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# Microbiological water quality in rivers of the Scheldt drainage network (Belgium): impact of urban wastewater release

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## Abstract

The Scheldt watershed is characterized by a high population density, intense industrial activities and intensive agriculture and breeding. Due to these anthropogenic pressures, microbiological water quality of the main rivers of the Scheldt drainage network is low as demonstrated by our recent monitoring survey. An evaluation of the sources of microbiological pollutions at the scale of the watershed showed that the release of treated urban wastewaters was the major cause of faecal contamination. The Zenne river, a small stream crossing the Brussels area where it receives the treated effluents of more than one million inhabitants, is a typical example of a river strongly impacted by wastewater release. Studies, as the present one, leading to understand the microbial contamination are important for both industrialized and developing countries where the low microbiological water quality is responsible for numerous waterborne diseases.

## Keywords

Microbiological water quality, rivers, wastewater, waterborne diseases.

## INTRODUCTION

Polluted surface waters can contain a large variety of pathogenic micro-organisms including bacteria, viruses and protozoa. The main origin of these pathogenic micro-organisms is the faeces of human and other warm-blooded animals; they are brought into aquatic environments through the release of wastewater effluents, surface runoff and soil leaching. The sanitary risk for man linked to the presence of these pathogens depends on the use of the water (drinking, recreational activities, bathing, irrigation, shellfish harvesting) and on the pathogen concentration in water. During the nineteenth century, waterborne pathogens were responsible, in western European countries, for severe outbreaks of dysentery, typhoid fever and cholera that caused thousands of deaths. In many developing countries, waterborne pathogens are still today the first cause of morbidity.

In aquatic systems, the detection and enumeration of all pathogenic micro-organisms potentially present is very difficult due to the large diversity of pathogens, the low abundance of each species and the absence of standardized and low-cost methods for the detection of each of them. Thus, for routine monitoring, indicators of faecal contamination are usually enumerated to evaluate the level of microbial water contamination. The abundance of these indicators is supposed to be correlated to the density of pathogenic micro-organisms from faecal origin and is thus an indication of the sanitary risk associated with the various water utilisations.

Today, *Escherichia coli* (*E. coli*) and intestinal enterococci (IE) are the most frequently used indicators of faecal pollution (Edberg et al. 2000; Fewtrell and Bartram, 2001) and the recent

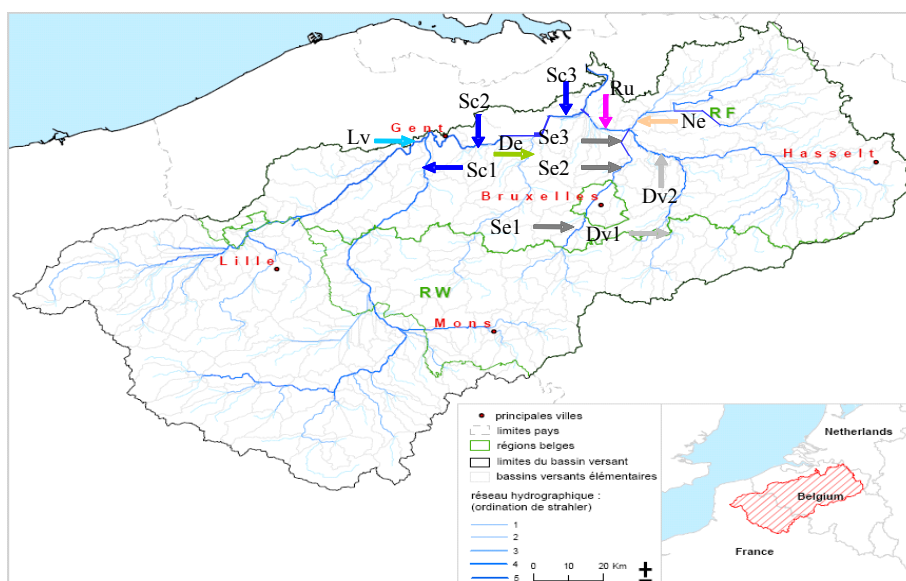
guidelines for assessing the water quality required for different water uses are based on their abundance. They were used in the present study to estimate microbiological water quality. The present study concerns the microbiological quality of the rivers of the Scheldt drainage network. The Scheldt watershed, which covers an area of 21,800 km<sup>2</sup> located from the North of France to the Belgian-Dutch border (Fig 1), is characterized by a high population density (around 500 inhabitants per km<sup>2</sup>), intense industrial activities and intensive agriculture and animal farming. Due to these anthropogenic pressures, one can assume that the microbiological water quality of the main rivers of the Scheldt drainage network is low. The objective of this study was to evaluate this water quality and to understand the dynamic of the microbiological contaminants in the rivers of the Scheldt basin. For this purpose, a monitoring survey was first organised in order to characterize the level of contamination of the main rivers of the watershed. A special attention was devoted to the highly contaminated Zenne river, a small river crossing the Brussels area. The sources of faecal bacteria to the rivers of the watershed were also studied; point sources (outfall of treated and untreated wastewaters) and non-point sources (surface runoff and soil leaching) of faecal pollution were quantified. Finally, some experiments were conducted in order to evaluate the disappearance rate of faecal bacteria once released in river water.

## MATERIAL & METHODS

### Sample collection

#### Monitoring faecal indicators in rivers of the Scheldt drainage network

During the monitoring survey conducted in the scope of this study, samples were collected in the downstream part of the main rivers of the Scheldt basin. Twelve sites were investigated monthly from March 2007 to June 2008 (Fig 1). Additionally, samples were collected at eight sites along the Zenne river (Fig 5 a) during a single campaign in October 2008. This longitudinal profile was performed during a low flow rate period. All samples were collected in 1-liter sterile bottles, kept at 4°C and analyzed within a maximum of 6 h after collection.



**Figure 1: Sampling sites localized in the downstream part of the main rivers of the Scheldt watershed. Lv: Lys river at St-Martens-Lerne; De: Dendre river at Gijzegem; Ne: Nethe river at Duffel; Dy1 and Dy2: Dyle river at Gastuche and Rijmenam; Se1, Se2 and Se3: Zenne river at Lot, Eppegem, and Leest; Ru: Rupel river at Boom; Sc1, Sc2 and Sc3: Scheldt river at Gavere, Uitbergen and Temse.**

### **Evaluation of point sources of faecal contamination**

In order to quantify the contribution of treated wastewaters to the faecal bacterial load to the rivers and to determine the treatment efficacy on bacterial reduction during the transit in wastewater treatment plants (WWTPs), samples were collected in raw and treated waters in WWTPs located in the Scheldt watershed. Accordingly, the Log removal of faecal bacteria was calculated (the difference between the log of indicators bacteria at the entrance and at the outlet of the WWTP). The studied WWTPs had a large range of treatment capacities (from 1,000 to 1,100,000 inhabitant-equivalents) and were characterized by various types of water treatment. The treatment in these plants included a pretreatment (screening, grease collection), settling and a biological treatment (activated sludge, with or without nitrification-denitrification). One of the studied WWTPs was a Lemna lagoon.

### **Evaluation of non-point sources of faecal contamination**

In order to assess the contribution of non-point sources of faecal pollution (runoff and soil leaching), small streams (stream order 1 or 2 according to the geomorphologic criteria defined by Strahler [1957]) located in rural areas were sampled upstream from any wastewater outfall. These small streams were classified on the basis of their watershed land use: forest areas, cultivated areas and pastured areas.

### **Enumeration of faecal bacterial indicators**

#### **Plate counts**

*E. coli* and IE were enumerated by standard plate counts on Chromocult Coliform Agar (CCA) and Chromocult Enterococci Agar (CEA) respectively. Both growth media are specific for their corresponding indicator bacteria. 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.

#### **$\beta$ -D-glucuronidase activity measurement**

The measurement of  $\beta$ -D-glucuronidase activity (an enzymatic activity specific of *E. coli*) has been proposed as an alternative to classical enumeration methods to investigate the abundance of *E. coli* in waters (Fiksdal et al. 1994). George et al. (2000) optimised a protocol for measuring the  $\beta$ -D-glucuronidase activity (GLUase) in rivers water using the substrate 4-methylumbelliferyl- $\beta$ -D-glucuronide (MUG) in a period as short as 30 minutes. This method was tested in this study in parallel with *E. coli* plate counts.

In this study, the protocol used for GLUase activity measurements was as follows (Servais et al. 2005): water samples (10 or 100 ml) were filtered through 0.2- $\mu$ m pore-size, 47-mm diameter polycarbonate filters (Nuclepore). The filters were placed in 200-mL sterile DURAN flasks containing 17 mL of sterile phosphate buffer (pH 6.9) and 3 mL of MUG solution (55 mg of MUG (Biosynth, Switzerland) and 20  $\mu$ l of Triton X-100 in 50 mL of sterile water) was added to each flask (final MUG concentration: 165 mg l<sup>-1</sup>). The flasks were incubated in a shaking water bath at 44°C. Every 5 min for 30 min, a 2.9 mL aliquot of the 20 mL was put in a quartz cell with 110  $\mu$ l of 1 M NaOH solution to obtain a pH of 10.7 (corresponding to the maximum of fluorescence of the methylumbelliferone (MUF)). The fluorescence intensity of the aliquot was measured with a SFM 25 spectrofluorometer (Kontron AG, Zürich, Switzerland) at an excitation wavelength of 362 nm and emission wavelength of 445 nm. The production rate of MUF (picomoles of MUF released per minute for 100 ml of sample filtered), expressing the enzymatic

activity, was determined by least-square linear regression when plotting MUF concentration versus incubation time.

### Batch survival experiment

A survival experiment was conducted in a microcosm to determine the disappearance rates of *E. coli* and IE. The microcosm consisted of a two-liter bottle (Duran) containing a sample of the Zenne river and was incubated for one week in the dark at 20°C. A daily measurement of indicator bacteria was performed during the microcosm incubation by plate count method.

## RESULTS AND DISCUSSION

### Monitoring of the level of faecal contamination in the rivers of the Scheldt basin

The geometric means of the abundance of both faecal indicators in the main rivers of the Scheldt drainage network ranged between  $1.3 \times 10^3$  and  $4.0 \times 10^5$  CFU/100 mL for *E. coli* and between  $2.0 \times 10^2$  and  $8.4 \times 10^4$  CFU/100 ml for IE (Fig. 2A and B). These abundances were compared to the values implemented as water quality criteria in the new EU directive on bathing water quality (EU, 2006). At all sites, the geometric means of abundance were higher than the maximum admissible level for sufficient bathing water quality ( $9.0 \times 10^2$  and  $3.3 \times 10^2$  CFU/100 ml for *E. coli* and IE, respectively), indicating that the main rivers of the basin have globally a poor microbiological water quality.

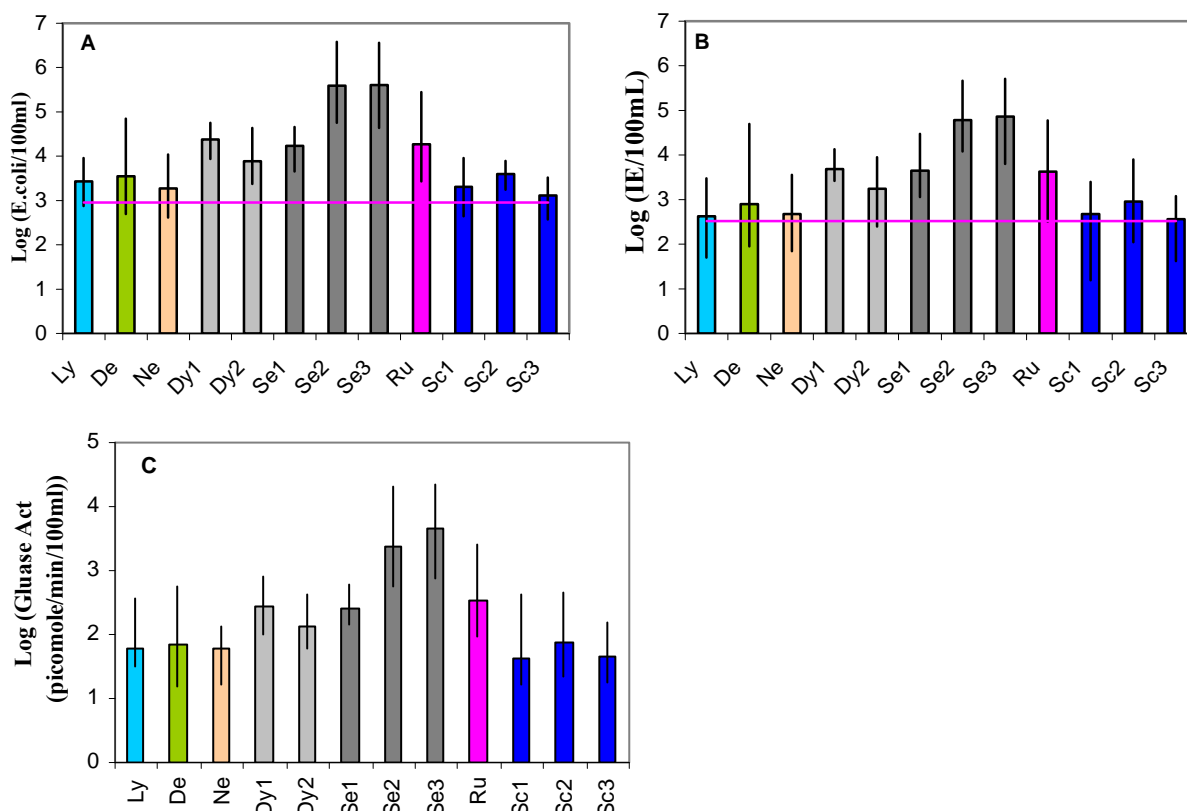
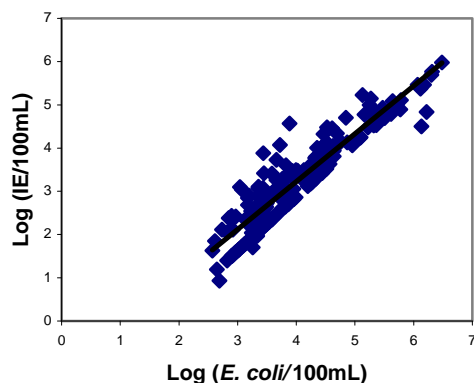


Figure 2: Abundances of *E. coli* (A) and IE (B) estimated by plate counts and GLUase activities (C) in the main rivers of the Scheldt basin. Data are presented as geometric mean values of 16 monthly measurements, and vertical bars represent the range between the minimal and maximal values. The horizontal bars on panels A and B represent the maximum admissible level for sufficient bathing water quality following the EU directive (EU, 2006).

The abundances of both faecal indicators followed very similar trends when comparing the sampling sites. Accordingly, when IE numbers were plotted against *E. coli* numbers in log-log scale, a significant correlation ( $r^2 = 0.86$ ) was found (Fig. 3). The level of faecal contamination in the rivers of the Scheldt basin can be thus equivalently assessed by *E. coli* and IE abundances. The fluctuations of the ratio of *E. coli* to IE numbers depending on the kind of samples are discussed below.



**Figure 3: Log –Log linear regression between IE and *E. coli* (EC) abundances in the main rivers of the Scheldt basin. Correlation:  $\text{Log (IE/100 ml)} = 1.11 \text{ Log (EC/100 ml)} - 1.20$  ( $r^2 = 0.86$ ;  $n = 180$ ).**

GLUase activities were measured in parallel with *E. coli* plate counts; they followed a trend between the sampling sites very similar to that observed for *E. coli* abundances (Fig. 2C). When GLUase activities were plotted against *E. coli* counts in log-log scale, a significant correlation ( $r^2 = 0.91$ ) was found (Fig. 4). Similar correlations were reported in previous studies for different types of aquatic systems: river waters (Farnleitner et al. 2001; Servais et al. 2005), marine waters (Lebaron et al. 2005) and wastewaters (Garcia-Armisen et al. 2005). The slope of the regression straight line obtained in this study was lower than 1 in agreement with previous studies. This indicates that the ratio of GLUase activity to culturable *E. coli* abundance increased in less polluted environments. A possible explanation for this observation was suggested by George et al. (2000): the higher enzymatic activities per culturable cells in less contaminated natural waters may be due to an underestimation of the number of faecal bacteria when enumerated by culture-based methods. This underestimation may be explained by a higher proportion of active but non culturable cells (cells presenting a detectable GLUase activity but unable to multiply in or on the specific media used in culture-based methods). The higher proportion of active but non culturable cells (ABNC) faecal bacteria in less polluted environments could be the result of more severe and/or longer environmental stress factors such as nutrient limitation and enhanced solar radiation effects due to deeper light penetration.

The quality of the correlation presented in Figure 4 demonstrated that GLUase activity measurement can be a valid alternative for monitoring *E. coli* in the rivers of the Scheldt basin. This method offers the advantage to give a result in less than 1 hour while the culture-based method requires a 24-hour incubation.

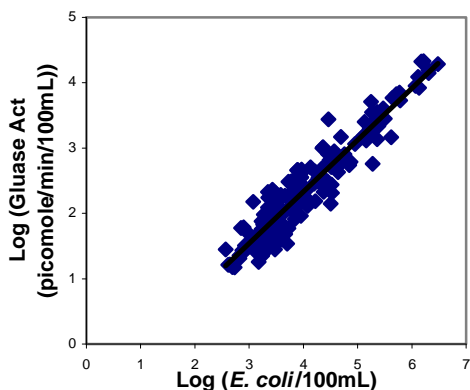


Figure 4: Log –Log linear regression between GLUase activities and *E. coli* plate counts in the main rivers of the Scheldt basin. Correlation:  $\text{Log (Glucose Act (picomole/min/100 ml))} = 0.79 \text{ Log (EC/100 ml)} - 0.83 (r^2 = 0.91; n = 180)$ .

As shown by the monitoring survey results, the Zenne river was the most contaminated of the studied rivers, particularly downstream from the Brussels area. This river is characterized by a low discharge (less than 4 m<sup>3</sup>/s in summer period) and received the treated wastewaters of two important WWTPs in the Brussels area: the Brussels-South WWTP (360,000 inhabitant-equivalents, active since 2000) and the Brussels-North WWTP (1.1 millions inhabitant-equivalents, active since early 2007). A longitudinal sampling profile was performed in the Zenne river in 2008 to determine the impact of the Brussels region on the microbiological quality of the river (Fig 5). The profile is characterized by two peaks of *E. coli* numbers observed downstream from both WWTPs and by concentrations close to that usually measured in treated wastewaters (10<sup>5</sup> *E. coli* / 100 ml) downstream from Brussels.

The 2008 profile was compared with another profile performed ten years ago (1998) in similar low flow conditions but before the implementation of both WWTPs. It showed that *E. coli* abundances downstream from Brussels were two orders of magnitude higher ten years ago. The implementation of the WWTPs had thus a major impact on microbiological water quality but efforts are still to be done.

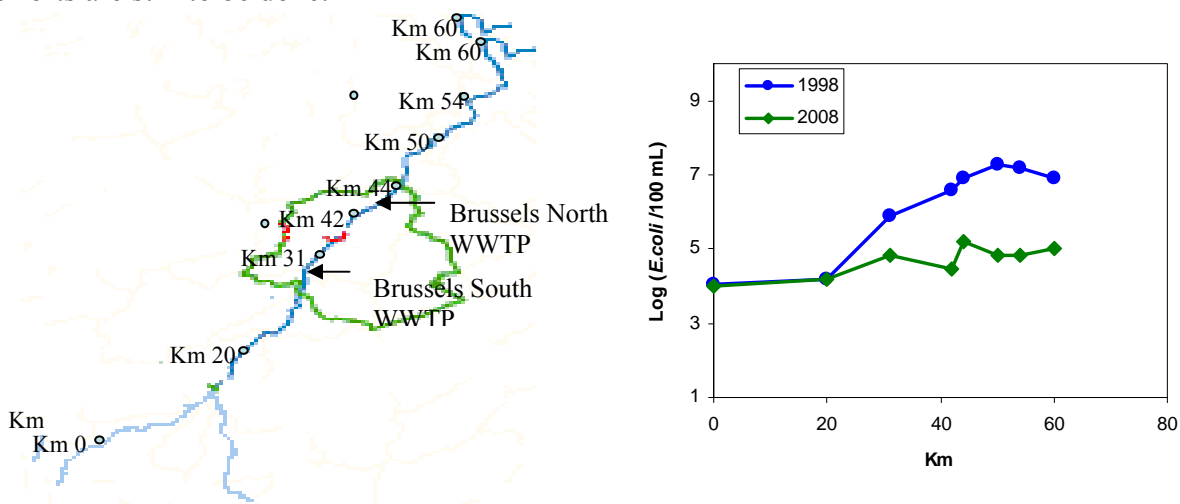
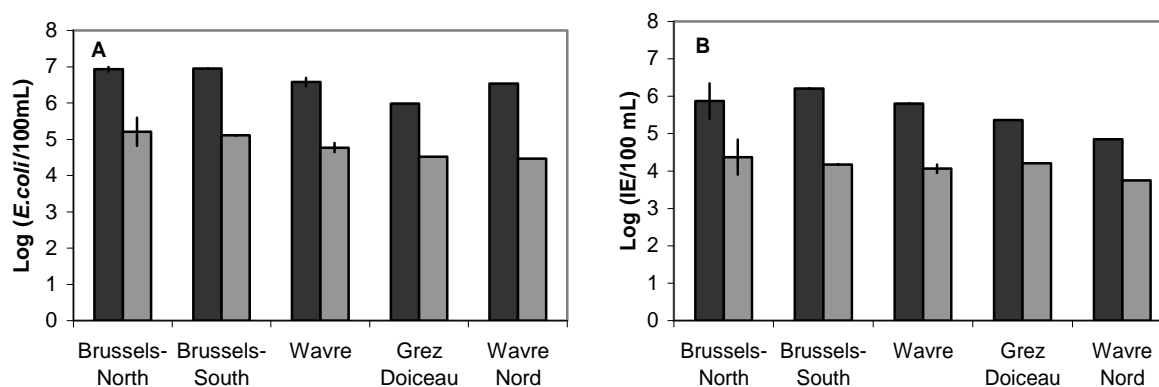


Figure 5: a) Map of the sampling sites in the Zenne river with localization of the two Brussels WWTPs and the limits of Brussels region (green). b) Longitudinal profiles of the abundance of *E. coli* in the Zenne river in 1998 and 2008 in low flow conditions. Origin of the x-axis (0 Km) is localized at Rebecq.

## Sources of faecal contamination of the rivers

### Point sources

Point sources are predominantly constituted by effluent releases from wastewater treatment plants (WWTPs). In the Scheldt watershed, there are more than 300 WWTPs and among these, five were sampled (Brussels-South and Brussels-North, Wavre, Wavre-Nord and Grez-Doiceau).

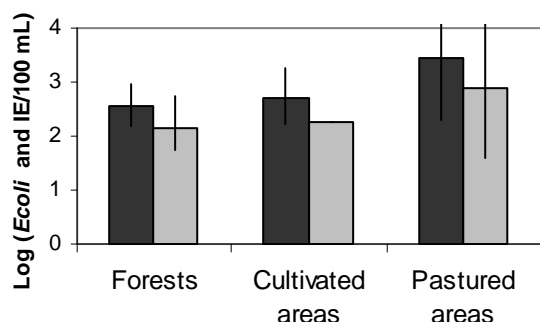


**Figure 6: Abundance of *E. coli* (A) and enterococci (B) in raw (black) and treated (grey) water in the five studied WWTPs located in the Scheldt watershed. Data are presented as geometric mean values and vertical bars represent the range between the minimal and maximal values.**

In raw waters, the geometric means of abundances ranged between  $9.6 \times 10^5$  and  $8.5 \times 10^6$  *E. coli* /100ml and  $7.1 \times 10^4$  and  $1.6 \times 10^5$  IE/100ml (Fig. 6). These values are in agreement with values reported in previous studies (Garcia-Armisen and Servais, 2007; Servais et al. 2007a; George et al. 2002). The log removal observed ranged between 1.46 and 2.07 for *E. coli* and between 1.10 and 2.03 for IE. Despite the efficient removal (between 90 and 99 % of faecal bacteria removed) the WWTPs effluents still contain a high abundance of indicator bacteria.

### Non-point sources

Non-point sources represent the part of faecal pollution brought to rivers by soil leaching and surface runoff. The origins of this microbial pollution are the faeces of wild animals and grazing livestock, and also cattle manure spread on cultivated areas. Therefore, the land use can have a major impact on the level of microbial pollution. The small streams sampled upstream any point source were classified according to the land use of their watershed: forests, cultivated areas and pastured areas.



**Figure 7: Abundance of *E. coli* (black) and IE (grey) in small streams flowing in forests, cultivated and pastured areas. Data are presented as geometric mean values and vertical bars represent the range between the minimal and maximal values.**



The level of faecal contamination of small streams was dependent on the land use of their watershed and the quantification of *E. coli* and IE revealed that streams from pastured areas were more contaminated than streams from forest and cultivated areas (Fig 7).

### **Respective contribution of point and non-point sources**

In order to roughly compare the respective contributions of point and non-point sources to faecal contamination in the Scheldt basin, fluxes of faecal bacteria loaded by both types of sources were calculated. For the point sources, calculation took into account: the mean concentrations of faecal bacteria in wastewaters (Fig 6), the average volume of wastewater produced per inhabitant (180 l/inh/day), the number of inhabitants connected to WWTPs and the number of inhabitants releasing their wastewaters in rivers without treatment. For the non-point sources, calculation took into account geometric means of *E. coli* and IE resulting from soil leaching and runoff on different types of land uses (forests, cultivated and pastured areas), the respective surface areas of these types of land uses and the average specific discharge in the Scheldt watershed ( $1 \text{ km}^2 \cdot \text{sec}^{-1}$ ). The result of these calculation showed that, at the scale of the Scheldt watershed, point sources of faecal bacteria were largely predominant (more than 50 fold) when compared to non-point sources. However, it should be kept in mind that these calculations do not give any information on the local impact of diffuse sources which can have a major importance on the microbiological quality of small rivers.

Several studies have shown that the composition of the faecal flora was different in animals and humans faeces; for example, the proportion of *E. coli* compared to IE was higher in human faeces than in animal faeces. Thus, several authors (Feachem 1975; Jagals *et al.*, 1995) have suggested that the ratio of *E. coli* to IE can be used to determine the source (human or animals faeces) of faecal pollution in aquatic systems. In this study, the ratio of *E. coli* to IE was calculated for runoff and soil leaching waters (small streams sampled upstream from any point source), main rivers and WWTP effluents. In soil leaching and runoff waters, faecal bacteria are mainly from animal origin while in WWTP effluents they are predominantly from human origin. In agreement with the origin of faecal bacteria, the ratio of *E. coli* to IE was significantly higher in WWTPs effluents than in soil leaching and runoff waters. The ratio of *E. coli* to IE estimated for the main rivers (9.05) was much closer to WWTP effluents one (10.43) than to small streams ratio (3.86) indicating that faecal bacteria in the main rivers are mainly from domestic origin. This confirms the results of the calculation showing that point sources of faecal bacteria were largely predominant at the scale of the basin.

### **Fate of Escherichia coli and IE in river water**

After their release into the rivers, faecal bacteria are exposed to the combined actions of various biological (grazing by protozoa, virus-induced cell lysis) and physico-chemical processes (stress due to nutrients depletion, sunlight intensity, and temperature decrease) leading to their disappearance. The decay of faecal bacteria due to these processes is usually described by a first order kinetics (Menon *et al.* 2003; Servais *et al.* 2007a). In the scope of this study, a batch experiment was conducted in order to determine the decay rate of *E. coli* and IE in the Zenne river. River water with initial concentrations of respectively  $1.03 \times 10^5$  *E. coli*/100 mL and  $4.73 \times 10^4$  IE/100mL was incubated in the dark at 20°C. These data indicated a less rapid decrease of IE compared to *E. coli* confirming the results of previous studies (Fujioka *et al.* 1981; Noble *et al.* 2004).

The estimated decay rates of *E. coli* and IE in this experiment were respectively of  $0.044 \text{ h}^{-1}$  and  $0.032 \text{ h}^{-1}$ . The *E. coli* decay rate was very close to the one used by Servais *et al.* (2007b) for modelling the dynamics of faecal bacteria in the Seine river drainage network ( $0.045 \text{ h}^{-1}$  at

Microbiological water quality in rivers of the Scheldt drainage network (Belgium)– Nouho.Koffi. Ouattara 20°C). A microcosm study performed with Onkapinga river water in conditions similar to those used in our study also showed a decay rate of *E. coli* in the same order of magnitude ( $0.050 \text{ h}^{-1}$ ) (Craig et al. 2004).

## CONCLUSIONS

- Globally, the rivers of the Scheldt basin have a poor microbiological water quality as shown by the monitoring study.
- The Zenne river is particularly contaminated downstream from Brussels due to the release impact of the treated wastewaters of the city.
- At the scale of the basin, the point sources (wastewaters) of faecal bacteria were largely predominant.
- Batch experiments showed that decay rate in rivers of IE was lower than that of *E. coli*.
- The final objective of this work is to develop a mathematical model describing the dynamic of *E. coli* and IE in the rivers of the whole Scheldt drainage network that could be used to evaluate the impact of wastewater management on microbiological water quality.
- Studies, as the present one, leading to understand the microbial contamination are important for both industrialized and developing countries where the low microbiological water quality is responsible for numerous waterborne diseases.

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