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CHEMICAL, MICROBIOLOGICAL, SPATIAL CHARACTERISTICS AND IMPACTS OF CONTAMINANTS FROM URBAN CATCHMENTS: CABRRES PROJECT

Variation of raw wastewater microbiological quality in dry and wet weather conditions

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Abstract The microbiological quality of urban wastewaters presents important environmental, sanitary, and political challenges. However, the variability of untreated wastewater quality is seldom known when it comes to microbial parameters. This study aims to evaluate the variability of microbiological quality in wastewater influents from different wastewater treatment plants connected to combined and partially separate sewer networks in the Parisian area and to evaluate the impact of this variability on the treatment efficiency and on the accuracy of wastewater effluent monitoring. The densities of fecal indicator bacteria (FIB), *Escherichia coli* and intestinal enterococci, and their partitioning on settleable particles were analyzed at the inlet of two wastewater treatment plants during dry weather (130 composite samples and 7 days sampled every 2 hours) and storm events (39 composite samples, and 7 rain courses) from 2008 to 2012. The results showed that fecal indicator densities vary according to the network characteristics and according to the meteorological conditions. During storm events, a significant dilution of *E. coli* and enterococci was observed, as well as a decrease in the settleable fraction of *E. coli* during the maximal impact of the storm. However, storm events did not significantly impact the regular FIB monitoring. FIB removals by primary and secondary treatment were significantly correlated with FIB densities in influent wastewater; however, meteorological conditions also influenced the removal of FIB.

Keywords Temporal and spatial variability · *Escherichia coli* · Intestinal enterococci · Fecal indicator bacteria · Storm event · Raw wastewater

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Introduction

Urban wastewater treatment plants (WWTPs) were originally designed to reduce the biological oxygen demand, total suspended solids (TSS), and nitrogen and phosphorus pollution. In general, the removal of pathogenic microorganisms has received less attention (Kay et al. 2007). Primary and secondary treatments are able to remove up to 99 % of fecal indicator bacteria (FIB) (Servais et al. 2007). Depending on the influent FIB concentrations, this extent of removal could be insufficient to achieve the quality required to use treated wastewaters for irrigation purposes and for allowing recreational activities in the receiving water bodies (Blumenthal et al. 2000; Zanetti et al. 2006). In order to obtain treated effluents safe for reuse, the addition of a final disinfection treatment (such as UV irradiation) or other advanced tertiary treatments may be necessary. The microbial standard required and the amount and type of wastewater treatment needed will depend on the final use of the effluent and legal requirements (Blumenthal et al. 2000). Disinfection treatments can significantly increase pathogen removal, but they are relatively costly and most WWTPs in Europe are not equipped with this kind of process (Bixio et al. 2005; Kristensen 2013). As a consequence, WWTP effluents can represent an important source of pathogens for natural water bodies (Wery et al. 2010). Improving our knowledge of the efficiency of treatment processes against microbiological contamination is thus necessary at present considering the current environmental and political contexts.

In Europe, the Water Framework Directive 60/2000/EU requires the establishment of management programs in order to insure the proper ecological status of water masses. It is of crucial importance to maintain appropriate water quality, at least in areas where water represents an important resource (water intake for drinking water production, recreational and bathing waters). The EU Water Framework Directive is accompanied by the Urban Waste Water Directive (EC 91/271) which defines the quality of treated effluents from WWTPs; however, microbiological parameters are not provided in this directive (CEU 1991, 2000; Blume and Neis 2004). With respect to the microbial quality of surface waters, there are only regulations for bathing water areas (European Union (EU) 2006). In light of this lack of regulation, not only the knowledge of pathogen concentration released by the different WWTPs is essential for restoring the quality of surface waters and for sanitary security, but also a better knowledge of the microbiological quality of untreated wastewaters is necessary.

Indeed, on one hand, WWTP efficiency and management could depend on the characteristics and the variability of influents. On the other hand, the characterization of untreated wastewater is important to evaluate the impact of combined sewer overflow (CSO) during intense storm events (Garcia-Armisen and Servais 2007; Kay et al. 2007; Passerat et al. 2011). The volume of mixed domestic effluents and rainwater circulating in combined sewer systems during storm events can sometimes exceed the capacity of the WWTP, and the excess volume is discharged into the receiving water bodies without any treatment (CSOs). As a consequence, samples of WWTP influents during storms can be considered as surrogates for CSOs and can provide useful information on the quality and potential impact of CSOs on aquatic ecosystems. Although CSOs can severely impact the microbial quality of the receiving surface waters, there are few available data in the scientific literature concerning the dynamics of fecal indicators or pathogens in CSOs (e.g., Passerat et al. 2011; Madoux-Humery et al. 2013). Most published papers deal with urban runoff and rivers (e.g., Auer and Niehaus 1993; Mahler et al. 2000; Characklis et al. 2005; Jeng et al. 2005; Krometis et al. 2007). In the rivers, FIB densities are usually 10 to 1,000 times higher during storm events as compared to dry periods, depending upon the storm intensity and the watershed characteristics (Characklis et al. 2005; Jeng et al. 2005; Krometis et al. 2007). Previous studies on rivers also found that the settleable fraction of FIB increased during the rain events (Jeng et al. 2005; Characklis et al. 2005). Generally, during storm events, chemical oxygen demand and TSS concentration increase in the sewers with regards to dry weather situations, whereas NH_4^+ and other dissolved pollutants decrease (Hurst et al. 2004). Runoff water input inside the combined sewers can also generate an important dilution of FIB densities (Passerat et al. 2011). As a consequence, studying FIB in raw wastewaters during wet weather would also provide valuable information on the variability range of these indicators within various matrices and meteorological conditions.

However, there are few detailed studies presenting the microbiological characterization of WWTP raw influents. Most of the published literature deals only rarely with the study of raw WWTP influents and generally focuses on treated waters quality, removal efficiencies, surface water contamination by WWTP effluents, or microbial diversity in activated sludges (e.g., Horan et al. 2004; Chen et al. 2004; Sahlström et al. 2004; Garcia-Armisen and Servais 2007; Liu et al. 2007; Wery et al. 2008; Samie et al. 2009; Foladori et al. 2010; McLellan et al. 2010; Ye and Zhang 2013). Currently, some fecal indicators (*Escherichia coli* and intestinal enterococci) are quantified in routine monitoring to evaluate the impact of urban effluents on surface water quality. For Instance in Australia and New Zealand, the Australian guidelines for sewerage systems (ARMCANZ, ANZECC 1997) specify the frequency and the procedure for monitoring the microbial quality of WWTP effluents and provide the required microbial standards for discharge in coastal and inland waters. Moreover, guidelines have been established by a number of countries to regulate the levels of FIB and the treatment requirements for safe wastewater reclamation and reuse (Blumenthal et al. 2000). Without some type of microbial monitoring, the performance of the required treatment processes cannot be assessed properly (Gerba and Rose 2003). How the spatial and temporal variation of raw sewage quality influence the accuracy of routine monitoring is a question that has been addressed rarely, although it could have potential impact on WWTP management.

This paper presents new insights on wastewater microbiological quality, with a substantial database analysis (monthly monitoring during dry and wet weather between 2008 and 2012 in two WWTPs). The originality of the paper resides in its focus on the microbial quality of raw wastewaters under dry and wet weather conditions and in considering the impact of the variability of this quality on WWTP monitoring and efficiencies regarding microbial removal. Some WWTPs can adapt their setup to storm conditions in order to increase their treatment capacity, particularly by increasing the number of process units. However, wastewaters do not only vary in terms of quantity but also in terms of quality, and these fluctuations may impact the performance of the treatments. This aspect has been seldom considered, particularly microbiological contaminant removal. In order to characterize the spatial and temporal variations of the concentration of *E. coli* and intestinal enterococci in raw wastewaters, two WWTPs receiving influents from contrasting Parisian sewer networks were selected for the study. The analysis was conducted over a large temporal range: from fine scale (daily and during storm events) to a larger scale (intra- and interannual).

This unique data set was collected and analyzed with several novel objectives: (1) to assess the dynamic behavior of the FIB during dry weather conditions and hydrological events, (2) to evaluate the impact of rain events on performance and treatment efficiency of WWTP regarding bacterial removal, and (3) to evaluate the impact of rain events and dry weather variability on the accuracy of wastewater routine monitoring.

Materials and methods

Sampling

Between 2008 and 2012, influent wastewaters were sampled in two WWTPs operated by the public agency of Paris conurbation sewage treatment (SIAAP) and located along the river Seine in the Parisian area (France): the Seine Amont (SAM) and Seine Centre (SCE) plants. In both plants, raw wastewaters are pretreated by screening and sand/grease catcher and processed through primary settling and secondary biological treatment after which the final effluent is released into the Seine River. These two plants differ in terms of types of sewer networks that are connected to the WWTP, their treatment capacities, and the types of primary and secondary processes as explained below.

The SAM plant (drained area, 104,673 ha; 2,196,253 persons) receives raw influents from a partially separate (80 %) sewer system and has a treatment capacity of 600, 000 $m^3.day^{-1}$ during dry periods and up to 1,500, 000 $m^3.day^{-1}$ during wet weather. Wastewater is first pretreated (screening and grit/oil removal) and then settled by primary settling tanks to remove a large amount of particles. An extended aeration-activated sludge unit (a biological reactor combined with a secondary settling tank) allows for carbon and nitrogen removal. The first zone operates under anoxic conditions to remove nitrates and the second step operates under aerobic conditions and allows the removal of carbon and total nitrification (Fig. 1).

The SCE plant (267 L. population equivalent $(PE)^{-1}$) receives raw influents from a combined sewer network and treats 240,000 $m^3.day^{-1}$ during dry periods and this capacity triples during storm events. Its design consists of a pretreatment (screening, grit/oil), a physicochemical lamellar settling tank (Densadeg®) with coagulant (ferric chloride) and flocculant (anionic polymer) injection, and a three-stage biofiltration unit. The first stage (Biofor®-type filters with biolite as the medium) is designed for carbon removal under aerated conditions, the second stage (Biostyr®-type filters with biostyrene as the medium) performs a total nitrification step under aerated conditions, and the third stage (Biofor®-type filters) consists of a denitrification step under anoxic conditions (Fig. 1). Compete details concerning these two WWTP are given by Gilbert et al. (2012), Radomski et al. (2011), and Rocher et al. (2012a, b).

In order to estimate the microbiological variability of raw influents, samples were collected in pipes during dry and wet weather conditions. For monitoring during the 2008–2012 period at the SCE and SAM plants, raw wastewaters were sampled before pretreatment (grid, sand, and fat removal; Fig. 1, point A). For sampling convenience, pretreated water was used as a proxy of raw wastewater to study daily variation during dry weather and to study intra-storm variability at the SCE plant (Fig. 1, point A′). A preliminary study at the SCE plant showed that the pretreatment had no significant influence on FIB densities. Samples were also collected in pipes after the primary settling step (point B for the SCE plant only) and after the biological treatment (point C) in order to evaluate the impact of raw sewage quality on FIB removal (Fig. 1).

During the 2008–2012 period, routine monitoring and 24-h composite samples were collected in the SAM and SCE plants using automated and refrigerated (4 °C) samplers using glass bottles and plastic tubing coated with Teflon. Routine monitoring is based on regular sampling dates (twice a month) and, as a consequence, can be conducted in dry and/or wet weather conditions. The 2008–2012 monitoring database (130 samples from the SAM plant and 113 samples from the SCE plant) was screened in order to separate dry weather from wet weather data. Days with rainfall, <0.5 mm/24 h on the day of the sampling and the previous day in Paris (Montsouris meteorological station), were considered as a “dry weather period.” For “wet weather conditions,” only the days of sampling with rainfall >2 mm/24 h were selected from the database. After screening, the final 2008–2012 database contained 63 days of dry weather and 20 days of wet weather for the SAM plant and 66 days of dry weather and 19 days of wet weather for the SCE plant. During dry periods, spatial variation of raw sewage quality was estimated by comparing the influents of the two WWTPs, and temporal variation (annual and seasonal) for both plants was estimated using the final 2008–2012 database. Composite samples from the 2008–2012 monitoring period were also compared to wet and dry weather periods for both plants in order to estimate the impact of rain events on regular monitoring. The impacts of spatial and temporal variations on FIB removal by the two plants were estimated by comparing raw, settled, and final effluents during dry and wet weather.

To study the daily variation of influent during dry weather, the FIB concentration was measured for 7 days at the SCE plant. Sequential samples were collected every 2 h during 22 h (from 2 am to 24 pm) using a multi-bottle sampler. In order to characterize the intra-storm variability, seven rain events were

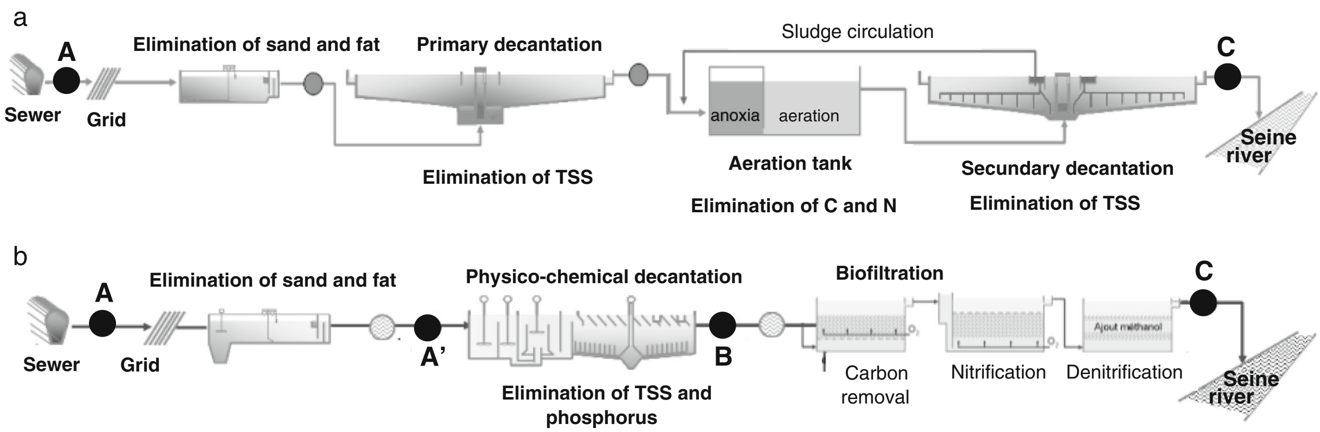

Fig. 1 Treatment lines and sampling points *A* and *C* at the SAM plant (**a**) and *A*, *B*, and *C* at the SCE plant (**b**)

sampled at the SCE plant. Sequential samples were collected every hour using an automated sampler during the course of each rain event. Since the rain length varied from one event to another, five to ten samples were collected per rain event.

Microbiological and chemical analyses

Electrical conductivity (in millisiemens per meter), TSS (in milligrams per liter), and ammonia nitrogen (N-NH_4^+, in milligram nitrogen per liter) were analyzed by the SIAAP analytical laboratory according to French Afnor standards (www.afnor.org).

To estimate *E. coli* and intestinal enterococci densities (most probable number (MPN).100 mL^{-1}), raw sewage samples (2008–2012 monitoring period, daily and intra-storm samples) were inoculated onto MUG/EC and MUD/SF microplates (AES Chemunex) following the French ISO standards NF EN ISO 9308-3 and NF EN ISO 7899-1. The FIB concentrations were estimated by the most probable number method using Excel data sheets (Jarvis et al. 2010). The percent of FIB associated with settleable particles was estimated only for the daily variation (5 days) and the intra-storm variation (5 days). Sequential samples collected during rain events and dry days were separated into settleable (density ≥1.05 $g.cm^{-3}$) and suspended fractions by centrifugation for 10 min at 1,164×g following Characklis et al. (2005). The supernatant contained the suspended fraction (free-living cells + FIB attached to non-settleable TSS). The pellet represented the settleable fraction (FIB attached to settleable TSS). For these samples, the supernatant and raw wastewater were inoculated onto UG/EC and MUD/SF microplates, and the settleable density was calculated from the difference.

Statistical analysis

Statistical analyses were carried out using the JMP 7.0.1 software (SAS Institute Inc.). The normality of the variables was checked with the Shapiro–Wilk W test, and, if necessary, the data were transformed to fit a normal distribution. The FIB densities were log transformed for the monitoring database. The removal of *E. coli* in the SAM plant and the percent of settleable *E. coli* were squared. Transformation was also performed for FIB densities of daily samples and *E. coli* densities from the intra-storm samples ($X^{0.2}$). ANOVA or Wilcoxon tests were performed in order to verify the relationship between the *E. coli* and enterococci concentrations and the environmental variables. Post hoc Tukey (HSD) tests followed the ANOVA tests. Wilcoxon p values were adjusted by a Bonferroni correction. The WWTP (SAM or SCE), weather condition (wet or dry), hour period (morning, afternoon, evening, night), year, season, TSS, and N-$NH4^+$ concentrations were initially included in the statistical models, and then, non-significant ($p>0.05$) terms were removed in the order of decreasing probability value by a backward stepwise procedure. Only the spatial and temporal variables are further discussed in this paper to comply with the focus of this study. To assess the effect of influent FIB concentration, influent TSS concentration, season and year on FIB log removal, ANOVA tests were performed. Since FIB log removal and influent FIB log concentration were not independently sampled, the density dependency was verified using a one-tailed t test to check if the slope of the linear regression calculated by the ANOVA test was significantly smaller than one (Varley and Gradwell 1960).

Results and discussion

Spatial variation during dry weather

The comparison of raw wastewaters (24-h composite samples, 2008–2012 monitoring period), collected during dry periods, showed significant differences between the two plants in terms of TSS, NH_4^+, and FIB concentrations (Table 1). TSS, NH_4^+ concentrations, and *E. coli* and enterococci densities

Table 1 Comparison of raw influent microbial and chemical parameters between the Seine Amont (SAM) and the Seine Centre (SCE) plants during dry weather (mean and standard deviations of 24-h composite samples)

Parameter	SAM	SCE	Statistical test	N, df	p value
TSS (mg.L^{-1})	344±51	241±45	ANOVA[a]	130.8	<0.001
N-NH^{4+} (mg.L^{-1})	46.5±4.9	27.7±4.3	Wilcoxon	130.1	<0.001
E. coli ×10^6 MPN/100 mL	14.0±8.3	9.1±4.7	ANOVA[a]	129.8	<0.001
Enterococci ×10^6 MPN/100 mL	3.4±2.6	1.9±1.3	ANOVA[a]	130.8	<0.001

N number of samples, *df* degrees of freedom

[a] Controlled by the year and the season effects

were significantly higher in the SAM raw wastewaters as compared to the SCE influents (Table 1).

The differences between the two WWTPs could be explained by the sewer network characteristics. Most of the WWTPs in the Parisian area (including the SCE plant) receive influents from a combined mesh network (which drains several main sewers) with a complex flow regulation. As a result, this huge spatial scale tends to smooth the local effects and to homogenize the quality of raw sewages. However, the sewer system connected to the SAM plant is, mostly, a separate network, and it receives a greater part of industrial effluents as compared to the SCE plant. The influence of effluent nature (industrial or domestic) is not very well documented. According to Manaia et al. (2010), the densities of enterobacteria (CFU/100 mL and CFU/inhabitant) were significantly higher in plants receiving industrial effluents as compared to plants receiving only domestic effluents. However, since these two types of plants also differed in terms of their dimensions and in terms of the demographic characteristics of the regions, Manaia et al. (2010) concluded that the nature of the raw wastewater seemed to have little influence on the enterobacterial levels in the influents. With respect to our study, it is also difficult to determine whether the industrial or domestic sources could impact the FIB levels in the SAM and the SCE raw wastewaters since these two plants also differ in terms of treated populations. The amount of treated waters per population equivalent is higher for SCE plant (267 L.PE^{-1}) as compared to the SAM plant (111 L PE^{-1}). This can be explained partly by the fact that the SCE domestic wastewaters are diluted by street washing waters. Such a dilution could induce a decrease in the microbiological contamination in the SCE raw sewages. The values found in the present study are congruent with several previous studies reporting values between 10^5 and 10^7/100 mL for *E. coli* in raw wastewaters (Miescer and Cabelli 1982; Koivunen et al. 2003; Garcia-Armisen and Servais 2007; Madoux-Humery et al. 2013). To our knowledge, only a few prior studies have compared the FIB concentrations of raw influents from several WWTPs (Samie et al. 2009; Madoux-Humery et al. 2013). Madoux-Humery et al. (2013) showed that two sewersheds with highly contrasting land uses presented very different *E. coli* concentrations in the raw sewage (measured under dry weather conditions). Samie et al. (2009) found a wide range of raw sewage qualities among 14 WWTPs in South Africa; however, they did not discuss this aspect.

High standard deviations (Table 1) suggest that the estimation of raw sewage quality requires a representative sampling effort, a campaign with only a few samples could greatly bias the interpretation. The high variability also suggests the presence of a temporal variability.

Temporal variation during dry periods

Within-day variation

The results we obtained from the SCE plant constitute a good illustration (Fig. 2) of the very well-known fluctuation of FIB densities during the day (Yaziz and Lloyd 1979). The samples collected every 2 h during 22 h showed that *E. coli* and enterococci abundances fluctuated during the day (Fig. 2). In order to analyze the results, the 22-h period was cut into four periods: 6–10 am (morning), 12–4 pm (afternoon), 6–10 pm (evening), and midnight–4 am (night). In the morning, *E. coli* densities were significantly lower (Fig. 2a; ANOVA, $F_{3,\ 61}$= 5.92, p=0.002). During the night and in the morning, intestinal enterococci densities were significantly lower as compared to the afternoon and the evening (Fig. 2b; ANOVA, $F_{3,\ 63}$= 7.76, p<0.001). This daily fluctuation with a low level of bacteria during the night and early morning is due to the diurnal defecation pattern (Duncan and Horan 2003). Depending upon the residence time in the sewer system, the FIB peak from morning defecation is generally observed in the early afternoon at the WWTP inlet (Madoux-Humery et al. 2013). As a consequence, it is important to keep in mind that the measured density range represents snapshots of raw sewage full variability (Duncan and Horan 2003), and that differences in location and timing of sample collection may greatly influence the FIB density from one measurement to another one. For instance, a sampling in the morning will show low levels of bacteria, whereas an afternoon sampling will give a higher level of bacteria.

The percent of settleable *E. coli* and enterococci showed no significant fluctuation during the day (data not shown).

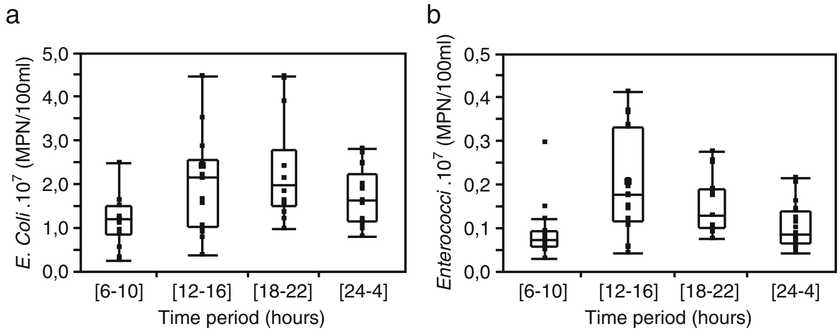

Fig. 2 Daily variations of *E. coli* (**a**) and intestinal enterococci (**b**) densities (median, 25th and 75th percentiles) in SCE raw influents during dry periods (7 days, sampled every 2 h during 22 h)

Bacterial association to particles depends upon several factors including the electrical charge and the hydrophobicity of the cell surface, the presence of extracellular polysaccharides, and the nature and abundance of the particles (Oliver et al. 2007; Pachepsky et al. 2008). As a consequence, bacterial affinity to particles could differ from one species to another (Pachepsky et al. 2008). For instance, intestinal enterococci seem to have a higher affinity for the settleable fraction or for particles with sizes >5 μm as compared to *E. coli* in soils, rivers, and rain runoff (Characklis et al. 2005; Plancherel and Cowen 2007). In a previous study, Gonçalves et al. (2009) showed that in the SCE raw sewages, 60 % of *E. coli* and 60 % to 90 % of intestinal enterococci were attached to TSS. However, the percents of attachment of these two FIB are still similar compared to other more hydrophobic bacteria like mycobacteria (Radomski et al. 2011). The different partitioning of bacterial species on particles could influence the efficiency of bacterial removal, especially in the settling process. According to Wery et al. (2008), the behaviors of *E. coli* and *Salmonella* in WWTP were similar; however, they differed from the behaviors of *Campylobacter jejuni* and *Clostridium perfringens*. Radomski et al. (2011) also demonstrated that non-tuberculous mycobacteria behaved differently along the SCE treatment line as compared to *E. coli* and intestinal enterococci. It would be interesting to choose indicators with different behaviors (hydrophilic, hydrophobic) in terms of attachment to settleable particles if the purpose is to estimate the efficiency of the WWTPs. The differences in particle associations among the different types of FIB and pathogens raise the question as to whether FIB could be a useful treatment efficiency indicator regarding pathogen removal. The traditional role for measurements of FIB has been as an index of fecal pollution and, therefore, it is a good predictor of potential health risks linked to the presence of pathogens (Payment and Locas 2011). However, FIB cannot predict, precisely, neither the level of occurrence of all pathogens nor their fate and transport in urban systems.

Intra- and interannual variation during dry periods

In addition to this daily pattern, FIB densities also varied from one season to another and from 1 year to another as suggested by the high standard deviations in Table 1. FIB densities during dry weather (composite samples from the 2008–2012 survey) significantly differed among years in both the SCE and SAM plants (ANOVA, $F_{8,\ 121}$=6.32, p=0.0001 for *E. coli*; ANOVA, $F_{8,\ 128}$=4.28, p=0.003 for enterococci, controlled by the WWTP and season effects). For both FIB, the coefficient of variation in the 2008–2012 database was high (51.6 to 59.1 % for *E. coli* and 71.9 to 75.6 % for enterococci, at the SCE and SAM plants, respectively). In both WWTPs, the *E. coli* densities were significantly lower in the winter as compared to the other seasons (Fig. 3; ANOVA, $F_{8,\ 121}$= 13.75, p<0.0001, controlled by the plant and year effects). For intestinal enterococci, there were also significant differences among the seasons, summer densities being lower as compared to the fall (Fig. 3; ANOVA, $F_{8,\ 122}$=3.93, p=0.010, controlled by the plant and year effects). A seasonal variation of nitrifying microbial communities was observed previously in the SCE plant raw sewages (Garnier et al. 2006). Seasonal variability of human activity such as summer vacations induces important variations in the quality of raw sewages. For example, nitrogen concentrations in raw sewages (generally considered as a marker of the human activity) are two times lower in the summer as compared to the rest of the year in the case of the Parisian sewer network. As a consequence, this seasonal variability of human activity can influence the FIB concentrations. Of course, a part of this temporal fluctuation could be due to the uncertainty of the measurements. However, the MPN microplate method to detect *E. coli* in sludge waste was evaluated as a very precise method by a European inter-laboratory method validation project (Maux et al. 2008).

Impact of storm events

Variation during storm events in SCE plant

During wet weather, domestic waters are diluted by the urban runoffs flowing in combined sewers. In the SCE plant, the conductivity of sampled raw influent decreased from 107± 4 mS m^{-1} (dry period) to 37±13 mS m^{-1} on the average for eight collected rain events. As a consequence, there was a dilution of FIB in the raw sewages (Fig. 4). Schematically, the

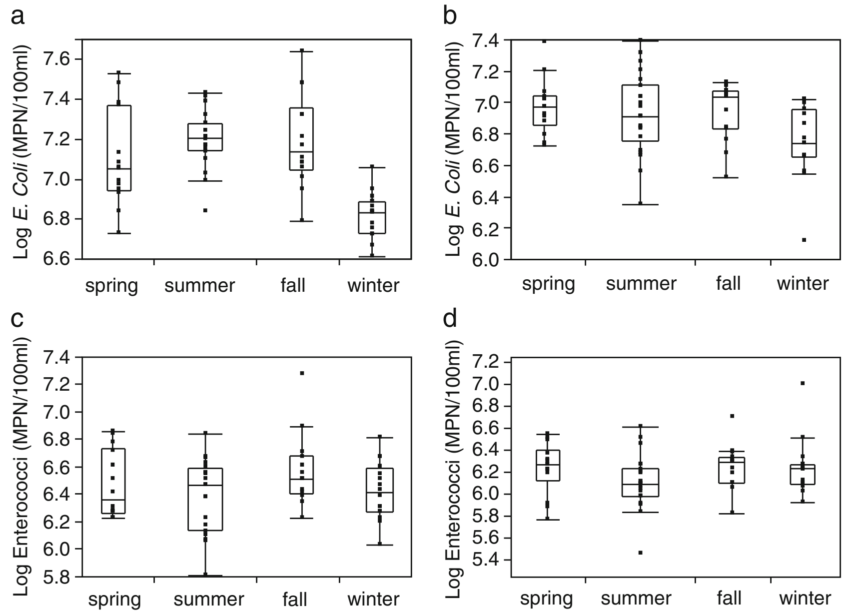

Fig. 3 Seasonal variations, during dry weather, of *E. coli* and intestinal enterococci densities (median, 25th and 75th percentiles) in the SAM (**a**, **c**) and the SCE (**b**, **d**) raw influents over the period 2008–2012. At the SAM plant, the number of samples was 14 in the spring, 20 in the summer, 14 in the fall, and 16 in the winter. At the SCE plant, the number of samples was 14 in the spring, 22 in the summer, 15 in the fall, and 15 in the winter

impact of a rain course on wastewaters was divided into three phases using the conductivity measurements of each individual rain event: the beginning of the impact (<1 h before minimum in the conductivity), the maximal impact (±1 h based on the minimal conductivity), and the end of the impact (>1 h after the minimum in the conductivity) (Fig. 4). In SCE raw sewages, FIB densities were significantly lower during the maximal impact as compared to the end of the impact (ANOVA, $F_{8,\ 36}=9.92$, $p=0.0004$ for *E. coli* and $F_{8,\ 36}=8.59$, $p<0.0001$ for enterococci, controlled by the storm event). The maximal impact produced a 0.4 to 1.8 log unit decrease for *E. coli* and 0.3 to 1.2 log units for enterococci as compared to the beginning of the impact. Although FIB are present in urban runoff waters (Characklis et al. 2005; McCarthy 2009), their concentrations remain very low as compared to FIB concentrations in wastewaters (McCarthy 2009; Madoux-Humery et al. 2013). As a consequence, the runoff water input inside the sewers results in the dilution of FIB. Madoux-Humery et al. (2013) found that FIB densities in CSOs were 2.7 to 3.4 times lower than those measured in raw wastewaters during dry weather conditions. Our results also show that attachment is an important factor that should be considered for the evaluation of CSO impact on rivers. The percent of settleable *E. coli* decreased significantly during the maximal impact phase (ANOVA, $F_{2,\ 26}=12.95$, $p=0.0008$, controlled by the storm event), while TSS usually increased (data not shown). Gasperi et al. (2010) demonstrated that during rain events, the TSS concentrations in the Parisian sewer system were mostly a reflection of resuspended sewer deposits which accounted for 47–69 %, whereas the runoff waters contributed only 7–12 % to the TSS. The dominant contribution of resuspended material to TSS in CSO waters was confirmed by Passerat et al. (2011) who sampled an intense storm at the outlet of the Clichy catchment (Paris, France). One can assume that less *E. coli* are attached to resuspended sewer deposits (old material) than to fresh TSS brought by wastewaters. This could explain the apparent contradiction between a decrease of the attached *E. coli* fraction and an increase of the TSS in the water collected during the maximum of the CSO discharge. These results show that the attachment of FIB to TSS is an important variable during CSOs that needs further investigation. Until recently, the prediction of surface water quality during storm events was conducted using models which considered all FIB to be free-living and not associated with particles (Jamieson et al. 2004). Currently, however, some models employ free-living and attached compartments for bacterial indicators (Garcia-Armisen et al. 2006; Gao et al. 2011; Ouattara et al. 2013).

Rain impact on regular monitoring data

The strong storm impact that we measured occurred mainly during the maximal impact phase. However, it would be interesting to evaluate the global impact of rain on 24-h regular monitoring samples and to compare the two WWTPs, since in the SAM plant, the sewers are partially separated while the SCE plant is fed only with combined sewers. For the SAM plant, the outfall sewer collects wastewaters from areas with strictly separated sewers (60 %), from mixed areas with both combined and separate sewers (30 %) and from areas with only combined sewers (10 %). Among the 2008–2012 monitoring data for the SCE and SAM plants, some 24-h composite samples were collected during wet weather. Using the 2008–2012 database, we compared the FIB densities measured during the dry periods (24 h rainfall <0.5 mm, the

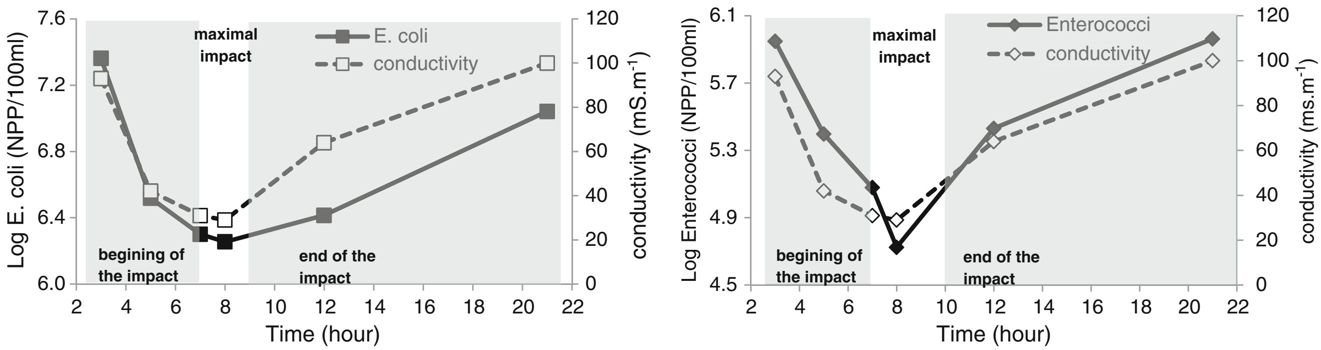

Fig. 4 Example of conductivity and FIB density fluctuation in the SCE plant raw wastewater during a storm event (5 November 2011)

sampling day, and the previous 24 h) with those measured during wet weather conditions (24 h rainfall >2 mm on the sampling day). For both plants, the NH_4^+ concentration was significantly lower during wet weather as compared to dry periods (Table 2, ANOVA, $F_{1,\ 92}$=36.14, p<0.0001 for the SAM plant and $F_{1,\ 96}$=15.52, p=0.0002 for the SCE plant, controlled by the year and season effects). The FIB concentrations were also lower during wet weather on the 24-h samples both in the SCE and the SAM plants; however, this dilution effect was not significant (Table 2). The lack of significance was probably linked to the fact that the 24-h composite samples integrate both the beginning and the end of the rain impact as well as some wastewater from the dry period. Moreover, the high variability of the rain intensity between the different sampled storms could explain the lack of significance. For the samples collected monthly during the 2008–2012 period, the 24-h rainfall on Paris (Montsouris meteorological station) ranged from 2.2 to 10.0 mm for the SCE plant and 2.4 to 24.8 mm for the SAM plant. Indeed, rain events are highly variable; there is no "characteristic" rainfall (Field et al. 1993). Madoux-Humery et al. (2013) also found a large variation in *E. coli* densities among CSO events. Our results suggest that rain events may influence the regular plant monitoring results, especially with respect to the NH_4^+ concentration. However, rainy weather did not seem to have a strong impact on the FIB regular monitoring, at least for the range of sampled storms.

What are the consequences for bacterial removal?

Flocculation and sedimentation, as well as filtration, are theoretically dependent of input particle concentration (Assavasilavasukul et al. 2008). Considering that most bacteria and protozoa can be considered as particles and most viruses as colloidal organic particles (Le Chevallier and Au 2004), it could be hypothesized that removal of pathogens by conventional water treatments should dependent on initial pathogen concentration. To test this hypothesis, we investigated the relationship between FIB removal by WWTPs with raw water FIB concentration during dry and wet weather conditions.

Effect of dry weather variability

During dry periods between 2008 and 2012, the *E. coli* removal (settling and biological treatment, Table 2) at the SCE and the SAM plants was not explained to a significant extent by the variation of the *E. coli* densities in raw sewages. Enterococci removal tended to be explained by their concentrations in raw sewages in the SAM plant (ANOVA, $F_{1,\ 59}$= 7.83, p<0.0007, controlled for the year and season and TSS influent concentration effects, slope test p value=0.052), although in the SCE plant, the relationship was not significant. In the SCE plant, the settling removal of *E. coli* (0.3±0.2 log units) was significantly related to the FIB concentrations (ANOVA, $F_{1,\ 57}$=9.51, p=0.0003, controlled for the year and season and TSS influent concentration effects, slope test p value<0.0001). Enterococci removal by settler (0.8±0.3 log units) also tended to be significantly related to the enterococci influent concentration (ANOVA, $F_{1,\ 59}$=36.15, p<0.0001, controlled for the year and season and TSS influent concentration effects, slope test p value=0.059). These results agree with the theory that higher particle loads lead to higher flocculation and sedimentation rates (Assavasilavasukul et al. 2008). The ratio of settling removal over total removal seemed higher when FIB influent densities were higher (ANOVA, $F_{1,\ 57}$=3.81, p=0.056 for *E. coli*; $F_{1,\ 59}$=13.75, p<0.001 for enterococci, controlled by the season, year, and TSS influent concentration), which means that the settler removal significantly increased compared to the total removal, when FIB influent concentration increased. This relationship does not seem to be related to the percent of settleable FIB, as there was no significant correlation between the percent of settleable fraction and the influent FIB concentration in the daily variation dataset. The relationship between FIB removal and FIB influent concentration is probably mainly due to particle interactions, collision rate, and flow pattern around particles during the flocculation process (Assavasilavasukul et al. 2008). However, in SCE, plant primary settling accounts only for a part of the

Table 2 Chemical parameters, FIB concentrations in raw sewages, and FIB log removal (mean and standard deviation, 24-h composite samples obtained in 2008–2012), Seine Amont (SAM) and Seine Centre (SCE) plant raw influents during wet and dry weather. Both plants were in dry weather setup

Station	Weather	Rain (mm/24 h)	*N*	*E. coli* (MPN.10^6/100 mL)	*E. coli* removal (log units)[a]	Enterococci (MPN 10^6/100 mL)	Enterococci removal (log units)[a]	TSS (mg L^{-1})	NH_4^+ (mg L^{-1})
SCE	Wet	5.1±2.5	19	7.5±3.6	2.1±0.9	1.7±0.9	3.0±1.3	246±71	24.1±4.5
SCE	Dry	0.1±0.2	66	9.1±4.7	2.7±0.6	1.9±1.3	3.5±0.8	241±45	27.7±4.3
SAM	Wet	8.1±5.6	20	10.7±5.1	2.3±0.6	3.4±3.1	2.5±0.5	325±72	35.4±8.9
SAM	Dry	0.1±0.1	64	14.0±8.3	3.2±0.4	3.4±2.6	3.0±0.5	344±51	46.5±4.9

FIB total removal (0.3 log vs 2.7 log). As a consequence, we cannot totally exclude that filtration was also dependent on the initial FIB concentration. Indeed, particle concentration is known to affect particle retention in filters, and as a consequence, log removal can be expected to increase with increasing influent particle concentration (Assavasilavasukul et al. 2008).

In SAM and SCE plants during dry periods, FIB removal was only partly explained by the FIB concentrations in raw sewages (10 to 26 % of the variability was explained). Other factors such as the raw water quality, fluctuations in the influent flow, and the plant management probably play important roles as well (Kay et al. 2007). It was previously shown that both raw water turbidity and initial pathogen concentration greatly influenced *Cryptosporidia* and *Giardia* removal rates during drinking water treatment (Assavasilavasukul et al. 2008). However, for SCE and SAM plants, TSS influent concentration had no significant influence on FIB removal.

All these results raise the question as to the amount of attention that should be given to the influent quality. Usually, only the level of effluent, quality is taken into consideration for WWTP management and for the evaluation of the impact of urban effluents on receiving surface waters (e.g., Australian guidelines; ARMCANZ, ANZECC 1997). Although Manaia et al. (2010) found that the homogeneity of the enterobacterial densities in treated effluents was independent of the inflow properties, our results show that this statement cannot be generalized.

Effect of storm events

During storm events, the higher influent flow can generate a decrease in the microbial removal as suggested by several studies (e.g., Rouleau et al. 1997; Hurst et al. 2004; Kay et al. 2007). Indeed, FIB log removal measured between 2008 and 2012 at the SCE plant was significantly lower during rain events as compared to dry periods (ANOVA, $F_{1, 83}=9.46$, $p=0.003$ for *E. coli*; $F_{1, 83}=7.02$, $p=0.011$ for enterococci, controlled for the year and season effects), as well as at the SAM plant (ANOVA, $F_{1, 81}=27.06$, $p<0.0001$ for *E. coli*; $F_{1, 80}=9.70$, $p=0.003$ for enterococci, controlled for the year and season effects). We explored whether the quality of raw sewage also could explain the difference in FIB removal efficiency. During wet weather, the log removals of FIB by the SAM and the SCE plants were not significantly explained by the FIB concentrations in raw sewages. The relationships between WWTP efficiency and raw sewage microbial quality that we found with the dry weather data were not observed during storms. The lack of relationship between the FIB removal and the FIB influent concentration could be explained by three possible parameters, which probably have cumulating effects. First, higher flow rates during wet weather probably reduced the residence time. We did not measure the variation of the influent flow or the variation of the residence time during the treatment process in 2008–2012 for each sampled day, so this hypothesis cannot be tested with our dataset. However, Rose et al. (2004) demonstrated that FIB concentrations in secondary treated waters were negatively correlated with the residence time in the activated sludge process. Also, it is well known that activated sludge processes are less efficient during wet weather due to shorter residence times (Rouleau et al. 1997; Kay et al. 2007). Second, FIB concentrations were lower during the storm events which could result in lower removal efficiency as demonstrated with the dry weather data. Third, the settleable fraction of FIB was lower during the maximum impact of the storms, which could also result in a decrease of the settler efficiency. It would be interesting to evaluate the relative importance of each these effects.

Conclusions

The microbial quality of raw sewage showed considerable spatial and temporal variability that could be linked to the characteristics of the sewer system and to the hydrological conditions. We demonstrated that the influents from two plants with contrasting sewersheds have significantly different dynamics and levels of FIB. However, it was not possible to conclude as to the relative influence of sewage type (domestic/industrial) and sewer type (combined/separate) on FIB concentrations in raw sewages. It would be interesting to conduct a wider sampling of different WWTPs in order to define the respective effects of these two factors. If the microbial quality of the influents affects the efficiency of the WWTP, as we showed for the SAM plant, then this could have consequences for WWTP management and the choice of treatments.

Our results show also that the microbial quality of the raw influents explains only partly the variability of FIB removal by the two WWTPs. The role of flow rate and residence time as possible explanatory variables for the removal of FIB also should be considered. We showed that FIB densities fluctuated significantly with the hydrological conditions by comparing dry and wet weather datasets and by studying intra-storm variations. Rain events certainly modify the flow rate and the residence time in the WWTP, thus influencing the FIB concentrations in influents and effluents. We did not measure these parameters; however, it would be interesting to analyze their relative roles with raw sewage of different microbial quality.

The influence of storm events on FIB densities in raw sewage is assessed rarely due to sampling difficulties; however, our results demonstrate the strong influence of weather conditions on the influent and the WWTP efficiency. Not only did the FIB densities fluctuate, but also their partitioning on settleable particles decreased during the maximal impact of the storm events. Our results also show that the association with settleable particles also differed between *E. coli* and intestinal enterococci. The potential role of FIB as treatment efficiency indicators can be questioned since their fate and transport in the sewer and throughout the different treatment process may be different depending upon the pathogenic species. However, they could be interesting surrogates for pathogens with similar behavior such as *Salmonella*.

Finally, our results also demonstrate the importance of sampling strategies. The very large variability in FIB densities should be taken into account when interpreting punctual samplings both in dry and wet weather situations, depending on the objectives of the study.

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