to affect the removal of DOC (the higher removal of 60% observed for 90min EBCT is due to a higher inlet DOC) but more PPCPs were detected at a concentration above 10 ng L^{-1} in the effluent for shorter contact times and the removal of gabapentin decreased (Figure 5b). However, the removal of baseline-TEQ_{bio} in BAC 2 was steady when the EBCT was reduced, showing that the ozonation by-products contributing to baseline-TEQ_{bio} are more easily biodegraded than the original compounds.

Overall, the performances of both BAC filters remained very similar to what was observed in the first campaign even for contact time as short as 30 minutes. The EBCT does not seem to strongly affect the removal of DOC in the range studied but the quantification of PPCPs and the baseline-TEQ_{bio} suggests that the removal of these compounds is affected, particularly in BAC 1. An increasing removal of phenol with increasing EBCT has also been observed by Seredynska-Sobecka *et al.* (2006) in a BAC treating ozonated river water.

The dissolved oxygen (DO) concentrations were measured in the influent and effluent of both filters at the beginning and end of each 24-hour sampling period and oxygen consumption across the filters was calculated (Table 5). The DO concentrations decreased by several mg L^{-1} through the filters, confirming that they are biologically active. The oxygen consumption per litre across the filters varied little from one EBCT to another in accordance with the observation that DOC removal also did not vary. The oxygen consumption in BAC 2 was higher than in BAC 1, which is consistent with a higher amount of DOC removed. Moreover, the DO concentrations in the effluent of both filters were low, below 0.8 and 1.5 mg L^{-1} for BAC 1 and BAC 2 respectively. This indicates that the DOC removal might be limited by the DO concentration and not by the reaction rate, explaining the fact that decreasing the EBCT did not affect the effectiveness of DOC removal in the BAC filters.

- Generally, the performance of the filters did not vary much when the EBCT was reduced from 120 to 30 minutes.
- However, the number of compounds detected in the effluent of the filters increased and the removal of gabapentin decreased with decreasing EBCT, suggesting that this parameter is important. Non-specific toxicity reduction also decreased with decreasing EBCT in the BAC filter before ozonation.
- The dissolved oxygen concentration in the effluent of the filters was low, suggesting that it could be a factor limiting the performance of the filters.

3.4. Long Term Performance

- Does the efficacy of BAC filtration decrease with time due to a saturation of adsorption capacity?
- How does sand filtration after ozonation perform?
- Does increasing the concentration of dissolved oxygen improve the efficacy of the BAC filters?

A third and a fourth sampling campaigns were carried out in October 2009 and July 2010, i.e. 14 and 24 months respectively after the first sampling campaign. The aim was primarily to verify the performance of the filters in the long term, but also to investigate the performance of sand filtration after ozonation and the impact of higher dissolved oxygen concentration. The EBCT of pilot scale filters was set at 60 minutes for both campaigns. The feed to the sand filter was changed from non-ozonated to ozonated water six months prior to the third campaign. Three months prior to the fourth campaign, the air supply at the top of BAC 1 and BAC 2 was switched to 90% oxygen used in the reclamation plant's ozone generators to ensure higher dissolved oxygen levels in the influent water. Two sets of 24-hour composite samples and three grab samples were collected and in the third and fourth campaign respectively.

Table 7: DOC, baseline-TEQ_{bio} and baseline-TEQ_{chem} in the third and fourth sampling campaigns (EBCT = 60 minutes). Removals are calculated based on the feed. For the third campaign, results are from one day only (24-h composite samples) as for the second day some samples were contaminated which affected DOC and baseline-TEQ_{bio}. Baseline-TEQ_{bio} is given with standard deviation from duplicates when available. Diclofenac was not included in baseline-TEQ_{chem} due to interference in the chemical analysis. For the fourth campaign, results are the mean of 3 grab samples ± standard deviation.

		DOC		Baseline-TEQ_{bio}		Baseline-TEQ_{chem}		TEQ_{chem}/ TEQ_{bio}
		mg L^{-1}	Removal	µg L^{-1}	Removal	µg L^{-1}	Removal	
3rd campaign	Feed	7.4	n/a	1000	n/a	1.92	n/a	0.192%
	BAC 1	4.6	36%	150±71	85%	0.02	99%	0.013%
	Ozonation	6.4	13%	700	30%	0.11	94%	0.015%
	O_3+SAND 2	4.7	37%	350±71	65%	0.08	96%	0.024%
	O_3+BAC 2	3.2	54%	400	60%	0.03	99%	0.007%
	O_3+BAC FS	4.8	36%	300	70%	0.02	99%	0.019%
4th campaign	Feed	7.0±1.0	n/a	2290±444	n/a	2.27±0.46	n/a	0.099±0.006%
	BAC 1	4.1±0.3	41±5%	909±168	60±4%	0.01±0.01	99±1%	0.002±0.001%
	Ozonation	6.4±0.7	7±7%	1493±139	33±18%	0.36±0.10	84±2%	0.025±0.009%
	O_3+SAND 2	5.0±0.3	27±7%	1175±221	49±3%	0.21±0.06	91±1%	0.018±0.002%
	O_3+BAC 2	3.3±0.3	53±2%	601±259	74±10%	0.02±0.01	99±1%	0.003±0.001%
	O_3+BAC FS	4.8±0.3	30±5%	685±165	70±4%	0.02±0.01	99±1%	0.003±0.001%

The DOC in the feed water was lower compared to the first and second campaigns; however, the PPCPs concentrations (Table 15, Appendix 4) and the baseline-TEQ_{bio} levels (Table 7) were still in the same range as for the two other campaigns. Overall, results were in agreement with observations made during the first and second campaign (Table 7 and Figure 6). This strongly supports the hypothesis that the removal of organic matter and PPCPs observed in the BAC filters is due to biodegradation (or adsorption followed by biodegradation) rather than adsorption alone as adsorption efficiency would typically decrease over time. The SAND 2 filter, placed after ozonation, showed limited removal of the PPCPs remaining after ozonation which is consistent with the findings of Hollender *et al.* (2009). Accordingly, filtration through SAND 2 did not improve the baseline-TEQ_{bio} level either, in agreement with the findings of Escher *et al.* (2009). After the switch to 90% oxygen supply, the dissolved oxygen concentration was measured in the effluent of both BAC 1 and BAC 2 (Table 8), showing that the filters were fully aerobic. This was accompanied by an increase in dissolved oxygen consumption across the filters compared to the third campaign which suggests that dissolved oxygen was a limiting factor in the other campaigns. However, no significant increase in DOC removal and baseline toxicity could be observed (Table 7). The removal of micropollutants was also similar in both campaigns (Figure 6). Further experiments are necessary to clearly identify the impact of dissolved oxygen concentration on the filters' performance.

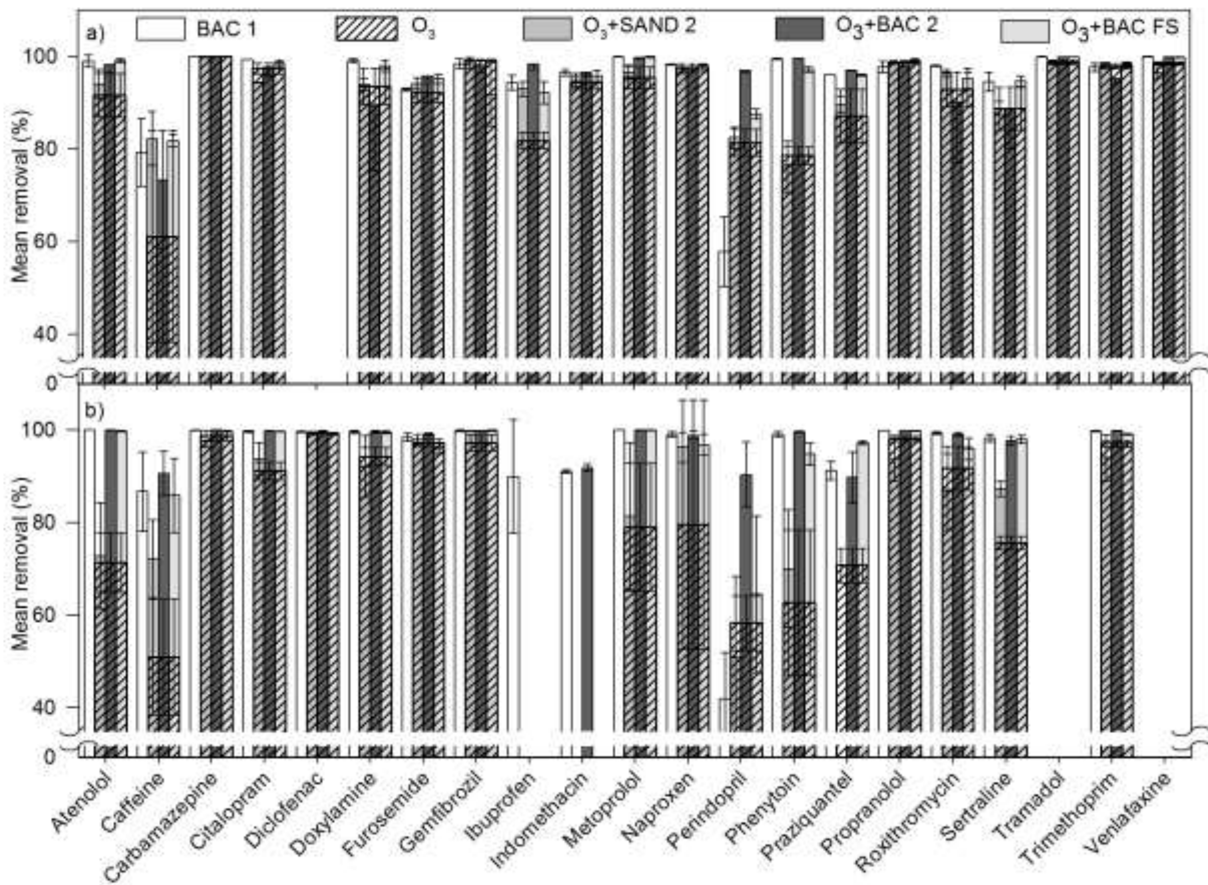

Figure 6: Removal of the PPCPs in the third (a) and fourth (b) sampling campaign compared to concentration in feed water. Bars represent the mean (n=2 or 3) and error bars show standard deviation. No bar means that no removal could be calculated (due to low concentration or interference).

Table 8: Dissolved oxygen concentrations in the influent (IN) and effluent (OUT) of the pilot scale biofilters and consumption across the filters during the fourth sampling campaign (mean ± standard deviation).

	IN ($mg\ L^{-1}$)	OUT ($mg\ L^{-1}$)	Consumption ($mg\ L^{-1}$)
SAND 2	8.2±0.9	5.6±0.5	2.7±0.7
BAC 1	9.7±0.7	2.7±1.2	9.6±1.8
BAC 2	10.3±1.1	2.0±1.0	9.9±2.0

Similarly to the first campaign, the baseline-TEQ_{chem} was calculated from the concentration of the PPCPs quantified by chemical analysis. Baseline-TEQ_{chem} explained less than 0.2% of baseline-TEQ_{bio} in the third and fourth sampling campaigns (Table 7).

- The efficacy of the BAC filters for DOC and PPCPs removal as well as non-specific toxicity reduction was stable over two years, suggesting the main mechanism is biodegradation and not adsorption.
- The sand filter placed after ozonation did not perform significantly better than the one placed before ozonation.
- Increasing the dissolved oxygen concentration did not lead to a significant increase of efficacy by the BAC filters.

3.5. Removal of Disinfection By-Product Precursors

- Can biofiltration alone remove DBP precursors in WWTP effluents?
- How does biofiltration alone compare with the combination of ozonation and biofiltration?
- What is the influence of the filtering media on the removal of DBP precursors?

3.5.1. NDMA and Other Nitrosamines Precursors

Nitrosamines (NDMA, NDEA, NMOR, N-Pip and NDBA) were quantified in the samples collected during the third sampling campaign (October 2009) and formation potential tests were performed. Two sets of time proportional 24-h composite samples were collected before ozonation, after ozonation and from the effluent of all pilot scale filters. Only NDMA and NMOR were detected but at levels below the LOQ (i.e. 5 ng L^{-1} for NDMA and 10 ng L^{-1} for NMOR).

The NDMA formation potential before ozonation was 260 ng L^{-1} and no other *N*-nitrosamines were formed above LOQ in the test. This level is in agreement with levels previously reported for SEQ treated effluents by Farré *et al.* (Farré *et al.*, 2010). BAC 1 reduced the NDMA formation potential by 81%, down to 48 ng L^{-1} (Figure 7). This is much higher than the observed removal of DOC (36%) and indicates that the NDMA precursors are preferentially removed or transformed to other compounds that do not form NDMA. Ozonation alone was also effective in removing and/or transforming NDMA precursors despite a limited removal of DOC; it reduced the formation potential by 64%, which is less than the BAC alone. After ozonation, the biofilters further removed the NDMA precursors but to varying degrees. In agreement with other parameters, SAND 2 did not improve the NDMA formation potential by much. BAC 2 further reduced the formation potential by 58%, bringing the overall reduction of O_3+BAC 2 to 85%, which is only slightly better than BAC 1. Finally the, full scale BAC filter further reduced the formation potential by 38% brining the overall O_3+BAC FS to 78%. The longer contact time in BAC 2 allowed further removal of NDMA precursors but the difference is marginal, considering that the contact time is three times longer in BAC 2. Again, the reduction of NDMA formation potential was higher than the observed removal of DOC. BAC 1 achieved similar results as the full scale combination of ozonation and BAC filtration.

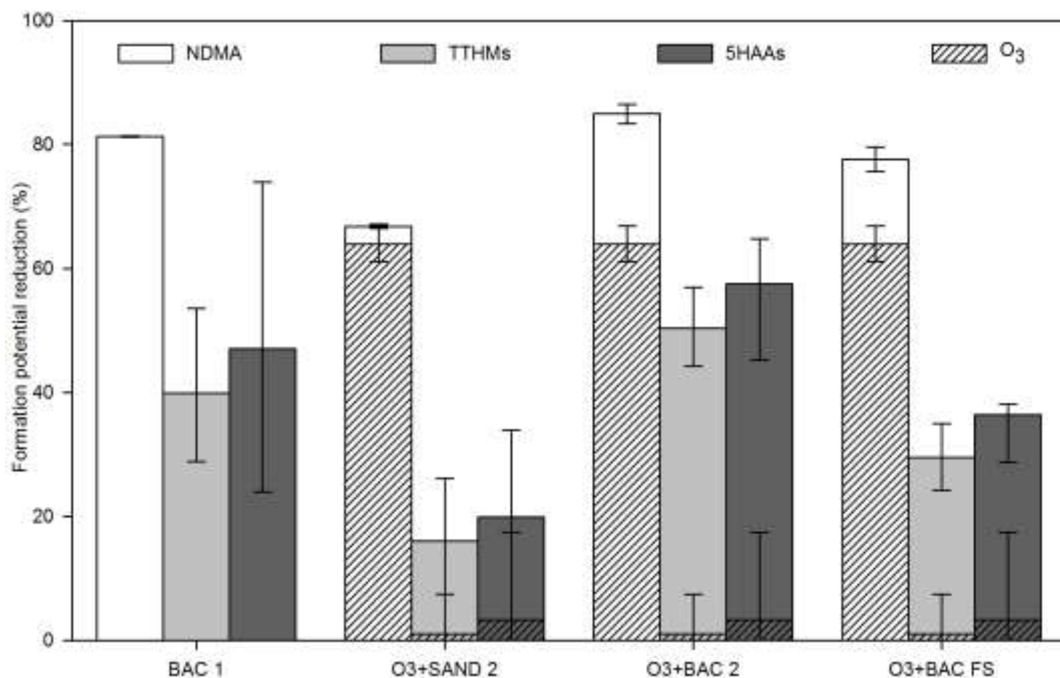

Figure 7: Reduction of formation potential of disinfection by-products after ozonation and biofiltration. Bars represent average values and error bars are minimum and maximum.

3.5.2. Trihalomethanes and Haloacetic Acids Precursors

TTHMs and 5HAAs were quantified in three sets of grab samples collected during the fourth sampling campaign (July 2010). A formation potential test was also applied to the same samples to estimate the removal of precursors by ozonation and biofiltration. The level of TTHMs was always below 11 µg L^{-1} after ozonation and no HAA was detected in the samples.

The formation potentials of TTHMs and 5HAAs in the feed water were 288 µg L^{-1} and 327 µg L^{-1} respectively. BAC 1 removed the DBP precursors and reduced the formation potential by 40% and 47% for TTHMs and 5HAAs respectively (Figure 7). Contrary to what was observed for NDMA, ozonation did not affect the THM and HAA precursors, or their transformation products have a similar formation potential. After ozonation, SAND 2 slightly reduced the TTHM and 5HAA formation potential by 16 and 20% respectively. BAC 2 reduced the formation potential by 50% and 57% for TTHMs and 5HAAs respectively. That is slightly better than BAC 1, indicating that ozonation may have increased the biodegradability of the precursors. Finally, BAC FS achieved 30 and 36% of TTHM and 5HAA formation potential reduction respectively. This is lower than BAC 2, highlighting again the influence of contact time. As observed previously, BAC 1 performed similarly to O_3+BAC 2 and O_3+BAC FS and better than O_3+SAND 2.

As seen in Figure 8, the formation of THMs and HAAs was largely dominated by chlorinated species in the feed. The reduction of TTHM and 5HAA formation potential observed after the various biofilters is explained almost exclusively by a reduction in chlorinated species formation whereas the brominated species do not seem to be affected. This induces a change in the relative distribution of DBPs, with a reduction of the chlorinated species importance and an increase of the brominated species relevance. The reduction in DBP formation potential is accompanied by a removal of DOC in the filters. On the contrary, the ion content remains constant as demonstrated by the constant conductivity measured in all samples. Since we did not observe bromate formation above the LOQ (i.e. 10 µg L^{-1}) across the treatment, we assumed the oxidation of Br^- to BrO_3^- by ozone was minimal. Therefore all Br^- was available to be oxidised to HOBr by HOCl during the formation potential test. The rate constant of bromide with HOCl to generate HOBr is 1.5×10^3 $M^{-1}s^{-1}$ (Kumar and Margerum, 1987) and the rate constant of THMs formation is in the range of 0.01 and 0.03 $M^{-1}s^{-1}$ (Gallard and von Gunten, 2002). It is known that, once formed, bromine reacts about 10 times faster than chlorine with natural organic matter, since the activities of electrophilic substitution for electron release to stabilise carbocation are more favourable for the Br atom due to its higher electron density and smaller bond strength relative to the Cl atom (Westerhoff *et al.*, 2004; Hua *et al.*, 2006). Hence, the formation of Br-DBPs is limited by the initial Br concentration whereas the Cl-DBPs is limited by the organic matter. Therefore, when organic matter decreases along the treatment train, the Cl-DBPs are reduced while the formation of Br-DBPs remains constant.

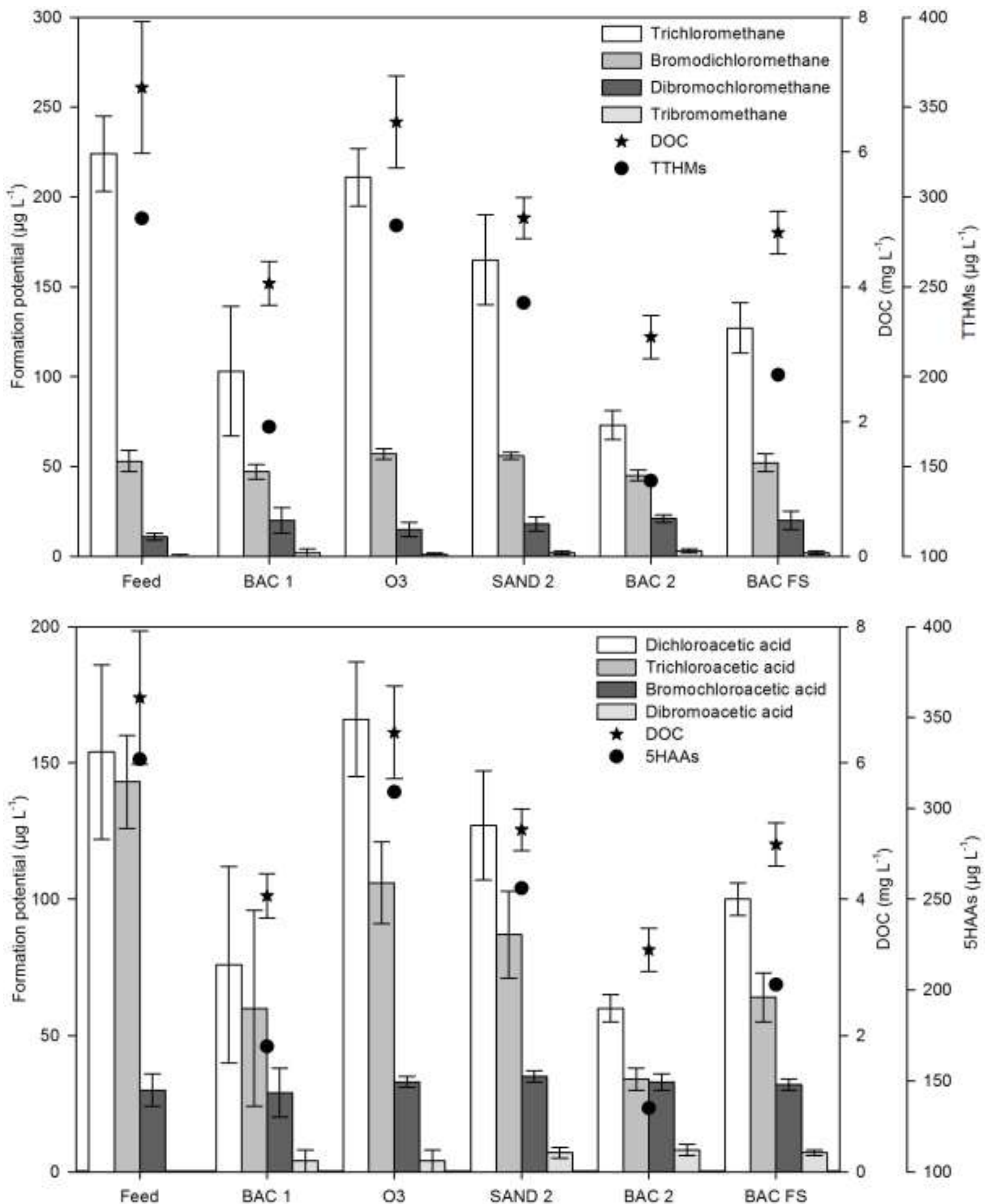

Figure 8: THMs (top) and HAAs (bottom) formation potential and DOC before (feed) and after treatment. Error bars correspond to standard deviation of three independent sampling campaigns (n=3).

- BAC filtration alone reduced the formation potential of NDMA, TTHMs and 5HAAs by 81, 40 and 47% respectively.
- BAC filtration alone performed similarly to ozonation followed by BAC filtration with the same EBCT and better than ozonation followed by BAC filtration with a shorter EBCT.
- The sand filter placed after ozonation removed less of the precursors compared to the BAC filter.

3.6. Influence of Filtering Media

Under similar operating conditions, BAC filtration is more effective than sand filtration to remove the EfOM and a wide range of PPCPs from a WWTP effluent as well as to reduce non-specific toxicity. This can be observed with or without prior ozonation. Whereas a sand filter can rely only on biodegradation to remove the EfOM and PPCPs, a BAC filter also has adsorption properties. However, after an initial period during which the removal of organic compound is due to adsorption, the adsorption efficiency decreases while biomass develops in the filter and eventually, the removal observed is due mainly to biodegradation or adsorption followed by biodegradation (Simpson, 2008). The higher effectiveness of BAC 1 and BAC 2 compared to SAND 1 and SAND 2, respectively, could therefore be due to more biomass attached on the surface of activated carbon or the combined effects of adsorption and biodegradation. Activated carbon typically has a surface area of several hundred square meters per gram due to its high porosity, but most of this surface is not accessible to bacteria as it is located in micropores with a diameter smaller than 2 nm. However, the external surface of the activated carbon grains is much rougher and uneven than the surface of sand grains, as can be seen in Figure 9, therefore potentially providing more sites for the bacteria to attach (Wang *et al.*, 2007). Some authors have also hypothesised that the biodegradation continuously regenerates adsorption sites by degrading adsorbed molecules (Herzberg *et al.*, 2003; Simpson, 2008). Adsorption of organic compounds onto the activated carbon surface could therefore increase their residence time within the filter and allow degradation by the bacteria, particularly for the compounds known to be poorly biodegradable. Another explanation of the higher removal observed in BAC filters compared to sand filters could be an increased flux of pollutants to the biofilm as mentioned by Herzberg *et al.* (2003).

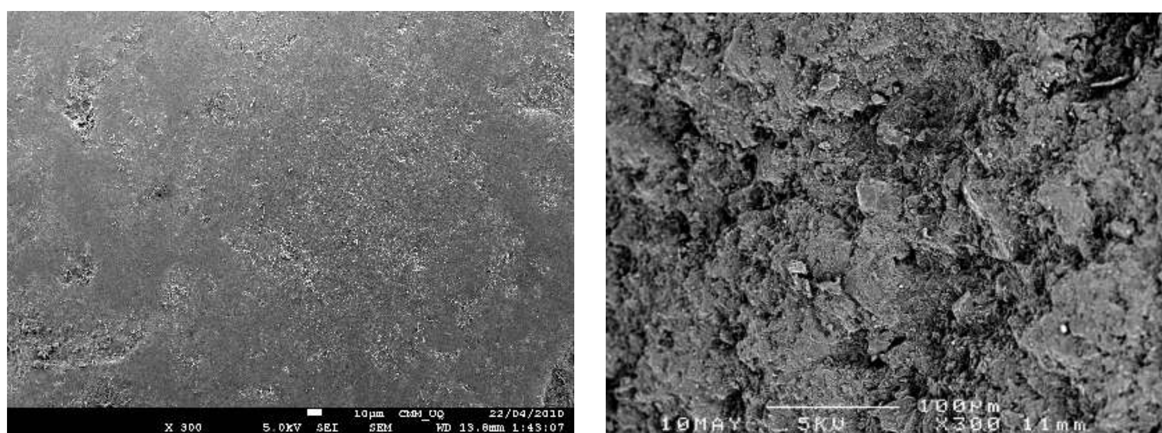

Figure 9: Scanning electron microscopy images of the surface of sand (left) and activated carbon (right) grains collected from the pilot scale filters.

3.7. Influence of Ozonation

Classically, in drinking water treatment, sand and BAC filters are placed after an ozonation process to degrade the by-products formed by oxidation of the natural organic matter. These transformation products have been shown to be more biodegradable than their parent compounds. The influence of ozonation on sand filtration is difficult to assess as SAND 1 and SAND 2 were investigated at different times with different influent water quality. Nevertheless, the combination of ozonation and sand filtration appears to be more effective for the removal of DOC compared to sand filtration alone, as could be expected. The overall removal of PPCPs observed after ozonation and filtration through SAND 2 is higher than the removal observed in SAND 1. However, this is mainly due to the effect of ozonation and SAND 2 in itself showed poor removal of PPCPs, similarly to SAND 1.

In the first campaign, there was no significant difference between the DOC concentrations in the effluents of both BAC filters, whereas, in the following sampling campaigns, the DOC levels were always lower after BAC 2 compared to BAC 1. The combination of ozonation and BAC 2 removed 15 to 20% more DOC compared to BAC 1, of which only 5 to 13% was directly due to ozonation alone.

This shows that ozonation increased the biodegradability of EfOM. Similarly, the baseline-TEQ_{bio} was generally lower after BAC 2 compared to BAC 1, by up to 54%. Removals of PPCPs by both BAC 1 and the combination of ozonation and BAC 2 were similar and no clear difference could be observed except for gabapentin, which was consistently detected at lower concentrations after BAC 2 compared to BAC 1, as well as perindopril in the third and fourth campaigns.

3.8. Microbial Community Characterisation

A preliminary study was conducted to determine whether there was some variability in the microbial communities present in the pilot scale filters and if that could be related to the operating conditions. Samples were collected from the pilot scale filters (Table 9), DNA was extracted and pyrotag sequencing performed to identify the organisms present in the samples and their relative abundance (Figure 10).

Table 9: Pilot scale biofilters media samples and DNA concentrations in the extracts.

Sample Name	Pilot Scale Filter	Depth	DNA Concentration (ng μl^{-1})
SAND 2 – top layer	SAND 2	Biofilm layer on top of bed	230.8
SAND 2 – top	SAND 2	5 cm	67.4
SAND 2 – middle	SAND 2	40 cm	62.4
BAC 1 – top layer	BAC 1	Biofilm layer on top of bed	464.5
BAC 1 – top	BAC 1	5 cm	225.0
BAC 1 – middle	BAC 1	40 cm	114.8
BAC 1 – bottom	BAC 1	80 cm	60.7
BAC 2 – top layer	BAC 2	Biofilm layer on top of bed	399.9
BAC 2 – top	BAC 2	5 cm	145.3
BAC 2 – middle	BAC 2	40 cm	236.6
BAC 2 – bottom	BAC 2	80 cm	225.8

The DNA concentration can be used as a semi-quantitative method to estimate biomass quantity, keeping in mind that it can be influenced by the presence of algae and the efficiency of the extraction (which is assumed to be similar for similar samples here). Table 9 shows that the DNA concentration generally decreased with increasing depth. This has been also observed in filters treating drinking water (Boon *et al.*, 2011; Velten *et al.*, 2011). It could be due to a lower availability of nutrients as the depth increases. However, a more accurate measurement of biomass quantity and intermediate nutrients concentrations would be required to confirm this hypothesis.

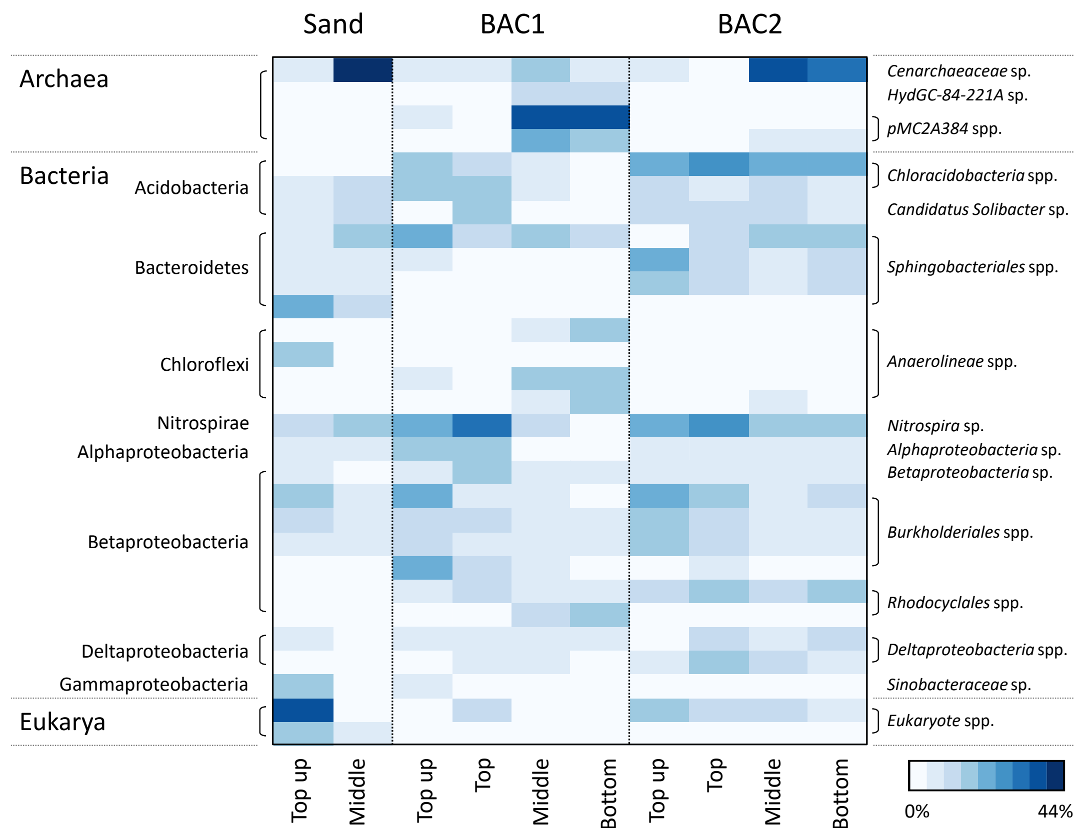

Figure 10: Heatmap summarising the relative abundances (%) of OTUs that were present at more than 1% in any of the filter-associated microbial communities.

3.8.1. BAC Filters Before and After Ozonation

The composition of microbial communities associated with the BAC filters was influenced by ozonation (P = 0.004, PERMANOVA) and depth (P = 0.043, PERMANOVA). Ozonation did not influence the numbers of species present in the filters (P > 0.05; GLM) but depth did (t = -5.078, P = 0.002, GLM). The number of species decreased with increasing depth (Figure 11). The effect of ozonation was most apparent in the deeper portions of the filter and manifested as an increased relative abundance of OTUs related to pMC2A384 spp., a Methyloversatalis sp., and an envOPS12 sp.

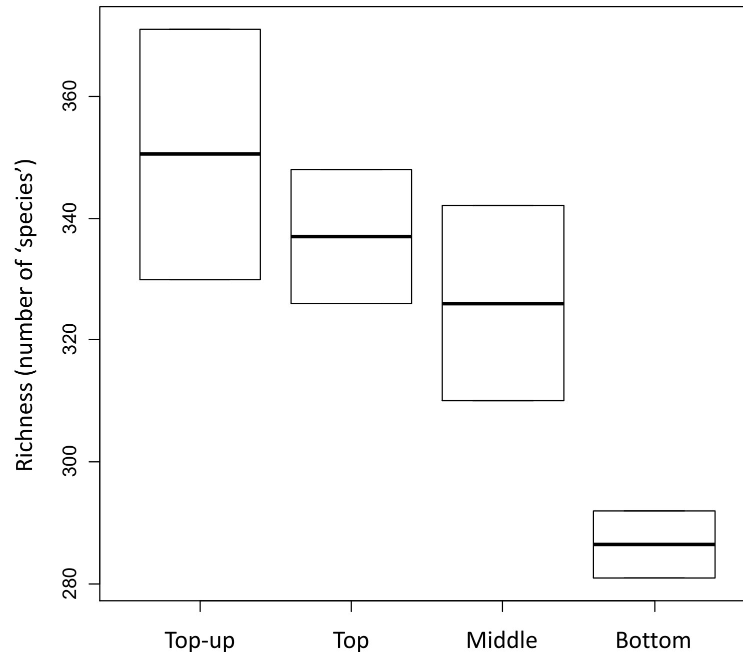

Figure 11: Number of species in the BAC filters at each depth (middle bar is the average of two samples, boxes show minimum and maximum).

Microbial community diversity and composition are affected by their environment. The variations observed here are likely due to a change of conditions along the BAC filters: organic matter becomes less available as its concentration and biodegradability decrease, nutrients and dissolved oxygen concentration also decrease along the filter. Yang *et al.* (2011) and Boon *et al.* (2011) also reported variations in microbial community diversity with depth in biofilters treating ozonated surface water. However, contrary to here, they observed a higher number of species in the bottom section of the filters. They hypothesised that residual ozone in the influent water at the top could inhibit the growth of some bacteria in the top section of the filters. Boon *et al.* (2011) showed that nutrient gradients drive microbial community organisation and dynamics but that microbial activity is not necessarily related to nutrient availability. They observed higher DOC removal efficiency in lower parts of BAC filters.

There was a clear shift in microbial community compositions in both BAC filters between the top and middle samples, as can be seen in Figure 10. The main difference is that deeper samples have higher PC2 values and are associated with more archaea, whereas the top layers are associated with bacteria (Figure 12). During the second sampling campaign, DO measurements in the influent and effluent of both BAC filters showed that there is a significant decrease in DO concentration across the filter even for EBCTs as short as 30 minutes (Table 5). When the samples for microbial community characterisation were collected, the EBCT was 60 minutes. It is therefore suggested that the top two samples (i.e. from the biofilm layer on the top of the bed and from the first 5cm of the bed) are exposed to high concentrations of DO but that the middle and bottom samples are exposed to very low DO levels or even no DO at all. It is know that different kind of bacteria thrive under aerobic and anoxic or anaerobic condition, we would therefore expect to observe a difference between the top samples and the middle and bottom samples. More research is required to investigate the link between the conditions in the filters and the microbial communities' composition.

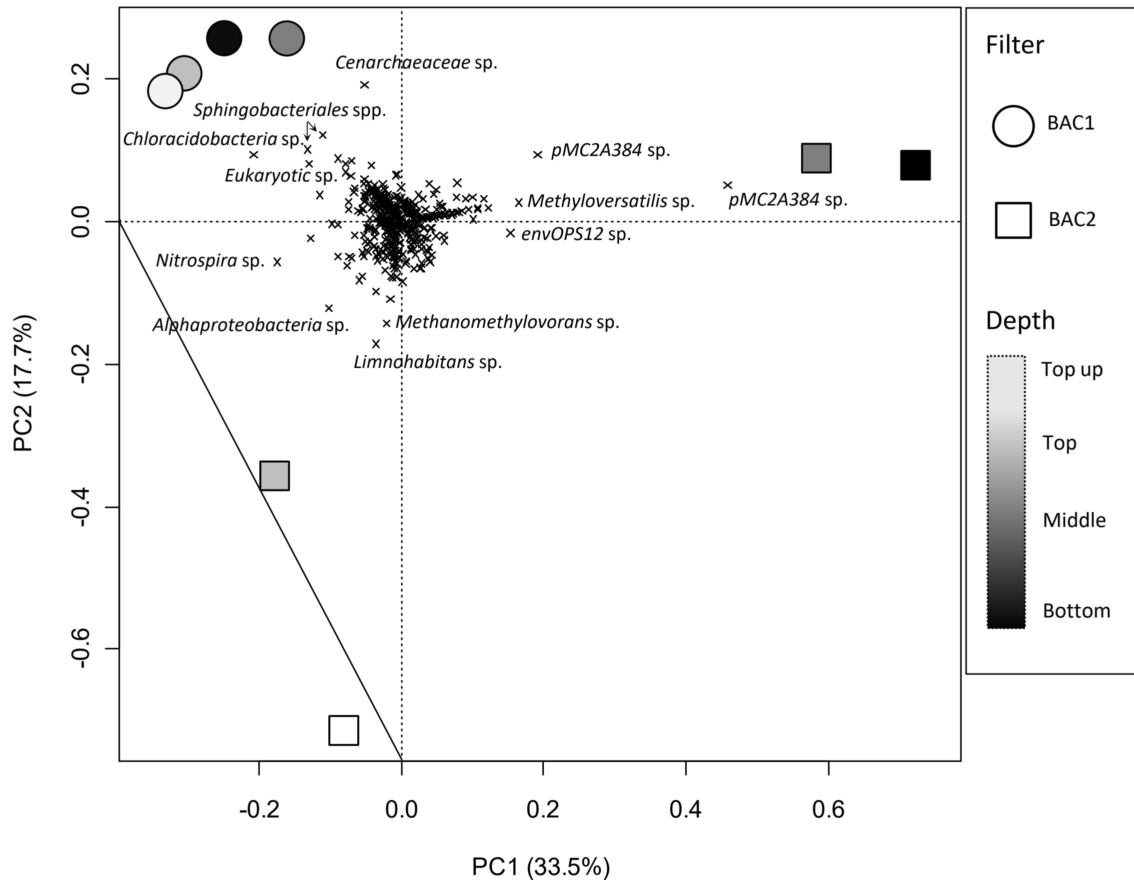

Figure 12: Principal component analysis summarising variation in the composition of microbial communities between samples. Samples are labelled to facilitate interpretation of the ozonation and sampling depth effects.

Several authors have previously reported an impact of ozonation on microbial communities' composition in biofilters (Moll *et al.*, 1998; Fonseca *et al.*, 2001; Magic-Knezev *et al.*, 2009; Wietz *et al.*, 2009). Ozone is a powerful disinfectant which would kill a lot of organisms present in the water, potentially giving the opportunity for new organisms to grow. Hess-Erga *et al.* (2010) observed that a different microbial community developed in batch systems after a once-off disinfection of seawater. Ozonation also alters the quality of the EfOM and typically leads to the production of more biodegradable products. As noted before, this could also influence microbial community composition. Surprisingly, the difference between BAC 1 and BAC 2 was minimal in the top layer and top samples and became more apparent in the middle and bottom samples. This warrants further research to understand the impact of ozonation on the development of microbial communities.

3.8.2. Sand and BAC

As it was observed that the sand filters were less effective in removing EfOM, PPCPs and at reducing toxicity compared to BAC filters, it was suggested that this could be (partially) due to different in microbial communities. Samples from SAND 2 and BAC 2 were compared to determine whether the filtering media influenced the bacterial communities. As there were only two samples from the SAND 2 filter, it was not possible to rigorously test differences in composition and richness. However, the richness of species did not seem to be affected by the filtering media but there appears to be more of some archaea and eukaryotes in SAND 2 (Figure 10).

The influence of supporting media on microbial communities' composition in biofilters has not been extensively studied. Yang *et al.* (2011) observed different communities in BAC and anthracite filters filtering ozonated surface water whereas Moll and Summers (1999) and Shoji *et al.* (2008) did not observe any influence of the filtering media. Wietz *et al.* (2009) also observed differences between biofilms grown on glass slides and fiberglass in ozonated seawater.

4. CONCLUSION AND RECOMMENDATIONS

The results of this study show that direct filtration (i.e. without ozonation) through biological activated carbon significantly improved the quality of WWTP effluents:

- It reduced the DOC concentration by 35 to 60% which potentially limits bacterial regrowth in the distribution system in the context of water reuse.
- It reduced the concentrations of a wide range of PPCPs by more than 90%, most of them down to levels below 10 ng L-1. This lowers the potential risk of environmental and/or human health impact.
- It reduced the baseline toxicity equivalent concentration, which is a measure of all chemicals present including PPCPs and small natural organic molecules, by 28 to 85%. This was less than the individual chemicals quantified by chemical analysis because transformation products formed during the biodegradation process and natural compounds may also contribute to the mixture toxicity measured with the bioassay.
- It reduced specific toxicity by more than 90% for five toxic modes of action: estrogenicity, AhR response, genotoxicity, neurotoxicity and phytotoxicity.
- It reduced the formation potential of NDMA, TTHMs and 5HAAs by 80%, 40% and 47% respectively.

On the contrary, under similar conditions, sand filtration showed limited improvement of water quality. Moreover, the long term study of the BAC filters showed steady performance which suggests that EfOM and PPCPs are biodegraded, the filters could therefore potentially be used for many years without replacing the media. BAC filtration could be implemented as an advanced treatment in WWTPs to reduce the impact of the effluent discharged into the environment and/or to produce a water of a higher quality for reuse. In the meantime, further investigations are necessary to fully understand the mechanisms involved in EfOM and PPCPs removal, particularly the role of adsorption, and to clearly identify the key parameters that have to be taken into account for the design of full-scale filters (e.g. initial DO concentration, EBCT). Also, a preliminary study showed some differences between the microbial communities of the various filters which warrant further research to elucidate the interactions between operating conditions, microbial communities and performance of the filters.

APPENDIX 1. QHFSS ORGANIC MICROPOLLUTANTS ANALYTICAL METHOD

Extraction Method

Solid phase extraction was performed using with Waters Oasis HLB 60 mg in 3 mL cartridges. The filled cartridges were conditioned with 1 mL dichloromethane, 5 mL methanol and 5 mL MilliQ water. The samples were extracted at neutral and acidic pH on an automated solid phase extraction equipment (Gilson Aspec); 40 mL of sample were loaded on the cartridge which was then dried for 10 minutes under a nitrogen flow. For acidic extraction, 1 mL of formic acid 98-100% purity was added to 50 mL of sample. The analytes of interest were eluted with 1 mL of acetonitrile followed by 2 mL of dichloromethane for neutral analysis; or 2% ammonium hydroxide/98% acetonitrile followed by 2 mL dichloromethane for acidic analysis. Extracts were gently blown to dryness using nitrogen. 400 µL of 15% acetonitrile/water was added to the dried extracts before transfer to LC/MS-MS vial with 400 µL glass inserts. For quality control, each batch included a blank sample and a standard mixture sample (both prepared with deionised water) which were extracted following the same method. The standard mixture sample contained 31 pharmaceuticals and personal care products. A mixture of five compounds (containing caffeine D_3, carbamazepine D_{10}, atrazine D_5, diclofenac D_4 and $^{13}C_6$ 2,4-dichlorophenoxy acetic acid) was added to each sample, including standard mixtures and blanks prior to extraction to monitor the extraction efficiency (Table 10). A 10 µL spike of internal standard containing 4 compounds (acetylsulfamethoxazole D_5, fluoxetine D_5, 2,4-dichlorophenyl acetic acid and simazine D_{10}) was added to each vial prior to analysis by LC/MS-MS. Long term mean recoveries are given in Table 11. Given the very low extraction recovery, gabapentin was quantified by direct injection of the sample (i.e. without extraction and concentration).

Quantification Method

Extracts were analysed by LC/MS-MS injecting 8 µL in a Shimadzu Prominence HPLC system (Shimadzu Corp., Kyoto, Japan) connected to an AB/Sciex API4000QTrap mass spectrometer equipped with an electrospray (TurboV) interface (MDS Sciex, Concord, Ont., Canada). The HPLC instrument was equipped with a 3 µm 150 × 2 mm Luna C18(2) column (Phenomenex, Torrance, CA) run at 45°C. Separation was achieved with a flow rate of 0.35 ml min^{-1} with a linear gradient starting at 15% B for 0.3 minutes, ramped to 100% B in 10 minutes, held for 4 minute and then to 15% B in 0.2 minutes and equilibrated for 4 minutes (A = 1% acetonitrile/99% HPLC grade water, B = 95% acetonitrile/5% HPLC grade water both containing 0.1% formic acid). Each sample extract was analysed separately in both positive and negative ion multiple reaction monitoring mode, LC/MS-MS parameters are given in Table 11. Positive samples were confirmed by retention time and by comparing transition intensity ratios between the sample and an appropriate concentration standard from the same run. Samples were only reported as positive if the two transitions are present, retention time is within 0.15 minutes of the standard and the relative intensity of the confirmation transition was within 20% of the expected value. Analyte concentrations were determined using the internal standard method and compared to a four point calibration curve using standard concentrations from 5 to 100 µg L^{-1}. Limits of quantification (LOQs) were set at a signal to noise ratio of 9.

Table 10: Long term mean recovery of surrogate chemicals from samples and standard deviation.

Surrogate Chemicals	Mean Recovery (%)	Standard Deviation (%)
Diclofenac D4	82	15
Carbamazepine D10	98	15
Caffeine D3	98	20
Atrazine D5	89	16
2,4-Dichlorophenoxyacetic acid 13C6	91	13

Table 11: Recoveries, liquid chromatography retention times and MS/MS parameters.

POSITIVE MODE (entrance potential = 10 volts)										
Compound Name	**Rec (%)**	**Rt (min)**	**DP (V)**	**Q1 (Da)**	**$Q3_{qant}$ (Da)**	**CE (V)**	**CXP (V)**	**$Q3_{conf}$ (Da)**	**CE (V)**	**CXP (V)**
Atenolol[1]	44	2.30	71	267.2	190.1	27	10	145.0	39	8
Ranitidine[1]	50	2.44	56	315.2	176.1	25	8	130.1	35	6
Codeine[1]	58	2.85	86	300.2	215.2	35	12	152.1	89	6
Gabapentin[1]	NA	2.90	66	172.1	154.0	19	8	137.0	23	6
Lincomycin[1]	51	3.02	60	407.3	126.1	44	8	359.3	28	20
Iopromide[1]	74	3.03	100	791.7	573.1	35	14	559.1	41	14
Oxycodone[1]	73	3.03	65	316.2	298.2	26	16	241.2	42	16
Paracetamol[1]	38	3.13	61	152.1	110.0	23	6	65.1	42	6
Cephalexin[1]	ND	3.17	45	348.3	158.1	13	8	174.1	21	10
Trimethoprim[1]	85	3.17	85	291.2	230.1	35	14	123.1	35	8
Norfloxacin[1]	22	3.21	70	320.3	276.2	26	14	233.2	35	14
Ciprofloxacin[1]	32	3.26	61	332.3	231.1	54	12	288.2	24	16
Oxytetracycline[1]	30	3.31	30	461.3	426.3	28	6	443.3	17	6
Doxylamine[1]	20	3.33	40	271.2	182.0	24	8	167.1	45	7
Enrofloxacin[1]	58	3.37	28	360.3	316.2	30	15	245.2	40	15
Tetracycline[1]	19	3.41	50	445.3	410.2	28	24	154.1	40	7
Caffeine D_3*[1]	100	3.50	60	198.1	138.0	27	8	110.0	35	8
Caffeine[1]	83	3.52	61	195.1	138.1	29	6	110.1	33	4
Metoprolol[1]	78	3.53	70	268.2	116.1	28	7	191.1	27	10
Sulfadiazine[1]	40	3.54	71	251.2	92.0	37	14	65.0	61	10
Tramadol[1]	87	3.54	45	264.2	58.0	44	8	42.0	125	3
Sulfathiazole[1]	69	3.58	51	256.2	156.1	22	10	92.1	40	8
Acetylsulfamethoxazole D_5	IS1	3.87	60	302.3	202.1	26	9	138.1	36	9
Chlortetracycline[1]	16	3.90	50	479.3	444.3	32	6	154.1	42	12
Venlafaxine[1]	79	3.98	45	278.2	58.0	50	7	121.0	40	10
Desisopropylatrazine[1]	90	4.02	70	174.0	104.0	34	10	132.0	27	10
Propranolol[1]	80	4.25	70	260.2	116.1	28	8	183.1	28	8
Desmethylcitalopram[1]	70	4.58	60	311.3	109.0	35	8	262.2	25	15
Citalopram[3]	86	4.67	70	325.3	109.0	38	4	262.2	28	4
Dapsone[3]	45	4.68	60	249.2	156.0	22	7	92.0	34	14
Erythromycin[3]	42	4.90	50	734.7	576.4	27	18	158.1	45	8
Sulfamethoxazole[3]	65	5.15	51	254.2	156.0	23	8	92.1	38	8
Tylosin[3]	12	5.36	10	916.7	174.1	58	5	101.1	72	12
Fluoxetine D_5	IS2	6.04	45	315.2	44.0	42	5	153.1	14	10
Fluoxetine[2]	44	6.09	51	310.1	44.0	37	8	148.0	13	7
Sertraline[3]	57	6.12	35	306.3	159.1	35	12	275.2	18	12
Roxithromycin[3]	21	6.39	10	837.6	679.5	32	9	158.0	52	5
Simazine D_{10}	IS3	6.9	60	212.0	137.0	40	10	134.0	38	10
Carbamazepine D_{10}*[3]	97	7.00	65	247.2	204.1	30	8	202.1	51	8
Carbamazepine[3]	90	7.06	96	237.2	194.0	31	16	193.0	47	12
Phenytoin[3]	98	7.07	66	253.2	182.0	29	18	104.0	48	14
Oxazepam[3]	96	7.42	60	287.2	241.2	32	10	104.0	52	10
Desmethyldiazepam[3]	92	7.80	70	271.2	140.1	41	15	165.1	41	15
Atrazine D_5*[3]	88	8.13	60	221.1	179.0	27	6	101.0	36	6
DEET[3]	79	8.27	86	192.1	119.0	26	10	91.0	44	6
Temazepam[3]	96	8.29	55	301.2	255.1	32	8	283.1	21	8
Naproxen[3]	100	8.49	61	231.2	185.1	19	10	170.1	37	8
Praziquantel[3]	70	8.62	70	313.3	203.2	25	10	55.0	72	8

Compound Name	Rec (%)	Rt (min)	DP (V)	Q1 (Da)	$Q3_{qant}$ (Da)	CE (V)	CXP (V)	$Q3_{conf}$ (Da)	CE (V)	CXP (V)
Diazepam[3]	84	8.89	76	285.2	154.1	36	12	193.2	42	14
Atorvastatin[3]	34	9.61	70	559.5	440.3	31	10	250.2	62	10
Diclofenac D_4*[3]	80	9.75	45	300.1	219.1	30	8	218.1	46	8
Indomethacin[3]	48	9.76	66	358.3	138.9	31	10	75.0	107	12
Diclofenac[3]	52	9.78	40	296.2	214.0	50	10	250.1	21	10
Simvastatin[3]	ND	11.8	62	419.3	285.2	16	15	199.1	18	15
NEGATIVE MODE (entrance potential = -10 volts)										
Hydrochlorothiazide[4]	91	3.98	-55	296.0	205.0	-34	-12	269.0	-28	-20
Acetylsalicylic acid[4]	67	5.78	-35	178.9	136.9	-9	-11	92.9	-30	-5
Chloramphenicol[4]	89	6.17	-70	321.0	152.0	-25	-13	257.0	-16	-13
Salicylic acid[4]	54	6.47	-45	137.0	93.0	-24	-6	65.0	-40	-6
Furosemide[4]	72	6.99	-57	329.0	285.0	-21	-13	205.0	-33	-13
2,4-Dichlorophenylacetic acid	IS4	7.95	-25	205.0	161.0	-10	-10	159.0	-10	-7
2,4-Dichlorophenoxyacetic acid $^{13}C_6$ *[4]	90	8.15	-36	225.0	167.0	-21	-10	169.0	-21	-10
Warfarin[4]	89	8.94	-170	307.0	161.0	-28	-11	250.0	-30	-9
Fluvastatin[4]	30	9.34	-10	410.3	348.1	-22	-26	210.1	-42	-11
Diclofenac D_4*[4]	80	9.58	-50	302.1	258.0	-16	-8	256.0	-16	-8
Ibuprofen[4]	101	9.85	-52	205.1	161.0	-11.5	-10	159.0	-11	-10
Gemfibrozil[4]	44	10.50	-60	249.1	121.0	-18	-8	127.0	-15	-9
Triclosan[4]	30	11.00	-50	287.0	35.0	-30	-3	35.0	-30	-3

Rec = recovery (per cent) at a concentration in the sample of 1 $\mu g\ L^{-1}$ for pharmaceuticals and 0.1 $\mu g\ L^{-1}$ for herbicides and pesticides (ND indicates insufficient data to determine); **Rt** = retention time; **DP** = declustering potential; **Q1** = parent ion; **$Q3_{quant}$** = fragment ion used for quantitation; **$Q3_{conf}$** = fragment ion used for confirmation; **CE** = collision energy; **CXP** = collision cell exit potential.

* surrogate compounds

[1] internal standard used for quantification = Acetylsulfamethoxazole D5 (IS1)

[2] internal standard used for quantification = Fluoxetine D5 (IS2)

[3] internal standard used for quantification = Simazine D10 (IS3)

[4] internal standard used for quantification = 2,4-Dichlorophenylacetic acid (IS4)

APPENDIX 2. AWMC ORGANIC MICROPOLLUTANTS ANALYTICAL METHOD

Extraction Method

Within 12 hours after collection, the samples were filtered (0.45µm Nylon filters, PM separation, Australia) and stored at 4°C before extraction which occurred within 3 days. The samples were then split in six 200 mL subsamples, three were extracted directly and the other three were spiked with 50 µL of a 200 µg L^{-1} mix of the target compounds (prepared in methanol) in order to evaluate losses during extraction and matrix interference during analysis. Solid phase extraction was performed using Waters Oasis HLB 60 mg (3 mL) cartridges. The cartridges were first conditioned with twice 3 mL of methanol and twice 3 mL of HPLC water. Subsamples were then extracted without any pH adjustement, under vacuum, at a flow rate of 1 mL min^{-1}. After extraction, cartridges were dried under vacuum for at least 30 minutes. The compounds of interest were eluted with 3 times 3 mL of methanol. Extracts were gently blown to dryness using nitrogen before being reconstituted in 1 mL of a water/methanol mixture (75/25, v/v).

Analytical Method

A volume of 20 µL of extract was injected in a Shimadzu UFLC connected to an AB Sciex 4000QTrap QLIT-MS equipped with a Turbo Spray source. The UFLC instrument was equipped with a SecurityGuard Gemini NX C18 4×20 mm (Phenomenex) pre-column and a 5 µm, 250×4.6 mm Altima C18 (Grace) column run at 40°C. Each sample extract was analysed separately in both positive and negative ion scheduled multiple reaction monitoring (SMRM) mode. The time window in the SMRM mode was set at 120 seconds with a target scan time of 0.5 seconds. The eluents compositions and gradients for each mode are detailed in Table 12. Two transitions were monitored in the SMRM mode using parameters detailed in Table 13. The first transition was used for quantification and the second one for confirmation purposes only. The retention time was also compared to the standards for confirmation.

Table 12: HPLC eluents gradients (total flow rate = 1 ml min^{-1}) and QLIT-MS parameters.

	Positive Mode		Negative Mode	
Eluent A (v/v)	95% acetonitrile / 5% HPLC grade water 26.5 mM formic acid		50% acetonitrile / 50% methanol	
Eluent B (v/v)	1% acetonitrile / 99% HPLC grade water 26.5 mM formic acid		95% HPLC grade water / 5% methanol 1mM sodium acetate	
Gradient	**Analysis Time (min)**	**% eluent A**	**Analysis Time (min)**	**% eluent A**
	0	15	0	0
	12.5	100	7	90
	15	100	10	90
	15.2	15	12	5
	21.2	15	15	5
			16	100
			21	100
Source Parameters	**Positive Mode**		**Negative Mode**	
Ion spray voltage (V)	5 500.0		- 4 500.0	
Source temperature (°C)	700		700	
Curtain gas (arbitrary units)	30.0		30.0	
Collision gas	High		High	
Q1 and Q3 mass resolution	Low, Unit		Unit, Unit	
Ion source gas 1 (arbitrary units)	62.0		55.0	
Ion source gas 2 (arbitrary units)	62.0		55.0	
Interface heater	ON		ON	

Table 13: Target compounds, retention times and optimized QLIT-MS parameters (Rt = retention time; DP = declustering potential; Q1 = parent ion; $Q3_{quant}$ = fragment ion used for quantitation; $Q3_{conf}$ = fragment ion used for confirmation; CE = collision energy; CXP = collision cell exit potential).

Compound Name	Rt (min)	DP (volts)	Q1 (Da)	$Q3_{quant}$ (Da)	CE (volts)	CXP (volts)	$Q3_{conf}$ (Da)	CE (volts)	CXP (volts)
Positive Mode (entrance potential = 10 volts)									
Atenolol	3.6	71	267.2	145.3	37	12	190.2	29	16
Caffeine	5.8	71	195.1	138.1	28	8	110.0	32	8
Carbamazepine	9.6	61	237.2	194.2	27	16	193.3	47	12
Citalopram	7.8	70	325.3	109.1	38	4	262.2	28	4
Dapsone	7.6	71	249.1	108.2	31	8	92.1	35	6
Doxylamine	5.4	40	271.2	182.2	24	8	167.2	45	7
Indomethacin	12.4	91	358.1	139.0	27	12	111.0	71	8
Lincomycin	4.9	91	407.3	126.2	39	10	359.3	27	10
Metoprolol	6.3	76	268.2	116.2	27	8	121.1	35	8
Perindopril	7.5	76	369.2	172.2	29	14	98.1	49	6
Phenytoin	9.4	61	253.1	182.2	27	10	104.1	51	8
Praziquantel	11.4	81	313.2	203.2	25	18	83.2	41	6
Propranolol	7.4	76	260.2	116.2	27	8	183.2	27	12
Ranitidine	3.7	61	315.3	176.1	25	14	102.2	51	16
Risperidone	6.8	96	411.2	191.2	41	12	109.9	71	6
Roxithromycin	8.8	96	837.6	679.5	31	12	158.0	49	12
Sertraline	8.9	56	306.1	159.1	39	12	275.1	19	18
Sulphathiazole	5.9	51	256.2	156.1	22	10	92.1	40	8
Tramadol	6.4	45	264.2	58.1	44	8	42.2	125	3
Trimethoprim	5.5	86	291.2	230.3	33	4	261.2	37	6
Tylosin	7.9	151	916.6	174.2	55	14	772.5	54	10
Venlafaxine	7.1	61	278.2	58.1	41	10	260.3	19	6
Negative Mode (entrance potential = - 10 volts)									
Chloramphenicol	7.6	-70	322.9	151.9	-26	-9	120.9	-48	-7
Diclofenac	10.1	-40	293.9	250.0	-16	-1	214.0	-30	-15
Furosemide	6.7	-70	329.0	284.8	-22	-7	204.8	-30	-11
Gemfibrozil	11.2	-85	249.0	121.0	-20	-7	127.0	-14	-5
Ibuprofen	10.4	-52	205.0	161.0	-11.5	-10			
Naproxen	9.4	-50	229.0	185.0	-10	-13	169.0	-38	-9
Warfarin	9.5	-85	307.0	161.0	-28	-11	250.0	-32	-1

Quantification Method

The quantification of the targeted compounds in the extract was performed using 10 points external calibration curves obtained from the injection of standard solutions ranging from 0.1 to 100 µg L^{-1}. Linear or quadratic regression was used depending on the compound, which gave good fits with $r^2 > 0.99$. The three non-spiked subsamples were averaged. The spiked subsamples were used to correct the concentrations obtained for losses occurring during the SPE and for matrix effect (ion-enhancement or -suppression) in the instrument. Each spiked sample was compared to the average of non-spiked samples allowing three determination of the overall recovery efficiency of the method. Overall recoveries were averaged and used with the average of non-spiked subsamples to calculate actual concentration. Overall recoveries were above 20% for all compounds in all samples. The LOQ was set at a signal to noise ratio of 10 and was determined using the spiked samples. Individual recoveries and LOQs are not reported here since they were determined for each compound and sample and varied from one to another as ion-suppression and enhancement depends largely on the matrix composition which varied with time and the treatment applied.

QA/QC

The calibration curve was renewed for each run, typically daily. Blank samples and 10 µg L^{-1} standards were injected regularly during each run to ensure there was no contamination of the column and the signal intensity remained steady for each compound.

APPENDIX 3. PROPERTIES OF THE PPCPS.

Table 14: Target compounds and some of their physico-chemical properties (pK_a: acidity constant, log K_{ow}: octanol-water partition coefficient, log D_{lipw}: liposome-water distribution ratio at pH 7) and relative potency (RP) of the compounds in the bioluminescence inhibition test with *Vibrio fischeri* (in relation to a reference virtual baseline toxicant); removal generally observed in full scale WWTP (P=poor, <20%; I=intermediate, 20-80%; G=good, >80%). NA = not applicable. NAv = not available.

Compound Name	Classification	Molecular Weight (g mol^{-1})	pK_a [i]	Charge pH=7 [ii]	Neutral Fraction at pH 7[ii]	log K_{ow} [i]	log D_{lipw}(pH 7)	Relative Potency RP	Removal in WWTP[iii]
Acetylsalicylic acid	Analgesic, antipyretic	180.16	3.63	-1	0.00	1.19	0.59	1.03E-02	G
Atenolol	Beta-blocker	266.30	9.6/9.05	+1	0.01	0.23	-0.24	1.40E-03	I
Atorvastatin	Hypolipidemic agent	558.64	4.46/4.18	-1	0.00	6.36	5.27	2.79E+01	
Caffeine		194.19	-	0	1.00	-0.07	0.45	7.31E-03	G
Carbamazepine	Anticonvulsant	236.27	-	0	1.00	2.45	2.73	4.89E-01	P
Cephalexin	Antibiotic (cephalosporin)	347.39	3.59/6.68	-1	0.32	0.65	0.69	6.50E-03	
Chloramphenicol	Antibiotic	323.13	12.66	0	1.00	1.14	1.55	3.63E-02	I
Chlortetracycline	Antibiotic (tetracycline)	478.84	1.31/5.7/9.03/9.7	-1	0.94	-0.62	-0.07	1.08E-03	
Ciprofloxacin	Antibiotic (quinolone)	331.34	6.0/5.68	0 (Z)	0.04	0.28	-0.10	1.48E-03	I-G
Citalopram	Antidepressant	324.39	9.63	+1	0.00	3.74	2.91	4.99E-01	I
Codeine	Analgesic	299.36	8.21	+1	0.06	1.19	0.77	8.84E-03	I
Dapsone	Antituberculotic, antileprotic	248.30	1.28/2.09	0	1.00	0.97	1.39	3.51E-02	
DEET	Insect repellent	191.27	0.91	0	1.00	2.18	2.49	3.77E-01	I
Desmethylcitalopram	Active citalopram metabolite	310.37	9.63	+1	0.00	3.74	2.91	4.99E-01	
Diazepam	Anxiolytic	184.74	3.4/3.62	0	1.00	2.82	3.07	7.76E-01	P
Diclofenac	NSAI*	296.15	4.15/4.12	-1	0.00	4.51	3.60	2.09E+00	P
Doxylamine	Sedative	270.37	8.73	+1	0.02	2.37	1.73	6.12E-02	
Enrofloxacin	Veterinary antibiotic (quinolone)	359.39	4	0 (Z)	0.00	0.70	0.15	2.22E-03	
Erythromycin	Antibiotic (macrolide)	733.95	8.88/8.23	+1	0.01	3.06	2.33	7.28E-02	P
Fluoxetine	Antidepressant	309.33	9.52	0	0.03	4.05	3.28	1.08E+00	I-G
Fluvastatin	Hypolipidemic agent	411.47	4.08	-1	0.00	4.85	3.90	2.69E+00	
Furosemide	Diuretic	330.74	3.5	-1	0.00	2.03	1.35	2.44E-02	I
Gabapentin	Anticonvulsant	171.24	3.68/4.86	0 (Z)	1.00	-1.10	-0.48	1.37E-03	G
Gemfibrozil	Hypolipidemic agent	250.33	4.9	-1	0.01	4.70	3.80	3.61E+00	I
Hydrochlorothiazide	Diuretic	297.74	9.76	0	1.00	-0.07	0.45	4.78E-03	I
Ibuprofen	NSAI*	206.28	4.91/4.53	-1	0.00	3.97	3.12	1.18E+00	G
Indomethacin	NSAI*	357-79	4.5	-1	0.00	4.27	3.39	1.15E+00	I
Iopromide	Radiographic agent	791.11	10.28/6.51	0	0.25	-2.05	-1.83	2.18E-05	P
Lincomycin	Antibiotic (lincosamide)	406.54	7.8	+1	0.14	0.20	0.04	1.59E-03	P
Metoprolol	Beta-blocker	267.36	9.6/9.08	+1	0.01	1.88	1.25	2.46E-02	I

Compound Name	Classification	Molecular Weight	pKa[i]	Charge pH=7 [ii]	Neutral Fraction	log Kow[i]	log Dlipw(pH 7)	Relative Potency	Removal in WWTP[iii]
Naproxen	NSAI*	230.27	4.15	-1	0.00	3.18	2.40	2.63E-01	I
Nordazepam (desmethyldiazepam)	Anxiolytic, active diazepam metabolite	270.71	3.75/11.02	0	1.00	0.00	0.51	5.92E-03	
Norfloxacin	Antibiotic (quinolone)	319.33	6.3/5.68	0 (Z)	1.00	-1.03	-0.42	8.32E-04	I
Oxazepam	Anxiolytic	286.71	10.28	0	1.00	2.24	2.54	2.80E-01	
Oxycodone	Narcotic analgesic	315.36	8.3	+1	0.05	0.66	0.27	3.15E-03	
Oxytetracycline	Antibiotic (tetracycline)	460.43	8.94/7.46/3.22	-1	0.74	-0.90	-0.41	5.80E-04	
Paracetamol	Analgesic, antipyretic	151.16	9.38/9.49	0	1.00	0.46	0.93	2.36E-02	G
Perindopril	ACE inhibitor	368.74	5.4	0	0.98	-2.42	-1.68	6.25E-05	
Phenytoin	Anticonvulsant	252.27	8.33	0	0.96	2.47	2.73	4.58E-01	I
Praziquantel	Anthelmintic	312.41	-	0	1.00	2.42	2.71	3.51E-01	
Propranolol	Beta-blocker	259.34	9.24	+1	0.01	3.48	2.69	4.11E-01	I
Ranitidine	Histamine-blocker	314.40	8.94	+1	0.01	0.27	-0.20	1.29E-03	I
Risperidone	Antipsychotic	410.50	9.59/7.99/7.06	+1	0.00	3.43	2.68	2.55E-01	
Roxithromycin	Antibiotic (macrolide)	837.10	8.3	+1	0.05	2.85	2.25	5.44E-02	P
Salicylic acid	Skin care ingredient	138.12	3.06	-1	0.00	2.26	1.56	8.73E-02	
Sertraline	Antidepressants	306.23	9.5	+1	0.00	5.97	4.93	2.62E+01	P
Simvastatin	Hypolipidemic agent	418.57	2.3	0	1.00	3.81	3.96	3.11E+00	
Sulfadiazine	Antibiotic (sulfonamide)	250.28	6.36	0	0.19	-0.99	-0.95	3.77E-04	I
Sulfamethoxazole	Antibiotic (sulfonamide)	253.28	1.8/1.84	+1	0.99	0.89	1.32	2.98E-02	P-I
Sulfasalazine	Anti-inflammatory	398.39	9.13/2.6/0.09	-1	0.00	3.81	2.96	4.53E-01	
Sulfathiazole	Antibiotic (sulfonamide)	255.32	1.5	0	0.05	-0.09	-0.41	1.07E-03	
Temazepam	Sedative	300.74	7.2	0	0.39	2.19	2.15	1.25E-01	
Tetracycline	Antibiotic (tetracycline)	444.35	1.32/5.74/9.27/10.11	-1	0.00	-1.30	-1.66	5.42E-05	G
Tramadol	Narcotic analgesic	263.38	9.61	+1	0.00	3.01	2.25	1.72E-01	
Triclosan	Biocide	289.54	8.1	0	0.93	4.75	4.78	2.09E+01	I
Trimethoprim	Antibiotic	290.32	7.12	0	0.43	0.91	1.03	1.48E-02	P
Tylosin	Antibiotic (macrolide)	916.10	7.02	0	0.49	1.63	1.72	1.80E-02	
Venlafaxine	Antidepressant	277.40	9.1	+1	0.01	3.28	2.51	2.73E-01	
Warfarin	Anticoagulant	308.33	4.8	-1	0.01	2.70	1.98	8.82E-02	

i) Search algorithm as described in Escher et al. (2011), preferentially experimental or estimated data taken from the Syracuse Research Physprop data base, http://esc.syrres.com/physprop/. If no experimental were available, SPARC (ii) was used to decide on a final value.

ii) calculated with SPARC (http://ibmlc2.chem.uga.edu/sparc/), September 2009 release w4.5.1529-s4.5.1529.

iii) estimated from Onesios et al. (2009).

*NSAI: non-steroidal anti-inflammatory

APPENDIX 4. PPCPS CONCENTRATION RANGES IN THE FEED WATER IN THE VARIOUS CAMPAIGNS

Table 15: PPCPs concentration ranges in the feed water in the various campaigns (ng L^{-1}). - = not quantified. OoR= out of range. N/Q= not quantifiable (due to interferences).

Compound name	First campaign			Second campaign			Third campaign		Fourth campaign	
	Min	Med	Max	Min	Med	Max	Min	Max	Min	Max
Acetylsalicylic acid	< 10	< 10	< 10	< 10	< 10	< 10	-	-	-	-
Atenolol	50	300	500	170	290	430	183	310	402	598
Atorvastatin	< 10	010	10	< 10	< 10	< 10	-	-	-	-
Caffeine	300	380	540	180	310	410	135	267	68	178
Carbamazepine	330	600	840	620	720	1 650	629	653	468	631
Cephalexin	< 10	50	90	20	40	350	-	-	-	-
Chloramphenicol	< 100	< 100	< 100	< 100	< 100	< 100	< 0.3	0.7	< 0.3	0.9
Chlortetracycline	< 10	< 10	< 10	< 10	< 10	< 10	-	-	-	-
Ciprofloxacin	< 10	< 10	< 10	< 10	< 10	< 10	-	-	-	-
Citalopram	60	70	110	40	60	70	115	134	168	208
Codeine	630	930	1 320	110	310	330	-	-	-	-
Dapsone	< 10	< 10	< 10	< 10	< 10	< 10	< 4.7	< 4.7	< 1.8	< 1.8
DEET	50	70	70	120	130	160	-	-	-	-
Desmethylcitalopram	40	50	80	< 10	10	20	-	-	-	-
Diazepam	< 10	< 10	< 10	< 10	< 10	< 10	-	-	-	-
Diclofenac	80	160	210	N/Q	N/Q	N/Q	221	290	194	240
Doxylamine	180	320	470	160	220	300	151	152	233	722
Enrofloxacin	< 10	< 10	< 10	< 10	< 10	< 10	-	-	-	-
Erythromycin	50	200	390	100	170	270	-	-	-	-
Fluoxetine	< 10	10	10	< 10	< 10	< 10	-	-	-	-
Fluvastatin	< 10	< 10	< 10	< 10	< 10	< 10	-	-	-	-
Furosemide	610	760	1 070	430	560	600	261	331	400	996
Gabapentin	2 200	3 050	3 400	1 860	2 790	4 620	-	-	-	-
Gemfibrozil	80	110	140	60	80	120	37	55	84	155
Hydrochlorothiazide	510	780	830	340	620	740	-	-	719	2508
Ibuprofen	< 10	< 10	< 10	< 10	< 10	< 10	111	161	< 21	88
Indomethacin	20	20	30	< 10	10	20	12	18	11	16
Iopromide	510	1 380	1 800	< 200	500	1 810	-	-	-	-
Lincomycin	< 10	30	50	< 10	20	70	< 0.2	0.6	< 0.2	3.1
Metoprolol	330	380	480	130	200	280	241	248	229	919
Naproxen	150	240	390	100	170	210	208	231	188	587
Nordazepam (desmethyldiazepam)	10	30	30	20	20	30	-	-	-	-
Norfloxacin	< 10	< 10	< 10	< 10	< 10	< 10	-	-	-	-
Oxazepam	410	820	960	560	700	870	-	-	-	-
Oxycodone	30	40	40	30	50	60	-	-	-	-
Oxytetracycline	< 100	< 100	< 100	< 100	< 100	< 100	-	-	-	-
Paracetamol	290	420	580	90	200	330	-	-	-	-
Perindopril	-	-	-	-	-	-	72	83	53	74
Phenytoin	90	200	250	110	170	200	221	251	161	373
Praziquantel	< 10	< 10	< 10	< 10	< 10	< 10	3.0	3.2	3.3	3.4
Propranolol	30	40	50	10	20	20	19	25	50	82
Ranitidine	110	130	220	20	30	70	< 3.4	8.4	< 0.3	6.6
Risperidone	-	-	-	-	-	-	< 0.3	2.4	0.5	0.9
Roxithromycin	140	170	220	40	50	80	97	109	77	153
Salicylic acid	< 10	10	20	< 10	< 10	10	-	-	-	-
Sertraline	< 10	20	20	< 10	< 10	< 10	8.9	12	22	33
Simvastatin	< 10	< 10	< 10	< 10	< 10	< 10	-	-	-	-
Sulfadiazine	< 10	< 10	20	< 10	< 10	< 10	-	-	-	-
Sulfamethoxazole	60	140	150	140	180	330	-	-	-	-
Sulfasalazine	< 10	10	10	< 10	< 10	10	-	-	-	-
Sulfathiazole	< 10	< 10	< 10	< 10	< 10	< 10	< 4.7	< 4.7	< 1.2	< 1.2
Temazepam	210	490	600	250	320	530	-	-	-	-
Tetracycline	< 10	< 10	< 10	< 10	< 10	< 10	-	-	-	-
Tramadol	800	1 210	1 520	520	860	940	1 146	1 338	639	OoR
Triclosan	10	10	10	< 10	< 10	< 10	-	-	-	-
Trimethoprim	110	150	180	10	40	60	14	15	27	49
Tylosin	< 10	< 10	< 10	< 10	< 10	< 10	< 0.8	< 0.8	< 0.4	< 0.4
Venlafaxine	960	1 470	1 760	630	860	1 060	1 144	1 692	471.1	OoR
Warfarin	< 10	< 10	< 10	< 10	< 10	< 10	< 0.1	0.3	< 0.4	< 0.4

GLOSSARY

AhR	arylhydrocarbon receptor
AWMC	Advanced Water Management Centre
BAC	biological activated carbon
BCAA	bromochloroacetic acid
BDCM	bromodichloromethane
CHF	swiss franc
DBAA	dibromoacetic acid
DBCM	dibromochloromethane
DBP	disinfection by-product
DCAA	dichloroacetic acid
DEET	N,N-Diethyl-meta-toluamide
DO	dissolved oxygen
DOC	dissolved organic carbon
EBCT	empty bed contact time
EfOM	effluent organic matter
EP	equivalent people
GAC	granular activated carbon
HAA	haloacetic acid
HPLC	high pressure liquid chromatography
IR	induction ratio
LC/MS-MS	liquid chromatography coupled with tandem mass spectrometry
LOQ	limit of quantification
MCAA	monochloroacetic acid
NDBA	N-nitrosodibutylamine
NDEA	N-nitrosodiethylamine
NDMA	N-nitrosodimethylamine
NMOR	N-nitrosomorpholine
N-Pip	N-nitrosopiperidine
OMP	organic micropollutant
OUT	operational taxonomic unit
PCR	polymerase chain reaction
PPCP	pharmaceutical and personal care products
QHFSS	Queensland Health Forensic and Scientific Services
RP	relative potency
TBM	tribromomethane (bromoform)
TCAA	trichloroacetic acid
TCM	trichloromethane (chloroform)
TEQ	toxic equivalent concentrations
THM	trihalomethane
WWTP	wastewater treatment plant

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Farré, M.J., Reungoat, J., Argaud, F.X., Rattier, M., Keller, J. and Gernjak, W. (2011). *Fate of N-nitrosodimethylamine, trihalomethane and haloacetic acid precursors in tertiary treatment including biofiltration.* Water Research 45(17), 5695-5704.

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