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This study aims at evaluating the influence of a heavily urbanized area such as the Paris city area on receiving water contamination by bisphenol A (BPA) and alkylphenol ethoxylate by-product (APE). Firstly, concentrations in urban sources were investigated. Therefore, in addition to wastewater treatment plant effluents commonly studied, wet-weather urban sources including combined sewer overflows, urban runoff and total atmospheric fallout were considered. The first results highlight significant contaminations of all urban sources (from few ng/L in atmospheric fallout to several µg/L in other sources) with clearly distinguishable distribution patterns. Secondly, the concentration changes along the Seine River from upstream to downstream of Paris conurbation were investigated. While concentrations of BPA and nonylphenoxy acetic acid (NP_1EC) significantly increase because of urban sources, 4-nonylphenol concentrations are homogeneous along the Seine River. These results suggest a global dissemination of 4-nonylphenol at the scale of the Seine River basin. Finally, the relationship between pollutant concentrations and Seine river flow were studied upstream and downstream of the Parisian conurbation. As a result, the clear decrease of NP_1EC dissolved concentrations according to Seine River flow underlines the influence of punctual urban pollution on Seine River contamination. Conversely, 4-nonylphenol dissolved concentrations reinforce the hypothesis of its widespread at the scale of the Seine River basin.

Keywords: Nonylphenol, bisphenol A, urban sources, Seine River, atmospheric fallout, heavily urbanized area

Introduction

Among the pollutants commonly pointed out in the literature for their endocrine disrupting properties, alkylphenol ethoxylates (APE) and the 2,2-(4,4 hydroxydiphenyl) propane commonly named bisphenol A (BPA) deserve special attention because of their global spread in the environment (Staples et al. 1998; Vethaak et al. 2005). APE, mainly composed of nonylphenol ethoxylate (NPE: 80%) and octylphenol ethoxylate (OPE: 20%), are widely used for industrial and domestic applications, such as lubricating, oil additives, detergents and antistatic agents (Ying et al. 2002). A reasonable estimate of the world annual production of NPE is around 500,000 tons (Ying et al. 2002). BPA is mainly used as a monomer in the manufacture of polycarbonate plastics known for its high resistance to shocks and temperature (plastic windows or greenhouses, car bumpers, baby bottles), and in epoxy resins used as food contact lacquer for cans and tin cans. Due to its uses, the world production of BPA was evaluated at about 3,000,000 tons (Vandenberg et al. 2007)

While BPA is recognized as an endocrine disrupting chemical (Wetherill et al. 2007), NPE and OPE have been of rising concern because of their biodegradation by-products. Indeed, in urban or natural environment NPE can be biodegraded through oxidative or non-oxidative pathways (John and White 1998; Jonkers et al. 2001). Under oxidative biodegradation pathway, NPE turn into carboxylic acids such as nonylphenol acetic acid (NP_1EC) while under non-oxidative biodegradation pathway, short chain ethoxylates are produced such as nonylphenol diethoxylate (NP_2EO) and nonylphenol monoethoxylate (NP_1EO) (Giger et al. 2009). Finally, NP_1EC and NP_1EO can be biodegraded into 4-nonylphenol (4-NP) known as the most toxic and persistent by-products of NPE in the aquatic environment (Servos 1999).

The occurrence of APE and BPA in environment is closely correlated with anthropogenic activities. Many studies suggest that these chemicals preferentially enter into the environment through urban sources (Sharma et al. 2009; Ying et al. 2002). Among the urban sources investigated in the literature, the effluents of wastewater treatment plants (WWTP) are frequently studied (Hohne and Puttmann 2008; Loyo-Rosales et al. 2007; Voutsa et al. 2006; Zhou et al. 2010). On the contrary, studies on wet weather urban sources such as combined sewer overflows (CSOs) and urban runoff are less available, despite their high contamination (Björklund et al. 2009; Gasperi et al. 2008).

The Seine Basin, located in North-West part of France, drains approximately a 32,000 km² area from its headwaters to Paris. It can be considered as representative of river basins exposed to the impacts of intense human activity (Parisian conurbation: 12 million of inhabitants, among the thirty most populous cities in the world and the third in Europe after Moscow and London) (Meybeck et al. 2007). This basin combines strong anthropogenic pressures with a very limited dilution factor in the Seine River, due to its low flow rate (Seine River median flow at Paris: 350 m^3/s). Actually, no comprehensive data on the occurrence of APE and BPA are presently available for the upper part of the Seine basin. As far as we are aware, only few studies deal with the influence of such heavily urbanized conurbations (more than 10 millions of

inhabitants) on their environment (Isobe et al. 2001) and none of them focus on watershed of European cities.

As a consequence, the study tackles several objectives. The first objective is to assess the occurrence of APE and BPA concentrations and their relative significance in different urban sources of the Parisian conurbation such as the effluents of the major WWTP, the main CSO outfall and urban runoff. Secondly, this study aims at evaluating the influence of the Parisian conurbation on receiving water between upstream and downstream sampling sites. Finally, the last objective is to better examine the concentration variation of target compounds according to the hydrological conditions on upstream and downstream sites.

Materials and methods

Sampling sites / campaigns

Seine River water was collected at three locations (n=11 for each sampling location; Table 1) from February 2010 to February 2011. The first site, Marnay, is located upstream of the Parisian conurbation (weakly urbanized). The second site, Bougival, is located just downstream of Paris City (heavily urbanized). Finally, the last site, Meulan, is located downstream of all Parisian wastewater effluent discharges (heavily urbanized) (Fig. 1). In addition, the Orgeval River in an agricultural experimental catchment (Vilain et al. 2012) was also monitored during year 2011 (n=9) (Fig. 1). All samples were manually collected in 2 L glass amber bottles previously pyrolysed at 500°C to avoid sample contaminations.

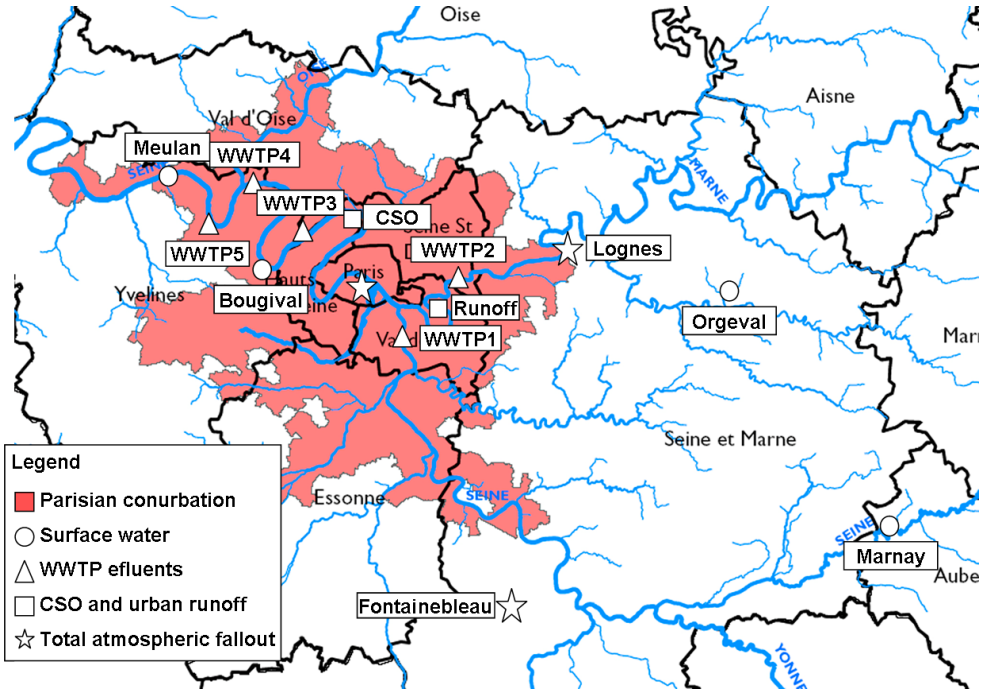

Fig. 1 Sampling sites within the Parisian conurbation

Urban sources investigated include WWTP effluents, total atmospheric fallout (TAF), urban runoff and CSOs. For WWTP effluents, the effluents of the 5 major WWTPs (WWTP1 to WWTP5 on Fig. 1) of the Parisian conurbation were monitored monthly from January 2011 to November 2011 (n=9). These WWTPs are supervised by the Parisian public sanitation service (SIAAP) and treat more than 2 millions m^3 of wastewater every day (70% of the Parisian conurbation inhabitants). Samples were collected in 2L glass amber bottles also pyrolysed at 500°C. Among these five WWTPs, three different processes of wastewater treatment are considered. Firstly, the WWTP1 use conventional activated sludge (AS) for carbon and nitrogen removal (AS-WWTP), WWTP4 use activated sludge for carbon removal and biofiltration (B) for nitrogen removal (ASB-WWTP), finally the more recent WWTP2, WWTP3 and WWTP5 use biofiltration units for both carbon and nitrogen removals (B-WWTP). The removal efficiencies on organic matter (evaluated with the Chemical Oxygen Demand: COD) and nitrogen (nitrification: Total Kjeldahl Nitrogen (TKN), denitrification: total nitrogen (TN)) are disclosed in Table 1. According to these efficiencies, WWTP1,2,3 and 5 reveal high removals for organic matter and nitrogen. Conversely, WWTP4 which is the largest WWTP of the Parisian conurbation and one of the biggest in the world (more than 1,600,000 m^3 of wastewater treated every day) highlights lower efficiencies for organic matter (COD: 85%), nitrogen (TKN: 70%; TN: 20%) and SS (90%). Measures for the modernization of this WWTP are under way to improve its efficiency for carbon and nitrogen removals.

Table 1 sampling site characteristics

Sample	n	period	comments / details
Orgeval River	9	Mar 2011 – Nov 2011	Upstream site ➔ Agricultural watershed Manually collected in 2 L glass bottles
Seine River	11	Feb 2010 - Feb 2011	3 stations (Marmay, Bougival, Meulan) increasing urbanization Manually collected in 2 L glass bottles
WWTP effluent	11	Jan 2011 – Nov 2011	**WWTP x (vol): process efficiency COD, NTK, TN, SS** WWTP 1 (385,000 m^3/d):AS, 96% , 97%, 74%, 98% WWTP 2 (48,000, m^3/d): B, 93%, 95%, 86%, 97% WWTP 3 (233,000 m^3/d): B , 95%, 96%, 70%, 98% WWTP 4 (1,612,000 m^3/d):AS + B, 86%, 70%, 21%, 91% WWTP 5 (92,000 m^3/d): B , 94%, 92%, 82%, 97%
CSO	8	June 2010 - Nov 2010	volume per event: 35,000 to 1,000,000 m^3 automatic samplers 12 bottles ➔ Weighted mean samples
Total atmospheric fallout	20	Feb 2011 - Nov 2011	3 sites (Paris City, Lognes, Fontainebleau) Total atmospheric fallout (dry + wet) (on 15 days)
Urban runoff	4	July 2011 - Oct 2011	samples collected at Sucy-en-Brie outfall, separated sewer automatic samplers 12 bottles ➔ Weighted mean samples

In addition to WWTP effluents, urban sources during wet weather period were also sampled, i.e. combined sewer overflows (CSOs) and urban runoff in separate sewer. CSOs were sampled at Clichy outfall, one of the biggest outfalls of the Parisian conurbation, for 8 events (discharged

volumes ranging from 35,000 m^3 to more than 1,000,000 m^3) (Fig. 1). Urban runoff was sampled at the outlet of small sub-urban catchment (Sucy-en-Brie) for 4 rain events (Fig. 1). For CSOs and runoff, samplings were performed by an automatic sampler. Flow-weighted composite samples representative of the entire discharges were considered for analyse. At last, TAF were collected at Paris city (heavily urbanized; n=10), Lognes (weakly urbanized; n=5) and at the Fontainebleau forest (n=5) in 10 L glass bottles fitted with 1 m² aluminium funnels (Fig. 1) during 7 periods (15 days) between January and November 2011.

Analytical procedure

Sample preparation

Bisphenol A (BPA), 4-nonylphenol (4-NP), nonylphenol mono- and diethoxylate ($NP_{1\&2}EO$), nonylphenol acetic acid (NP_1EC), 4-tert-octylphenol (4-t-OP) and octylphenol mono- and diethoxylate ($OP_{1\&2}EO$) were analyzed in dissolved and SS. After filtration (GF/F, Whatman), 100 mL of dissolved phase for CSOs or 250 mL for all other water samples were spiked with a surrogate standard mixture (BPA-d6, NP_1EO-d2, OP-d17) before extraction.

For dissolved phase, samples were extracted by solid phase extraction (Autotrace SPE Workstation, Caliper LifeScience) using OASIS® HLB cartridges (200 mg, 6 mL). After conditioning with 10 mL of methanol and 10 mL of ultrapure water, dissolved samples were extracted at 5 mL/min, under neutral pH. After drying, elutions were performed with 12 mL of a mixture methanol (MeOH)/Dichloromethane (DCM)/Ethyl acetate (Etace) (40/40/20, v/v).

After freeze-drying, the SS were extracted by microwave assisted extraction using a multiwave 3000 (Antonn Paar). The filters, were extracted using 20 mL of MeOH/DCM (60/40, v/v) mixture at 100°C and 7 bars during 30 min. After extraction, samples were cleaned up by SPE using OASIS® HLB (200 mg, 6 mL). Basically, after conditioning (3 mL MeOH and 3 mL MeOH/H_2O (20/80 v/v), the samples dissolved in 500 µL of MeOH/H_2O (50/50 v/v) mixture, were deposited on the top of the cartridges. Then, the cartridges were washed with 3 mL of H_2O prior to elution with 9 mL of MeOH/DCM/Etace (40/40/20, v/v/v) mixture.

UPLC-MS-MS analysis

Before analysis, both extracts were concentrated and spiked with internal standard for quantification (BPA-d16, n-NP and n-NP_1EO). The analysis was performed by liquid chromatography coupled to a tandem mass spectrometry, LC-MS-MS (AQUITY UPLC / TQD, Waters). APEOs and BPA were separated on an AQUITY UPLC / BEH C_{18} column, heated at 40 °C, with ultrapure water (A) and methanol (B) each containing 4.5 mM NH_4OH. Equilibration takes place with 50 % B at 0.4 mL/min and 10 µL of sample were injected. The mass spectrometer is equipped with an electrospray interface used in positive ionization mode (ESI+) for $NP_{1\&2}EO$ and $OP_{1\&2}EO$ and negative ionization mode (ESI-) for all other compounds. The compounds were detected in multiple reactions monitoring mode (MRM) with two mass transitions.

Analytical variability

In order to assess the variability of the analytical protocol, three surface water samples from the same sampling location (downstream of WWTP 4) were individually analyzed. This variability was only studied for the dissolved phase extraction and UPLC-MS-MS analysis and not for the suspended solids. Actually, in receiving water, alkylphenols and BPA are mostly present in the dissolved phase (4-NP: 80%; 4-t-OP: 90% and BPA: 95%; Cladiere et al. 2010; Isobe et al. 2001). The dissolved phases of the three water samples were extracted in triplicates and each extraction was also analyzed in triplicates. Thus, nine concentration values were obtained for each sample and every target compound. The nine values were compared to the median in order to calculate relative deviations that are representative of the analytical variability. Finally, the absolute values of relative deviations generated for the three samples (9x3=27) were represented on Fig. 2 as histograms for BPA, 4-NP and NP_1EC.

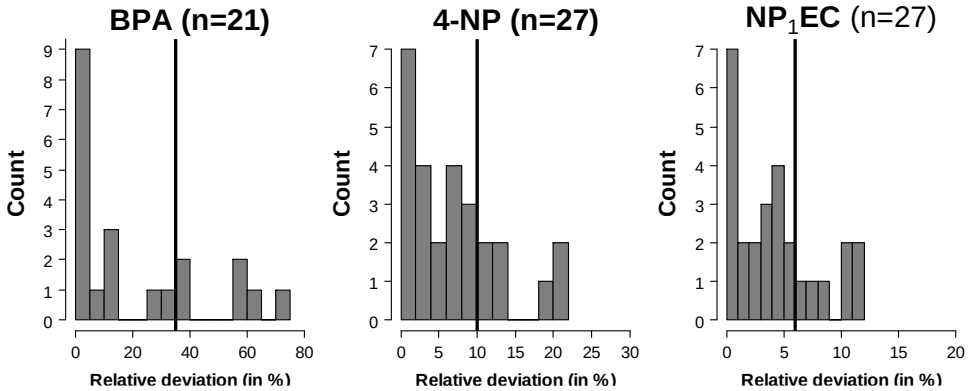

Fig. 2 Distribution histograms of relative deviations of BPA, 4-NP, and NP_1EC to the median value. The bold lines represent the third quartile of the relative deviations.

For 4-NP, NP_1EC and NP_2EO, the relative deviations globally lie within the 0 - 15% range while maximum values reach 20% for 4-NP. Thus, in order to account 75% of the relative deviation values (bold lines), the analytical variabilities (±AV) of 4-NP, NP_1EC and NP_2EO were respectively estimated at 10%, 6% and 13%. For BPA and NP_1EO, the relative deviations were calculated only for 21 analyses since their concentrations were close to their limit of quantification (LOQ: BPA: 11 ng/L; NP_1EO: 10 ng/L). Similarly to previous compounds and in order to account 75% of the relative deviation values (bold lines), the analytical variabilities of BPA and NP_1EO were respectively evaluated at 35% and 31%. For OPE, the analytical variabilities were not evaluated since these compound concentrations were mostly under their limit of quantification (4-t-OP: 4 ng/L; OP_1EO: 12 ng/L and OP_2EO: 3 ng/L) in investigated surface water.

Data exploitation

The concentrations presented in this study are total concentrations (expressed in ng/L) calculated by summing dissolved concentrations (ng/L) and SS concentrations (ng/L). For the variations of concentrations with hydrological conditions, only the dissolved phase was considered in order to better assess the origins of target compounds (punctual or diffuse sources) within the Seine River.

Results are displayed in box plots which extend from the 1st quartile (Q1) to the 3rd quartile (Q3) and thus contain the central half of the distribution score. Dotted lines symbolize 1.5 times the interquartile range while outliers are indicated as points above or below the dotted lines.

Since concentration distributions of each sampling point cannot be assumed to be normally distributed; non parametric tests were performed to compare concentrations found on different sites. The Wilcoxon signed-rank test was selected to compare the Marnay, Bougival and Meulan sites since samplings were collected on the same day at the three sites. For the same reason, the Wilcoxon signed-rank test was also used to compare the WWTP effluents. For all other matrices and sampling sites (TAF, CSO, runoff and Orgeval), the Mann-Whitney U test was selected to compare distributions of different sizes. In accordance with the analytical variabilities, statistical significance was accepted at $p < 0.1$ for all comparisons.

Results and discussions

Urban sources

Wastewater treatment plants

Results found in WWTP effluents for BPA and NPE are illustrated in Fig. 3. Three groups corresponding to AS-WWTP, ASB-WWTP and B-WWTP as described earlier are considered.

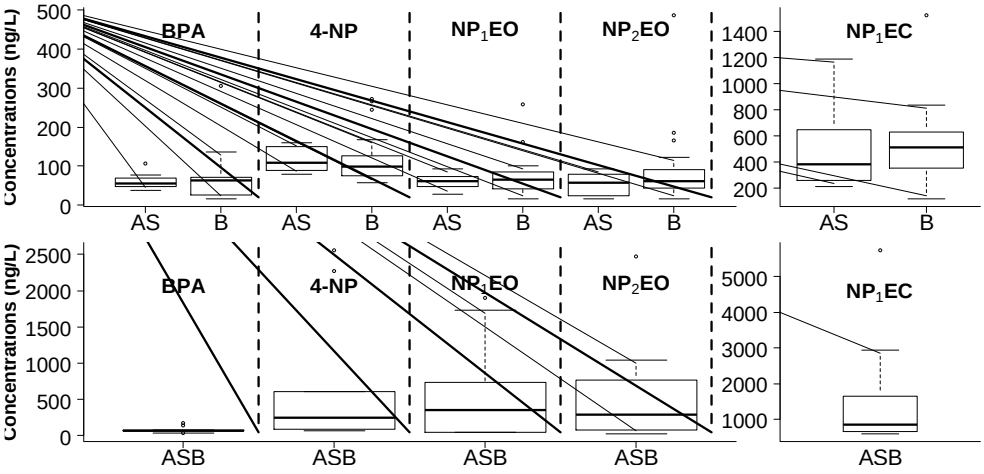

Fig. 3 Concentrations of target compounds (ng/L) in WWTP effluents. AS: activated sludges; B: biofiltration; ASB: activated sludge (carbon removal) + biofiltration (nitrogen removal)

For AS-WWTP and B-WWTP considered, the median concentrations (±AV) of effluents were evaluated at 67(±24) ng/L for BPA, 114(±12) ng/L for 4-NP, 74(±23) ng/L for NP_1EO, 74(±9) ng/L for NP_2EO and 573(±34) ng/L for NP_1EC. For ASB-WWTP (WWTP4) the median concentrations (±AV) found in the effluent were higher and evaluated at 76(±27) ng/L for BPA, 244(±24) ng/L for 4-NP, 304(±109) for NP_1EO, 295(±38) ng/L for NP_2EO and 861(±62) ng/L for NP_1EC. The OPE were less concentrated (<54 ng/L) and only account for 10% of target alkylphenols in accordance with their use distributions (Ying et al. 2002). Globally, these median

concentrations are similar to those reported in the literature in Europe (Hohne and Puttmann 2008; Jonkers et al. 2009), USA (Loyo-Rosales et al. 2007), China (Zhou et al. 2010) and in a previous study in the Parisian conurbation (Gilbert et al. 2012).

The WWTP effluents were characterized by the predominance of NPE (4-NP and NP_1EC) comparatively to BPA. The low concentrations of BPA in WWTP effluents (medians being 76(±27) ng/L for ASB-WWTP and 67(±24) ng/L for B-WWTP and AS-WWTP) result from the high efficiency of wastewater treatment and the high BPA biodegradation during wastewater treatment processes (about 90% according to Zhou et al. (2010)). Concerning NPE, the main by-product of nonylphenol ethoxylate surfactants found in all WWTP effluents is NP_1EC, median concentrations being 861(±39) ng/L for ASB-WWTP and 512(±31) ng/L for B-WWTP and AS-WWTP. The NP_1EC has already been claimed as the major by-product of NPE during wastewater treatment processes essentially based on aerobic biodegradation (Ahel et al. 1994). In contrast to results reported by Loyo-Rosales et al. (2007), but in accordance with those quoted by Hohne and Puttmann (2008) no seasonal trend of concentrations could be drawn for all WWTP effluents from January 2011 to November 2011.

By comparing the AS-WWTP and B-WWTP effluents (Wilcoxon signed-rank test), no significant differences of concentrations appear for all target compounds ($p > 0.1$). The ASB-WWTP effluent does not exhibit any statistical difference compared to AS-WWTP and B-WWTP effluent for BPA and 4-NP ($p > 0.1$). Conversely, for NPE by-product (NP_1EC, NP_1EO and NP_2EO) significant differences were found between ASB-WWTP and AS-WWTP + B-WWTP effluents ($p < 0.05$). The higher concentrations found in ASB-WWTP effluents can be certainly explained by the lower biodegradation rate on organic and nitrogenous matter (COD: 86%, NTK: 70%, TN: 21%) and to a lesser extent by the higher concentration of SS (23 mg/L for WWTP4, against 5 and 10 mg/L for other WWTPs).

Urban sources during wet weather period

Concentrations found in CSOs, runoff and TAF are exhibited in Table 2.

Table 2 Total atmospheric fallout, CSO and runoff concentrations (ng/L)

Compound	TAF n=20 (ng/L) min-max (median)	CSO n=8 (ng/L) min – max (median)	Runoff n=4 (ng/L) min – max (mean)
BPA	10 – 180 (40)	917 – 2098 (1410)	287 – 1224 (635)
4-NP	<LQ[1] – 167 (80)	445 – 1208 (668)	272 – 533 (400)
NP_1EO	<LQ[1] – 65 (21)	357 – 1257 (598)	47 – 401 (184)
NP_2EO	<LQ[1] – 301 (10)	138 – 401 (250)	146 – 847 (402)
NP_1EC	2 – 63 (15)	220 – 591 (251)	193 – 426 (128)

[1] : <LQ: lower than limit of quantification

The concentrations reported in the Table 2 disclose significant differences between TAF, on the one hand, and CSOs and runoff on the other hand ($p < 0.01$). For example, while concentrations of BPA range between 10(±4) and 180(±63) ng/L in TAF, concentrations vary between 917 and 2098 ng/L (about 32 times higher) in CSOs and between 287 and 1224 ng/L (about 13 times

higher) in runoff. In addition, changes of pattern are clearly noticeable in Table 2. Indeed, while 4-NP has the highest median concentration in TAF (80(±8) ng/L), BPA is the predominant compound in CSOs and runoff (CSO: 1410(±495) ng/L; runoff: 635(±222) ng/L).

TAF concentrations are generally low. Minimal concentrations are lower or close to the limits of quantification whereas median concentrations are always lower than 100 ng/L (Table 2). The non parametric Mann-Whitney U test, performed for the three sampling locations, points out that the concentrations of BPA are statistically greater in Paris (heavily urbanized) and in Lognes (weakly urbanized) than in Fontainebleau (forest) while no significant difference between the three sites is found for NPE.

Higher concentrations in CSOs and runoff compared to those measured in TAF highlight a clear enrichment from atmosphere to catchment outlet. These enrichments could originate from wastewater for CSOs or road and buildings leaching (Björklund et al. 2009; Bressy et al. 2011) for runoff since NPE and BPA are used in building material (concrete, plastics). For both CSOs and runoff, the concentrations measured were close to those reported in untreated wastewaters (Zhou et al. 2010). These high concentrations underline necessity to treat wet weather effluents such as CSOs and runoff in order to avoid short-term pollutions of receiving water during wet weather periods especially for BPA.

Comparing CSOs and urban runoff, BPA, 4-NP and NP_1EO revealed significant differences of concentrations while NP_1EC and NP_2EO concentrations were statistically equivalent. Although these results suggest that the wastewater contained in CSOs may play a role in 4-NP, BPA and NP_1EO concentrations, no correlation could be drawn between wastewater proportion and total concentrations of compounds in CSOs ($R^2 < 0.25$ for all investigated compounds). However, a good correlation between maximum flows discharged by CSOs (Q_{max}: $m^3.s^{-1}$) and particulate contents could be drawn for all target compounds ($R^2 \approx 0.80$). The higher the Q_{max}, the higher the particulate contents. These results may confirm the existence of in-sewer sources such as sewer deposit erosion during high-intensity events. For some events, Gasperi et al. (2010) demonstrated that in-sewer deposit erosion can actually play a major role in the polycyclic aromatic hydrocarbon or suspended solid contaminations of CSOs.

Surface water

Concentration change in relation to urbanization gradient

In order to track the influence of urban sources (WWTPs, CSOs and runoff) on the Seine River, the dissolved organic carbon (DOC) concentrations in Marnay, Bougival and Meulan were analysed in addition to target compounds. As expected, whatever the measurement campaign, a significant increase of DOC concentrations was noticed between Marnay (median: 2.0 mgC/L), Bougival (2.8 mgC/L) and Meulan (3.3 mgC/L) (Wilcoxon signed-rank test; $p < 0.05$).

At the scale of all investigated sites, the concentrations ranged from < 11 ng/L to 154(±54) ng/L for BPA, from 28(±3) ng/L to 157(±16) ng/L for 4-NP and from 2.1(±0.1) ng/L to 274(±16) ng/L for NP_1EC. These concentrations found in surface water within the Parisian conurbation were similar to concentrations reported in Europe (Brix et al. 2010; Jonkers et al. 2010), but lower than

in the Seine River estuary (4-NP: 168 ng/L and NP_1EC: 544 ng/L; Cailleaud et al. 2007). Concerning the upstream sites Orgeval (representative of rivers not influenced by urban activities) and Marnay (weakly urbanized) the concentrations of target compounds generally lie close to concentrations found in TAF. As a result, these sites could be considered as representative of the background pollution of the Seine River basin by target compounds. Moreover, the environmental quality standards established by the directive 2008/105/EC (European commission 2008) (4-NP: 300 ng/L; 4-t-OP: 100 ng/L) were never exceeded in the Seine River since the highest concentrations found in Meulan (the most impacted site) were 157 ng/L for 4-NP and 30 ng/L for 4-t-OP.

Total concentrations are represented according to the urbanization gradient (from agricultural site to heavily urbanized areas) on Fig. 4.

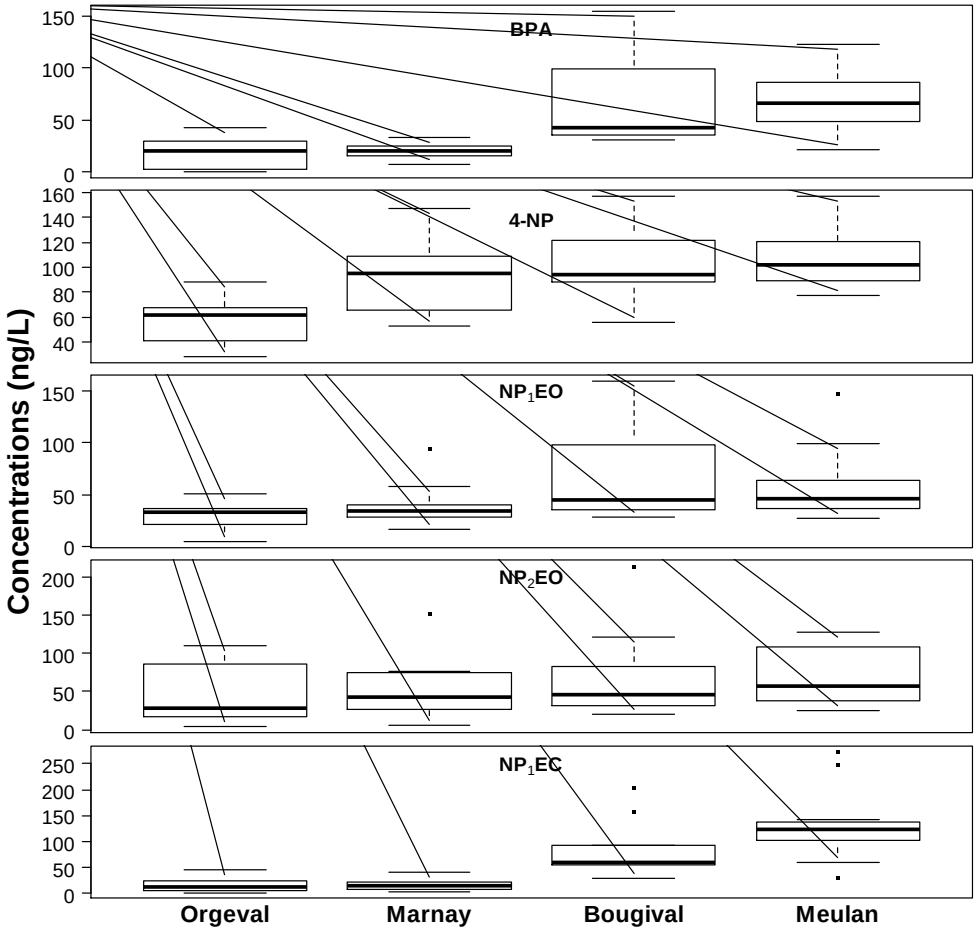

Fig. 4 Change of NPE and BPA concentrations according to urbanization gradient

Three groups of compounds may be distinguished.

In the first group including BPA and NP_1EC, significant differences ($p < 0.05$) of concentrations between upstream sites (medians at Orgeval and Marnay: BPA: 20(±7) ng/L; NP_1EC: 16(±1) ng/L) and downstream sites (Bougival and Meulan BPA: 66(±23) ng/L; NP_1EC: 124(±7) ng/L) are noticed (Fig. 4). A similar evolution of NP_1EC concentrations from upstream to downstream stations of the Glatt River have been pointed out by Jonkers et al. (2009). These significant differences underline the influence of urban sources on receiving water for BPA and NP_1EC.

However, while Bougival and Meulan sites exhibit similar concentrations of BPA, a significant difference could be noticed for NP_1EC between both sites ($p < 0.05$). This difference is linked to the large volume discharged by WWTP4 coupled with high concentrations of NP_1EC (median: 861±51 ng/L). Thus, whereas the NP_1EC was the lowest NPE by-product in upstream sites, it becomes predominant at Meulan. Similarly, the prevalence of NP_1EC on a large range of organic pollutants in the Danube River has been reported by Loos et al. (2010).

The second group of compounds (NP_1EO and NP_2EO) reveals no significant difference of concentrations between all sites (NP_1EO: 45(±14) ng/L and NP_2EO: 50(±6) ng/L). The concentrations found in the Orgeval River (agricultural basin) are statistically similar to those found at Meulan site ($p > 0.5$). A large variation of NP_1EO concentrations is observable at Bougival site (min: 20(±6) ng/L; max: 213(±66) ng/L) (Fig. 4). The highest concentrations found at Bougival could be the consequence of the activities generated by Gennevilliers Harbour (the greatest of Île-de-France) located upstream of the sampling site.

Finally, the last group (4-NP and 4-t-OP) reveals a contrasted pattern. A significant difference ($p < 0.05$) of concentrations between Orgeval River (agricultural basin) and Seine River sites (Marnay, Bougival and Meulan) could be noticed on Fig. 4. The occurrence of 4-NP in the Orgeval River (median: 61(±6) ng/L) could be imputed either to mixture of diffuse sources such as TAF (median: 80(±8) ng/L) and groundwater (Latorre et al. 2003), and/or to agricultural activities (Zgola-Grzeskowiak et al. 2009). Conversely, Marnay site could be partially impacted by urban sources and by biodegradation of precursors (NP_1EC, $NP_{1\&2}EO$) into 4-NP along the Seine River. Nonetheless, no statistical difference between Marnay (weakly urbanized), Bougival and Meulan (heavily urbanized) sites could be drawn ($p > 1$). This homogeneity of concentrations along the Seine River suggests that urban sources are probably not predominant sources of 4-NP on receiving water. Thus, diffuse sources such as TAF, groundwater and biodegradation of 4-NP precursors could play a key role in the occurrence of 4-NP in the environment and the homogeneity of its concentrations along the Seine River. This observation is in agreement with a previous study reporting the same observation on the Glatt River in Switzerland (Jonkers et al. 2009).

The concentrations found in the Seine River are far lower than concentrations leading to an acute lethal effect on aquatic wildlife (lethal concentration (LC50) for rosy barb: 344 µg/L; Bhattacharya et al. 2008). However, these authors have claimed that concentration closer to those reported in the Seine River could lead to histopathological effects on gills, liver and kidney of the same fish. In addition Brian et al. (2007) have highlighted the mixture effects of 4-NP, 4-t-OP and BPA. They have reported that the mixture of these three compounds has a greater estrogenic effect on fish's reproduction than each compound separately. Therefore, even the low concentrations found in the Seine River may have chronic effects en aquatic wildlife.

Variation of pollutant concentrations according to hydrological conditions

The dissolved concentrations of BPA, 4-NP and NP_1EC measured at Marnay and Meulan were compared to the Seine River flow on the Fig. 5. During the sampling period, from February 2010 to February 2011, the Seine River flow ranged from 13 to 228 m^3/s at Marnay and from 157 to

1050 m^3/s at Meulan. Only the dissolved phase was investigated since the distributions of BPA and NPE between dissolved and particulate phases are mostly in favour of the dissolved phase as previously mentioned. The prevalence of the dissolved phase could be imputed to the low SS concentrations in the Seine River (from 6 mg/L to 20 mg/L at Meulan). Furthermore, and depending on the group of compounds, the relationship between the dissolved concentrations and the Seine River flow enables to better understand the origins (spot or diffuse sources) of NPE and BPA in the Seine River.

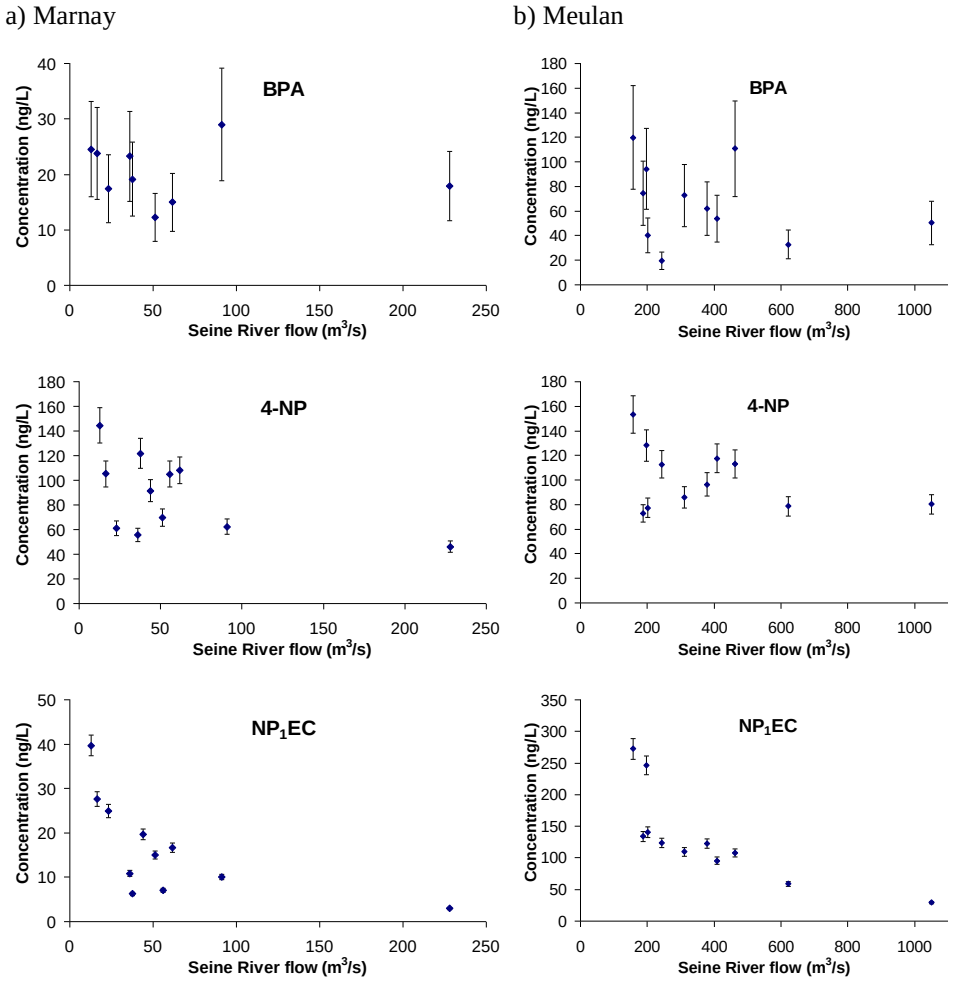

Fig. 5 Dissolved concentrations of target compounds (ng/L) versus Seine River flow (m^3/s) at Marnay (a) and Meulan (b)

The first pattern, found for NP_1EC, shows a clear decrease of concentrations according to Seine River flow increase suggesting that the NP_1EC comes from constant and punctual sources diluted by the Seine River. This hypothesis is reinforced by the increase of NP_1EC concentrations according to the urbanization gradient as mentioned before. Therefore, NP_1EC probably mostly originates from punctual urban sources such as WWTP effluents. While NP_1EC found at Meulan could originate from the urban sources of the Parisian conurbation, the concentrations found at Marnay are probably impacted by Troyes City (about 60,000 inhabitants) located about 50 km upstream of Marnay site.

The second pattern, as reflected by the 4-NP and NP_2EO trends, shows a decrease of concentration according to Seine River flow increase. However, this decrease of concentration is significantly less marked than the trend observed for NP_1EC. While the ratios between the highest and the lowest concentrations of NP_1EC are about 14 and 10 respectively at Marnay and Meulan, the ratios for 4-NP do not exceed 3 at Marnay and 2 at Meulan. Thus, the influence of the punctual urban sources could be partially hide by the global contamination of the Seine River basin by 4-NP. This global contamination of the Seine River probably originates from the large uses of NPE since 1960 and the persistence of 4-NP in the environment. Consequently, the diffuse sources mentioned before (TAF and groundwater) as well as biodegradation of precursors (NP_1EC, $NP_{1\&2}EO$ originate from urban sources) along the Seine River probably avoid high decreases of concentration according to hydrological conditions (lowest concentrations: 46 ng/L at Marnay and 73 ng/L at Meulan).

The last pattern, including BPA and NP_1EO, is difficult to interpret since the analytical variabilites of these two compounds are important and do not enable to draw any relationship between dissolved concentrations and Seine River flow. Therefore, it is impossible to conclude on the predominance of punctual urban sources or diffuse sources for BPA and NP_1EO. Nonetheless, in the case of BPA, the two highest concentrations at Meulan (111 ng/L in January 2010 and 120 ng/L in July 2010) were found consecutively to rainy periods with large CSO discharges (January: 250,000 m^3 dumped, July: 990,000 m^3 dumped). Consequently, the wet weather urban sources such as CSOs or urban runoff discharged into the Seine River probably play a key role on short-term contamination of the Seine River by BPA.

Conclusions

This study aimed at investigating the APE and BPA in urban sources and evaluating the impact of a heavily urbanized area such as the Parisian conurbation on the receiving water. Hence, urban sources such as WWTP effluents, combined sewer overflows, urban runoff and total atmospheric fallout were studied. Depending on the urban sources considered, differences of concentrations and distribution patterns appear. WWTP effluents are featured by the predominance of NPE, especially NP_1EC, due to aerobic biological treatments of wastewater. No seasonal trend of concentration as well as no significant difference of concentration according to the biological treatment of wastewater was noticed. Only the effluent of WWTP4 (one of the biggest WWTP in the world) revealed higher concentrations of NPE and BPA due to lower efficiencies on carbon and nitrogen removals than the other WWTPs. Conversely, wet weather urban sources (CSOs and runoff) are dominated by BPA probably due to wastewater contribution and leaching of building materials.

In this study, a significant influence of the Parisian conurbation on NP_1EC and BPA concentrations along the Seine River (respectively from 17(±1) ng/L to 124(±7) ng/L and from 20(±7) ng/L to 66(±23) ng/L) was observed. For all other NPE, no significant evolution of concentrations from upstream to downstream sites was noticed suggesting that urban sources of

the Parisian conurbation do not have major effect on receiving water and underlining the existence of diffuse sources at the scale of the Seine River basin. Finally, the variation of dissolved concentrations according to the Seine River flow at Meulan and Marnay sites exhibits the importance of spot urban sources for NP_1EC, and reinforces the hypothesis of the global dissemination of 4-NP at the scale of the Seine River basin. Unfortunately, the cases of BPA and NP_1EO could not be interpreted because of their high analytical variabilities.

In order to better understand the homogeneity of 4-NP concentrations along the Seine River as well as the dynamic of NPE within the Seine River, a further study should be carried out to asses the biodegradation rates of these compounds in receiving water. Finally, the diffuse sources probably linked to river flow origins have to be clearly identified and better assessed in order to understand the widespread of NPE and BPA at the scale of the Seine River Basin.

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