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Characterisation of dissolved organic matter in Parisian urban aquatic systems: predominance of hydrophilic and proteinaceous structures

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Abstract Understanding the nature of organic matter is a necessary first step in assessing contaminant bioavailability and allowing water supply managers to optimise the treatment train in the aim of providing safe and inexpensive drinking water. This study provides further insight into the composition, structure and functional groups of dissolved organic matter (DOM) (both hydrophobic and hydrophilic) from urban aquatic systems by means of various analytical techniques (DAX-8/XAD-4 fractionation, elemental analysis, UV and FTIR spectroscopies, ^{13}C and ^{15}N isotopic analysis, size exclusion chromatography and Pyrolysis-GC-MS). The analytical range chosen for this study constitutes a powerful tool in the characterisation of DOM in urban water. The inclusion of information from one technique to the next might not only serve as a support to each one, but also as a complement. The DOM fraction from treated effluent and, more generally, DOM from urban water (i.e. receiving treated effluent) display a strong hydrophilic characteristic [i.e. low humic substance (HS) content, low SUVA], along with a high distribution in molecular weights observed by SEC and low average molecular weight. Due to the origin of this DOM, proteinaceous structures constitute the main compounds, as observed by FTIR and Py-GC-MS. Such characteristics (i.e. heterogeneity, low average molecular weight and diverse functional groups, which make up a total of N) could explain that DOM from treated effluent displayed a strong reactive potential metals pollutants as previously demonstrated.

Keywords Dissolved organic matter · Isolation · Characterisation · Composition · Hydrophilic/hydrophobic

Abbreviations

DEHP	Diethylhexyl phthalate
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
FTIR	Fourier transformed infrared

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HPI	Hydrophilic
HPO	Hydrophobic
HS	Humic substances
NHS	Non-humic substances
Py-GC-MS	Pyrolysis associated with gas chromatography and mass spectrometry
RO	Reverse osmosis
SRFA	Suwannee River fulvic acid
SEC	Size exclusion chromatography
SUVA	Specific ultraviolet absorbance
TPI	Transphilic
WWTP	Wastewater treatment plant

Introduction

Dissolved organic matter (DOM) is a major widespread component in aquatic systems as well as an intermediary element in global carbon cycles (Hedges et al. 1997); moreover, it plays a significant role within the aquatic food web (Thingstad 2003), regulates the bioavailability of nutrients, metals and organic pollutants (Buffle 1988; Guo et al. 2004; Kukkonen and Oikari 1991) and alters the optical properties of natural waters (Schindler et al. 1997). The extent of the presence of natural organic matter (NOM) in surface waters and its potential impact on health are also well known (Gjessing 1997). For this reason, it becomes necessary to understand the nature of this material in assessing the bioavailability of pollutants as well as in helping managers in the water supply industry to adopt an appropriate, cost-effective balance in the treatment methods introduced to provide safe and inexpensive drinking water to the world's population. DOM composition and transport depend primarily on whether the origin is in soil and plant material, in situ or discharged from human activity. The sources of dissolved organic carbon (DOC) can indeed be described as: (1) natural autochthonous, i.e. derived from biota (e.g. algae, bacteria, macrophytes) growing in bodies of water; (2a) natural allochtonous, i.e. entering the system from the terrestrial watershed; and (2b) anthropogenic allochtonous, i.e. water streaming (street runoff and discharge leachate) and urban sewage (household and industrial), whether treated or not. Over the past few decades, many studies have been published regarding the characterisation of DOM from freshwater. It is helpful to note, however, that these published data pertain mainly to so-called 'humic substances' (HS), which make up the most hydrophobic fraction of DOM, in isolation from natural water and based on XAD-8 resin adsorption at an acidic pH. HS are heterogeneous polyelectrolyte organic materials (Stevenson 1994) and typically constitute 40–60% of DOC in most natural surface water (Cabaniss and Shuman 1988; Determan 1968; Hertkorn et al. 2002; Hyne et al. 2005; Martin-Mousset et al. 1997; McDonald et al. 2004; Robards et al. 1994). Nevertheless, in freshwater where DOM is derived primarily from autochthonous algal/microbial precursor material, HS generally account for a smaller proportion of DOM compared to freshwater, where DOM is derived from plants and organic soil matter (McKnight et al. 1994). In urbanised water, the hydrophobic nature of DOM is weaker as a result of various urban DOM discharges and the high primary productivity induced by these discharges (Imai et al. 2002; Ma et al. 2001; Pernet-Coudrier et al. 2008). Moreover, studies carried out on wastewater in urban area (Buzier et al. 2006; Sarathy and Allen 2005; Pernet-Coudrier et al. 2008) have emphasised the great potential to complex metals despite the expected large amount of hydrophilic substances it contains. Because of the difficulty involved in isolating the hydrophilic fraction of DOM, very little information is available in the literature regarding this fraction. To date, scientists have been unable to describe the exact structure of DOM due to its complexity, and this is true even for HS macromolecules, which constitute the most widely studied fraction of DOM. By taking into account the diversity of the natural processes of synthesis and degradation, the number of components in the complex mixture called DOM can be considered as infinite. The prospect therefore of completely separating and identifying these components remains very limited. To better cope with this complexity, it is advisable to apply relevant independent methods in order to gather jointly structural information relative to DOM.

In this particular context, our objective consists of gaining additional insight into the composition, structure and functional groups of DOM (both hydrophobic and hydrophilic) from urban aquatic systems to improve understanding of trace metal binding and more generally their speciation in receiving water. This goal was achieved using strategically a panel of various analytical techniques like: DAX-8/XAD-4 fractionation to get the proportion of humic and non humic; elemental analysis, UV and FTIR spectroscopies to understand the composition and the functional groups, ^{13}C and ^{15}N isotopic analysis: to have information about origin and processes that affect DOM; size exclusion chromatography: to evaluate the molecular size and Pyrolysis-GC-MS: to assess which kind of molecules are making up this DOM.

Materials and methods

Sample collection

Treated effluent (A1 and A2) from the *Seine-Aval* wastewater treatment plant (WWTP) was sampled (quantity: 500 l) in April and December 2006 during a dry weather period. This WWTP was chosen for the present study since it is representative of urban DOM in the Parisian area, collecting over 70% of the dry weather (combined sewer) flows from the Paris metropolitan areas (11 million inhabitants). At the time of sampling, raw wastewater was mainly treated at this WWTP by means of primary settling and aerobic activated sludge. The Marne and Seine Rivers were sampled in June 2007 during a dry weather period (see Fig. 1). The Marne River site (M), upstream of Paris, was considered as sparsely urbanised (our 'natural' reference site), whereas the Seine River site, downstream from Paris at Andrésy (An), had experienced significant impacts. An IHSS standard fulvic acid (Suwannee River Fulvic Acid SRFA 1S101F) was also used as a reference NOM. The water sample was collected using a double-membrane pump (PD002P, IR ARO®) and filtered onsite through subsequent 10 and 0.45-µm polypropylene cartridge filters (Pre-d'Pure, Predel®).

DOM isolation: the RO/DAX resin protocol

The sample was then softened on a sodium cation-exchange resin in order to eliminate the calcium and magnesium ions that co-precipitate with DOM and could foul the reverse osmosis (RO) membranes. The RO step enables reducing sample volume by one order of magnitude. During the concentration step, conductivity was monitored in the permeate so as to stop the concentration step before any major DOM breaktrough occured. The aliquots for DOC measurement and UV absorbance were also sampled before and after softening and in RO permeate in order to perform mass balance. After the concentration step, a final rinsing of the membranes (Filmtech TW 30) with a 0.05 M sodium hydroxide solution allowed recovering adsorbed DOM as a sodic eluate (Croué 2004). The steps of sample filtration, softening and concentration were carried out in line and onsite so as to limit process duration and potential DOM biodegradation and the complete process have never been longer than 15 h. The obtained sodic eluate and RO concentrate, once added and collected in a (50 l) stainless steel bottle, were acidified (HCl, 0.01 mol L^{-1}) and filtered back at the laboratory on nonionic, macroporous Amberlite® DAX-8 resins [substitute for XAD-8, according to Peuravuori et al. 2001, 2002 (also known as acrylate ester)] as well as

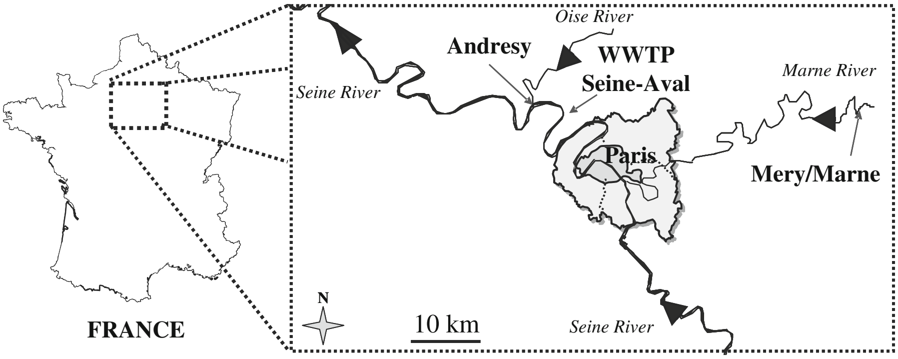


Fig. 1 Location of the sampling sites

on Supelite® XAD-4 (divinylbenzene) used in series. According to Leenheer (1981), a column capacity factor k', equal to 50, was applied to isolate HS. DOC measurements were conducted in both the influent and effluent of DAX-8 and XAD-4 resins in order to assess the proportion of DOM retained on each column. The hydrophobic (HPO, retained on DAX-8) and transphilic (TPI, retained on XAD-4) fractions were derived following the procedure described in Pernet-Coudrier et al. (2008). All Teflon and stainless steel materials used for sampling and isolation were washed several times with ultrapure deionised water. The glass material was washed with a detergent (TFD4, 5%), rinsed thoroughly with ultrapure deionised water and then precombusted for 5 h at 500°C. Prior to application, the resins were washed using soxhlet extractions and successive acid and sodic rinsings, in accordance with a protocol described by Leenheer (1981). Trial experiments were carried out by implementing this protocol with ultrapure deionised water throughout the system (i.e. pipes, pump, filters and resins all prewashed using ultrapure deionised water). The DOC values for these trial tests never reached the DOC quantification limit of 0.5 mgC l^{-1}.

Purification of the hydrophilic fraction

The HPI fraction, i.e. the fraction of DOM not adsorbed on the DAX-8 and XAD-4 resins, contains all salts initially present in the sample and requires further purification given that salts would interfere during the various investigations. The separation of inorganic salts from the HPI fraction was performed according to a protocol described by Leenheer et al. (2000) and based on several zeotrophic distillation steps with acetic acid, in order to precipitate the inorganic salts. Due to the presence of remaining salts in the resultant HPI fraction, this protocol was implemented using a second zeotrophic distillation with acetonitrile. After the several first distillation step, the sample was dry evaporated, dissolved in methanol and acetonitrile (50%/50%) and vacuum evaporated to precipitate the remaining inorganic salts that co-precipitate with HPI DOM during the first zeotrophic distillation. The sample was spiked with barium acetate to precipitate the remaining sulphates, which were then separated by centrifugation. Residual cations, such as barium, were softened on cationic exchange resin (H^+), and residual solvents and acids could be eliminated by successive vacuum evaporation steps in acetonitrile, formic acid and methanol.

DOM characterisation

A variety of analytical tools were employed to characterise the composition, the size and the structure of the isolated organic material.

DOC content was determined using the carbon analyser developed by O.I. Analytical (quantification limit = 0.5 mgC l^{-1}).

Elemental analyses were carried out by the *Service Central des Analyses*, a unit with the CNRS Scientific Research Laboratory (Solaize, France) and enabled determining the C, H, O, N and S contents to within a precision of 0.3% and a standard deviation of 0.2%.

Specific UV absorbance (SUVA) is defined as the UV absorbance of a given sample, determined at 254 nm and divided by the organic carbon content of the solution, in accordance with U.S. EPA recommended method 415.3 (United State Environmental Protection Agency, 2005). UV absorbance was obtained using a Lambda Perkin Elmer spectrophotometer with 1-cm long quartz cells. A DOM solution was prepared the night before analysis by dissolving 10 mgC l^{-1} of each fraction in ultrapure deionised water buffered with 0.05 mol l^{-1} $NaHCO_3$ and Suprapur HCl (pH 7.9). The standard deviation on the measurement is equal to 0.1%.

Infrared spectra of 2–5 mg of the DOM fraction isolated in potassium bromide pellets were determined on a Fourier Transform InfraRed spectrometer (Perkin Elmer Spectrum BX). All spectra were normalised after acquisition with a maximum absorbance set at 1.0 for comparative purposes.

Stable isotopic signatures of ^{13}C and ^{15}N were measured by automated recordings of the ^{13}C and ^{15}N contents of CO_2 and nitrogen oxide reduced in N_2, evolved from a CHN auto-analyser (CHN NA 1500, Carlo Erba), which had been coupled with an isotopic ratio mass spectrometer (VG Sira 10). The laboratory reference was calibrated against the international standards: PDB (Pee Dee Belemnite) for the carbon isotope, and CDT (Cañon Diablo Troilite) for the nitrogen isotope. Results were expressed as: $\delta^{13}C$ and $\delta^{15}N$ (in ‰ terms), and the isotopic ratio (R: $^{13}C/^{12}C$

and $^{15}N/^{14}N$) relative to the respective standards (PDB and CTD): $\delta = \left(\left(R_{\text{sample}} - R_{\text{standard}}\right)/R_{\text{standard}}\right) \times 1000$. The standard error on this measurement was equal to 0.1‰.

The molecular size distribution for organic materials was evaluated at the Water Chemistry and Microbiology Laboratory (Poitiers, France) by high-performance, size-exclusion chromatography. The chromatographic system consisted of a Waters 717 plus Autosampler and a Waters 1525 Binary HPLC pump, followed by a UV–Vis detector: a Waters 486 Tunable Absorbance Detector set at 254 nm. Size-exclusion separation took place via an AIT SEP-SEC 2000S column (lengh: 300 mm, diameter: 8 mm) preceded by a guard column with the same stationary phase. The overall molecular weight range of calibration with this system equalled 400–13000 Da with indigo carmine and polystyrene sulphonate. For each sample, fractions were dissolved in ultrapure deionised water. The injection volume of all samples was 500 µl, the eluent used was sodium acetate (0.82 g l^{-1}) buffered at pH 7 with acetic acid (Vartiainen et al. 1987), and the flow rate was 1 ml min^{-1}. For each analysed fraction, the peaks were integrated and divided over the total integrated area for comparison purpose. Both the number (M_n) and weight-averaged (M_w) molecular weights for the solutes were calculated using the following equations:

$$M_n = \frac{\sum_i n_i M_i}{\sum_i n_i}$$

and

$$M_w = \frac{\sum_i n_i M_i^2}{\sum_i n_i M_i}$$

with n the number of molecules, having a molecular weight M, *t*he subscript i is an index representing all molecular weights present in the ensemble of chains (Determan 1968). Indeed, these ‘average’ molecular weights do not have a physical meaning for DOM because they present different molecular weight classes. Nevertheless, these average molecular weights allow an easy comparison between each fraction. The quotient M_w/M_n can be used to estimate the degree of polydispersity I_p.

Molecular analysis was performed by means of pyrolysis coupled with gas chromatography (GC) and mass spectrometry. Approximately 0.5–1 mg of sample were loaded in tubular ferromagnetic wires and inductively heated to their Curie temperature of 650°C in 0.15 s (with a 10-s hold) by a pyrolysis unit (GSG Curie-Point Pyrolyser 1040 PSC). Pyrolysis products were separated using a Hewlett Packard HP-5890 GC on a 60-m fused silica capillary column (SolGelWax a bonded polyethylene glycol stationary phase, 0.32-mm inner diameter, 0.5-µm film thickness), with helium as the carrier gas. A polar capillary column was chosen for the GC separation of pyrolysis products, to yield a better separation of polar compounds originating from sugars, proteins and lignins. The less polar compounds, such as alkanes/alkenes, were not as easily observed on this column (Dignac et al. 2006). The temperature program of the GC oven was set with a temperature increase from 30 to 280°C at a rate of 2°C min^{-1} and then maintained at 280°C for another 15 min. The GC was coupled to a Hewlett Packard HP-5889 mass spectrometer (electron energy: 70 eV). Compounds were identified based on: their mass spectra, GC retention times and comparison with mass spectra from the literature (Wiley). A number of pyrolysis products were identified from each sample, and some of these were specific to a macromolecular source. Peaks were integrated using the HP MS Chemstation (Version C.01.05) over the total current ion trace.

Results

Extraction

The carbon balances both before and after each extraction and purification step enabled reconstituting the initial DOM sample composition and estimating DOM loss. The DOM recovered as a brownish powder after lyophilisation ranged from 61 to 75% of the bulk DOC (in the raw sample, i.e. 0.45 µm filtered sample), depending on the particular sample (see Table 1). The majority of the DOC loss relates to the HPI fraction and is due to the co-precipitation of organic matter with inorganic salts during the purification step. As a matter of fact, only approximately 62% of this fraction was recovered at the maximum point, thus revealing the difficulty of HPI isolation. The other minor DOC losses stem from the lack of complete elution of the HPO and TPI fractions as

Table 1 Characteristics of sampled water

Site	Symbol	Date	Flow (m^3 s^{-1})	pH	Conductivity (µS cm^{-1})	DOC (mgC l^{-1})	Chlorophyll *a* (µg l^{-1})	DOM extraction yield (%)
Méry/Marne	M	27/06/07	Austerlitz: 120	8.3	517	2.0	171	67
Achères	A1	06/04/06	20	7.8	1580	38.7	nd	75
	A2	15/12/06	20	8.1	1261	17.4	nd	62
Andrésy	An	29/06/07	Austerlitz: 120	7.7	606	4.1	197	61

well as adsorption on the RO membranes, despite an ultimate membrane rinsing step that uses a sodium hydroxide solution.

Distribution of DOC

Fractionation of the RO concentrated sample on DAX-8/XAD-4 resins has led to three fractions (see Fig. 2). The Méry/Marne DOC distribution is quite typical of natural water, with a predominant HPO fraction (62% of DOC). As opposed to Méry/Marne, in the treated effluent, the hydrophobic fraction represents less than 40% of DOC and the sum of the most hydrophilic fractions (TPI and HPI) varies between 60 and 75%, a finding also observed in other studies on WWTP effluent (Imai et al. 2002; Pernet-Coudrier et al. 2008). Given the load of DOM from the WWTP in Paris conurbation, DOM in Andrésy, downstream from Paris, results from an approximate 50/50 mixing of carbon from upstream river water and from WWTP (during the low water season). The characterisation analysis, of DOM from Andrésy will not be presented in the following section given that it also displays a mix of characteristics between DOM from treated effluent and DOM upstream of Paris.

Elemental analysis

Table 2 lists the results from an elemental analysis along with SUVA of the HPO, TPI and HPI fractions of each sample. Except for HPOM and HPIA2, the sums of elemental masses exceed 94% in each of the fractions, which indicates that the isolation protocol has efficiently removed inorganic constituents from the sample. The inorganic cation and anion contents were measured by means of ICP-AES and ionic chromatography; taken together, they contribute 1% or less (unpublished results). The carbon contents of DOM fractions are close to those observed in the literature for surface water (Croué 2004; Ma et al. 2001; Peuravuori et al. 2001; Violleau 1999). The hydrogen, nitrogen and sulphur contents, however, are especially high, while on the other hand, oxygen content is lower for DOM from treated effluent (Croué et al. 2003; Pernet-Coudrier et al. 2008). As previously observed (Croué 2004; Pernet-Coudrier et al. 2008; Violleau 1999), C/H, C/O and C/N ratios all decrease with the hydrophilic nature of the samples (HPO < TPI < HPI), thus indicating a lower degree of unsaturation combined with an enrichment in nitrogenous structures and oxygenated functional groups. The C/N ratio of the HPI fraction is very low

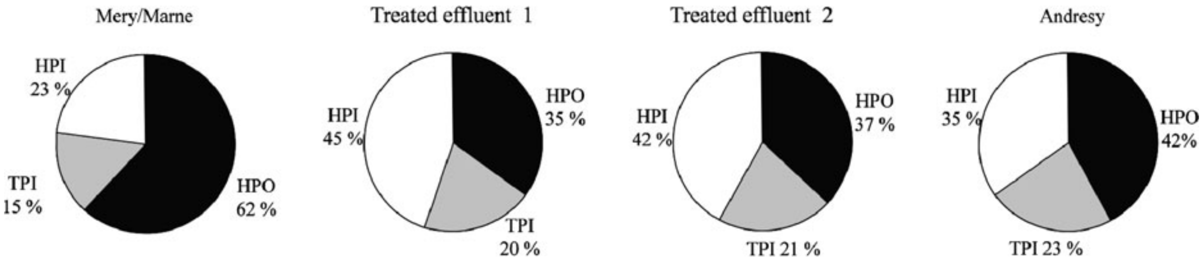


Fig. 2 DOC distribution at each sampling site

Table 2 Elemental composition and SUVA of DOM fractions (% of weight)

Fraction	C (%)	H (%)	O (%)	N (%)	S (%)	Residue (%)	Total (%)	C/H	C/O	C/N	C/S	SUVA (m^{-1} l mgC^{-1})
SRFA	52.3	4.4	43.0	0.7	0.5	0.6	101.4	1.0	1.6	91.1	303.8	4.3
HPOM	40.4	4.5	29.7	2.8	1.6	12.5	91.5	0.8	1.8	17.0	67.0	2.3
TPIM	47.3	5.6	37.0	4.9	1.5	4.0	100.3	0.7	1.7	11.3	84.8	1.8
HPOA1	53.6	6.5	27.9	5.7	2.6	2.9	99.1	0.7	2.6	11.1	55.9	2.1
TPIA1	48.4	6.4	28.8	8.4	2.1	4.7	98.7	0.6	2.2	6.7	62.7	1.4
HPIA1	43.8	7.1	29.4	12.3	2.1	5.0	99.8	0.5	2.0	4.2	54.7	0.8
HPOA2	54.1	6.4	29.2	4.4	2.7	4.5	101.2	0.7	2.5	14.3	53.7	1.8
TPIA2	47.3	6.1	34.1	6.9	2.2	2.5	99.0	0.7	1.8	8.0	57.6	1.3
HPIA2	40.1	5.9	34.0	10.6	2.7	10.4	103.7	0.6	1.6	4.4	39.1	0.7

HPIM was not purified and analysed because of the extremely limited quantities involved

compared to that of the other fractions, which suggests the abundance of nitrogenous structures in this particular fraction.

UV absorbance

The likely presence of more aromatic structures in HPO and, to a lesser extent in TPI than in HPI, has once again been confirmed by SUVA, with absorption at 254 nm being an indicator of aromatic structures (Leenheer and Croué 2003). SUVA obtained for these fractions range between 2.3 and 0.7 m^{-1} l mgC^{-1} (Table 2) and remain close to those obtained from fractions of treated effluent by Imai et al. (2002). Nevertheless, SUVA of DOM from treated effluent remains weaker than that for SRFA (4.3 m^{-1} l mgC^{-1}), like with fractions from Méry/Marne and DOM in natural water, since HS can reach values of 5 m^{-1} l mgC^{-1} (Croué 2004; Imai et al. 2001; Violleau 1999; Weishaar et al. 2003). This finding denotes a low aromatic characteristic of DOM from treated effluent, most likely due to the presence of fewer degradation and condensation processes. The fractions in the Méry/Marne sample, which was taken during a phytoplanktonic bloom [(Chlorophyll *a*) = 171 µg l^{-1}], also present low aromaticity compared to DOM in natural water as a probable result of algal production (Her et al. 2004).

FTIR spectra

Figure 3 exhibits the FTIR spectra of: the three DOM fractions from A1 (samples A1 and A2, taken in April and December 2006, respectively, yielded the same trends), the HPO and TPI fractions from Méry/Marne and the SRFA. The first observation is that the absence of bond vibration for inorganic salts (like carbonate: 2506 cm^{-1}, or silicate: 1100 cm^{-1}) (Dignac et al. 2000; Tatzber et al. 2007; Violleau 1999) confirms once again the purity of DOM fractions and isolation protocol efficiency in removing inorganic salts. Eleven bands can be attributed to the various chemical bond vibrations. The first is very broad and can be attributed to the O–H bond in alcohols and carboxylic acids. Bands 2, 6 and 8 are all related to amides and amines, which are characteristic of proteins and amino sugars (Leenheer and Rostad 2004), thus explaining the high nitrogen content previously determined DOM from treated effluent. Because of the low nitrogen content in SRFA and DOM fractions from Méry/Marne, but most of all because of the slight shift in its wavelength, band 7 for SRFA, HPOM and TPIM is mainly attributed to aromatic carboxylic acid. Bands 3 and 9 reveal the presence of aliphatic chains: at 2960 cm^{-1} with a small shoulder (C–H asymetric stretching in CH_2 and CH_3), and at 2890 cm^{-1} (C–H symetric stretching in CH_2) (Dignac et al. 2000). The variation in intensity of the aliphatic peaks confirms the differences in alkyl chain abundance between these fractions. Bands 4 and 5 are associated with the carboxylic acid bonds. Peak 5, at approximately 1700 cm^{-1}, is very intense; this feature highlights the acidic character of organic matter (Croué 2004). Band 10 is attributed to the C–O of alcohols and sugars (Croué 2004) and the high intensity of this peak for the HPI fraction suggests an

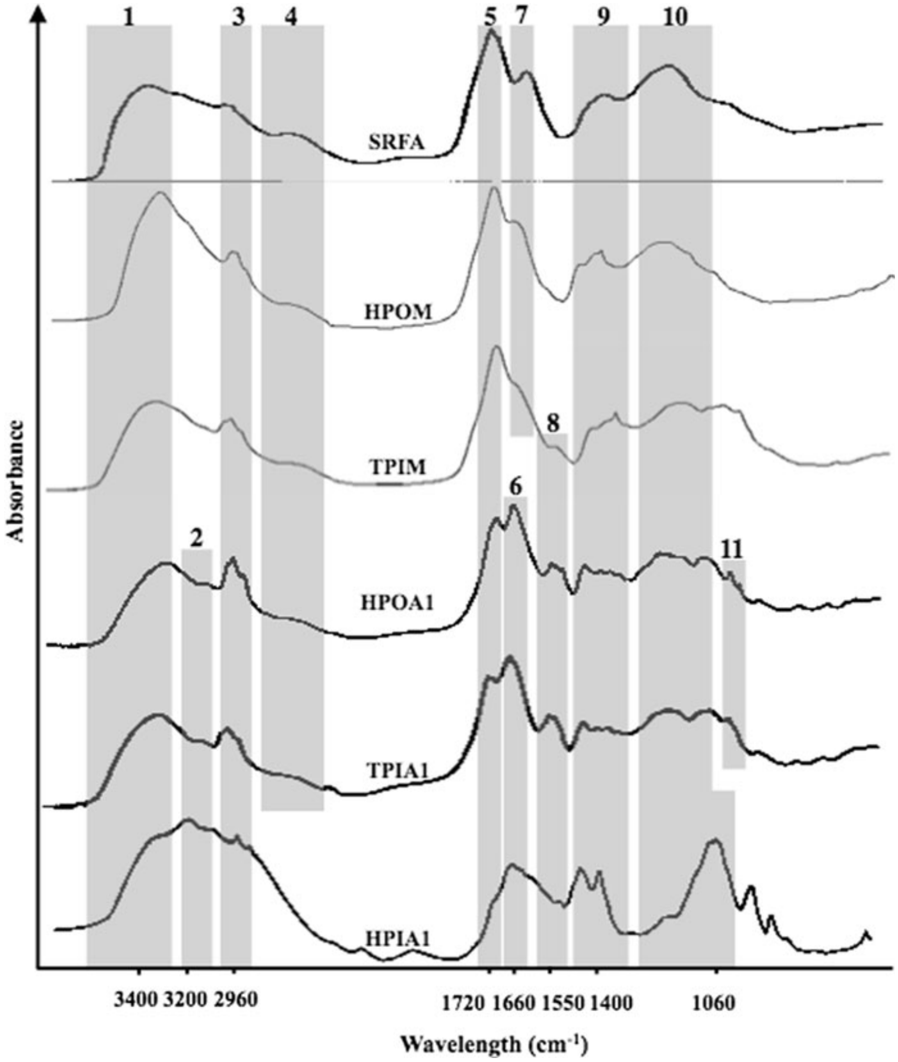


Fig. 3 FTIR spectra of the DOM fraction

abundance of sugars in the HPI fraction (Leenheer and Rostad 2004; Jarusutthirak et al. 2000). Band 11 may be correlated with the presence of a sulphonic group associated with the carboxylic aromatic cycles. These compounds are probably breakdown products (metabolites) of a surfactant (LAS: linear alkyl sulfonates), as previously observed in municipal wastewater (Barber et al. 1997, 2001; Drewes and Croue 2002).

C and N isotopic contents

Results of the isotopic analysis (δ^{13}C and δ^{15}N) are listed in Table 3. These values are similar to those reported for freshwater and marine organic matter samples (Deines 1980) and for effluent organic matter (Drewes and Croue 2002). Soil organic matter and terrestrial plants are expected to be the major sources of DOC carried by the Marne River. Freshwater algae, with similar δ^{13}C values to terrestrial plants displaying in a wide range (Deegan and Garritt 1997), might also influence the carbon isotopic contents of DOC. Regarding DOM fractions from treated effluent, the HPO fractions reveal an enrichment in ^{13}C compared to TPI and HPI. This situation underscores a higher degree of maturation in HPO fractions since the breakdown of compounds as a step in the nutritive series of an ecosystem results in an enrichment of the heavier isotope within the organism (^{13}C) due to a loss of $^{12}CO_2$ in metabolic processes (DeNiro and Epstein 1978; Hall et al. 1999; Drewes and Croue 2002; Templetona et al. 2006).

Table 3 δ^{13}C, δ^{15}N and molecular weights of DOM fractions

Fraction	Isotopic analysis		SEC		
	δ^{13}C (‰)	δ^{15}N (‰)	MW$_n$ (Da)	MW$_w$ (Da)	I_p
SRFA	−28.1	0.7	2278	3,308	1.5
HPOM	−28.4	3.2	781	1463	1.9
TPIM	−27.9	3.6	461	915	2.0
HPOA1	−24.1	2.5	678	1838	2.7
TPIA1	−24.8	2.8	400	1454	3.6
HPIA1					
With colloids	−26.7	–	1738	18707	10.8
Without colloids			529	2132	4.0
HPOA2	−24.9	2.1	700	1789	2.6
TPIA2	−28.6	2.2	424	1212	2.9
HPIA2	−38.1	–	420	1478	3.5

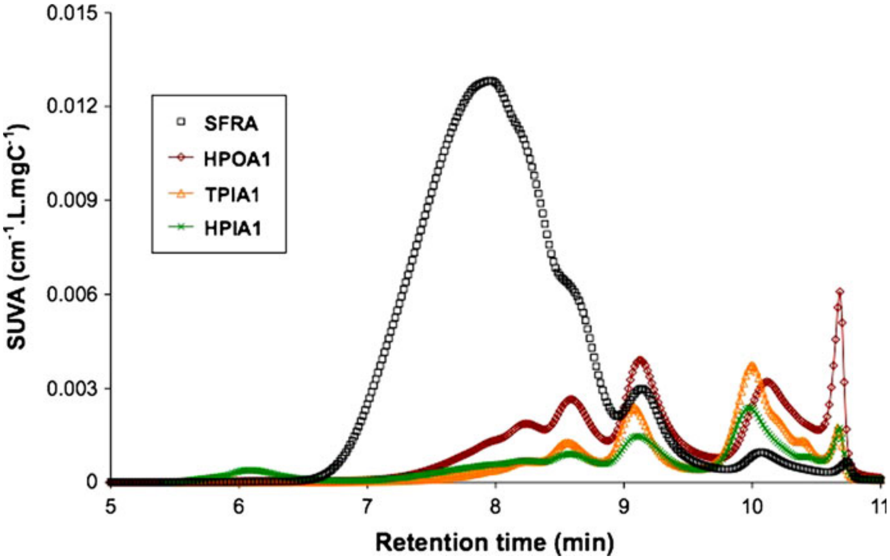
The values of δ^{15}N varied from 0.7 to 3.6‰ in the DOM fractions. It is generally accepted that the biogeochemical cycling of nitrogen is more dynamic compared to carbon in aquatic environments, with trophic transfer, microbial processes and isotopic fractionation all being important factors in influencing the N-isotope distribution (Schell et al. 1998). Kendall (1998) reports that the TPI fraction typically posts a heavier δ^{15}N value than the hydrophobic fraction. Our results at Méry/Marne are consistent with this observation, although the reason for the difference in δ^{15}N between the two fractions remains unclear. Because DOM derived from plant material has a high hydrophobic (or fulvic acid) character in comparison with DOM derived from algal material (McKnight et al. 1994), the fact of being derived at least partially from different sources might explain the difference in δ^{15}N values between the two fractions. This explanation would further imply that algal material is enriched in δ^{15}N with respect to terrestrial plant material. While it cannot be verified that such is the case in our system, other studies have shown that planktonic material in both freshwater (Kendall 1998) and saltwater (Peterson and Howarth 1987) ecosystems is typically enriched in δ^{15}N compared to terrestrial plant material. Concerning the δ^{15}N in the DOM fraction from treated effluent, we are able to observe the same trend yet for a different reason: TPI fractions are more highly enriched in ^{15}N than HPO fractions. During biological treatment in WWTP, heterotrophic microorganisms grow in order to eliminate OM; they are also responsible for a strong N-isotopic fractionation due to their deaminating enzymes, which cause a depletion in ^{15}N after the uptake of amino acids and their transfer into proteins (Macko and Estep 1984). This aspect once again underscores a higher contribution of microbial products in DOM from treated effluent as compared to natural DOM. It highlights also the higher degree of ageing in HPO fractions than TPI fractions, in accordance with the δ^{13}C results.

HPSEC-UV analysis

Figure 4 presents the size exclusion chromatogram of the DOM fractions. The SRFA chromatogram showed one peak of high molecular weight (>3000 Da). Many peaks have been observed in the other DOM fractions, which correspond to various compounds with distinct molecular weights. The HPI fraction from treated effluent displays a small peak at about 6 min, attributable to the very high molecular weight compounds that could be colloidal polysaccharides and proteins due to the negligible corresponding UVA peaks (Croué 2004; Nam and Amy 2008). These colloids are not adsorbed on DAX/XAD resins due to their high molecular size and remain present in the effluent; note, however, that they do not exhibit necessary a hydrophilic character and could be isolated by dialysis before fractionation.

The averaged M_n and M_w molecular weights, determined using HPSEC for the various isolated fractions, are listed in Table 3. Taking into account that the UV detector can biased the results since some molecules can be invisible at 254 nm, the SRFA could contain the higher molecular weight (3308 Da),

Fig. 4 Size-exclusion chromatograms of fractions from treated effluent and SRFA



compared to our fractions (ranging between 915 and 2132 Da). In this study, the SRFA present a higher average molecular weight than in other studies (≈2000 Da), whose results were obtained using the same SRFA and detector (UV) (Chi and Amy 2004; Her et al. 2004; Hur and Schlautman 2003). This feature may be explained by many reasons, the most critical of which being solution preparation. In the other studies, SRFA was dissolved in ultrapure water at an acidic pH and then filtered through a 0.2 or 0.45-µm filter in order to ensure the dissolved characteristic. Yet according to the isolation procedure, all of the obtained originated from dissolved organic substances. For this reason, it was decided in the present work not to filter the solution before SEC injection, for the purpose of avoiding artefacts; moreover, it was preferred to dissolve, to the greatest extent possible, SRFA powder while maintaining the solution for 1 h at neutral pH, under soft ultrasound and with stirring. The SRFA polydispersity coefficient is less than the other fractions due to a higher degree of fractionation, given that it has been isolated from the DOM matrix via resin adsorption and pH separation. The HPO fractions systematically reveal higher molecular weights than either TPI or HPI. The degree of polydispersity varies, as opposed to molecular weight: the higher the molecular weight, the lower the polydispersity coefficient, which in this case corresponds to the HPO fractions. The TPI and HPI fractions thus display a wide range of molecular weights and have hence given rise to a great diversity of compounds.

Py-GC-MS chromatograms

The goal of the Py-GC-MS analysis was to evaluate which kinds of molecule are making DOM from treated effluent and to have further insight into the proteinaceous structure. The main compounds identified following pyrolysis have been grouped according to origin into three major classes (Table 4): compounds originating from the pyrolysis of anthropogenic compounds (A), lipids (LIP) and N-containing compounds (N). Compounds that may have resulted from the pyrolysis of several distinct biological sources were designated as unspecific (U). All chemicals identified during this step have resulted to some extent from anthropogenic activities since DOM actually stems from treated effluent.

The pyrolysates of DOM fractions from treated effluent (Fig. 5) were dominated by N-containing compounds (Table 4). Acetonitrile (N1) and acetamide (N6) were present in the pyrolysis products. While both compounds may be derived from peptides and proteins (Tsuge and Matsubara 1985), pyrrole (N4) and its derivatives (N5, N14) can be formed upon pyrolysis of proteins containing proline, hydroxyproline, glycine and glutamic acid (Tsuge and Matsubara 1985) or might also be released from pigments, such as chlorophyll (Bracewell et al. 1987;

Table 4 Identified pyrolysis products in fractions from treated effluent

No.	Name	HPOA1	TPIA1	HPIA1	HPOA2	TPIA2
N-containing compounds						
N1	Acetonitrile	X	X	X	X	X
N2	Pyridine	X		X	X	X
N3	?-Methyl-pyridine			X		X
N4	1H-Pyrrole	X	X	X	X	X
N5	2-Methyl-1H-pyrrole		X	X		X
N6	Acetamide	X	X	X	X	X
N7	3,4-Ethylmethyl-1H-pyrrole-2,5-dione				X	
N8	2-Pyrrolidinone	X	X	X		X
N9	2-Piperidinone		X	X		
N10	2-Pyridinone		X	X		
N11	3-Methyl-pyrrolidine-2,5-dione					X
N12	1H-Indole	X			X	
N13	Pyrrolidine-2,5-dione	X	X	X	X	X
N14	Diketodipyrrole		X			
N15	Diketopiperazine	X	X	X	X	X
N16	2,4-Methylamino-pyrimidine					X
N17	1-Methylpyrido[3,4-b]-9H-indole	X				
N18	Thymine			X		
N19	1H,3H-Pyrimidine-2,4-dione			X		
N20	1H,3H-Pyrimidine-2,4-dione? (derivative)			X		
Compounds derived from anthropogenic activities						
A1	1,3,7-Trimethylxanthine = caffeine	X	X			X
A2	*N*-Diisooctyl phthalate = DEHP	X	X	X	X	X
A3	1H-Quinolin-2-one	X	X	X		
A4	3,7-Dimethylxanthine = caffeine derivative		X	X		
Compounds derived from lipids						
LIP1	Dipropyleneglycerol		X			X
LIP2	Diethyleneglycerol		X	X		
LIP3	Triethyleneglycerol	X	X	X	X	X
LIP4	Dodecanoic acid = lauric acid	X			X	
LIP5	Hexanoic acid = caproic acid			X	X	X
LIP6	Hexanoic acid? (derivative)			X	X	
LIP7	Octanoic acid = caprilic acid			X	X	X
LIP8	Octanoic acid? (derivative)			X		
LIP9	Decandioic acid? (derivative)	X			X	
Compounds of unspecified origin						
U1	Methyl-benzene = toluene	X	X		X	X
U2	Acetic acid	X	X	X	X	X
U3	Phenol	X	X	X	X	X
U4	4-Methyl-phenol	X	X		X	X

X detected, *Blank* not detected

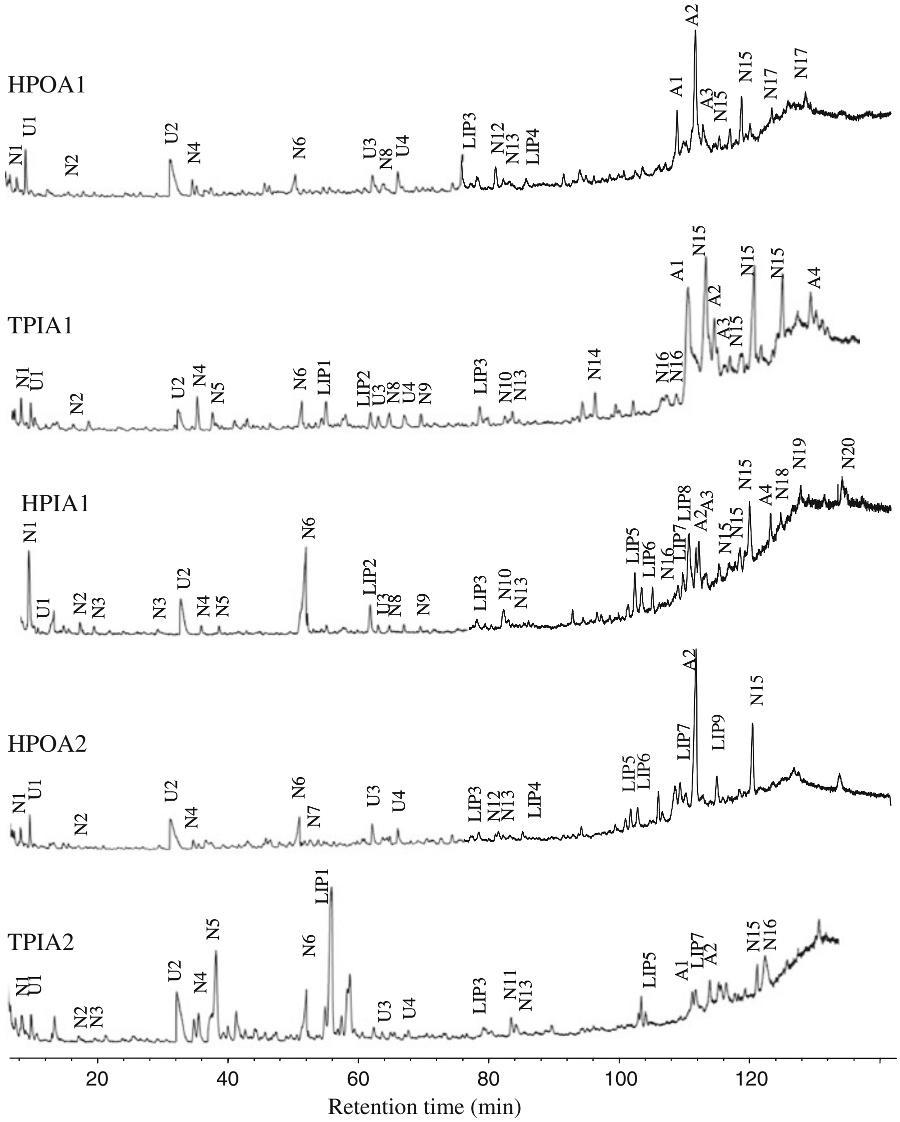


Fig. 5 Pyrograms of fractions from treated effluent (compound names and numbers are listed in Table 4)

Sinninghe Damsté et al. 1992). Given that no other chlorophyll-derived compound, such as pristene or phytadiene, were identified, the pyrrole and derivatives in these pyrolysates were attributed to proteins. Indole (N12) is an indicator of tryptophane-containing peptides (Tsuge and Matsubara 1985), while pyridine (N2) and derivatives (N3, N10) can be attributed to alanine-containing proteins and peptides (Bracewell et al. 1987; Chiavari and Galletti 1992; Ishiwatari et al. 1995; Tsuge and Matsubara 1985). The prominent N-heterocyclics in TPI and HPI fractions were diketopiperazine (N16): the indicators of sporulation material, proteins, amino acids and nucleic acids (Voorhees et al. 1992). Lastly, pyrrolidinone (N8), its derivatives (N11, N13, N17) and piperidinone (N9) may indicate the presence of asparagine-containing peptides (Tsuge and Matsubara 1985). The N-heterocycles like pyrimidine (N16, N19, N20, N23) and 1H-indole (N12) in the DOM fractions result from proteinaceous materials (Schnitzer et al. 2007).

Three compounds derived from anthropogenic activities were identified in the DOM from treated effluent. The predominant compound is caffeine (A1, and its derivative A4), which is well known as a potential chemical marker for domestic wastewater contamination (Buerge et al. 2003). Despite its generally efficient elimination (>99%) at WWTP sites, given its high concentration in WWTP influent, caffeine was found to be ubiquitous in lakes and rivers (Buerge et al. 2003), thus explaining its appearance in DOM from treated effluent. Diethylhexyl phthalate (DEHP, A2) constitutes another major compound found in the HPO fraction. Because of its widespread use throughout industry and in households, particularly as additives in plastics, DEHP was expected to occur at high content levels (Gasperi et al. 2008). The dominance of DEHP in the HPO fraction may be due to its highly hydrophobic character ($\log(K_{\mathrm{OW}}) = 7.5$). The last anthropogenic compound is 1*H*-quinolin-2-one (A3), i.e. the aromatic structure of a large family of antibacterial products detected in both wastewater influent and effluent (Nakata et al. 2005).

Many usual C_6–C_{12} fatty acids (LIP4–LIP9) were present in pyrolysates of DOM from treated effluent. The diversity of these acids could be better evaluated by the selective ion monitoring of *m/z* 60, which is characteristic for acids (data not shown). The C_6–C_{12} acids produced large, unresolved peaks. Acids with longer chains may also have been present in the pyrolysate, but could not be GC separated without derivatisation. Moreover, acids with long chains were often coeluted with other compounds, and their distributions could not be determined accurately by the polar chromatographic column used to separate pyrolysis products. A non-polar column would therefore be better adapted to separating these compounds (Dignac et al. 2006). Nevertheless, these fatty acids accompanied with substituted glycerol compounds (LIP1–LIP3) reveal the presence of triglycerides (Decker et al. 2000) in DOM from treated effluent. Triglycerides are mainly well known for constituting vegetable oils and animal fats (Maher and Bressler 2007); they are also widely used in human dietary-related products (Ackroff et al. 2005) and have been detected in both wastewater and effluent (Chipasa and Mdrzycka 2008).

Some products of unspecific origin were found in the pyrolysates (Table 4). Toluene (U1), phenol (U3) and methylphenol (U4) may originate from tyrosine-containing peptides and proteins (Chiavari and Galletti 1992; Ishiwatari et al. 1995; Tsuge and Matsubara 1985), as well as from lignins (Saiz-Jimenez and De Leeuw 1986), tannins (Galletti and Reeves 1992) and polysaccharides (Pouwels et al. 1987); consequently, they are unspecific compounds. Neither lignin- nor polysaccharide-derived products, however, were identified in the pyrolysates of DOM from treated effluent. Non-hydrolysable tannins (catechins) are known to release catechol (1,2-benzenediol) upon pyrolysis (van Bergen et al. 1996). The presence of tannins in the DOM from treated effluent may be ruled out since this product was not detected in its pyrolysate. The unspecific compounds in this sample, i.e. toluene, phenol and methylphenol, were thus derived primarily from proteins. Acetic acid (U2), one of the major peaks, also features a ubiquitous origin since it can be formed by cleavage of the acetyl groups during the pyrolysis of both hemicelluloses (Pouwels et al. 1987) and fatty acids (Wilson et al. 1983).

Discussion

Contrasting compositions of DOM from urban and natural water

DOM from treated effluent and, more generally, DOM in urban water receiving treated effluent differ sharply from natural HS, which even when from different origins display a number of similar characteristics (Frimmel and Abbt-Braun 1999; Kalbitz et al. 1999; Lam and Simpson 2008; Leenheer and Croué 2003; Leenheer et al. 2003; McDonald et al. 2004; Pettersson et al. 1994). For starters and as previously observed (Nam and Amy 2008), urban DOM presents a low average molecular weight along with a high coefficient of polydispersity (Table 3), as opposed to HS; this finding indicates the presence of fast biodegradation due to strong microbial activity (Nam and Amy 2008; Pettersson and Rahm 1996). The SUVA (Table 2) of urban DOM is much lower (between two and five times) than HS, thus revealing a very limited aromatic characteristic even for the most hydrophobic fraction from treated effluent. Moreover, SUVA is not correlated with M_{w} for urban DOM ($n = 11$, $r^2 = 0.17$), in contrast with what had typically been observed for HS (Chin et al. 1994; Imai et al. 2002; Pettersson et al. 1994; Peuravuori and Pihlaja 1997; Swietlik and Sikorska 2006). The weak aromatic characteristic of urban DOM has also been underscored by a low C/H ratio, ranging between 0.8 and 0.4, hence indicating a lower degree of maturation (Stevenson 1994) which was also highlighted by isotopic results. The hydrophilic nature of urban DOM results from a high content of polar functional groups, especially N-containing groups, as suggested by the abundant proteinaceous structures observed by FTIR, fluorescence (data not published) and Py-GC-MS (to be discussed below), and possibly from the sulphur-containing groups, as suggested by the high elemental sulphur contents. Due to its low aromatic characteristic and isotopic hallmark, urban DOM seems to be less mature, e.g. more recent than HS. These features are mainly due to the source (human faeces and bacteria versus lignin) and processes (both short and fast biological degradation in WWTP versus long natural biogeochemical processes) that affect and transform urban DOM, in comparison with natural DOM (Imai et al. 2002; Nam and Amy 2008). It must be pointed out that the DOM fraction from Méry/Marne exhibits some characteristics close to those of urban DOM (low SUVA, low M_w, etc.) that are explained by high planktonic productivity (Table 1). These characteristics will not be discussed herein. Also note that the high concentration of chlorophyll measured in river water has not been detected in the isolated fraction (by fluorescence and Py-GC-MS); this lack of detection is probably due to the chlorophyll photodegradation occurring during DOM extraction.

Origin and consequences of the predominant proteinaceous structures

Compared to natural DOM, we have demonstrated again that DOM from treated effluent are enriched in nitrogen (Barber et al. 2001; Drewes and Croue 2002; Nam and Amy 2008) and sulphur (Drewes and Croue 2002), both of which are well known to constitute proteins. Nitrogenous organic compounds originate mainly from microbial growth/death cycles (DNA, RNA, cell materials, etc.). The main N-containing compounds observed by means of Py-GC-MS could therefore be attributed to peptides and proteins containing a variety of amino acids. This finding indicates that DOM from treated effluent could demonstrate considerable binding potential for trace metals since proteins are well known to play a key role in the transport mechanisms of trace metals into organisms (Roesijadi 1992) and since studies carried out on wastewater in urban area (Buzier et al. 2006; Sarathy and Allen 2005; Pernet-Coudrier et al. 2008) have emphasised the great potential of DOM to complex metals despite the expected large amount of hydrophilic substances it contains. The proteins in this DOM may originate from bacterial cells or their exopolymers, whose main components include these proteins (Dignac et al. 2006). They may also be derived from other constituents, such as faeces and plant cells. Amino sugars, major components of peptidoglycans in bacterial cell walls, are revealed by: the occurrence of acetamide (N6) (Eudy et al. 1985) and the presence of amine, amide groups and sugars highlighted by FTIR. Proteinaceous structures are also observed for the HPO fraction of wastewater treatment effluents (by FTIR and Py-GC-MS), and these observations are interpreted as an indicator of intense microbial activity (Drewes and Croue 2002). This strong presence of proteinaceous structures once again indicates the recent character (probably produced during the treatment) of urban DOM given that proteins are part of most biodegradable compounds (Kiikkilä et al. 2006; Ogawa et al. 2001; Wiegner and Seitzinger 2001). Although their elemental composition significantly evolves to a much higher N-content, and therefore more proteinaceous material, in the more hydrophilic HPI fraction, our Py-GC-data could not demonstrate any strong and systematic difference in the composition of the HPO, TPI and HPI fractions from treated effluent, except for DEHP, a highly hydrophobic contaminant, which was prominent in HPO fractions and almost from the other fractions. Some other components are present in the HPO fraction only (N12, LIP4 and LIP9), LIP4 and LIP9 are the heavier organic acids we could identified, their hydrophobic character is also an argument to explain their detection in the HPO fraction.

Analytical range: resisting the infinite complexity of DOM

Due to the sources and various processes affecting DOM, this organic mixture features great complexity. Whatever the used technique there are advantages but also limits mainly because of the limited analytical window or the need to extract DOM which could induce biased results. Strongest and weakest points of each analytical methods used in this study are summarised in Table 5. To better cope with this complexity, different techniques can be used depending mainly on the expected level of understanding. The use of such analytical ranges ensures also greater

Table 5 Advantages and limits of the used analytical techniques

Techniques	Interests	Advantages	Limits
DAX	Proportion of humic or hydrophobic versus hydrophillic	Allow extraction of DOM	Could change DOM and biased the next analysis
Elemental	Elemental composition	Total elemental composition of DOM, allow to see inorganic impurities	Need extraction
UV	Aromatic character	Fast and easy to measure	Limited information
FTIR	Chemical bonds and groups	Easy, lot of information on chemical groups, allow to see inorganic impurities	Need extraction
Isotopic	Origin of DOM	Signature of the origin of DOM and of the processes that affect DOM	Need extraction, difficulties to interpret data due to many processes that could affect DOM
SEC	Molecular weight	Size of the molecules	Affected by pH, ionic strengh, and charge for very low molecular weight
Py-GC-MS	Molecular composition	Lot of information, molecular identification, chemical structure and groups	Need extraction, could only show what is pyrolysed and chromatographable

reliability and insight into the DOM composition. For example, the use of Py-GC-MS has confirmed the presence of amines and amide groups already observed by FTIR and allows assigning a proteinaceous origin of N through elemental analysis measurement. The minor aromatic character, as described by the C/H ratio, observed through SUVA was once again confirmed by Py-GC-MS in the few presence of aromatic structures. Even though incorporation of these techniques one after the next may not only support but also strengthen each other, the DOM composition is still incomplete, especially regarding sulphur characterisation. Sulphur was indeed detected by elemental analysis and could be attributed to sulphonic groups by FTIR; however, the S-containing compounds and their origins remain poorly identified.

Conclusion

In conclusion, the analytical range used in this study offers a powerful tool for characterising DOM in urban water. The incorporation of information between one another may serve not only as a support, but as a reinforcement as well. The fraction from treated effluent and more broadly DOM from urban water (receiving treated effluent) display a strong hydrophilic nature (low HS content, low SUVA), along with a high degree of heterogeneity (as observed by SEC) and a low average molecular weight. Due to the origin of this DOM, proteinaceous structures constitute the main compounds. Such characteristics (i.e. heterogeneity, low average molecular weight and large functional groups, in particular N groups) could explain that DOM from treated effluent might have a strong reactive potential with metals and organic pollutants as it was previously demonstrated. Future work is needed to determine to what extent and under what conditions the DOM from treated effluent could bind metal ions.

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