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Impact of an intense combined sewer overflow event on the microbiological water quality of the Seine River

Julien Passerat[a], *Nouho Koffi Ouattara*[a], *Jean-Marie Mouchel*[b], *Vincent Rocher*[c], *Pierre Servais*[a,*]

[a] *Ecologie des Systèmes Aquatiques, Université Libre de Bruxelles, Campus de la Plaine, CP 221, Boulevard du Triomphe, B-1050 Bruxelles, Belgium*

[b] *UMR 7619 Sisyphe, Université Pierre et Marie Curie, Case 105, 4 place Jussieu, 75005 Paris, France*

[c] *Syndicat Interdépartemental pour l'Assainissement de l'Agglomération Parisienne, Direction du Développement et de la Prospective, 82 avenue Kléber, 92700 Colombes, France*

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ABSTRACT

For a better understanding of the short and mid-term impacts of a combined sewer overflow (CSO) on the microbiological quality of the receiving river, we studied the composition of a CSO discharge and monitored during several hours the changes in the concentration of fecal indicator bacteria (FIB) in the impacted river water mass. The CSO occurred at the Clichy outfall (Paris agglomeration, France) in summer 2008 as a result of the most intense rainfall of the year. In 6h, 578, 705 m^3 of sewage and 124 t of suspended matter (SM) were discharged into the Seine River. The CSO contained 1.5×10^6 *E. coli* and 4.0×10^5 intestinal enterococci per 100 mL on average, and 77% of the *E. coli* were attached to SM. It was estimated that 89% of the CSO discharge was contributed by surface water runoff, and that resuspension of sewer sediment contributed to ~75% of the SM, 10–70% of the *E. coli* and 40–80% of the intestinal enterococci. Directly downstream from the CSO outfall, FIB concentrations in the impacted water mass of the Seine River (2.9×10^5 *E. coli* and 7.6×10^4 intestinal enterococci per 100 mL) exceeded by two orders of magnitude the usual dry weather concentrations. After 13–14 h of transit, these concentrations had decreased by 66% for *E. coli* and 79% for intestinal enterococci. This decline was well accounted for by our estimations of dilution, decay resulting from mortality or loss of culturability and sedimentation of the attached fraction of FIB.

1. Introduction

Many urban centers are drained by a unique sewer network in which wastewater is mixed with urban runoff water in wet weather. When rainfalls are intense, the transport capacity of the sewer system can be insufficient to allow all the water flow to reach the wastewater treatment plant (WWTP) or the treatment capacity of the WWTP can be insufficient to treat all the water flow. In such cases, combined sewer overflows (CSOs) occur, resulting in the discharge without any treatment of a mixture of wastewater and runoff water, loaded with urban surface pollution, into the receiving waters. CSO impacts on aquatic environments are multiple in terms of pollution types and dynamics in time and space, and encompass: (i) oxygen depletion due to the biodegradation of the high load of organic matter brought by the untreated wastewater, (ii) turbidity increase leading to the reduction of photosynthetic primary production, (iii) increase in the concentration of some

* *Corresponding author*. Tel.: +32 2 650 5995; fax: +32 2 650 5993.
E-mail address: pservais@ulb.ac.be (P. Servais).

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organic micro-pollutants, (iv) increase in metal concentrations and (v) increase in the concentration of pathogenic and fecal indicator micro-organisms.

Concerning this latter topic, a lot of studies have reported degraded microbiological water quality due to the release of stormwater runoff and CSO in various kinds of receiving natural waters as coastal zones (Hall et al., 1998; Noble et al., 2003), lakes (McLellan et al., 2007) and rivers (Rechenburg et al., 2006; Ham et al., 2009). Other researches were conducted on the microbiological quality of the CSO (Jefferies et al., 1990; Ashley and Dabrowski, 1995) but only few studies (Donovan et al., 2008) reported data on both microbiological CSO quality and its impact on the receiving water.

The aim of the present work was to study in parallel the fecal microbial contamination of a large CSO in the Parisian area and its impact on the Seine River. The Paris agglomeration is equipped with a combined sewer system and the Seine River is the receiving environment of the wastewater treated in WWTPs and of CSOs in wet weather conditions. This river is a typical example of an aquatic system severely impacted by wastewaters due to the large size of the conurbation (10 million inhabitants) and the relatively low discharge of the river (328 m^3 s^{-1} on average at its entry into Paris). In the framework of the PIREN-Seine program (Meybeck et al., 1998), previous studies of CSO impacts on the Seine River had been mainly devoted to the problem of oxygen depletion. Anoxic conditions created in the river after CSO events used to cause fish mortality in the 90 s and before. Measurements were performed in CSOs (Seidl et al., 1998a; Servais et al., 1999) and downstream in the receiving Seine River (Seidl et al., 1998b) in order to improve the understanding of all the processes involved in oxygen depletion. This allowed the building of an ecological model, ProSe, able to describe and predict the impact of a CSO on the Seine River oxygen concentration (Even et al., 2004, 2007). CSO impacts on metal contamination were also investigated in the Parisian area (Estebe et al., 1998).

In the last ten years, the SIAAP (Syndicat Interdépartemental pour l'Assainissement de l'Agglomération Parisienne), which is in charge of the management of the sewer network and the WWTPs in the Paris agglomeration, has developed significant efforts to reduce the frequency and the volume of the CSO spill events into the Seine River. Many facilities, allowing the storage or the treatment of wastewater during wet weather periods, have been built inside the sewer network (storage tunnels, detention basins) or in the WWTPs (ballasted flocculation processes). In addition, significant efforts have been made by the SIAAP operators to improve the control of the wastewater flow in the sewer system during wet weather periods, using hydrological and meteorological predictive models.

Despite this progress, CSO discharges still occur in the Parisian area and one of them was studied in August 2008. Up to now, no studies were devoted to the impact of CSOs on the microbiological water quality in the Parisian area. In the present study, we monitored the fecal microbial contamination discharged during an intense CSO occurring at the Clichy outfall, and we investigated the impacts on the microbiological quality of the receiving waters of the Seine River. The fecal indicator bacteria (FIB) *Escherichia coli* and intestinal enterococci were used to assess fecal contamination. FIB enumeration is commonly used to control microbiological water quality, as the search for the presence of all types of pathogens in aquatic systems is not feasible. Today, *E. coli* and intestinal enterococci are considered as the best FIB to predict the sanitary risk associated with freshwaters (Edberg et al., 2000; Kay et al., 2004).

2. Materials and methods

2.1. Study site

2.1.1. The combined sewer system of the Paris agglomeration and the Clichy CSO outfall

More than 75% of the sewage water from Paris and its suburb are collected and transported by gravitation towards three wastewater treatment plants (WWTPs) located in the western part of Paris agglomeration: Seine Centre (240,000 m^3 d^{-1}, hereafter referred as WWTP 1), Seine Aval 1,700,000 m^3 d^{-1}, WWTP 2) and Seine-Grésillons (1,00,000 m^3 d^{-1}, WWTP 3) (Fig. 1A). Treatment process at WWTP 1 and 3 consists of primary treatment, biofiltration for carbon and nitrogen removal and physico-chemical phosphorus removal. Treatment process at WWTP 2 consists of primary treatment, activated sludge for carbon removal, biofiltration for nitrogen removal and physico-chemical phosphorus removal. In their course, due to the presence of meanders, collectors leading to these WWTPs cross the Seine River by means of siphon systems. During rainstorm events their transport capacity may be exceeded and the water overload is discharged to the river.

A major wet weather outlet in this system is located on the right bank of the river, at the Clichy pretreatment plant. This plant is a major node in the sewer system: in dry weather conditions it collects and pre-treats (screening and grit removal) approximately 600,000 m^3 d^{-1} of wastewater which are transferred towards the three WWTPs. WWTP 1 receives no other water except from the Clichy pretreatment plant, located 2 km upstream. Therefore, the quality of the influent water at WWTP 1 can be considered as well representative of the quality of the sewage water transiting at the Clichy site.

2.1.2. The Seine River

The Seine River has been canalized for more than a century and is nowadays equipped with navigation dams in order to maintain a constant water level between 4 and 5 m. Summer flow is regulated by reservoirs constructed on the upstream part of the Seine, Marne and Aube rivers. The average summer flow in Paris is 144 m^3 s^{-1} (measured at the Austerlitz Bridge during the 1974–2009 period).

Positions in the Seine River are administratively identified by their kilometric point (KP), which is their distance in km from the reference bridge Pont Marie in downtown Paris. The Clichy CSO outfall is located at KP 23.4. Twice downstream from the Clichy outfall, the Seine River is divided into two arms by central longitudinal islands: first between KP 25.5 and KP 32.6 by the Île St-Denis, second between KP 40.3 and KP 50.6 by a continuous succession of islands beginning with the Île de Chatou and ending with the Île de la Loge (Fig. 1B). At KP 25.5, because the Clichy outfall is located on the right bank and the lateral dispersion is still limited, most of the water mass impacted by a Clichy CSO flows into the right arm. At KP 40.3, the impacted water mass flows into both arms, and we chose to

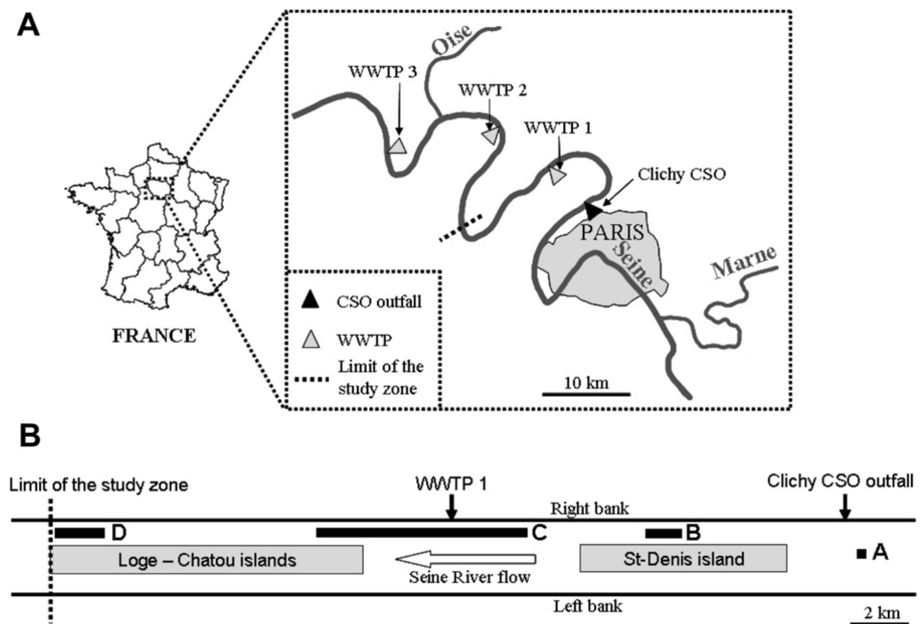

Fig. 1 – (A) Map of the study area and (B) schematic localization of the sample series in the Seine River.

follow the right arm. Because transit times are different in the two arms, their confluence results in the mixing of two water masses with distinct ages. We chose not to sample this new heterogeneous water mass, and the end of the Île de la Loge marked therefore the downstream limit of our study zone.

2.2. *Sample collection*

The CSO discharge was sampled with a Bühler 1029 automatic sampler (Hach Lange) equipped with a cooled (4 °C) sample compartment as described in Kafi et al. (2008). Successive composite samples were constituted by the assembling of subsamples collected every 6 min over 30 min into detergent-washed 1-L PE bottles. The sampling system was purged between each subsample. In parallel, the water mass of the Seine River impacted by the CSO was followed during its flow with a small motorized inflatable boat and was sampled. Before each sampling series, the impacted water mass was located by in situ measurements of conductivity and oxygen concentration. A water mass impacted by a CSO has indeed a lower oxygen concentration than the surrounding non-impacted water due the degradation of the organic matter contained in the CSO; it has a higher conductivity due to the high conductivity of a CSO in comparison to the Seine River. Three series of samples (B, C and D) were collected, from directly downstream from the outfall to the end of the Île de la Loge (Fig. 1B). For each series, a subsurface sample and a bottom sample were collected in the same water column at two to three different locations in the impacted water mass. In addition, a fourth series of samples (A) was collected directly upstream from the Clichy CSO outfall. Distances between sampling stations and the CSO outfall as well as the time of sampling are given in Table 1. Bottom samples were collected half a meter above the bottom with a length of rubber tubing connected to a pump. In order to rinse the sampling system between two samples, 2 to 3 L of the new sample were pumped and discarded before collecting 1 L in a sterile polypropylene bottle. Subsurface samples were grabbed by opening then closing the cap of a sterile 1-L polypropylene bottle 30 cm below the surface. All samples were kept cold until returned to the laboratory where they were refrigerated (4 °C). River samples were processed within 4 h after collection, CSO samples within 3 h after collection of the last sample.

2.3. E. coli *and intestinal enterococci enumeration*

E. coli and intestinal enterococci were enumerated by plate counting on Chromocult Coliform Agar (CCA) and Chromocult Enterococci Agar (CEA) respectively (Merck KGaA, Darmstadt,

Table 1 – Time and location of the four series of samples collected in the Seine River on the day of the CSO event (the Clichy CSO outfall is located at KP 23.4).

Sample series	Date	Collection time	Kilometric point (KP)	Distance from the Clichy outfall (km)
A	2009-08-07	8:55–10:10	22.7–23.0	– 0.66 to –0.42
B	2009-08-07	12:10–14:45	29.0–30.2	5.7–6.8
C	2009-08-07	18:35–21:50	34.5–41.7	11.1–18.3
D	2009-08-08	2:00–3:35	48.8–50.5	25.4–27.1

Germany). Both growth media are specific to their corresponding indicator bacteria. Plate counting on CCA has been shown to be equivalent to the reference method ISO 9308-1 recommended in the UE Directive 2006/7/EC on bathing water quality (Mavridou et al., 2010). Depending on the expected concentration, different volumes of each water sample were plated in duplicate on the agar plates after ten-fold serial dilution in sterile Ringer solution or after filtration through a 0.45-μm GN-6 membrane filter (Pall Corporation, Ann Arbor, MI, USA). CCA and CEA plates were incubated at 36 °C for respectively 24 h and 48 h. Plate counts were expressed as colony-forming units (CFU) per 100 mL of sample.

2.4. Determination of the fraction of E. coli attached to suspended matter

In this study, the approach proposed by Garcia-Armisen and Servais (2009) to estimate the fraction of *E. coli* attached to suspended matter (SM) was used. It is based on measurements of the β-D-glucuronidase (GLUase) activity (an enzymatic activity specific to *E. coli*) in two particle size fractions. GLUase activity measurements have been shown to be a good surrogate to *E. coli* enumeration by plate counts in different types of aquatic systems (Servais et al., 2005; Lebaron et al., 2005; Garcia-Armisen et al., 2005). Briefly, GLUase activity retained on a 0.2-μm pore-size membrane is used to quantify the GLUase activity of the total population of *E. coli* in a sample while the GLUase activity retained on a 5-μm pore-size membrane is used to quantify the activity of the fraction of *E. coli* attached to SM. The ratio of both activities gives an estimate of the proportion of *E. coli* attached to SM. Measurements of GLUase activities were performed following the protocol proposed by George et al. (2000) slightly modified. A known volume of river water was filtered through a 0.2-μm or a 5-μm pore-size filter (47 mm-diameter polycarbonate membrane). Each filter was placed in a flask with 17 mL of a 67 mM phosphate buffer (pH 6.9) and incubated in a water bath at 44 °C. The reaction was started by adding 3 mL of a 2.83 mM 4-methylumbelliferyl-β-D-glucuronide solution (Biosynth AG, Staad, Switzerland). Every 5 min for 30 min, a 2.9-mL aliquot was poured in a quartz cell with 110 μL of a 1 M sodium hydroxide solution to increase the pH to 10.7. The fluorescence intensity of the aliquot was measured with an SFM 25 spectrofluorometer (Kontron AG, Zürich, Switzerland) at an excitation wavelength of 362 nm and an emission wavelength of 445 nm. GLUase activity is measured by the production rate of 4-methylumbelliferone (MUF), determined by a linear least squares regression of the MUF concentration on the incubation time.

3. Results

3.1. Characterization of the CSO

3.1.1. The CSO event

The Clichy CSO event that was studied occurred on August 7, 2008. It resulted from a summer rainstorm on the Parisian area, during which 39 mm of precipitation were measured (average precipitation calculated from the measurements of 32 rain gauges located on the catchment area managed by the SIAAP). This was the highest daily rainfall measured on the area in 2008. Most of the rain fell between 5:00 a.m. and 9:00 a.m. The resulting CSO at the Clichy site was the first since 12 days.

The CSO lasted 6 h (from 5:50 a.m. to 11:50 a.m.) and resulted in the discharge of 5,78,705 m^3 into the Seine River. This was the largest discharge observed at Clichy in 2008, the third largest discharge of the 2006–2008 period. The average flow rate of 26.8 $m^3\ s^{-1}$ was the highest observed for this period. The flow peaked at 7:31 a.m. at 45.4 $m^3\ s^{-1}$ (Fig. 2A). Twelve successive samples were collected automatically in the CSO, each sample being a time-proportional composite of the waters discharged during a 30-min period.

3.1.2. Conductivity

The conductivity of the 12 samples evidenced a strong variation of the CSO composition over time (Fig. 2A). The conductivity peaked during the first half-hour (518 $\mu S\ cm^{-1}$) and rapidly decreased to reach a minimum during the third half-hour (143 $\mu S\ cm^{-1}$), after which it progressively increased until the end of the CSO discharge to 327 $\mu S\ cm^{-1}$.

During an 18-day survey of the raw wastewater quality at the entrance of WWTP 1, carried out by the SIAAP in spring 2008, a conductivity of 1175 ± 30 $\mu S\ cm^{-1}$ was observed on average in dry weather conditions (SIAAP, unpublished data). This conductivity is a good estimate of the conductivity expected for dry weather wastewater at the Clichy site, located on the same collector 2 km upstream. Therefore all CSO samples displayed conductivities much lower than dry weather wastewater. These low conductivities were the consequence of the dilution of wastewater by urban stormwater runoff with a much lower conductivity.

In an attempt to estimate the respective proportions of wastewater and runoff water composing the CSO, we hypothesized the mixing of an average dry weather wastewater with an average runoff water. The conductivity of the dry weather wastewater was set to 1175 $\mu S\ cm^{-1}$ as discussed above. Previous measurements inside the Clichy catchment had shown that the average conductivity of runoff water during a rainstorm event ranged between 56 $\mu S\ cm^{-1}$ and 141 $\mu S\ cm^{-1}$ (Kafi-Benyahia, 2006). Fig. 2B shows the mixing proportions estimated if runoff conductivity was set to the minimum, the centre (100 $\mu S\ cm^{-1}$) or the maximum of this range. According to these hypotheses, runoff water was estimated to represent 85%, 89% and 92% of the total CSO respectively. Since the different hypotheses resulted in minor differences, we considered in the rest of the study an average conductivity of 100 $\mu S\ cm^{-1}$ for the runoff water constituting the CSO discharge. The lowest proportion of runoff water was estimated at 61% for sample 1 and the highest at 96% for sample 3 (Fig. 2B). The proportion of runoff water began to decrease during the half-hour preceding the peak flow, probably as a result of an increase in the volume of wastewater arriving at the Clichy pretreatment station after 7 a.m.

3.1.3. Suspended matter

In 2008, the average concentration of SM in wastewater at the entrance of WWTP 1 was 264 ± 48 $mg\ L^{-1}$ in dry weather conditions (SIAAP, unpublished data). In comparison, water discharged during the first 30 min of the CSO carried a very high load of SM (830 $mg\ L^{-1}$) (Fig. 2C). During the following 30 min, the concentration dropped rapidly to an average of 290 $mg\ L^{-1}$, and then went on decreasing at a slower pace

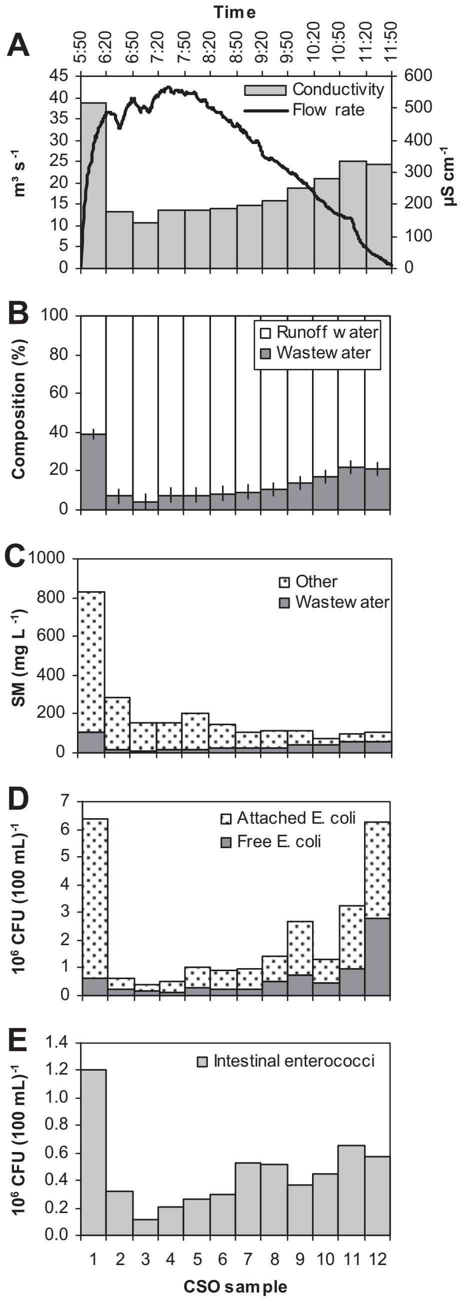

Fig. 2 – Variation of some CSO parameters over time. (A) Flow rate (moving 5-min average, line) and conductivity of the 12 CSO samples collected during successive intervals of 30 min (bars). (B) CSO composition in wastewater and runoff water as estimated by conductivity measurements. (C) SM concentration and fraction estimated to originate from wastewater. Vertical bars represent the variation of the estimation according to the assumptions made about urban runoff conductivity, as described in the text. (D) Concentrations of *E. coli* attached to SM and free *E. coli*. (E) Intestinal enterococci concentration.

towards a final concentration of 110 mg L^{-1} approximately. Considering that wastewater was estimated to represent only 11% of the average CSO, SM carried by wastewater was highly insufficient to account for the concentrations observed in the 12 samples. Fig. 2C shows an estimation of the proportion of SM that was not directly brought by wastewater, based on a calculation where wastewater carries 264 mg L^{-1} and is mixed with runoff water in the proportions derived above. For the whole CSO, 86% of the total SM discharge (1,24,000 kg) was estimated to originate from another source than wastewater. Two sources can be proposed for it: (i) the particles washed by the stormwater runoff on urban surfaces or (ii) the resuspended sewer sediment.

3.1.4. *Fecal indicator bacteria*

The concentrations of *E. coli* and intestinal enterococci in the CSO followed a trend similar to conductivity (Fig. 2D and E). The concentrations of both FIB were the highest during the first 30 min of discharge (6.4×10^6 *E. coli* and 1.2×10^6 intestinal enterococci per 100 mL), next they dropped to reach a minimum in sample 3 (3.8×10^5 *E. coli* and 1.2×10^5 intestinal enterococci per 100 mL), when the proportion of runoff water peaked in the CSO discharge, and then they increased progressively until the end of the CSO. The increase was more pronounced for *E. coli* than for intestinal enterococci, leading to a final *E. coli* concentration close to what was observed in the first sample. Maximal concentrations were similar to those measured in raw wastewater, since the influent at WWTP 1 contains on average 6.5×10^6 *E. coli* and 1.1×10^6 intestinal enterococci per 100 mL (median values of bimonthly measurements between 2003 and 2007) (Gonçalves et al., 2009). Minimal concentrations were at least one order of magnitude higher than concentrations usually observed in the treated effluents of the WWTPs from Paris agglomeration (Gonçalves et al., 2009).

FIB concentrations were positively correlated to conductivity ($R^2 = 0.79$ for *E. coli* and $R^2 = 0.88$ for intestinal enterococci, $p < 0.001$), suggesting that they were primarily driven by the proportion of wastewater in the CSO. We analyzed more closely if FIB concentrations followed a strict dilution pattern. We compared them to the theoretical concentrations that would be observed if wastewater, carrying a load of FIB corresponding to dry weather conditions as measured in the WWTP 1 influent, was diluted with runoff water carrying no FIB, in the proportions derived above (Fig. 3). In six out of the twelve CSO samples for *E. coli*, and in all samples for intestinal enterococci, the measured concentrations were higher than those expected if wastewater was the only source of FIB. Another source of FIB can therefore be suspected, the two possibilities being again resuspended sewer sediment and runoff water.

Finally, it was estimated that 77% of the *E. coli* discharged during the whole CSO were attached to SM. A marked difference in the proportion of attached *E. coli* was observed between the first half-hour of the CSO and the rest of the discharge, as it was already observed for conductivity and for the concentrations of SM and FIB. The proportion of attached *E. coli* was the highest in the first sample (91%) while it was on average 68 ± 7% in the other samples (Fig. 2D).

3.2. *Impact on the microbiological water quality of the Seine River*

3.2.1. *Characterization of the Seine upstream from the Clichy CSO outfall*

On the day of the rainstorm event at 6:00, the Seine flow rate was of 157 $m^3 s^{-1}$ in downtown Paris (Austerlitz bridge), which

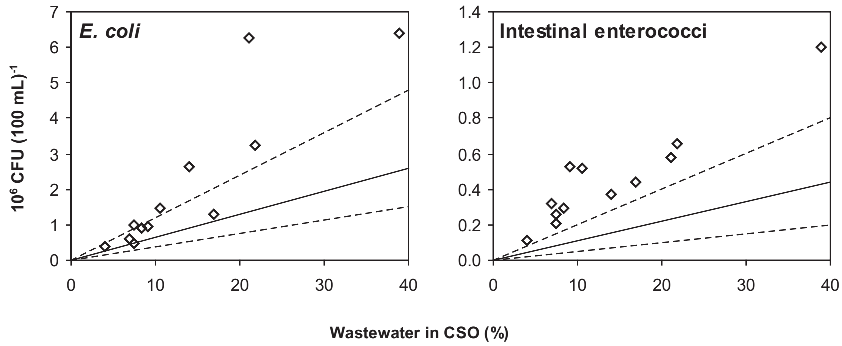

Fig. 3 – Comparison of the actual concentrations of FIB in the CSO (white symbols) with their expected concentrations if wastewater was the only source of FIB. Expected concentrations were calculated on the basis of the 1st decile ("low hypothesis", lower dashed line), the median ("medium hypothesis", plain line) and the 9th decile ("high hypothesis", upper dashed line) of the concentrations measured bimonthly between 2003 and 2007 in dry weather wastewater at the entry of WWTP 1 (Gonçalves et al., 2009).

is close to its average summer flow rate of 144 $m^3 s^{-1}$. In the following 3 h, the flow rate dramatically increased and peaked at 10:00 at 367 $m^3 s^{-1}$ (Fig. 4). Therefore, the flow of the Seine River was already strongly impacted by the rainstorm upstream from the Clichy CSO outfall.

To have an estimate of the microbiological quality of the Seine River before being impacted by the Clichy CSO, the Seine was sampled directly upstream from the Clichy outfall once the CSO had begun (sample series A). Mean concentrations of 4.0×10^4 *E. coli* and 8.6×10^3 intestinal enterococci per 100 mL were observed (Fig. 5). These wet weather concentrations were compared with two different estimates of the microbiological quality expected in dry weather conditions, obtained from (i) a longitudinal profile in the same river portion six days later, and (ii) a bimonthly monitoring at one location over the year 2008 (SIAAP, unpublished data). The two data sets gave very similar estimates: 2×10^3 *E. coli* and 2×10^2 intestinal enterococci per 100 mL (Fig. 5). The concentrations in series A were more than one order of magnitude higher. Therefore, as seen with the flow rate, the Seine River was already impacted by the rainstorm and its microbiological quality was already impaired upstream from the Clichy CSO outfall. This deterioration could result from the discharges of several minor CSOs located upstream from the Clichy outfall, from sediment resuspension from the river bed due to the increase in the flow rate or from increased runoff from rural areas due to the rain event.

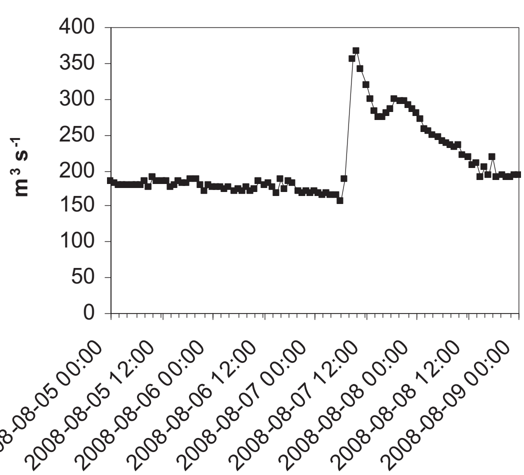

Fig. 4 – Seine River flow rate as measured under the Austerlitz Bridge in the City of Paris on the days around the CSO event.

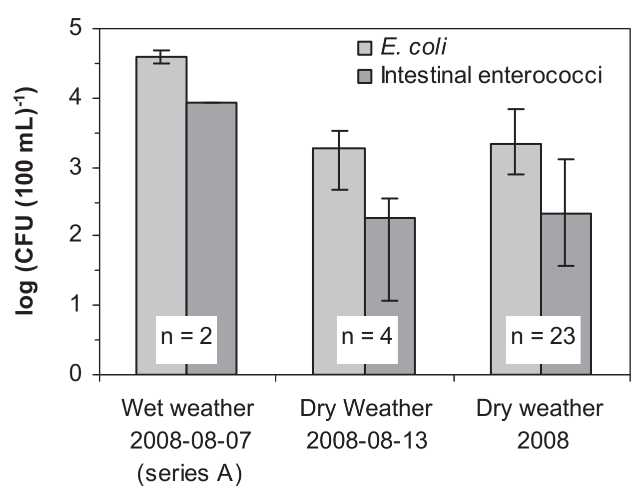

Fig. 5 – Comparison of the microbiological water quality of the Seine River, as observed directly upstream from the Clichy outfall on the day of the CSO event, with dry weather data. *Left*: FIB concentrations as measured on the day of the CSO event directly upstream from the Clichy outfall (sample series A); bars represent the arithmetic mean; error bars represent the range bewteen the two samples. *Centre*: FIB concentrations as measured during a longitudinal profile (four locations between KP 16.4 and KP 37.3) carried out in dry weather conditions six days after the CSO event; bars represent the arithmetic mean; error bars represent the standard deviation. *Right*: FIB concentrations as measured on average in 2008 at the level of the Argenteuil Bridge (KP 36.0) during dry weather conditions; bars represent the medians of bimonthly measurements; error bars represent the 1st and 9th deciles.

3.2.2. Microbiological water quality downstream from the Clichy CSO outfall

Downstream from the Clichy outfall, the water mass of the Seine River impacted by the Clichy CSO was followed and successive series of samples were collected in it. As a result of the dramatic increase in the Seine flow rate, the impacted water mass reached the downstream limit of our study zone in only 20 h (see Section 2.1.2). In dry weather conditions, the transit time of the Seine River between the Clichy outfall and this limit is on average of 30 h. Therefore, only three series of samples were collected in the impacted water mass (sample series B, C and D) (Table 1).

For each series an average FIB concentration was calculated for the whole impacted water mass. In series B, directly downstream from the outfall, *E. coli* and intestinal enterococci concentrations were almost 1 log higher than those measured directly upstream in series A (Fig. 6). They subsequently decreased in C and D, representing in D 34% and 21% of the concentrations measured in B for *E. coli* and intestinal enterococci respectively.

Because of the limited number of samples, only general qualitative trends describing the spatial (longitudinal and vertical) distribution of the FIB within the impacted water mass can be suggested. For series D, no difference in the FIB concentration of the samples was observed at the longitudinal or the vertical level, suggesting that the impacted water mass was homogenous. In series B and C, the samples collected in the downstream part of the water mass were more contaminated than the samples collected in the central and upstream parts (data not shown). This was consistent with the temporal variation in the CSO composition, since the FIB discharge was the highest during the first half-hour. As regards the vertical distribution, very weak differences or no differences at all were observed between subsurface samples and bottom samples from the same water column. Nevertheless, when the differences were significant ($p < 0.05$), the FIB concentration was higher in the bottom sample.

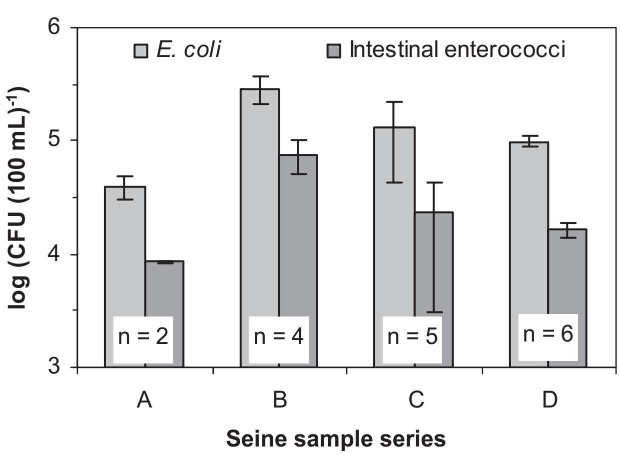

Fig. 6 – FIB concentrations in the Seine River on the day of the CSO event. FIB concentrations were measured directly upstream from the Clichy outfall (sample series A) and in the downstream water mass impacted by the CSO (sample series B, C and D). Bars represent the arithmetic mean of the sample series; error bars represent the standard deviation.

4. Discussion

Few studies have monitored in parallel the composition of a CSO and its impacts on the receiving waters over time in order to explore the dynamics of microbial contamination. Important logistics have to be deployed at the precise day and hour of the CSO event in order to sample simultaneously the CSO and the river, to carry out the in situ monitoring of the pollutant dynamics in the river during several hours and to perform the laboratory analyses at the same time. The present study focusing on microbiological aspects was possible because it was part of a larger campaign aimed at investigating a large range of pollutants. Due to the logistical difficulties, only one single CSO event was monitored during this study. This CSO event resulted from the most intense rainfall on the Paris agglomeration in 2008. As a consequence, the volume discharged was exceptional. Therefore, the data gained from this event may not be completely representative of more ordinary CSO events and their impact on the Seine River. It is not easy to tell how the exceptional intensity of the rainstorm has affected the parameters we measured, since it may have resulted in at least two antagonistic effects: sources of SM and FIB may have been increased (higher resuspension of sewer and river bed sediments, higher duration of the discharge) but the contaminants may have been more diluted (larger amount of stormwater in the CSO discharge and in the Seine River).

4.1. Dynamics of the CSO

Due to the intensity of the rainfall event, a very high proportion of the CSO was estimated to be constituted of runoff water (89%), and the wastewater constituting the remaining 11% was apparently insufficient to account for the observed SM and FIB concentrations. The composition of the CSO varied over time, particularly between the first half-hour and the rest of the discharge.

4.1.1. Suspended matter

After the study of 16 rain events between 2003 and 2006, Gasperi et al. (2010) have estimated the mean contributions of wastewater, runoff and sewer deposit resuspension to the wet weather load of SM in sewage at the Clichy catchment. Depending on the sub-catchment, wastewater contributed between 22% and 44% of the total SM load, while runoff only contributed between 7% and 12%. The major contributor was the resuspension of sewer deposits that accounted for 47–69%. Additionally, it was shown that the contribution of sewer deposits tended to increase with the intensity of the rain event while wastewater contribution tended to decrease; runoff contribution appeared less related to rainfall intensity. Considering the exceptionally high intensity of the rain event we studied, our estimation that only 14% of the SM discharged during the CSO was attributable to wastewater seems realistic. If we admit a contribution of ~10% for runoff water, then ~75% of the SM must have originated from the resuspension of sewer sediment. A large part of the sewer sediment stock seems to have been resuspended at the beginning of the event since 32% of the total SM load was discharged during the first half-hour of the CSO.

4.1.2. Fecal indicator bacteria

As regards FIB, it was estimated that 8.7×10^{15} *E. coli* and 2.3×10^{15} intestinal enterococci were discharged during the whole CSO event (Table 2). These fluxes can be compared with the amount of FIB discharged during the same time by the three WWTPs that represent the main impact on the Seine microbiological quality in dry weather conditions downstream from the city of Paris. It was estimated that the CSO discharged 79 times more *E. coli* and 100 times more intestinal enterococci than the WWTPs (Table 2). Although very large, the Clichy CSO is only one of the CSOs in the combined sewer system of Paris. Accordingly, these factors of 79 or 100 do underestimate the total load of FIB the CSOs discharged to the Seine River during this rainstorm event.

Among the discharged FIB, 9–71% *E. coli* and 43–83% intestinal enterococci was estimated to originate from another source than wastewater, depending on the assumptions made on the dry weather load of wastewater (low hypothesis: 1st decile; high hypothesis: 9th decile). A review of numerous measurements in separated stormwater sewers has shown that typical *E. coli* concentrations in runoff water are in the order of magnitude of 10^3–10^4 CFU per 100 mL (Marsalek and Rochfort, 2004). *E. coli* concentration in the dry weather wastewater at Clichy is in the order of magnitude of 10^6–10^7 CFU per 100 mL. As runoff water constituted ~90% of the CSO, its contribution to the load of *E. coli* was therefore at the very least ten times lower than wastewater. Resuspension of FIB from sewer deposits was therefore the most plausible additional source. It is consistent with: (i) the large contribution of sewer deposits to the SM load (~75%), (ii) the previous observation in sewers from the Clichy catchment that the resuspended fraction of the deposits consisted mainly of the organic layer found at the interface between water and the gross bed sediment (Gasperi et al., 2010), (iii) the fact that this layer is favorable for the survival of fecal bacteria (Ellis and Yu, 1995) and (iv) the observation that the proportion of attached *E. coli* was notably higher during the first half-hour of the discharge. Indeed, since the major part of the very high SM load of the first half-hour was estimated to originate from the resuspension of sewer sediment and since *E. coli* is expected to be in an attached form in this sediment, an increase in the ratio of attached to free *E. coli* would be expected. If the medium hypothesis for the dry weather load of wastewater (median FIB concentrations) is retained, sewer deposits are estimated to have contributed ~45% and ~65% to the discharge of *E. coli* and intestinal enterococci respectively.

4.2. Dynamics of the discharged FIB in the Seine River

In the Seine River water mass impacted by the CSO, a decrease in concentration of 66% for *E. coli* and 79% for intestinal enterococci was observed between the sample series B and D, collected at an interval of 13–14 h. Three in-stream processes could account for this decline, namely dilution in the waters of the Seine River not impacted by the Clichy CSO, sedimentation and decay (meaning here mortality or loss of culturability). In order to explore the respective contribution of these processes, we first estimated for each river sample to what level the CSO waters were diluted in the Seine River waters. For that purpose, an average non-impacted Seine River water and an average CSO water were hypothesized. The parameters of the average Seine River water (conductivity, SM concentration, FIB concentrations and the proportion of attached *E. coli*) were estimated by the arithmetic mean of their values measured directly upstream from the Clichy outfall (samples A). The parameters of the average CSO water were estimated by the weighted mean of their values in the twelve successive samples of the discharge; the sample weight represented the proportion of the total CSO volume that was discharged during the 30 min of its collection. Then we calculated for each river sample the proportions of average Seine River water and average CSO water that, when mixed together, would have resulted in its conductivity. Conductivity was used for this calculation as it can be considered as a conservative tracer. That way, the proportion of average Seine River water was an estimate of the dilution rate of the CSO in the samples: a value of 0 signifies the sample is only composed of average CSO water and a value of 1 signifies it is only composed of average Seine River water.

On the basis of these dilution rates and the FIB concentrations in both average waters, it was possible to calculate the FIB concentrations that would have resulted from their mixing. These values were thus estimates of the FIB concentrations that would have been observed in the river samples if dilution was the only decline process ("dilution-only" concentrations). They were systematically higher than the actual concentrations, for both FIB (Fig. 7). Therefore dilution alone was not sufficient to account for the decrease in the FIB concentrations.

Table 2 – Comparison of the FIB loads discharged into the Seine River downstream from the City of Paris by the studied CSO and the three WWTPs of the area.

	Discharge (m^3 per 6 h)	*E. coli*		Intestinal enterococci	
		Concentration[b] (CFU (100 mL)$^{-1}$)	Load (CFU per 6 h)	Concentration (CFU/100 mL)	Load (CFU per 6 h)
WWTP 1[a]	60,000	1.7×10^4	1.0×10^{13}	9.9×10^2	5.9×10^{11}
WWTP 2	425,000	2.4×10^4	1.0×10^{14}	5.2×10^3	2.2×10^{13}
WWTP 3	25,000	3.4×10^3	8.5×10^{11}	2.5×10^2	6.3×10^{10}
WWTP 1 + 2 + 3	510,000		1.1×10^{14}		2.3×10^{13}
CSO	578,705	1.5×10^6	8.7×10^{15}	4.0×10^5	2.3×10^{15}

a For the identification of the three WWTPs and the description of their treatment process, see Section 2.1.1.
b For WWTPs, median concentrations measured in the effluent in 2008. WWTP 1: $n = 25$, WWTP 2: $n = 50$, WWTP 3: $n = 26$.

We subsequently explored the possible contribution of decay. The decay of culturable fecal bacteria in aquatic environments results from the combined actions of various biological and physico-chemical processes (grazing by protozoa, virus-induced cell lysis or autolysis, stress due to nutrient depletion, solar radiation or low temperature, all of them inducing mortality or loss of culturability). This decay is usually modeled by a first-order kinetics (Kashefipour et al., 2002; Tian et al., 2002; Menon et al., 2003; Collins and Rutherford, 2004). In our calculation, we set the decay constant to 0.045 h^{-1} for *E. coli*, as proposed by Servais et al. (2007a,b), and to 0.032 h^{-1} for intestinal enterococci on the basis of previous experimental measurements (Passerat, unpublished data). The time during which decay was considered was the interval between 8:50 a.m. (time at the half of the CSO duration) and the collection time of the river samples. The new estimates, incorporating both dilution and decay contributions ("dilution + decay" concentrations), matched better the actual FIB concentrations but were nevertheless generally slightly higher than them (Fig. 7). This could suggest that dilution and decay are not sufficient to account for the observed decrease in the FIB concentrations.

Finally, we explored the role of sedimentation. For that purpose, we compared the dynamics of free and attached *E. coli*. Indeed, Garcia-Armisen and Servais (2009) have shown that only *E. coli* attached to SM is settleable. First we compared the actual concentrations with the "dilution + decay" concentrations. Free *E. coli* concentrations were rather well accounted for or even slightly underestimated, but attached *E. coli* concentrations were systematically overestimated (Fig. 7). An additional process could therefore contribute specifically to the decline of attached *E. coli*, and sedimentation was the most relevant candidate. For each river sample, we then tried to estimate the sedimentation that had occurred since the discharge of the CSO. It was done on the basis of the difference between the sample's actual SM concentration and its "dilution-only" estimate: the higher the discrepancy, the more SM was expected to have settled since its discharge. A sedimentation factor was calculated as the ratio of this difference to the "dilution-only" estimate: a factor of 0 meant no sedimentation and a factor of 1 meant a complete sedimentation of SM. By deducting the proportion equal to this factor from the "dilution + decay" concentration of attached *E. coli*, a "dilution + decay + sedimentation" estimate was obtained. The new estimates matched much better the actual concentrations (Fig. 7). Therefore sedimentation could be a major driver of the fate of attached FIB in the waters impacted by the CSO. Nevertheless, new calculated concentrations of total *E. coli*, incorporating dilution, decay and, for its attached fraction

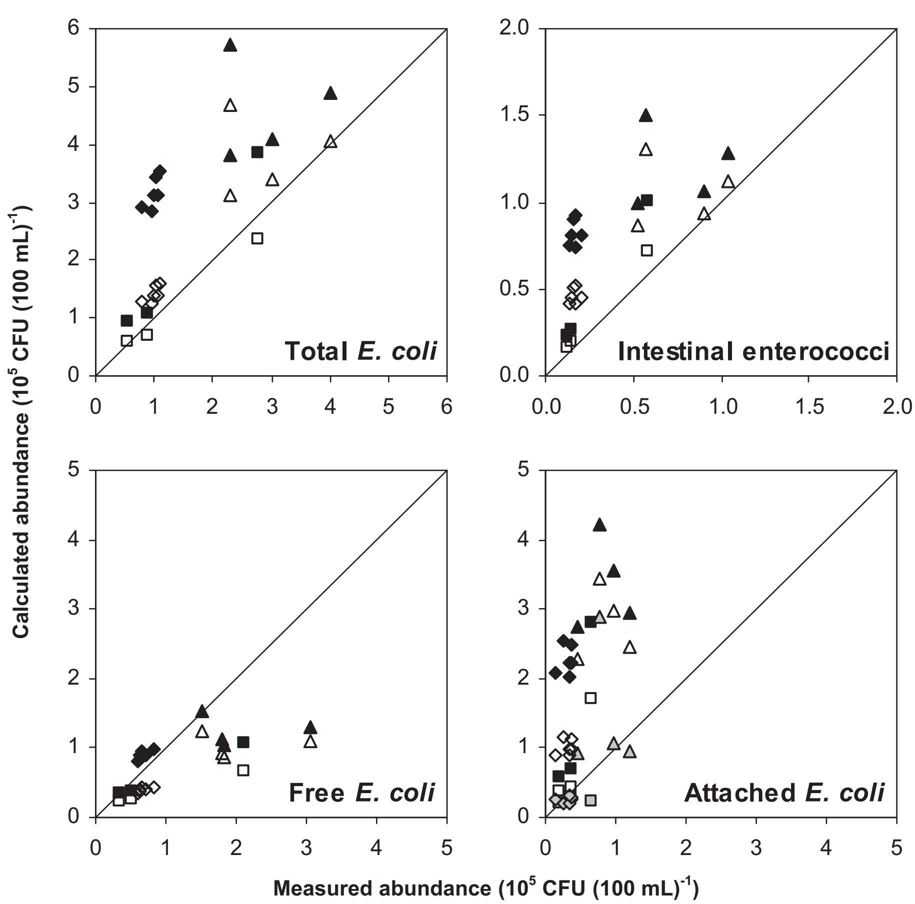

Fig. 7 – Scatter plot of calculated vs. measured concentrations of FIB in the Seine River impacted water mass. For each river sample, calculated concentrations were estimated on the basis of a modeling of dilution only (black symbols), dilution + decay (white symbols) or dilution + decay + sedimentation (grey symbols). Sample origin: series B (triangles), C (squares) and D (diamonds).

only, sedimentation, were on majority slightly inferior to the actual concentrations (data not shown), suggesting that the way we modeled FIB decline by combining the three processes lead to a slight overestimation.

5. Conclusion

The CSO we monitored was a major discharge event, due to very intense rainfall. Its study revealed that:

- the FIB discharged during the CSO represented 80–100 times the dry weather discharge that the Seine River receives in this area due to WWTPs,
- by their resuspension, sewer sediments were estimated to contribute to ∼75% of the SM, 10–70% of the *E. coli* and 40–80% of the intestinal enterococci that were discharged,
- 77% of the discharged *E. coli* were attached to SM,
- directly downstream from the CSO outfall, the FIB concentrations in the impacted water mass of the Seine River were 7–9 times higher than directly upstream,
- these concentrations had decreased by 66% for *E. coli* and 79% for intestinal enterococci after 13–14 h of transit in the Seine River.

These results stress that a CSO at the Clichy site can have considerable impacts, although limited in time, on the microbiological quality of the Seine River. It justifies, if needed, the efforts undertaken by the SIAAP in the past decade to reduce them, and is an incentive to pursue these efforts for better understanding and management.

By combining the analyses on both the CSO and the receiving river, it was possible to compare what was actually measured in the impacted water mass with what was expected on the basis of the CSO parameters and our knowledge of the in-stream processes affecting the contaminants. Regarding fecal bacteria, the processes of dilution, decay and sedimentation of the attached fraction, as modeled in this study, gave a reasonably good explanation for the in situ observations.

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