

Impact of Wastewater Release on the Faecal Contamination of a Small Urban River: The Zenne River in Brussels (Belgium)

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Abstract In this study, we investigated the microbiological quality of the small urban Zenne River which flows through the city of Brussels (Belgium). The abundances of faecal indicator bacteria (FIB) (*Escherichia coli* and intestinal enterococci) revealed high levels of faecal contamination of the Zenne River, especially in Brussels area and downstream from this area where FIB were in the range of what is usually observed in secondary treated wastewaters. The origin of this faecal contamination is mainly attributed to the effluents of the two wastewater treatment plants (WWTPs) located along the river in Brussels. Comparison of the present situation with the 1990s situation (before the implementation of Brussels WWTPs) showed a sharp improvement of the river microbiological quality. This improvement should however be regarded with care as it was also observed that, during rain events, combined sewer overflows (CSO) outfalls were responsible of a strong increase of faecal contamination in the river downstream from Brussels, and such CSO occur frequently in Brussels. Altogether, these results document the variations of the microbiological quality of a sewage-polluted urban river in relation with long-term changes (implementation of WWTP) and the short-term disturbances (CSO).

Keywords Microbiological water quality · River · Wastewater · Faecal indicator bacteria · *Escherichia coli* · Intestinal enterococci

1 Introduction

When a river flows through a large city, the quality of its water is often negatively impacted due to the release of domestic and industrial wastewaters. The importance of the impact depends primarily on the size of the city (number of inhabitants), the importance of industrial activities, the type of treatment applied to wastewater and the flow of the river; the higher the flow is, the higher the dilution capacity of the river is.

The present paper deals with the huge impact of a city (Brussels, Belgium) on the water quality of the small Zenne River which crosses the city. The Zenne River which has an annual average discharge upstream from Brussels of $4 \text{ m}^3 \text{ s}^{-1}$ is an example of surface water highly disturbed by urban wastewater. Indeed, in Brussels area, the river receives the wastewater released by the two wastewater treatment plants (WWTPs) of Brussels (treating together more than 1.4 million equivalent inhabitants); the average flow of treated wastewaters is thus in the same order of magnitude ($3.9 \text{ m}^3 \text{ s}^{-1}$) than the Zenne River flow rate upstream from Brussels. The river waters downstream from Brussels is thus roughly half composed of treated wastewaters, this proportion being even higher during the low flow periods of the river. In addition, during rain events, combined sewer overflows (CSO) occur in the Brussels area and

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release in the river a mixture of untreated wastewater and surface runoff water.

Among the negative impacts of an urban area on the river water quality can be mentioned: the downstream decrease of dissolved oxygen concentration due to biodegradation of organic matter and nitrification of ammonia, eutrophication due to phosphorus and nitrogen inputs, increase of turbidity leading to a reduction of the photosynthesis, presence of high levels of mineral (metals, for example) and organic pollutants and the decrease of the microbiological water quality due to the presence of high level of enteric microorganisms in wastewaters. This last point is a concern for public health as among these enteric microorganisms, human pathogens could be present. Significant increases in microbiological water contamination were already reported in rivers downstream from large cities as, for example, the Seine River downstream Paris (Servais et al. 2007) and the Thames River downstream London (Tryland et al. 2002).

The objectives of our research were to evaluate the impact of Brussels' wastewaters discharge on the Zenne River microbiological water quality and to quantify the fluctuations of the microbiological quality of the river over space and time. Longitudinal profiles were performed in dry weather conditions at different seasons to evaluate the spatial distribution level of faecal contamination along the river. Faecal indicator bacteria (FIB) abundances were also quantified in the effluents of both Brussels WWTPs and in tributaries in order to evaluate their contribution as sources of river contamination. Present levels of contamination in the river were compared with data collected before the implementation of Brussels WWTPs. Samples were also collected downstream from Brussels during rain events in order to investigate the impact of combined sewer overflows, which occur frequently in Brussels, and during a period of treatment failure in the largest of the Brussels WWTP in order to investigate the impact of massive contamination by untreated sewage. Due to the limited discharge of the river and the size of Brussels city, the Zenne River could be considered as a model site to investigate the microbiological water quality of a river highly disturbed by urban wastewater.

The evaluation of the microbiological quality of surface waters is currently based on the abundance of faecal indicator bacteria (FIB). Today, the FIB primarily used to evaluate the level of faecal contamination of waters are *Escherichia coli* (*E. coli*) and intestinal enterococci (IE) (Edberg et al. 2000; Fewtrell and Bartram 2001;

UWWD 1991). The concentrations of these two FIB were measured during the present study in order to evaluate the level of faecal contamination.

2 Material and Methods

2.1 Study Area

The Zenne River is located in the Scheldt watershed and flows in Belgium (Fig. 1); it is a tributary of the Dijle River. The Zenne watershed (surface 991 km²) is characterised by agricultural activities in its upstream part and an important urbanisation in its downstream part. The population density in the watershed is very high (on average 1,260 inhabitants km⁻²) and mostly located in Brussels city and suburbs. The Zenne River has a total length of 103 km and crosses the city of Brussels from south to north over a distance of about 20 km. For sanitation purposes, the Zenne River was covered in the nineteenth century (1867–1871) in most of the stretch located in the Brussels-Capital area. In this area, the Zenne River receives the sewage waters from two large WWTPs: the Brussels South WWTP (360,000 equivalent inhabitants) in operation since the year 2000 and the Brussels North WWTP (1.1 million equivalent inhabitants) in operation since 2007. The two WWTPs function according to different technologies. The Brussels South WWTP treatment line includes a primary settling stage (to remove suspended solids) and a secondary biological treatment (activated sludge process to remove biodegradable organic matter). Thus, this WWTP does not yet fulfil the European Directive on the treatment of urban wastewater (UWWD 1991) requiring, for the Zenne basin, tertiary treatment (removal of N and P) of sewage waters in all WWTPs of more than 10,000 equivalent inhabitants. At Brussels North WWTP, there are two treatment lines. The first one (biological line) includes a primary settling stage followed by a modern tertiary treatment technology (simultaneous removal of biodegradable organic carbon, nitrogen and phosphorus by an activated sludge process). The other treatment line (rain line) runs in parallel to the biological line when the discharge reaching the WWTP is too high in wet weather situations; this rain line uses only a primary settling process. On an annual basis, the volume treated in the biological line accounts for roughly 90 % of the total volume reaching the WWTP.

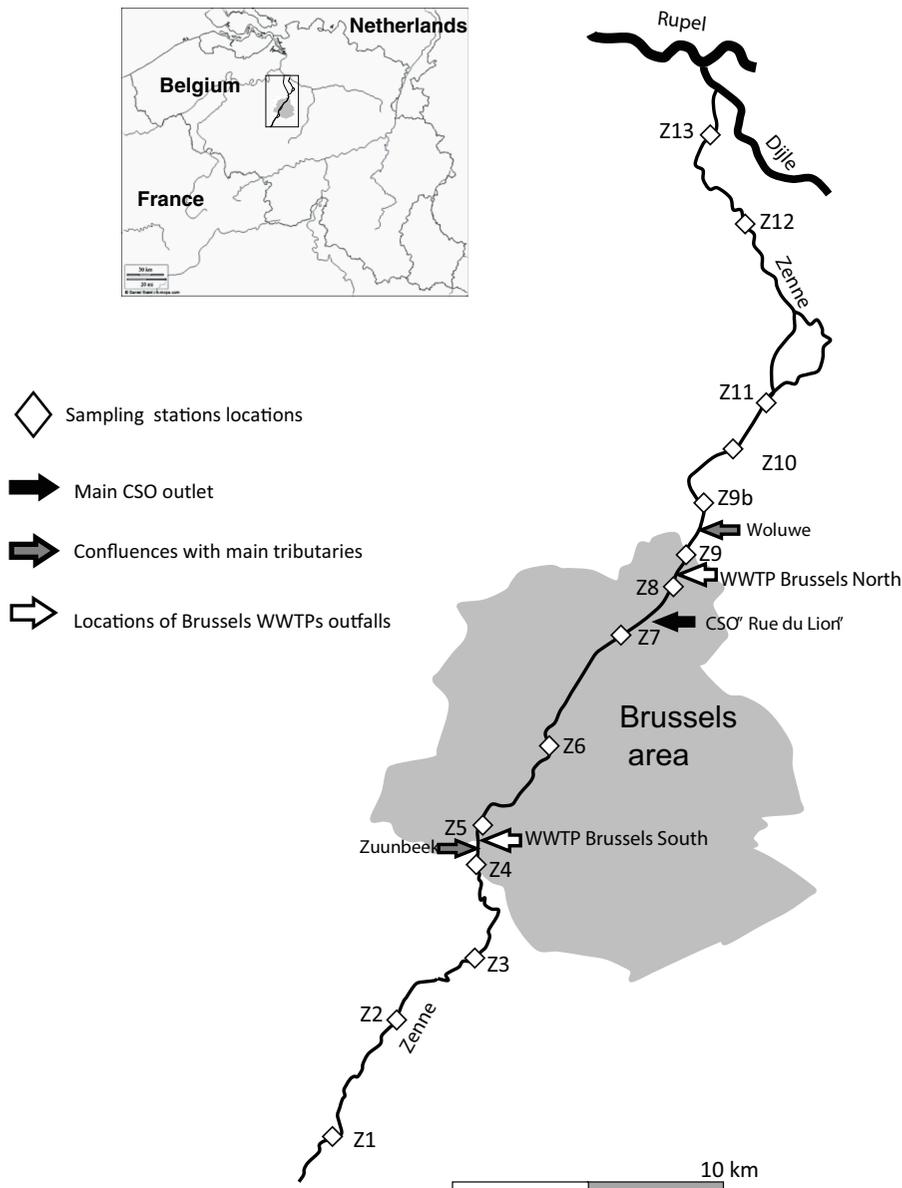


Fig. 1 Map of the Zenne basin. Brussels-Capital region is indicated in grey; location of sampling stations in the Zenne River are presented by white symbols; locations of the effluent releases of both Brussels WWTPs effluents in the Zenne River are indicated

by white arrows; confluences with tributaries (Zuunbeek and Woluwe) are indicated by grey arrows and the CSO outlet at “Rue du Lion” is indicated by a black arrow

The Zenne River also receives waters from two tributaries in the Brussels-Capital region: the Zuunbeek and the Woluwe (Fig. 1). Some other small tributaries located in the Brussels area are diverted in the sewer collectors so that their waters reach Brussels WWTPs. The annual average discharge of the Zenne River arriving in Brussels from the upstream part of the watershed is $4 \text{ m}^3 \text{ s}^{-1}$ (as deduced from water flow data of the last

20 years as monitored by the Hydrologisch Informatiecentrum—HIC).

2.2 Sampling Strategy

Five longitudinal sampling campaigns were performed in dry weather periods; the discharge of the Zenne River just upstream from Brussels was always low during

these campaigns, $1.4 \text{ m}^3 \text{ s}^{-1}$ in September 2009, $2.5 \text{ m}^3 \text{ s}^{-1}$ in January 2010, $1.4 \text{ m}^3 \text{ s}^{-1}$ in April 2010, $2.0 \text{ m}^3 \text{ s}^{-1}$ in July 2010 and $2.9 \text{ m}^3 \text{ s}^{-1}$ in October 2010. During these campaigns, water samples were collected at 13 sites in the Zenne River between Lembeek (Z1) and Heffen (Z13) which is located just upstream from the confluence with the Dijle River (Fig. 1); samples were also collected at the outlet of two tributaries, the Zuunbeek and the Woluwe. A kilometric scale along the longitudinal course of the river was defined; it is arbitrarily set at zero at Lembeek (Z1) and increases from upstream to downstream (km 56 in Z13).

During the sampling campaigns in the Zenne River, samples were also collected in the inlet and outlet of both Brussels WWTPs. In these two WWTPs, average daily samples were collected with refrigerated automatic samplers in order to integrate the daily fluctuations of FIB abundance in sewage due to human activities. Data on FIB concentrations in the treated waters of Brussels North WWTP presented in this paper concern the biological line.

In December 2009, due to technical problems, the treatment was interrupted at Brussels North WWTP during 10 days, and thus, the whole volume of wastewaters reaching this WWTP was released in the Zenne River without any treatment. In order to evaluate the impact of such a major release of untreated wastewater on the microbiological water quality of the river, samples were collected in the downstream stations of the Zenne River during the period of treatment interruption.

As CSO are known to occur frequently in Brussels in wet weather situations (Le et al. 2014), the impact of rain events over Brussels on the Zenne River microbiological water quality downstream from Brussels was studied on June 16, 2011 and October 3, 2012. For this, samples were collected in the Zenne River on an hourly or bihourly basis for a 12-h period (from 8 a.m. to 8 p.m.) in June 2011 and a 13-h period (from 10 a.m. to 11 p.m.) in October 2012 at the outlet of the Brussels-Capital region (sampling station Z9b) (Fig. 1). In addition, a sampling campaign (with hourly sampling during 24 h) was performed on September 16, 2010 at the same station in dry weather conditions to serve as a reference.

All the river water samples were collected with a plastic bucket from bridges, halfway between the banks, and were stored in 1-L sterile bottles, kept at $4 \text{ }^\circ\text{C}$ and analysed within a maximum of 6 h after collection.

2.3 Enumeration of *E. coli* and Intestinal Enterococci by Plate Counts

E. coli and IE were enumerated in the water samples by plate counts on Chromocult coliform agar (CCA) and Chromocult enterococci agar (CEA), respectively (Merck KGaA, Darmstadt, Germany). These two chromogenic growth media were shown to be highly specific to their corresponding indicator bacteria. High percentages of specificity were reported in the literature for CCA enumeration of *E. coli* (96 %) (Prats et al. 2008) and for CEA enumeration of enterococci (98 %) (Miranda et al. 2005) in water samples. These high levels of specificity were confirmed on samples from Zenne River at the beginning of the present study. Depending on the expected concentration, 1, 10 or 100 mL of samples were filtered through sterile $0.45\text{-}\mu\text{m}$ GN-6 membrane filters (Pall Corporation, Ann Arbor, MI, USA) or 100 μL of samples (or tenfold serial dilutions in sterile Ringer's solution) were plated on the agar plates. When the volume to be filtered was 1 or 10 mL, it was mixed with Ringer's solution to ensure a uniform repartition of the bacteria on the filter. CCA and CEA plates were performed in triplicates and incubated at $36 \text{ }^\circ\text{C}$ for, respectively, 24 and 48 h. Plate counts were expressed as colony-forming units (CFU) per 100 mL of sample.

2.4 Physicochemical Parameters Measurements

Chemical oxygen demand (COD) was determined by oxidation of the sample using potassium dichromate in acid conditions during 1 h at $150 \text{ }^\circ\text{C}$ under pressure. The reading of the result was made by colorimetry; the potassium hydrogen phthalate was used as organic matter reference for the calibration. Ammonium (NH_4) concentrations were determined by automatic colorimetric method using a QuAAtro (Seal) segmented flow analyser system.

2.5 Statistical Analysis

The relationship between FIB concentrations and physicochemical water quality parameters (COD and NH_4) were examined using linear regression analysis. The coefficient of determination (r^2) was determined. The probability test (p value) associated to linear regression was performed using Student test and significance was determined at 95 % confidence level. Kruskal-Wallis

test completed with Tukey's honestly significant difference (Tukey's HSD) test was performed to analyse the difference in FIB concentrations in the influents and the effluents of the two Brussels WWTPs. Test significances were determined at 95 % confidence level. Statistical analyses were performed using R Software.

3 Results and Discussion

3.1 Faecal Contamination of the Zenne River in Dry Weather Conditions

3.1.1 Longitudinal Distribution of the Contamination

The spatial distribution of *E. coli* and IE along the Zenne River during five longitudinal profiles performed in dry weather conditions is presented in Fig. 2. Dry weather conditions mean that no significant precipitation ($<1 \text{ mm d}^{-1}$) occurred 2 days before and during the sampling campaign.

The spatial distribution of FIB abundances in the river Zenne shows the following: (1) a relatively high level of FIB in stations located upstream Brussels area, between Lembeek (Z1) and Drogenbos (Z4), (2) a strong increase of FIB concentrations in Brussels area (stations Z5 to Z9) and (3) a general decrease of the abundance of FIB downstream from Brussels area (stations Z10 to Z13).

Even upstream from Brussels area, the Zenne River is already contaminated by faecal pollution. When considering the stations Z1 to Z4 for the five sampling campaigns, the geometric means of *E. coli* and IE were, respectively, $1.1 \times 10^4 \text{ E. coli}(100 \text{ mL})^{-1}$ and $3.6 \times 10^3 \text{ IE}(100 \text{ mL})^{-1}$. The origins of this pollution are the release of the effluents from three relatively small WWTPs (with a total capacity of 103,300 equivalent inhabitants), the runoff on pastured areas, which is known to contaminate rivers with FIB (Kay et al. 2008), and the effluents from farms performing intense breeding activities in the upstream watershed.

At the entrance of the Brussels area, between stations Z4 and Z5, a strong increase in FIB concentrations was observed; in this stretch, the river receives the treated effluents of the Brussels South WWTP (see next section for FIB concentrations in effluents) and the small but highly contaminated tributary Zuunbeek. The FIB levels observed in the Zuunbeek were up to $4.1 \times 10^6 \text{ E. coli}(100 \text{ mL})^{-1}$ and $3.4 \times 10^5 \text{ IE}(100 \text{ mL})^{-1}$; such

concentrations in this small stream can only be explained by the release of raw wastewaters in this tributary. Indeed, in the urban area surrounding the Zuunbeek, some of the sewers were still not connected to the regional WWTP of Sint-Pieters-Leeuw, and thus, untreated sewage was released into the Zuunbeek until 2011 (AQUAFIN, personal communication). Between Z5 and Z8 stations, the behaviour of the FIB concentrations differed depending on the sampling campaign considered. Variable contribution of different "in situ" processes and the variations in sewage discharges can explain these varying behaviours. Concerning the "in situ" processes, FIB can decrease due to mortality and sedimentation (Garcia-Armisen and Servais 2009). On the other hand, releases of water from the sewer system were shown to occur sometimes even in dry weather situations in Brussels downtown (Petrovic et al. 2012); such release can be a significant source of FIB in the covered part of the Zenne River. Between Z8 and Z9 stations, the effluents of Brussels North WWTP are discharged in the river. This had different impacts depending on the FIB concentrations in the river upstream from these effluents. When FIB concentrations in the river were higher at Z8 station than in the treated effluents, a decrease of concentrations was observed in the river. These situations are surprising examples of improvement of the microbiological quality of a river downstream from the discharge of WWTP effluents. At the opposite, when the concentrations were lower at Z8 than in the effluents, an increase of concentrations in the river was observed between Z8 and Z9 stations.

In the downstream part of the river, a decrease of FIB concentrations was generally observed (except in April 2010); it can be explained by mortality of FIB and sedimentation. Between Z9 and Z10, the waters of the Woluwe River are discharged into the Zenne River. As the level of contamination is very high in Z9 station, the impact of the Woluwe River on the contamination of the Zenne River seems negligible. In January 2010, a peak of contamination was observed in Z10 which can be due to some release of untreated wastewater as works were performed on the sewer network at this period.

Overall, FIB concentrations were the lowest upstream from Brussels (geometric means for the five campaigns in the stretch Z1 to Z4, $1.1 \times 10^4 \text{ E. coli}(100 \text{ mL})^{-1}$ and $3.6 \times 10^3 \text{ IE}(100 \text{ mL})^{-1}$) and the highest in the Brussels-Capital region (geometric means for the five campaigns in the stretch Z5 to Z9, $2.3 \times 10^5 \text{ E. coli}(100 \text{ mL})^{-1}$ and $5.4 \times 10^4 \text{ IE}(100 \text{ mL})^{-1}$). The

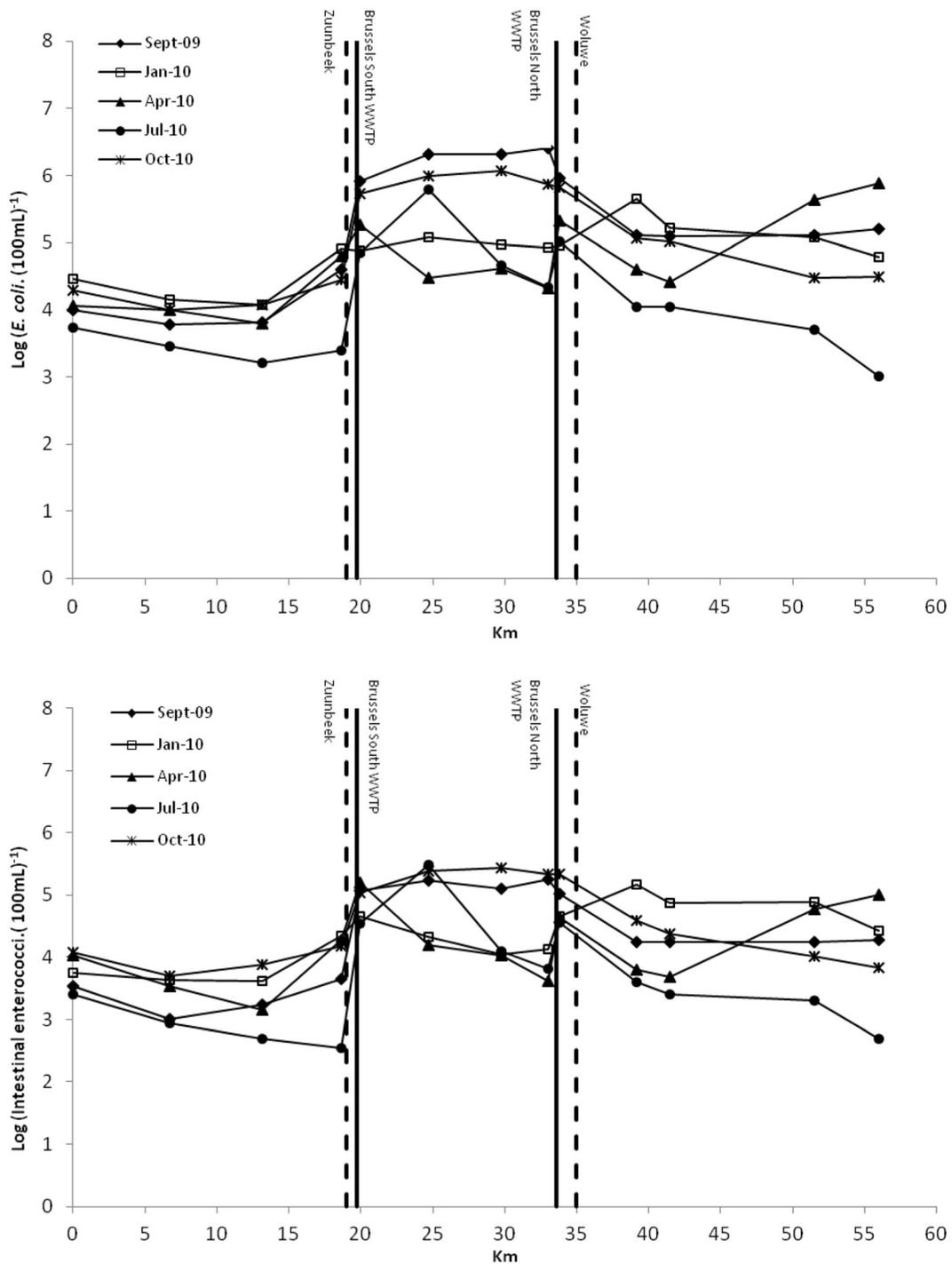


Fig. 2 Longitudinal profiles of *E. coli* and IE abundances (in log scale) in the Zenne River. The x-axis is a kilometric unit that is set at zero at Lembeek and increases from upstream to downstream. Vertical black lines represent the location of the WWTPs and the tributaries

major impact on the contamination level of the river seems to be due to Brussels South WWTP, but other sources of FIB contamination are of non-negligible

importance: Brussels North WWTP, the tributaries Zuunbeek and Woluwe, raw wastewaters release from the Brussels old sewer system. According to Petrovic et al.

(2012), there are still illicit sewer connections discharging untreated sewage water directly to the river even in dry weather conditions.

Overall, wastewaters (treated and untreated) were responsible for the contamination of the river, it is why positive correlations were found between FIB concentrations in the Zenne River and physicochemical water quality parameters known to be present in high concentrations in urban effluents (chemical oxygen demand (COD) and ammonium (NH_4)). *E. coli* and IE concentrations were significantly and positively correlated with COD ($r^2=0.2$, $n=55$, $p<0.001$ for *E. coli*; $r^2=0.2$, $n=55$, $p<0.001$ for IE) and NH_4 ($r^2=0.5$, $n=67$, $p<0.001$ for *E. coli*; $r^2=0.3$, $n=67$, $p<0.001$ for IE) concentrations. Such significant correlations between FIB, on one hand, and COD and NH_4 , on the other hand, in rivers contaminated by domestic wastewater were already reported in literature (Kirschner et al. 2009; Lyautey et al. 2011; Suter et al. 2011).

The measured FIB abundances in the Zenne River were compared with the requirements of the new EU Directive for bathing water quality (EU Directive 2006). This directive considers that bathing activities can be allowed in freshwaters when the 90th percentiles of the FIB abundances measured during the bathing period do not exceed 9.0×10^2 *E. coli*(100 mL) $^{-1}$ and 3.3×10^2 IE(100 mL) $^{-1}$. The FIB concentrations at each location, even upstream from Brussels, exceeded the level of the directive, indicating that none of the sampling sites had a microbiological water quality sufficient for bathing activities.

When all data were considered together, a significant relationship between *E. coli* and IE concentrations (in log-log plot) was observed ($r^2=0.92$; $n=146$; p value <0.01) (data not shown), indicating that the information on level of faecal contamination of water samples was quite similar whatever the faecal indicator used, *E. coli* or IE.

3.1.2 Faecal Contamination of Brussels WWTPs Effluents

Figure 3 presents the abundances of *E. coli* and IE in the raw waters and the treated waters of Brussels North (biological treatment line) and Brussels South WWTPs. In the raw waters of both WWTPs, the abundances ranged between 7.41×10^6 and 1.80×10^7 *E. coli*(100 mL) $^{-1}$ (geometric mean 1.09×10^7 *E. coli*(100 mL) $^{-1}$) and between 1.91×10^6 and 4.15×10^6 IE(100 mL) $^{-1}$ (geometric mean

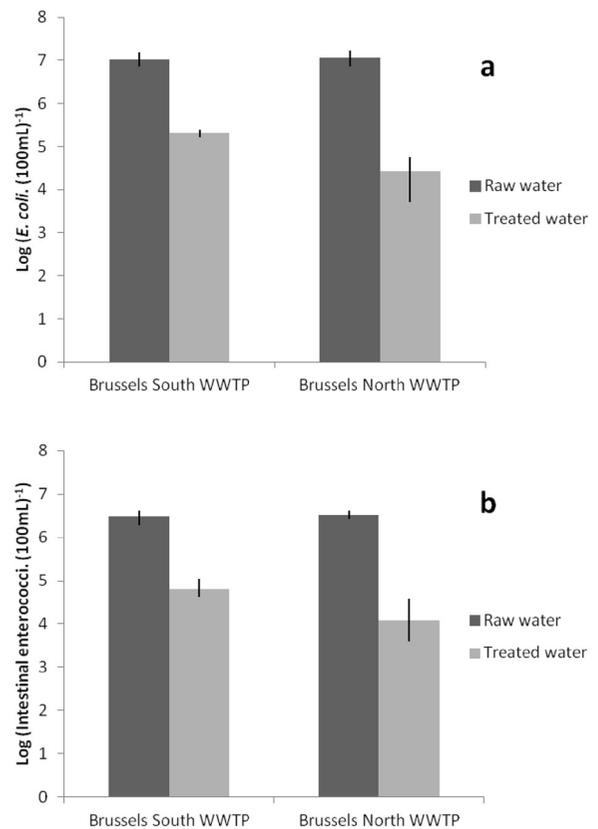


Fig. 3 Abundances of *E. coli* and IE in the raw waters (black bars) and treated effluents (grey bars) of Brussels North and Brussels South WWTPs. Data are expressed as geometric means, and vertical bars represent the range between minimal and maximal values

3.22×10^6 IE(100 mL) $^{-1}$). There was no significant difference between the abundances of FIB ($p<0.001$) in raw waters of both WWTPs. The values measured in Brussels raw waters are in complete agreement with the range of values reported in previous studies for raw domestic wastewaters (Rose et al. 1996; Garcia-Armisen and Servais 2007; Kay et al. 2008; Kistemann et al. 2008; Ouattara et al. 2011; Lucas et al. 2012, 2014). In the treated effluents of the WWTPs, the abundances of both FIB were significantly reduced while it must be noted that these WWTPs do not include a disinfection stage aiming to specifically remove microbiological pollutants. The difference observed between the FIB concentrations in the effluents of both WWTPs was significantly different ($p<0.001$). The log removal of FIB (calculated as the difference between the log of the FIB concentrations before and after treatment) ranged between 1.59 and 1.79 (average 1.70) for *E. coli* and between 1.41 and 2.00 (average 1.69) for IE at Brussels South WWTP. At Brussels North WWTP, the log

removal of FIB ranged between 1.89 and 3.09 (average 2.62) for *E. coli* and between 1.84 and 2.92 (average 2.43) for IE. The average log removals of *E. coli* and IE observed at Brussels North WWTP (tertiary treatment) were significantly higher than that at Brussels South WWTP (secondary treatment). Higher removals of FIB in WWTPs using tertiary treatment than in WWTPs using secondary treatment were already reported in the literature (George et al. 2002; Servais et al. 2007). Despite the removal of FIB by both WWTPs of Brussels city, the abundances of *E. coli* observed in the treated effluent remain very high especially for the Brussels South WWTP (geometric means 2.04×10^5 *E. coli*(100 mL)⁻¹ and 6.21×10^4 IE(100 mL)⁻¹) and thus represent a major source of faecal pollution of the Zenne River.

3.1.3 Impact of the WWTPs Implementation on the Microbiological Quality of the Zenne River

Before the year 2000, domestic sewage waters from Brussels area were directly discharged into the river without any treatment. In 1998, our laboratory investigated for the first time the microbiological quality of the Zenne River; two longitudinal sampling profiles were performed in dry weather conditions in January and July 1998. At this time, faecal coliforms (FC) were enumerated on lactose agar with Tergitol (0.095% wt/vol final

concentration) and triphenyl 2,3,5-tetrazolium chloride (TTC) (0.024% wt/vol final concentration) (Petit, personal communication). In order to compare the old data with the recent data, the faecal coliform concentrations were converted into *E. coli* concentrations by multiplying the faecal coliform data by 0.77; this value is the average ratio between *E. coli* and faecal coliform numbers measured in river water samples collected in Belgium and France (Garcia-Armisen et al. 2007).

Figure 4 presents the longitudinal profiles of faecal contamination (log of the geometric means of *E. coli* concentrations) in 1998 (2 sampling campaigns) and in 2009–2010 (five sampling campaigns). Overall, the levels of *E. coli* were higher in 1998 than those recorded in 2009–2010 along the whole course of the Zenne River. The lower values of *E. coli* abundances observed in 2009–2010 with regards to the abundances measured in 1998 in the upstream part of the longitudinal profiles is mainly due to the implementation of several small WWTPs in the upstream part of the watershed. Downstream from Brussels city, the level of faecal contamination was really huge in 1998 with concentrations in *E. coli* close to what is usually measured in raw wastewaters. A decrease of around 1.5 log unit was observed in *E. coli* abundances between the 1998 and the 2009–2010 period; this is the result of the implementation of both Brussels WWTPs. Even if the

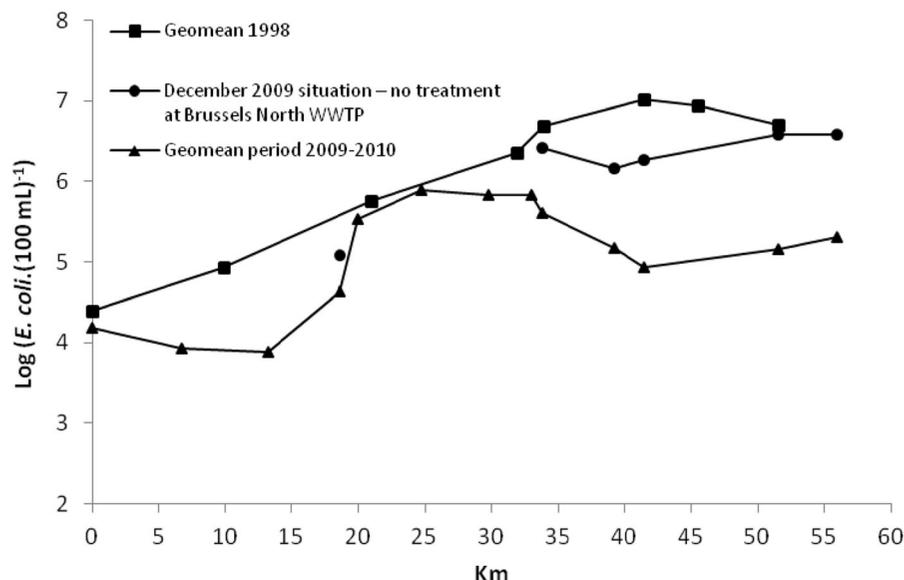


Fig. 4 Longitudinal profiles of geometric means of *E. coli* abundances along the Zenne River in 1998 (two sampling campaigns) (filled squares) and 2009–2010 (five sampling

campaign) (filled triangles) and during the treatment interruption at Brussels North WWTP in December 2009 (filled circles)

microbiological quality of the Zenne River downstream from Brussels is still poor today, it is significantly better than in the 1990s.

In December 2009, due to a technical problem, the treatment was interrupted at Brussels North WWTP and thus the whole volume of wastewaters reaching this WWTP was released in the Zenne River without any treatment. Downstream from Brussels area, the measured *E. coli* concentrations during the interruption of Brussels North WWTP in December 2009 were very close to those observed in the 1990s (Fig. 4). This result confirmed that Brussels North WWTP contributed significantly to the improvement of the microbiological of the downstream part of the Zenne River.

3.2 Faecal Contamination of the Zenne River During Rain Events

In rainy periods, the water circulating in combined sewer systems is a mixture of raw wastewater and runoff water; its flow can exceed the transport capacity of the sewer system or the treatment capacity of the WWTP so that the excess volume, combined sewer overflow (CSO), is released into the receiving water bodies without any treatment. Our interest for microbiological water quality in the Zenne River downstream from Brussels in rainy situations was due to the fact that CSO are known to occur frequently in Brussels (Le et al. 2014). Indeed, Le et al. (2014) recorded the occurrence of 75 CSO events over a 1-year period (from November 2011 to October 2012) at the outlet of one of Brussels main sewers (CSO outlet station called “Rue du Lion”, located about 2.5 km upstream from the Brussels North WWTP outlet) with an average discharge of $6 \text{ m}^3 \text{ s}^{-1}$ releasing for an average period of 6.5 h per event. This means that, on an annual average basis, a significant CSO occurs each 5 days at this main CSO outlet in Brussels. Concerning FIB concentrations in CSO, recent studies (Passerat et al. 2011; Madoux-Humery et al. 2013; Lucas et al. 2014) have shown that the runoff water input inside the sewers results usually in the dilution of FIB present in wastewaters. In these studies, FIB concentrations in CSO were 2.5 to 10 times (depending on the rain intensity, the sampling location, the moment of the day,...) lower than those measured in raw wastewaters during dry weather conditions at the same location. This means that minimal concentrations in CSO were still at least one order of magnitude higher than FIB concentrations usually observed in secondary

treated effluents. Thus, one can assume major impact of CSO on the microbiological quality of the receiving natural environment; such impact was investigated in the Zenne River in the present study.

During the reference dry period, the concentrations of *E. coli* measured ranged between 2.45×10^4 and $1.06 \times 10^5 E. coli(100 \text{ mL})^{-1}$ (geometric mean $4.62 \times 10^4 E. coli(100 \text{ mL})^{-1}$) (Fig. 5b). The fluctuations of *E. coli* abundances observed in the Zenne River (4.3-fold between the maximum and the minimum values) can be due to small fluctuations in the flow rates of effluents from both Brussels WWTPs and in the *E. coli* concentrations in treated effluents. Fluctuations in raw and treated wastewater content of faecal microorganisms are known to occur during a day due to diurnal profile of defecation (Yaziz and Lloyd 1979) and also to varying retention time of the sewage in the sewers system and in the WWTPs (Lucas et al. 2014).

The first studied rain event (June 16, 2011) was characterised by the succession of two rain periods. The first one occurred in the end of the morning and the second one was an intense local storm event occurring between 4 and 5 p.m. The first rain episode induced an increase of discharge at the monitoring station, from $6 \text{ m}^3 \text{ s}^{-1}$ before the starting of the rain to $10 \text{ m}^3 \text{ s}^{-1}$ between 12 a.m. and 2 p.m. (Fig. 5a). It corresponded to an increase of the discharge at the outlet of Brussels WWTPs. Due to the increase of volume to be treated at Brussels North WWTP, the rain line was used in parallel to the biological line; the maximum flow rate passing through the rain line ($1.8 \text{ m}^3 \text{ s}^{-1}$) was observed just after 12 a.m. (AQUIRIS, personal communication). This leads to an increase of *E. coli* concentration in the river (from 8.98×10^4 to $3.60 \times 10^5 E. coli(100 \text{ mL})^{-1}$) (Fig. 5b). Even if no microbiological quality measurements were available at the outlet of the rain line in Brussels North WWTP, one can assume that the *E. coli* concentrations were much higher at the outlet of the rain line than at the outlet of the biological line. Log removals of FIB around 0.5 were reported in the literature when a primary settling treatment alone was applied (George et al. 2002; Kay et al. 2008), while an average log removal close to 2.5 was measured for the biological line at Brussels North WWTP (see above). Due to the afternoon intense rain episode, the river discharge increased rapidly from $8.5 \text{ m}^3 \text{ s}^{-1}$ at 5 p.m. to reach a maximum value ($15.2 \text{ m}^3 \text{ s}^{-1}$) at 6 p.m. (Fig. 5a). The flow rate increase in the river occurring at the end of the afternoon corresponded not only to a second increase of the discharge at the outlet of Brussels WWTPs but also to

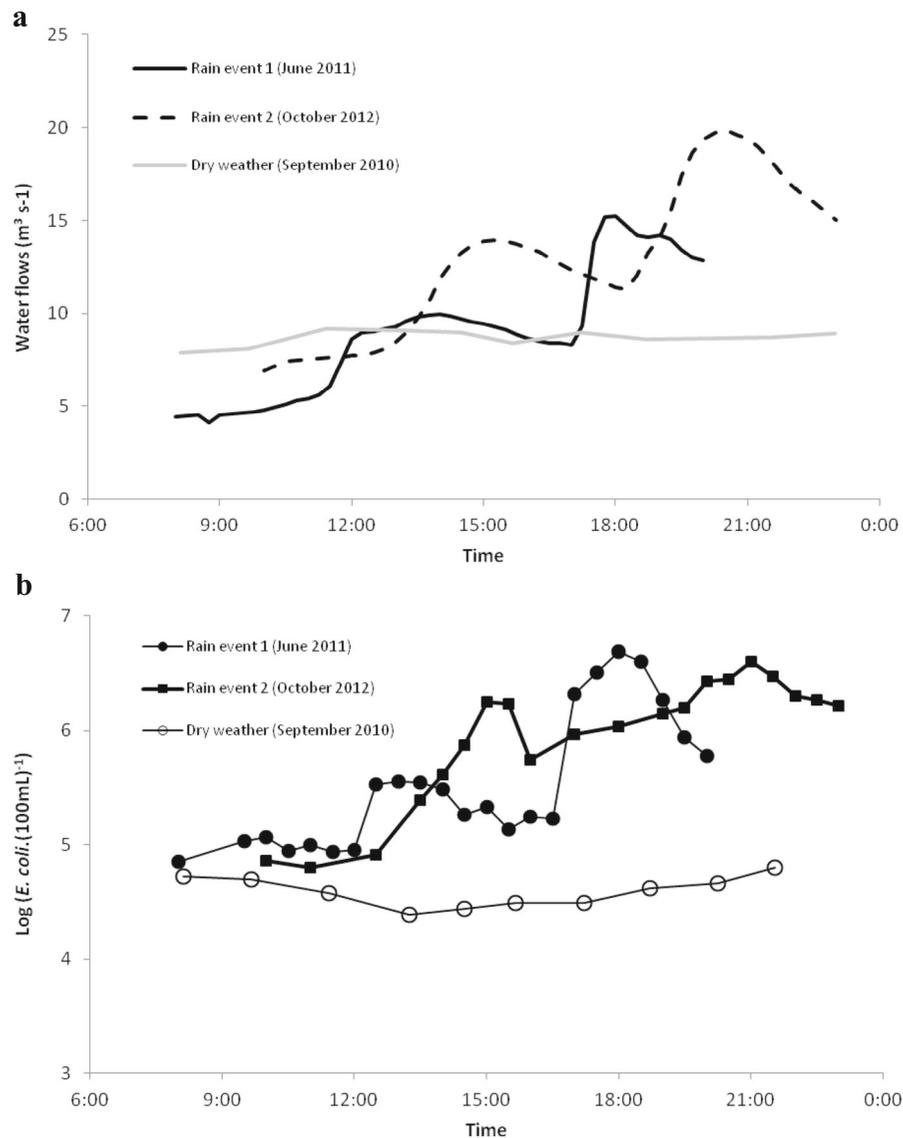


Fig. 5 **a** Temporal fluctuations of the discharge (HIC data) at Vilvoorde station (station Z9b) during three sampling campaigns, two during rainy days (June 16, 2011: *black line*; October 3, 2012: *dashed line*) and one in dry weather conditions (September 16,

2010: *grey line*). **b** Short-term temporal fluctuations of *E. coli* abundances at Vilvoorde station during two rain events (June 16, 2011: *filled circles*; October 3, 2012: *filled squares*) and in dry weather conditions (September 16, 2010: *empty circles*)

CSO occurring in Brussels area (maximum flow rate of the main CSO $3.5 \text{ m}^3 \text{ s}^{-1}$ at 6 p.m. (Petrovic et al. 2012)). The resulting maximum *E. coli* concentration, $4.85 \times 10^6 \text{ E. coli}(100 \text{ mL})^{-1}$, was recorded at 6 p.m. (Fig. 5b).

The second studied rain event (October 3, 2012) was also characterised by the succession of two rain periods. The first rain episode occurred in the morning with a maximum rain intensity recorded at 11 a.m. (data not shown). The second one was more intense and occurred in the afternoon with maximum rain intensity recorded in

Brussels between 6 and 7 p.m. (data not shown). After the first rain episode, an important increase of the flow rate was observed at the monitoring station (Fig. 5a). The flow rate recorded at 10 a.m. ($7 \text{ m}^3 \text{ s}^{-1}$) increased gradually to reach the value of $14 \text{ m}^3 \text{ s}^{-1}$ at 3 p.m. (Fig. 5a). At this moment, the flow rate measured at the outlet of Brussels North WWTP was $10 \text{ m}^3 \text{ s}^{-1}$; $2.5 \text{ m}^3 \text{ s}^{-1}$ was passing through the rain line (AQUIRIS, personal communication). In addition, CSO occurred between 2 and 3 p.m. (maximum discharge $3.5 \text{ m}^3 \text{ s}^{-1}$). The consequence of the

utilisation of the rain line at Brussels WWTPs and of the CSO was a significant increase of the abundance of *E. coli* in the river (from $7.2 \times 10^4 E. coli(100 \text{ mL})^{-1}$ at 10 a.m. to $1.75 \times 10^6 E. coli(100 \text{ mL})^{-1}$ at the 3 pm) (Fig. 5b). The second rain episode occurred at the end of the afternoon, and consequently, CSO occurred in Brussels area between 6 and 10 p.m. (maximum discharge of the main CSO in Brussels $7.5 \text{ m}^3 \text{ s}^{-1}$ around 8 p.m.). The flow rate measured at the outlet of Brussels North WWTP was maximum at 8 p.m. ($12 \text{ m}^3 \text{ s}^{-1}$ with $5 \text{ m}^3 \text{ s}^{-1}$ passing through the rain line). The CSO and WWTPs discharge contributed to increase the flow rate of the river at the monitoring station. The maximum value of flow rate at the monitoring station ($20 \text{ m}^3 \text{ s}^{-1}$) was recorded at 8:30 p.m. During this second rain episode, we also observed an important increase of the abundance of *E. coli* in the river (with a maximum $4.0 \times 10^6 E. coli(100 \text{ mL})^{-1}$ measured at 9 p.m.) (Fig. 5b).

The analysis of *E. coli* concentrations observed during dry and wet weather conditions indicates that the microbiological quality of the Zenne River is strongly impacted by rain events. Rainy period results showed that CSO and WWTP effluents (especially those from the rain line of Brussels North WWTP) constituted major sources of faecal contamination of the river. During the studied rain events, the *E. coli* concentrations increased by more than a two log factor. This factor is assumed to depend on the discharge of CSOs and partly treated waters and thus on rain intensity.

Some studies already reported a negative impact of CSOs on the microbiological quality of receiving natural waters (Rechenburg et al. 2006; Passerat et al. 2011; Madoux-Humery et al. 2013). For example, Passerat et al. (2011) observed that downstream from a very intense CSO outfall, FIB concentrations in the impacted water mass of the Seine River (France) exceeded by two orders of magnitude the usual dry weather concentrations. Marsalek et al. (1994) studied the impact of urban sources on faecal pollution of the St. Clair River (Canada) in both dry and wet weather conditions and observed an increase of the density of *E. coli* (2.5–5.6 times) attributed to storm sewer and CSO discharged.

4 Conclusions

The microbiological water quality of the Zenne River was improved during the last decade, when comparing the present situation and the past

situation. However, even the microbiological quality is better than in the past, the FIB concentrations measured at each location along the river (from upstream to downstream from Brussels) are still high with values exceeding the level of microbiological water quality sufficient for bathing activities, the stretch of the river crossing Brussels area being the most contaminated. The analyses of the faecal contamination brought by Brussels WWTPs indicated that the effluents of Brussels North and Brussels South WWTPs represent the major sources of faecal pollution of the Zenne River by dry weather, but the release of some still untreated wastewaters directly in the Zenne River or in its tributaries also contributes to the river contamination.

Situations in rainy periods are still more problematic as downstream from Brussels, *E. coli* concentrations can be multiplied by more than a two log factor during rain events with regards to dry weather situations due to combined sewer overflows and to the increase of the flow rate coming out both from Brussels WWTPs.

The remediation solutions to improve the microbiological quality of the Zenne River should be as follows: (1) implementation of disinfection systems in the important WWTPs located in Brussels area to reduce load of microbial pollution coming out in dry weather conditions, (2) to increase the capacity of storm basins to collect storm water in order to avoid too frequent CSO and (3) to limit the urban runoff by limiting the water proofing of some surfaces in the city by promoting the development of green or living roofs.

Altogether, these results document the variations of the microbiological quality of a sewage-polluted urban river in relation with long-term changes (implementation of WWTP) and the short-term disturbances (CSO).

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