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Step-by-step analysis of drinking water treatment trains using size-exclusion chromatography to fingerprint and track protein-like and humic/fulvic-like fractions of dissolved organic matter†

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This paper provides a glimpse into the removal of dissolved organic matter (DOM) during conventional drinking water treatment and evaluates the potential of high-performance size-exclusion chromatography (HPSEC) as a supplementary tool for routine monitoring of drinking water treatment plants (DWTPs). Two DWTPs in Central Finland were systematically evaluated using HPSEC with simultaneous UV and fluorescence detection. For tyrosine-like, tryptophan-like, and humic/fulvic-like DOM fractions of various molecular weight (MW) values, the total and step-by-step removal efficiencies were estimated along the treatment trains. Overall, both DWTPs removed ~70% of dissolved organic carbon (DOC) and reduced by 80–90% the total fluorescence and total UV absorbance (UVA). DOM fractions of high MW $>$ 1500 Da were efficiently $>$95% removed. Fractions of intermediate MW 750–1500 Da were 80–90% removed, whereas the removal efficiency for fractions of low MW $<$ 600 Da was in the range of 60–70%. The lowest removal efficiency across all fractions and detection was observed by UVA_{210} for the DOM fraction of small MW $<$ 300 Da, for which only 20–30% was removed. In one of the DWTPs, the chromatographic area of this fraction occasionally increased, indicating the formation of degradation and/or oxidation products. Pre-ozonation of raw water reduced total tyrosine- and tryptophan-like fluorescence by ~30%, humic/fulvic-like fluorescence by ~20%, and total UVA_{254} by ~25%. In the conventional coagulation/flocculation, high MW fractions were removed almost completely, whereas the removal of low MW fractions was only ~20%. The coagulability of individual fractions was correlated with their hydrophobicity/hydrophilicity estimated using the ratio of UVA_{210}/UVA_{254}. In one of the DWTPs, oxidation with ClO_2 induced the formation of DOM with MW 750–1500 Da due to the polymerization or release of DOM from colloidal matter. This new DOM was partly removed in the subsequent sand and activated carbon (AC) filtration and partly ended up in the treated water. In the AC filters, 20–60% of DOM fractions of low MW $<$ 600 Da were removed, and fluorescent compounds exhibited two-fold higher removal efficiencies compared to UV absorbing compounds. Analyses of SUVA and the ratio of UVA_{210}/UVA_{254} provided surrogate quantification of the aromatic character and hydrophobic/hydrophilic properties of unfractionated and fractionated DOM.

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Water impact

This is one the first studies where size-exclusion chromatography coupled with both UV and fluorescence detection is evaluated as a routine tool for advanced monitoring of the removal of dissolved organic matter in conventional drinking water treatment plants. To determine what is removed and what is not removed, protein-like compounds and humic substances were monitored step-by-step and fraction-by-fraction.

1. Introduction

Accelerating anthropogenic activity, climate change, and other megatrends lead to deterioration of water quality globally through chemical contamination, eutrophication, algal bloom, *etc.*[1] Particularly, soil acidification, intensifying rain and drought events are causing a rapid increase of dissolved organic matter (DOM) content in lakes and rivers.[2] For example, the dissolved organic carbon (DOC) concentration in Lake Päijänne, which is the second largest lake in Finland, increased from ~5.5 to ~7.5 mg C per L between 2000 and 2015.[3]

The quality and quantity of DOM in raw water sources influence the choice of appropriate treatment methods and

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determine the demands of coagulants, oxidants, and disinfectants in drinking water treatment plants (DWTPs). Efficient engineering design and operational control are further challenged by possible seasonal variations of DOM composition.[4] Unremoved DOM can cause adverse effects, such as formation of toxic and mutagenic disinfection by-products (DBPs) upon chlorination,[5,6] microbial growth in distribution networks,[7] and unpleasant color, taste, and odor of tap water.[8] To prevent these problems, routine monitoring of DOM quality and quantity is needed in all the steps from the source to the tap.

DOM in surface water is a highly complex system of humic and fulvic compounds, lignins, carbohydrates, proteins, and other heterogeneous components of not only mostly natural, but also anthropogenic origin. Thus, a comprehensive characterization of DOM requires a multi-method analytical approach.[9]

High performance size-exclusion chromatography (HPSEC) is an established technique to evaluate the removal of DOM along drinking water treatment trains.[10] Relatively fast, easy and affordable HPSEC analysis requires small sample volume and minimal pre-treatment.[11] In HPSEC, molecules are separated according to their apparent molecular weights (MWs) into fractions, which can be simultaneously characterized by various detectors. Typically, UV and online DOC detectors are used. While DOC detection is universal, it does not provide insights into the chemistry of individual fractions. Monitoring UV absorbance at 254 nm (UVA_{254}) allows robust detection of conjugated double bonds and aromatic structures. At the same time, UVA_{254} detection cannot discriminate different classes of aromatic compounds, such as humic substances and aromatic proteins. Sometimes wavelengths other than 254 nm are explored. For example, a correlation between UVA_{210} and microbiological water quality parameters was reported.[12]

Humic substances, which comprise 40–80% of raw water DOC,[13,14] have featureless UV-vis spectra that provide scarce information.[15] Moreover, a rather low sensitivity of UV detection limits the applicability of HPSEC-UV for analysis of samples with low DOC content, such as drinking water. Thus, additional detectors are necessary to obtain more information about DOM fractions.[16]

The high sensitivity and selectivity of fluorescence detection allow determination of various organic compounds at low concentrations, particularly, in drinking water.[17] Besides humic/fulvic-like fluorescent compounds, a diverse pool of protein-like compounds can be monitored by their characteristic tyrosine- and tryptophan-like fluorescence. Recently, tryptophan-like fluorescence was suggested as a surrogate measure of microbial contamination risk.[18] Usually, excitation–emission matrix (EEM) fluorescence spectroscopy with parallel factor analysis (PARAFAC) is suggested to characterize the removal of fluorescent DOM.[19,20] At the same time, the combination of HPSEC fractionation and fluorescence detection is overlooked in the field of water analysis and technology. To the best of our knowledge, only a few studies have applied HPSEC-fluorescence to study drinking water treatment[21,22] and none of them undertook systematic comparative characterization of DOM fractions at different excitation/emission wavelengths ($\lambda_{ex}/\lambda_{em}$).

Thus, we aimed to evaluate HPSEC-UV-fluorescence as a supplementary tool for routine (and, potentially, online and on-site) monitoring of DOM along drinking water treatment trains. For this, samples of raw, processed, and treated water were collected from two conventional DWTPs and systematically analyzed to identify removable and refractory DOM fractions and to assess the treatment performance – overall, step-by-step, and fraction-by-fraction.

2. Materials and methods

2.1. Design of DWTPs

Principal schemes of the DWTPs assessed in this study are given in Fig. 1.

DWTP A (city of Jyväskylä, Finland) pumps raw water from the nearby Lake Tuomiojärvi and has an average water flow of ~300 $m^3\ h^{-1}$. The DWTP employs pre-ozonation to oxidize manganese present in the raw water. The ozonation is followed by coagulation–flocculation (with the addition of polyaluminum coagulant KEMIRA PAX-XL 100), flotation combined with sand filtration, intermediate oxidation with NaOCl, activated carbon (AC) filtration (eight parallel AC tanks), intermediate low-power UV treatment, post-oxidation, and final high-power UV treatment.

DWTP B (city of Tampere, Finland) pumps raw water from Lake Roine, located at a distance of 7 km from the DWTP, and has an average water flow of ~1700 $m^3\ h^{-1}$. Conventional coagulation–flocculation (with the addition of ferric sulphate) is followed by flotation, intermediate oxidation with ClO_2, sand filtration, AC filtration, and final post-chlorination with Cl_2. Alkalinization with lime is used to adjust hardness and alkalinity. Over a decade ago, the performance of DWTP B was studied using HPSEC-UV.[23–25]

2.2. Sampling and sample preparation

Each DWTP was sampled four times in total: DWTP A – in 2017 (September, October, and November) and 2018 (September), DWTP B – in 2017 (October) and 2018 (March, June, and August). Locations of the sampling points are indicated in Fig. 1.

Water samples were collected into polypropylene bottles (pre-washed and rinsed three times with ultrapure water) and transported in cool boxes. Upon delivery to the laboratory, the samples were filtered through pre-washed 0.45 μm syringe filters (cellulose acetate from VWR, USA, or regenerated cellulose from Phenomenex, USA). HPSEC analyses were completed within a few days. Filtered aliquots for DOC analysis were frozen at −20 °C in polypropylene screw cap tubes (Sarstedt, Germany) and analyzed within several weeks.

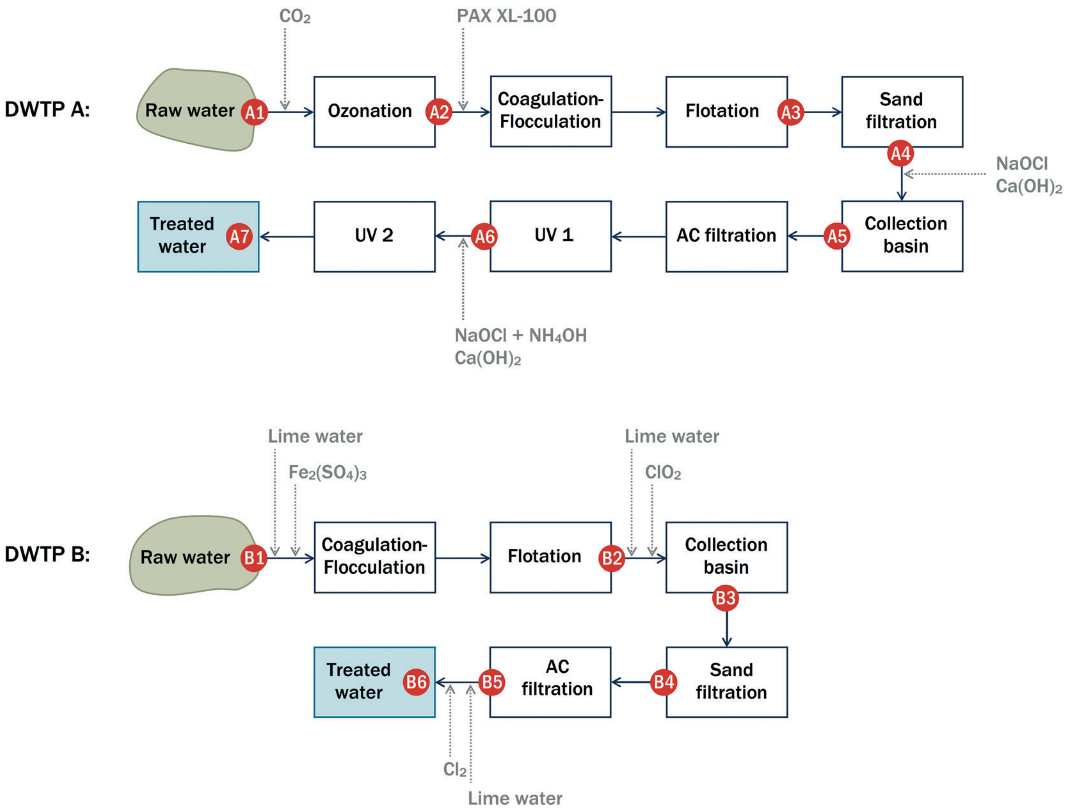

Fig. 1 Principal schemes of DWTPS A and B and indicative locations of sampling points A1–A7 and B1–B6.

2.3. HPSEC method

All the samples were analyzed using an HPLC Shimadzu LC-30AD equipped with online degassing units Shimadzu DGU-20A5R and DGU-20A3R, a column oven Shimadzu CTO-20AC, an autosampler Shimadzu SIL-30AC, a photodiode array (PDA) detector Shimadzu SPD-M20A, and a fluorescence detector Shimadzu RF-20A XS. The separation column was a silica-based Yarra SEC-3000 (300 × 7.6 mm, Phenomenex, USA).

The eluent was 5 mmol L^{-1} phosphate buffer (pH 6.8, ionic strength 10 mmol L^{-1}) with $\beta(Na_2HPO_4 \cdot 2H_2O)$ = 0.45 g L^{-1} and $\beta(NaH_2PO_4 \cdot 2H_2O)$ = 0.39 g L^{-1} at a flow rate of 1 mL min^{-1}. The eluent composition was chosen based on previous studies[11,23,26,27] to achieve high chromatographic resolution. Analytical grade $Na_2HPO_4 \cdot 2H_2O$ and $NaH_2PO_4 \cdot 2H_2O$ were purchased from VWR, Belgium, and Merck, Germany, respectively. Ultrapure water was generated using an "Ultra Clear UV plus TM" system (SG Water, Germany). The eluent was pre-filtered through 0.2 μm cellulose acetate membrane filters (Whatman, Germany).

The main parameters of the HPSEC method are summarized in Table S1.† The monitored $\lambda_{ex}/\lambda_{em}$ values were chosen according to typical EEM fluorescence spectra of raw water (Fig. S1†) to represent tyrosine-like (region B_1: 220/310 nm), tryptophan-like (region T_2: 270/355 nm), and humic/fulvic-like (region C_1: 330/425 nm and region C_2: 390/500 nm) fluorescent compounds. Region A of humic/fulvic-like fluorescence and region T_1 of tryptophan-like fluorescence were not monitored to avoid possible inner filter effects due to intense UV absorption below 250 nm and to minimize the total number of HPSEC analyses. Region B_1 of tyrosine-like fluorescence was chosen due to the lower noise and higher intensity of tyrosine-like fluorescence at the shorter excitation wavelengths compared to those at the longer excitation wavelengths of region B_2 (Fig. S2†).

The void volume, determined with blue dextran (Sigma-Aldrich, Sweden), was ~5.4 mL (elution time ~5.4 min), and the permeation volume, determined with acetone, was ~12.2 mL (elution time ~12.2 min). The size-exclusion column was calibrated with polystyrene sulfonate (PSS) standards of 210, 1600, 3200, 4800, 6400, 17 000, and 32 000 Da (Sigma-Aldrich, Germany). The calibration curve is shown in Fig. S3.†

Recently, the same HPSEC-UV-fluorescence method was used to characterize treated and untreated wastewater and to evaluate the efficiency of wastewater treatment.[11]

2.4. Processing of HPSEC data

Raw chromatographic data were exported from proprietary software Shimadzu LabSolutions LC/GC Version 5.51 to ASCII text files and processed in MathWorks MATLAB R2016b. In-house scripts were used to automatically correct baseline, recognize individual fractions, and integrate each chromatogram.

Total fluorescence and total UVA were obtained by integrating the HPSEC chromatograms in the elution time range of 4.5–15.0 min to combine all the peaks eluted within fractions I–VI. Thus, total fluorescence and total UVA represent the properties of unfractionated (whole) water samples.

Removal efficiencies were calculated for DOM fractions I–VI and for whole water for each treatment step according to the following equation

$$\text{Removal efficiency} = (1 - A_{\text{effluent}}/A_{\text{influent}}) \times 100\% \qquad (1)$$

where A_{influent} and A_{effluent} are the chromatographic areas (fractional or total) of the water samples collected before (influent) and after (effluent) a treatment step. To calculate the total efficiency of a drinking water treatment train, raw water was compared with treated water after final post-oxidation.

The SUVA index of the whole water samples was calculated as follows:

$$\text{SUVA} = (\text{total UVA}_{254}/\text{DOC}) \times (u/V_{\text{inj}}) \times 100 \qquad (2)$$

where total UVA_{254} in [mAU min] is calculated from the HPSEC-UV_{254} chromatograms, V_{inj} is the injection volume in [L], u is the eluent flow rate in [L min^{-1}], and DOC in [mg C per L] was measured with the total organic carbon analyzer.

The weight average MW (M_{W}), number average MW (M_{N}), and polydispersity (ρ) were calculated from the HPSEC-UV and HPSEC-fluorescence chromatograms according to (3)–(5).[28]

$$M_{\text{W}} = \sum_{i=1}^{n} \left(h_i \times \text{MW}_i\right) \Big/ \sum_{i=1}^{n} h_i \qquad (3)$$

$$M_{\text{N}} = \sum_{i=1}^{n} h_i \Big/ \sum_{i=1}^{n} \left(h_i / \text{MW}_i\right) \qquad (4)$$

$$\rho = M_{\text{W}}/M_{\text{N}} \qquad (5)$$

where h_i is the height of a chromatogram at elution time i, MW_i is the apparent MW in [Da] corresponding to elution time i, and n is the number of data points. The apparent MWs were estimated from the calibration curve (Fig. S3†) for the elution time range of 6.0–11.0 min where a satisfactory fitting equation was obtained. Thus, the calculated M_{W}, M_{N}, and ρ do not account for fraction I (biopolymers) and a minor part of fraction VI (low MW neutrals) eluted correspondingly before the 6th min and after the 11th min.

2.5. DOC analysis

A total organic carbon analyzer Shimadzu TOC-L with an autosampler ASI-L was used to determine the DOC content. The non-purgeable organic carbon method was selected. The analyzer was calibrated in the DOC range of 0–30 mg C per L with standard solutions of potassium phthalate prepared by automatic dilution of a fresh stock. Prior to analysis, vials were calcined at 400 °C for 4 h in air. All the samples were acidified to pH < 2 with HCl and purged with N_2 to strip dissolved inorganic carbon. The injection volume was 100 μL. The number or replicate injections (2 or 3) was chosen automatically using the analyzer.

3. Results and discussion

3.1. Characterization of raw water DOM

3.1.1. DOM fingerprints. General raw water quality parameters that are routinely monitored at both DWTPs are given in Tables S2 and S3.† Additional characteristics obtained using the HPSEC-UV-fluorescence approach are discussed below.

The SUVA of the raw water was in the range of 2–4 at both DWTPs (Tables 1 and S4†) indicating the complex DOM composition of humic and non-humic and hydrophobic and hydrophilic fractions of various MWs.[29] Depending on the detector (fluorescence or UV), six to eight peaks were observed in the HPSEC chromatograms of raw water, which is typical for Finnish surface waters.[26,30] These peaks were deliberately combined into six fractions as shown in Fig. 2 for DWTP A and in Fig. S4† for DWTP B. MW decreases from fraction I to fraction VI.

Fraction I, eluted around the void volume, contributed to ∼15% of the total tyrosine-like and ∼5% of the total tryptophan-like fluorescence of the whole raw water samples. It is thought that this fraction consists of organic colloids (MW ≥ 50 kDa) and biopolymers (MW ≥ 10 kDa), such as polysaccharides, with some contribution from proteinic matter and amino sugars.[2,31,32] Some researchers suggest that fraction I contains aggregates from self-association of humic substances.[26,33] However, in this study, fraction I did not exhibit characteristic humic/fulvic-like fluorescence.

The MWs of DOM fractions II–VI were estimated using the calibration curve obtained with PSS standards (Fig. S3†). The PSS standards are considered similar to humic substances in terms of charge density and behavior in size-exclusion columns. However, due to debatable structural similarity and various secondary interactions, MWs estimated using PSS calibration should be considered apparent and indicative rather than true.[34,35]

Humic substances of high MW 1500–3200 Da were eluted in fraction II. Humic substances of low MW 750–1500 Da were eluted in fractions III and IV. Building blocks (breakdown products of humic substances) had a MW of 500–600 Da and were eluted in fraction V.

Fractions II–VI simultaneously exhibited tyrosine-like, tryptophan-like, and humic/fulvic-like fluorescence, suggesting that protein-like compounds and humic/fulvic-like substances were eluted together. However, HPSEC analysis alone does not allow determination of whether the observed fluorophores are present side-by-side in the same molecules or belong to different molecules that co-elute due to similar MWs or intermolecular association. Moreover, it is known that humic structures can incorporate protein-like fractions as a result of weak interactions based on π–π and/or van der

Table 1 Properties of unfractionated (whole) water sampled at different steps of DWTP A (mean ± SD, $n = 4$)

	A1	A2	A3	A4	A5	A6	A7
	Raw water	Ozonation effluent	Flotation effluent	Sand filtration effluent	Chlorination effluent	AC filtration effluent	Treated water
DOC (mg C per L)	6.9 ± 0.6	6.5 ± 0.3	3.1 ± 0.4	2.5 ± 0.4	2.7 ± 0.4	2.3 ± 0.4	2.0 ± 0.2
SUVA (L mg^{-1} m^{-1})	3.4 ± 0.4	2.8 ± 0.4	1.2 ± 0.2	1.3 ± 0.4	1.1 ± 0.2	0.9 ± 0.1	1.1 ± 0.3
UVA_{210}/UVA_{254}	1.8 ± 0.1	2.0 ± 0.1	2.6 ± 0.3	2.7 ± 0.4	2.8 ± 0.2	3.2 ± 0.1	3.3 ± 0.4
Total UVA (mAU min)							
254 nm	11.6 ± 1.1	9.0 ± 1.4	1.8 ± 0.1	1.6 ± 0.5	1.5 ± 0.2	1.1 ± 0.1	1.1 ± 0.2
210 nm	20.8 ± 2.7	17.9 ± 2.9	4.9 ± 0.7	4.3 ± 1.0	4.4 ± 0.9	3.3 ± 0.2	3.5 ± 0.3
Total fluorescence (mV min)							
Tyrosine-like (220/310 nm)	44.0 ± 1.6	30.2 ± 2.0	14.6 ± 2.7	13.0 ± 2.7	10.3 ± 1.2	7.5 ± 0.5	7.4 ± 0.5
Tryptophan-like (270/355 nm)	88.8 ± 7.1	57.3 ± 5.3	24.7 ± 2.4	22.5 ± 3.2	20.6 ± 2.3	10.8 ± 1.4	10.9 ± 1.5
Humic/fulvic-like (330/425 nm)	259.3 ± 21.4	214.4 ± 25.3	65.1 ± 6.8	58.4 ± 8.5	53.5 ± 6.2	27.9 ± 3.3	28.2 ± 3.1
Humic/fulvic-like (390/500 nm)	66.9 ± 8.8	55.6 ± 8.8	13.4 ± 1.8	11.6 ± 2.2	10.0 ± 1.3	6.0 ± 0.8	5.9 ± 0.7

Waals forces between the DOM components.[36,37] Recent studies indicate that not only proteins but also humic supramolecules containing certain structures (derived from phenol or aniline) can contribute to protein-like fluorescence.[38]

"Red-shifted" humic/fulvic-like fluorescence at 390/500 nm (emission at longer wavelengths) can be related to highly conjugated aromatic compounds of high MW, whereas "blue-shifted" humic/fulvic-like fluorescence at 330/425 nm (emission at shorter wavelengths) can be assigned to compounds with lower aromaticity and lower MWs.[39] Thus, fluorescence at 390/500 nm may be indicative of humic-like compounds, whereas fluorescence at 330/425 nm may be indicative of fulvic-like compounds. However, the same fluorophores are present in both humic and fulvic compounds and a clear differentiation between them based on fluorescence properties is not always possible.[40]

Tyrosine- and tryptophan-like fluorescence of raw water is attributed to amino acids, free, bound to proteins, or associated with high MW organic compounds, such as humic substances.[39] Tyrosine residues in proteins and peptides do not emit fluorescence in the presence of tryptophan, because the emission energy of tyrosine residues is transferred to the excitation energy of the neighboring tryptophan residues.[41] Thus, tyrosine-like fluorescence may indicate more degraded peptide materials, while tryptophan-like fluorescence may represent intact proteins and less degraded peptide materials.[39]

Fraction VI with apparent MW < 300 Da represented a diverse pool of low MW acids and neutrals (carboxylic acids, amino acids, sugars, purines, aldehydes, ketones, *etc.*).[31,42]

The humic/fulvic-like fluorescence of the raw water was evenly distributed across fractions II–VI, whereas tryptophan-like and tyrosine-like fluorescence was more pronounced in fractions IV–VI with lower MW. These fractions accounted for ~70% of the total tyrosine-like and total tryptophan-like fluorescence in the raw water (Fig. 3 for DWTP A and Fig. S5† for DWTP B).

3.1.2. MW distributions. MW distributions, calculated with eqn (3)–(5) from the HPSEC chromatograms, were notably different for the fluorescent and UV absorbing DOM. The weight-average MW (M_W) and number-average MW (M_N) calculated from the fluorescence signals were ~50% lower than M_W and M_N calculated from UVA_{254} (Tables S5 and S6†). The values of M_W and M_N could be slightly underestimated because biopolymer fraction I, eluted outside the calibration range, was not included in the calculations. Biopolymers contribute up to ~7% of raw water DOC.[21,43,44]

Calculated from the HPSEC-UV chromatograms, M_W was ~1500 Da and M_N was ~800 Da for both raw water samples at DWTPs A and B. These are similar to the values reported for several drinking water sources in Norway and Australia,[2] but considerably smaller than the M_W of 2114 Da and M_N of 1385 Da reported for reference Suwannee River fulvic acid[28] and the M_W of 6102 Da and M_N of 3873 Da determined for Nordic reference fulvic acid.[45] These discrepancies could stem from the different eluents, size-exclusion standards and columns used in the studies.

The polydispersity (ρ) of the raw water DOM was ~1.8 regardless of detection. $\rho \sim 1$ indicates that the DOM is made up of compounds with similar MWs, while greater ρ indicates more complex mixtures of heterogeneous organic compounds.[46]

3.2. Overall DWTP performance

At both DWTPs, raw water and treated water are routinely sampled and analyzed by accredited laboratories, as required by operational protocols and regulations. Selected general water quality parameters are summarized in Tables S2 and S3† to complement data obtained in this study.

Treatment efficiency was evaluated in terms of DOC removal, the decrease of UVA_{210} and UVA_{254}, reduction of fluorescence signals at different $\lambda_{ex}/\lambda_{em}$, changes of SUVA and the ratio of UVA_{210}/UVA_{254} for whole water samples collected along the treatment trains (Table 1 for DWTP A and Table S4† for DWTP B).

Both DWTPs reduced the DOC content by ~70%. The total UVA_{254} and total humic/fulvic-like fluorescence were reduced by ~90% at DWTP A and by ~85% at DWTP B. The total tyrosine- and tryptophan-like fluorescence signals were

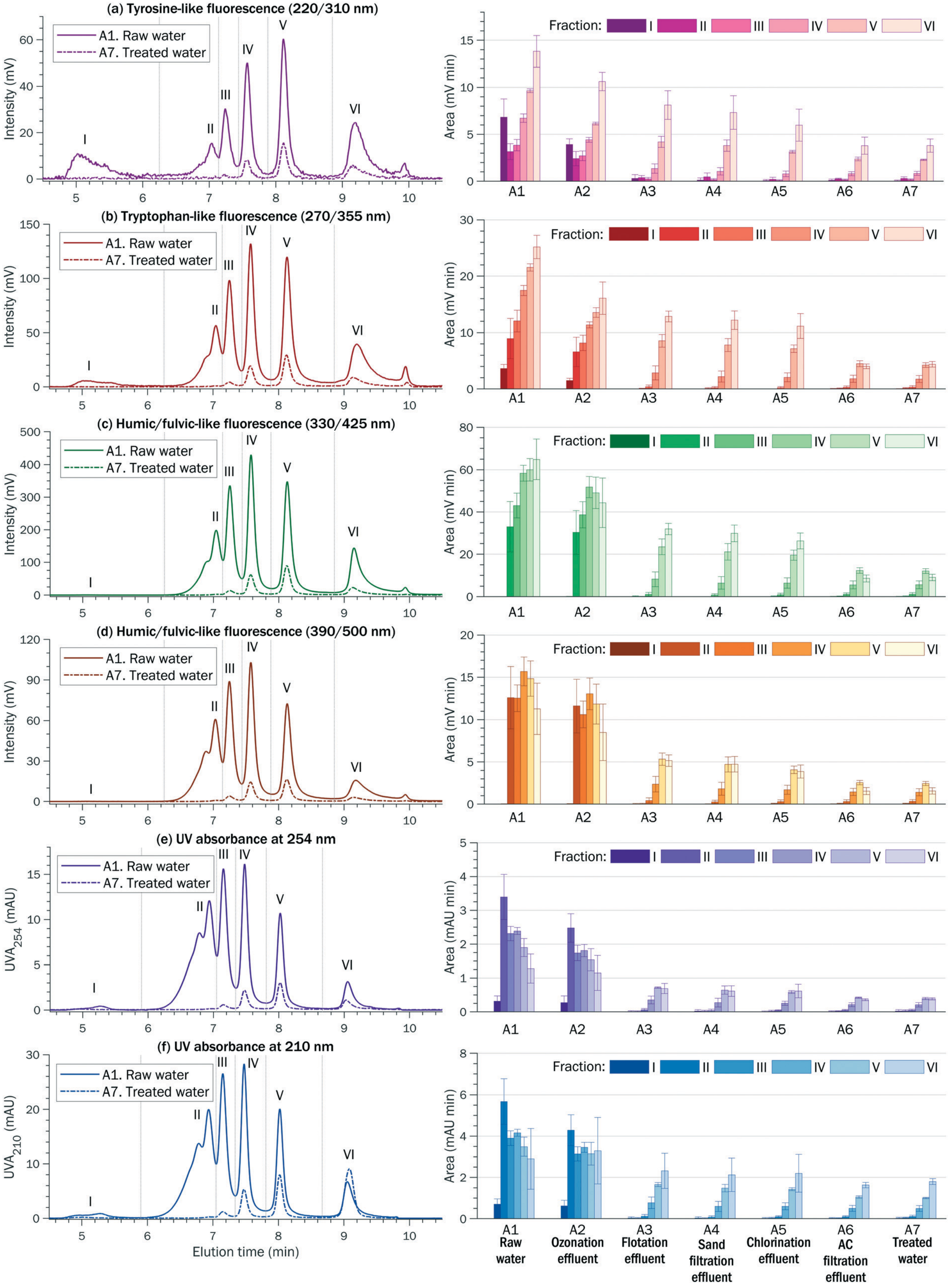

Fig. 2 Characterization of DOM at DWTP A. Left column: superimposed HPSEC chromatograms of raw water and treated water. Right column: evolution of DOM fractions I–VI along the treatment train (mean area ± sd, *n* = 4).

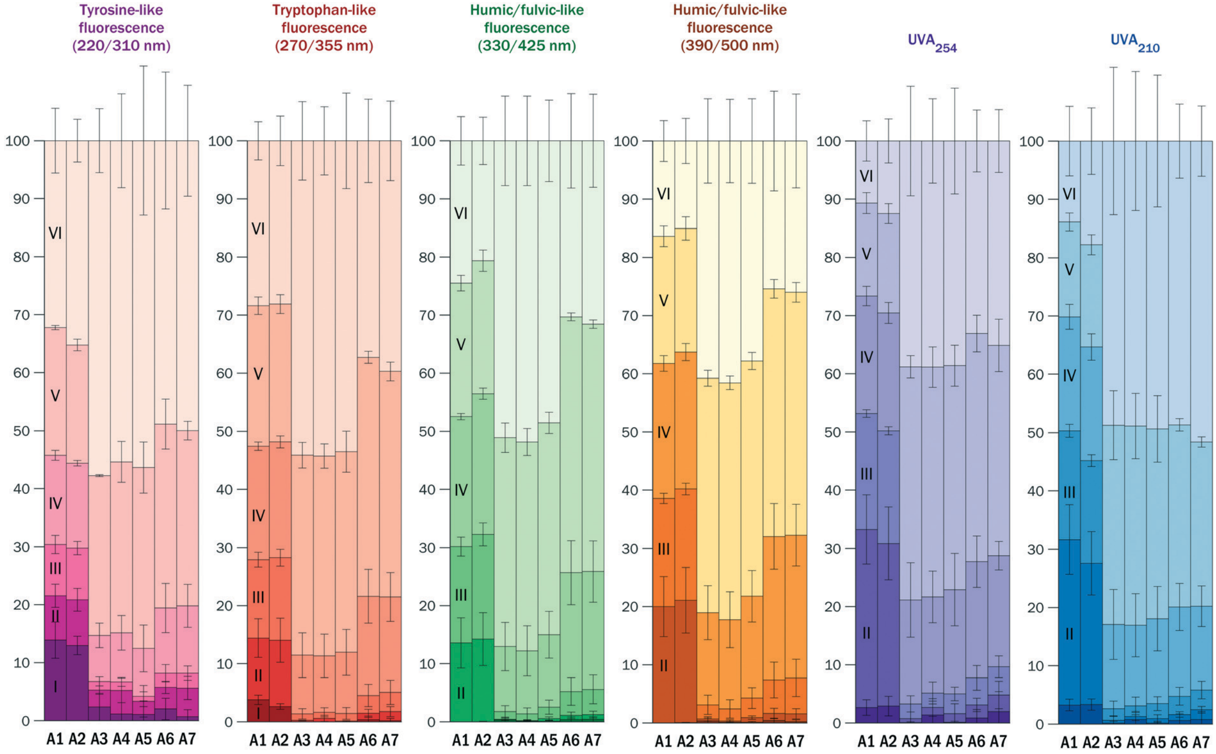

Fig. 3 Relative abundance of fluorescent and UV absorbing DOM fractions I–VI in raw water (A1), processed water (A2–A6), and treated water (A7) at DWTP A (mean ± sd, *n* = 4). Locations of sampling points A1–A7 are shown in Fig.1.

reduced by ~86% at DWTP A and by ~77% at DWTP B. The lowest removal efficiency was observed for the total UVA_{210}: ~83% reduction at DWTP A and ~68% reduction at DWTP B. Aromatic and many aliphatic compounds as well as some inorganic ions (for example, nitrate anion) absorb at 210 nm and thus, UVA_{210} cannot be attributed to a particular category of compounds.

The decreasing SUVA and increasing ratio of UVA_{210}/UVA_{254} (Tables 1 and S4†) indicate that the residual DOM in the treated water samples had a significantly lower aromatic character compared to the raw water samples. For surface raw water, higher SUVA corresponds to higher aromaticity, higher hydrophobicity and higher humic/fulvic content.

The ratio of UVA_{210}/UVA_{254} was suggested as a surrogate indicator of the relative density of aliphatic functional groups and conjugated double bonds.[47] Aliphatic functional groups typically have higher UVA_{210} and lower UVA_{254} whereas conjugated double bonds and aromatic structures have high UVA_{210} and high UVA_{254}. Thus, the ratio of UVA_{210}/UVA_{254} decreases with the increase in aromatic character. For example, the UVA_{210}/UVA_{254} values of Suwannee River standard humic and fulvic acids are correspondingly 1.59 and 1.88,[48] while for proteins of bovine serum albumin with low aromaticity the ratio is 13.50.[49] The ratio of UVA_{210}/UVA_{254} of fractions II–V indicates the fulvic character of the raw water DOM (Fig. 5 and S7†).

The HPSEC separation eliminated the inorganic component from all the fractions except fraction VI where inorganic ions, which can be UV absorbing, co-eluted with low MW DOM components. Due to this possible interference, the ratio of UVA_{210}/UVA_{254} may not reflect the properties of low MW organic compounds in fraction VI.

The specific (DOC normalized) fluorescence decreased during the treatment by ~45% for tyrosine-like and by ~60% for tryptophan-like fluorescence, which are lower than the ~70% decrease of SUVA and the specific humic/fulvic-like fluorescence at $\lambda_{ex}/\lambda_{em}$ of 390/500 nm. This indicates that protein-like DOM components are harder to remove than humic/fulvic-like compounds.

At both DWTPs, the average MW decreased two-fold (Tables S5 and S6†) and the change in polydispersity ($\Delta\rho$) was ~0.3. $\Delta\rho$ was suggested as both an indicator of coagulation efficiency and a measure of treatability of particular water.[2] A large $\Delta\rho$ corresponds to significant narrowing of the MW distribution and thus, indicates efficient removal of compounds in specific MW ranges (for example, large molecules). The values of $\Delta\rho$ calculated for DWTPs A and B were considerably higher than the values reported elsewhere for DWTPs in Norway and Australia.[2]

The treatment almost completely removed biopolymers (fraction I) and high MW fractions II and III, and reduced fraction IV by ~90% (see the right columns in Fig. 4 and S6†). Low MW fractions V and VI were removed by ~80%. Across all the fractions and detection, the lowest removal efficiency (~20%) was observed for fraction VI monitored by UVA_{210}.

In the samples of treated water, fractions V and VI (MW < 600 Da) contributed to ~80% of the total tyrosine- and

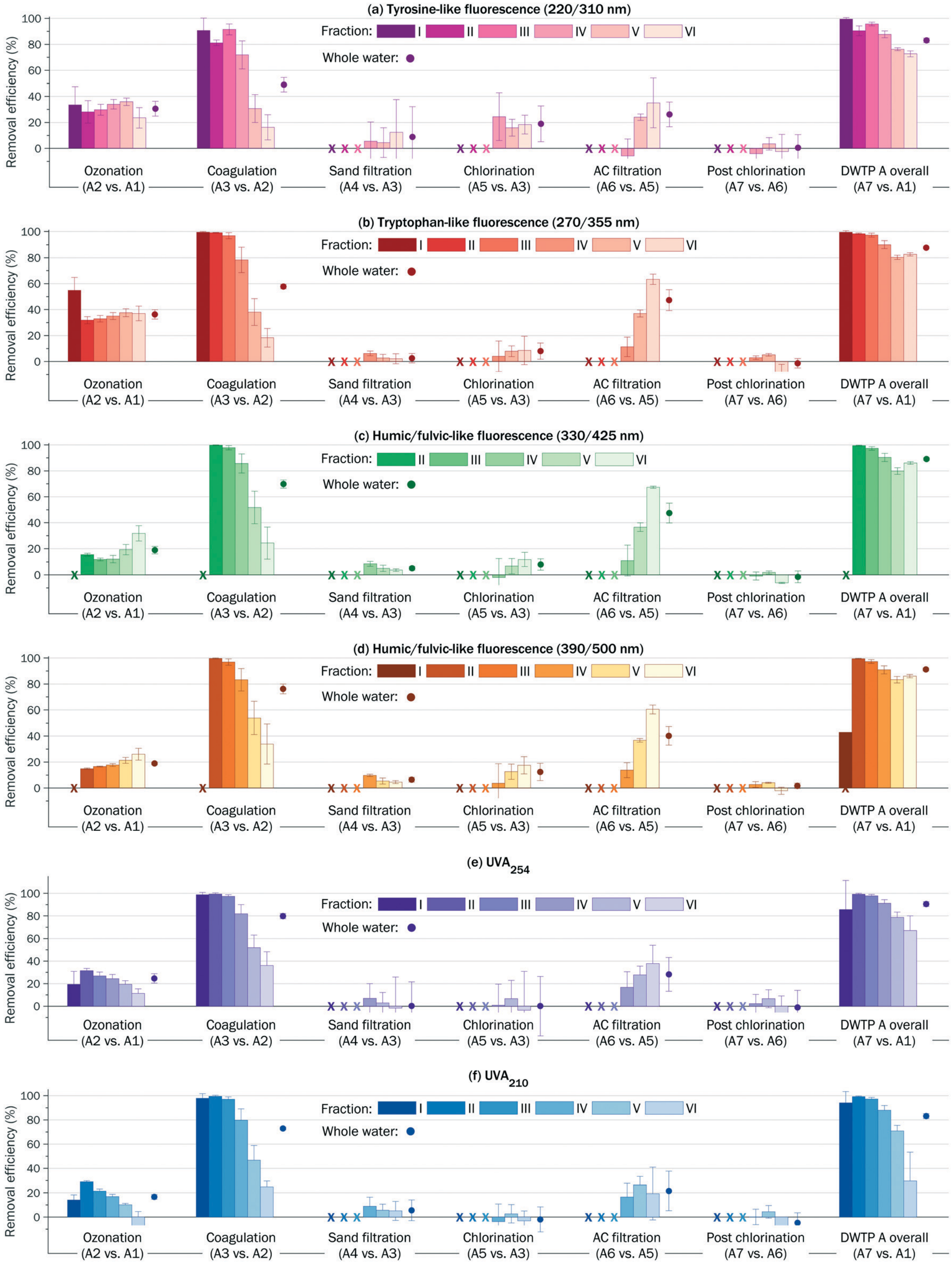

Fig. 4 Step-by-step, fraction-by-fraction, and overall efficiency of DWTP A (mean ± sd, *n* = 4). Completely removed fractions are denoted with 'x'.

tryptophan-like fluorescence and to ~70% of the total humic/fulvic-like fluorescence and total UVA (Fig. 3 and S5†). Fraction VI alone contributed to ~60% of the total UVA$_{210}$ of the treated waters. This means that low MW compounds represent a major share of DOM in treated water.

For both DWTPs, the chromatographic area of fraction VI detected by UVA$_{210}$ occasionally was higher for the treated water samples than for the raw water samples, indicating the formation of low MW by-products. Recent studies have demonstrated that DOM of low MW < 1 kDa has DBP formation

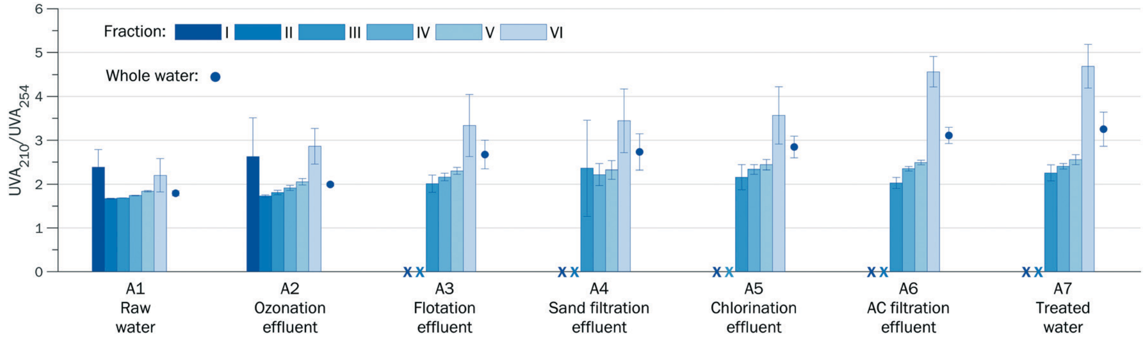

Fig. 5 Ratio of UVA_{210}/UVA_{254} of DOM fractions and whole water sampled at DWTP A (mean ± sd, $n = 4$). Lower values indicate higher aromatic character. Completely removed fractions are denoted with "x".

potential similar to that of high MW humic substances.[50–52] And while large hydrophobic humic molecules are readily removed in coagulation, low MW hydrophilic compounds exhibit refractory behavior and pass through conventional water treatment systems. Although DBP identification was out of the scope of this work, the HPSEC-UV-fluorescence approach may be useful in developing surrogate indirect methods to assess DBP formation potential.

3.3. Step-by-step analysis of DWTP performance

3.3.1. Pre-ozonation. Pre-ozonation at DWTP A slightly reduced DOC and significantly decreased the fluorescence and UVA of raw water. A 30–40% decrease in tyrosine- and tryptophan-like fluorescence was observed across all the DOM fractions. The reduction of the total humic/fulvic-like fluorescence was lower (~20%) and reciprocal correlations were observed between MWs of DOM fractions and the corresponding removal efficiencies. The humic/fulvic-like fluorescence of low MW fractions declined more compared to that of high MW fractions. This can be explained in terms of steric effects: fluorophores located inside large humic structures are shielded from ozone attack.

The stronger reduction of tyrosine- and tryptophan-like fluorescence (compared to humic/fulvic-like fluorescence), at first glance, contradicts a study where preferential removal of humic-like PARAFAC components was observed during ozonation.[19] However, in that study[19] ozonation followed coagulation and sand filtration, whereas at DWTP A ozonation is the first pre-treatment step. Hence, the discrepancy may be attributed to differences in DOM composition of the ozonation influents.

Electron withdrawing carboxyl groups formed during pre-ozonation reduce fluorescence quantum yields in aromatic molecules.[53] Thus, the observed reduction of fluorescence should not be linearly correlated with possible chemical transformations.

The pre-ozonation did not significantly change the relative abundance of DOM fractions I–VI (compare columns A1 and A2 in Fig. 3). This means that larger molecules were not split into smaller fragments, which would occur at higher ozone doses. However, ozone-induced cleavage of conjugated double bonds (Criegee mechanism) reduced UVA_{254} of all DOM fractions. The decline of UVA_{254} was ~30% for high MW fraction II and ~15% for low MW fraction VI (Fig. 4e), which reflects the higher aromatic character of humic and fulvic compounds of high MW. The SUVA of whole water after preozonation decreased by ~20%. A moderate increase in the ratio of UVA_{210}/UVA_{254} across all the fractions points to formation of aliphatic functional groups (Fig. 5).

3.3.2. Coagulation. The coagulation influents had SUVA in the range of 2.5–2.8 L mg^{-1} m^{-1}, predicting coagulation efficiency (in terms of DOC removal) in the range of 25–50%.[29] The observed DOC removals were ~50% for both DWTPs (Table 1 for DWTP A and Table S4† for DWTP B), pointing to the correct choice and dosage of the coagulants.

The reduction of the total fluorescence and total UVA varied in the range of 60–80%. Fig. 4 and S6† illustrate that high MW fractions I–III were removed almost completely, while intermediate and low MW fractions V and VI were removed by 30–50% and 10–20%, respectively.

Preferential removal of high MW fractions and incomplete removal of low MW fractions in coagulation/flocculation are well-known.[24,31,43,44,54–56] In conventional coagulation, insoluble particles are formed as the result of diverse interactions (destabilisation, complexation, entrapment, adsorption) between DOM components and mononuclear, polynuclear, and colloidal species of coagulants. Hydrophobic humic and fulvic compounds, which carry high levels of negative charge due to the presence of ionized carboxyl and phenolic groups, are removed, mainly, through the charge neutralization mechanism. Coagulation of low MW compounds, which are more hydrophilic, occurs, mainly, through adsorption onto colloidal metal hydroxides, which are present at lower concentrations. Thus, hydrophobic high MW fractions are generally more coagulable than hydrophilic low MW fractions.[57]

The ratio of UVA_{210}/UVA_{254} can be used as a surrogate indicator of relative hydrophobicity/hydrophilicity to predict the coagulation performance. For both DWTPs, similar correlations were observed between the ratios of UVA_{210}/UVA_{254} and coagulation efficiencies (Fig. S8†). The most hydrophobic fractions with ratio of UVA_{210}/UVA_{254} in the range of 1.8–1.9

were readily removed in coagulation by >80%. For fractions with ratio of $UVA_{210}/UVA_{254} \sim 2.0$, the coagulation efficiency was ~50%. The coagulation efficiency for the most hydrophilic fraction VI ($UVA_{210}/UVA_{254} > 2.8$) was <30%.

At DWTP A, the removal efficiencies observed for tyrosine- and tryptophan-like fluorescent compounds in low MW fraction V were ~10% lower than those at DWTP B. This can be explained in terms of the higher hydrophilicity of the coagulation influent at DWTP A due to the formation of polar carboxyl groups during the pre-ozonation. Alternatively, it is possible that some coagulable compounds in fraction V were removed in the pre-ozonation.

Our results demonstrate that MW determines the coagulation efficiency of both UV absorbing DOM and fluorescent DOM.

3.3.3. Sand filtration. Sand filtration is used to remove residual flocs and particulate impurities. At DWTP A, only a slight reduction (<10%) of humic/fulvic-like fluorescence and DOC content was observed at this step without any statistically significant changes in tyrosine-like, tryptophan-like fluorescence and UVA. At DWTP B, sand filtration did not change the DOM composition.

3.3.4. Intermediate oxidation. At DWTP A, the intermediate oxidation with NaOCl resulted in a 10–15% decrease of fluorescence of low MW fractions V and VI. UVA_{254} and UVA_{210} slightly changed without a particular pattern.

At DWTP B, fractions II–III, which were almost completely removed in the preceding coagulation, reappeared after the oxidation with ClO_2. The area of fraction IV also increased, however the areas of low MW fractions V and VI did not change or slightly decreased. The apparent formation of high MW DOM is reflected by the negative removal efficiencies in Fig. S6.† This phenomenon was observed at DWTP B a decade ago.[23] It is hypothesized that low and intermediate MW compounds could aggregate or undergo polymerization induced by active species formed during the oxidation.[23,58] Another possibility is the release of organic compounds during partial oxidation of colloidal matter.[43] However, no final explanation has been suggested.

3.3.5. Activated carbon (AC) filtration. Comparison of water samples collected at points A5 and A6 of DWTP A (Fig. 1) demonstrated that AC filtration (and the following low-power UV treatment) reduced by 40–50% the total fluorescence, by 20–30% the total UVA_{254}, and by 10–20% the total UVA_{210} (Fig. 4 and S6†). At DWTP A, ~70% of low MW tryptophan-like and humic/fulvic-like fluorescent compounds in fraction VI (MW < 300 Da) were removed, and ~40% of building blocks and protein-like compounds in fraction V (MW 500–600 Da) were removed, whereas 10–20% of fluorescent compounds in fraction IV (MW 750–1500 Da) were removed. At DWTP B, the pattern was similar, but the fractional removal efficiencies were 10–30% lower.

The preferential adsorption of low MW fractions onto AC can be explained on the basis of steric effects: smaller molecules more easily diffuse into pores of AC, while larger molecules have limited access to the pores.[59,60]

At the same time, fraction VI (MW < 300 Da) exhibited relatively low reduction of UVA_{210} (~20% at DWTP A and <10% at DWTP B). This can be attributed to the hydrophilic character of this fraction, which also had the highest ratio of $UVA_{210}/UVA_{254} > 3$. In general, hydrophilic molecules do not adsorb well onto AC.[23,58,59] Low MW compounds in AC filtration effluents may also represent metabolic products of microbes living in the filters.[23,61] However, the microbes would also excrete high MW biopolymers that are poorly retained by AC filters.[59,60] And since biopolymer fraction I was not detected in the AC filtration effluents, an intense microbial activity in the AC filters was ruled out.

3.3.6. Decline of AC filtration efficiency. The AC filtration unit at DWTP A consisted of eight parallel tanks, which were sampled individually. The removal efficiencies, calculated for each tank, showed strong negative correlations with the time passed since renewal or regeneration of AC (Fig. 6). High fluctuations of the efficiencies, observed for AC of the same age, resulted from the sampling being unsynchronized with the schedule of AC backwash. The backwash is a routine procedure that removes reversibly adsorbed compounds and temporarily restores the adsorption capacity. Over time, the backwash performance declines due to the slow accumulation of irreversibly adsorbed compounds and the decrease of the AC microporosity.[59] Consequently, restored filtration

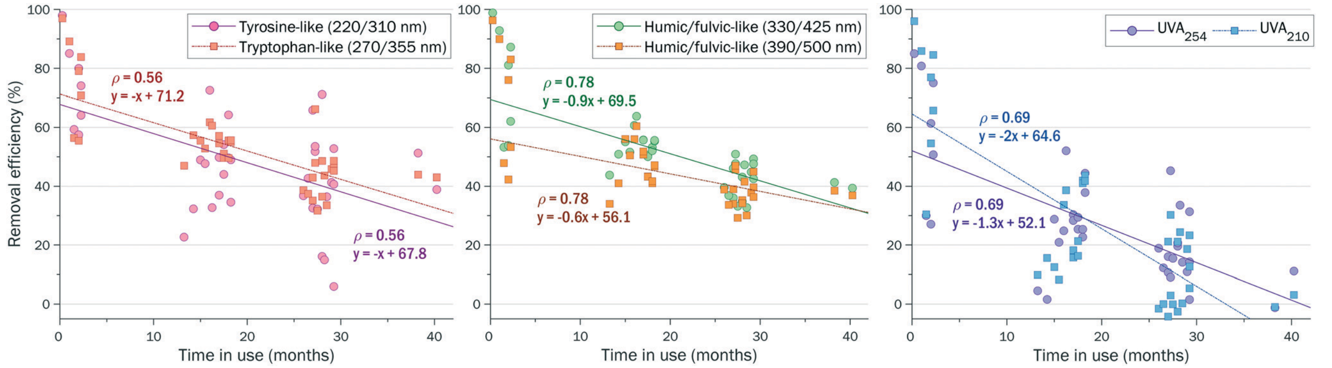

Fig. 6 Decline of AC filtration efficiency at DWTP A over time. Each point represents a pair of AC filtration influent and effluent sampled from one of the eight parallel AC tanks. ρ is the Pearson correlation coefficient (n = 38).

efficiency, achieved after each backwash cycle, becomes lower and lower over months and years of continuous use of AC.

The efficiency of AC filtration declined faster for UV absorbing compounds than for fluorescent compounds (Fig. 6). New or recently regenerated AC retained 50–100% of DOM components. After 30 months, the AC was able to remove <60% of tyrosine- and tryptophan-like compounds, <50% of humic/fulvic-like fluorescent compounds and <30% of UV absorbing compounds.

The efficiency of AC filtration declined simultaneously for fractions IV–VI (Fig. S9†). The fastest decline of AC filtration efficiency (from >80% to <20% in 30 months) was observed for low MW fraction VI detected by UVA_{210}.

This quick assessment demonstrates that HPSEC-UV-fluorescence analysis may be helpful to predict removal efficiencies based on the spectroscopic properties of DOM fractions, to schedule maintenance of AC filters and to monitor possible microbial activity in AC filters (by following tyrosine- and tryptophan-like fluorescence). For long-term monitoring, sampling of the AC filtration effluent should be done immediately after the AC backwash.

3.3.7. Final oxidation. The final treatment steps at DWTP A (post-oxidation with NaOCl, followed by high power UV treatment) and DWTP B (post-chlorination with Cl_2) did not cause statistically significant changes in the DOM composition. Most of the DOM had been removed in the previous steps, and the amounts of added reagents were too low to oxidize the residual refractory DOM.

4. Conclusions

HPSEC-UV-fluorescence was applied to investigate the transformations of DOM along two conventional DWTPs and to evaluate the removal of protein-like and humic/fulvic-like DOM fractions of various MWs in the main treatment processes: pre-ozonation, coagulation/flocculation, intermediate oxidation, sand filtration, AC filtration, *etc.*

Fluorescent protein-like compounds and humic substances exhibited different removal efficiencies. MW was the main factor determining the efficiency of coagulation/flocculation and AC filtration. While larger molecules were readily removed in the coagulation, the AC filtration favoured removal of smaller molecules. Pre-ozonation of raw water led to a higher decrease in tyrosine- and tryptophan-like fluorescence than in humic/fulvic-like fluorescence. The refractory DOM, which passed through the DWTPs, was present, mainly, in two fractions of MW 500–600 Da and 100–300 Da.

The HPSEC-UV-fluorescence approach allows rapid, robust and sensitive detection, characterization, and tracking of protein-like compounds and humic substances. However, a better understanding of the molecular structures responsible for protein-like and humic/fulvic-like fluorescence is needed to unambiguously assign observed signals to the chemical structures of DOM components. Automatic processing of HPSEC chromatograms, used in this work, allowed fast calculation of various water quality parameters and demonstrated high potential of the HPSEC approach for future on-line monitoring and early warning systems.

Abbreviation

DOM	Dissolved organic matter
HPSEC	High-pressure size-exclusion chromatography
DWTP	Drinking water treatment plant
MW	Molecular weight
DOC	Dissolved organic carbon
UVA	UV absorbance
AC	Activated carbon
EEM	Excitation–emission matrix
PARAFAC	Parallel factor analysis
$\lambda_{ex}/\lambda_{em}$	Excitation/emission wavelength pair
M_W	Weight average molecular weight
M_N	Number average molecular weight
ρ	Polydispersity

Conflicts of interest

There are no conflicts to declare.

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