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Faecal contamination of water and sediment in the rivers of the Scheldt drainage network

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Abstract The Scheldt watershed is characterized by a high population density, intense industrial activities and intensive agriculture and breeding. A monthly monitoring ($n = 16$) of the abundance of two faecal indicator bacteria (FIB), *Escherichia coli* and intestinal enterococci (IE), showed that microbiological water quality of the main rivers of the Scheldt drainage network was poor (median values ranging between 1.4×10^3 and 4.0×10^5 *E. coli* (100 mL)$^{-1}$ and between 3.4×10^2 and 7.6×10^4 IE (100 mL)$^{-1}$). The Zenne River downstream from Brussels was particularly contaminated. Glucuronidase activity was measured in parallel and was demonstrated to be a valid surrogate for a rapid evaluation of *E. coli* concentration in the river waters. FIB were also investigated in the river sediments; their abundance was sometimes high (average values ranging between 2.1×10^2 and 3.3×10^5 *E. coli* g^{-1} and between 1.0×10^2 and 1.7×10^5 IE g^{-1}) but was not sufficient to contribute significantly to the river water contamination during resuspension events, except for the Scheldt and the Nethe Rivers. FIB were also quantified in representative point sources (wastewater treatment plants) and non-point sources (runoff water and soil leaching on different types of land use) of faecal contamination. The comparison of the respective contribution of point and non-point sources at the scale of the Scheldt watershed showed that point sources were largely predominant.

Keywords Microbiological water quality · Rivers · Faecal indicator bacteria · Sediment contamination · Wastewater

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Introduction

Polluted surface waters can contain a large variety of pathogenic microorganisms including bacteria, viruses and protozoa. The main origin of these pathogenic microorganisms is the faeces of human and warm-blooded animals; they are brought to the aquatic environments through the release of wastewater effluents, surface runoff and soil leaching. The human sanitary risk 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.

In aquatic systems, the detection and enumeration of all pathogenic microorganisms potentially

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present is impossible due to the large diversity of the 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, faecal indicator bacteria (FIB) are usually enumerated to evaluate the level of microbial water contamination. The abundance of these FIB is supposed to be correlated with the density of pathogenic microorganisms from faecal origin and is thus an indication of the sanitary risk associated with the various water utilisations. For more than a century, total coliforms and faecal (also called thermotolerant) coliforms were the main organisms used as bacterial indicators. Nowadays, *Escherichia coli* and intestinal enterococci (IE) are the most frequently used indicators of faecal pollution as it was demonstrated by epidemiological studies that they were better indicators of the human risk associated with waters than coliforms (Edberg et al. 2000; Fewtrell and Bartram 2001). Recent guidelines for assessing the water quality required for different water uses are based on their abundance, i.e. the recent European Union Bathing water Directive (EU 2006). In the present study, abundances of *E. coli* and IE were used to estimate microbiological quality. *E. coli* and IE were enumerated using classical culture-based methods (plate count on agar media); in addition, a direct (without cultivation) and rapid enzymatic method, the measurement of the glucuronidase activity (George et al. 2000), was tested as a surrogate of the culture-based method for estimating *E. coli* concentration.

In the present study, the microbiological quality of the rivers of the Scheldt drainage network was investigated. The Scheldt watershed, which covers an area of 22,000 km^2 located from the North of France to the Belgian–Dutch border (Fig. 1), is characterized by a high population density (around 500 inhabitants/km^2), intense industrial activities and intensive agriculture and breeding. Due to these anthropogenic pressures, one can assume that the microbiological water quality of the main rivers of the Scheldt drainage network is low. A first objective of this study was to evaluate the level of faecal contamination of the water of the main rivers. For this purpose, a monitoring survey was organized in the main rivers of the watershed.

Sediments of aquatic environments may constitute a reservoir for FIB. Indeed, different studies reported high FIB concentrations in sediments from streams and rivers (Crabill et al. 1999; Craig et al. 2002a; Smith et al. 2008), lakes (An et al. 2002), estuaries and coastal areas (Craig et al. 2002a; Roslev et al. 2008). High concentration of faecal bacteria in sediments is usually explained by the longer survival of FIB in sediments than in the overlying water (Davies et al. 1995; Craig et al. 2004; Lee et al. 2006). A part of the present study was devoted to evaluate the FIB level in the sediments of the rivers of the Scheldt watershed and to assess the possible impact of sediment resuspension on the microbiological water quality.

Another objective of this study was to determine the main sources of the faecal contamination of the rivers. The sources of FIB to the rivers of the watershed were thus studied in order to quantify and compare the point sources (outfall of treated and untreated wastewaters) and non-point sources (surface runoff and soil leaching) of faecal pollution at the scale of the whole drainage network.

Materials and methods

Water and sediment sampling

During the monitoring survey conducted in the scope of this study, water samples were collected in the downstream part of the main rivers of the Scheldt watershed. Twelve sites were investigated monthly from March 2007 to June 2008 (Fig. 1); a total of 16 sampling campaigns were thus performed. During these campaigns, water samples were collected in the rivers with a plastic bucket from bridges, halfway between the banks. Some of the sampling locations were under the tide influence; during the survey, these sites were systematically sampled during the low tide period. Samples were stored in 1-L sterile bottles, kept at 4°C and analysed within a maximum of 6 h after collection.

Sediments were collected from the upper layer of the river sediment (approximately 10 cm from the surface of the sediment) using a hand trowel. Sediment samples were collected at the same

sampling sites than water samples (Fig. 1) during two of the 16 sampling campaigns. Sediment samples were placed into a sterile plastic container, transported to the laboratory and kept at 4°C and analysed within a maximum of 6 h after collection.

Evaluation of point sources of faecal contamination

In order to quantify the contribution of wastewater releases to the FIB load and to determine the treatment efficiency on FIB removal, samples were collected in raw and treated waters of various wastewater treatment plants (WWTPs) located in the Scheldt watershed. 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 types of treatment considered in the present study were primary treatment (PT; including screening, grease collection and settling), PT followed by trickling filtration, PT followed by activated sludge (AS) for carbon removal, PT followed by AS for carbon removal and nitrification (Nit), PT followed by AS for carbon removal, nitrification and denitrification (Denit), PT followed by AS for carbon removal, nitrification and denitrification associated with phosphorus removal (P), and stabilisation pond. For each of these treatment types, samples were collected during three sampling campaigns in different WWTPs in the Scheldt basin excepted for the treatment with PT followed by trickling filtration for which only one sampling campaign was performed.

Evaluation of non-point sources of faecal contamination

Faecal pollution brought to the rivers through soil leaching or surface runoff represents the non-point sources; its origin can be the faeces from wild animals and grazing livestock and also the cattle manure spread on cultivated areas. Therefore, the land use can have a major impact on the level of microbial pollution brought to rivers by soil leaching and surface runoff. Quantification of faecal contamination of rivers through non-point sources is relatively difficult. In this study, the approach proposed by George et al. (2004) and Garcia-Armisen and Servais (2007) was adopted to quantify the contribution of non-point sources of faecal pollution: FIB concentrations were measured in small streams (stream order 1 or 2 according to the geomorphologic criteria defined by

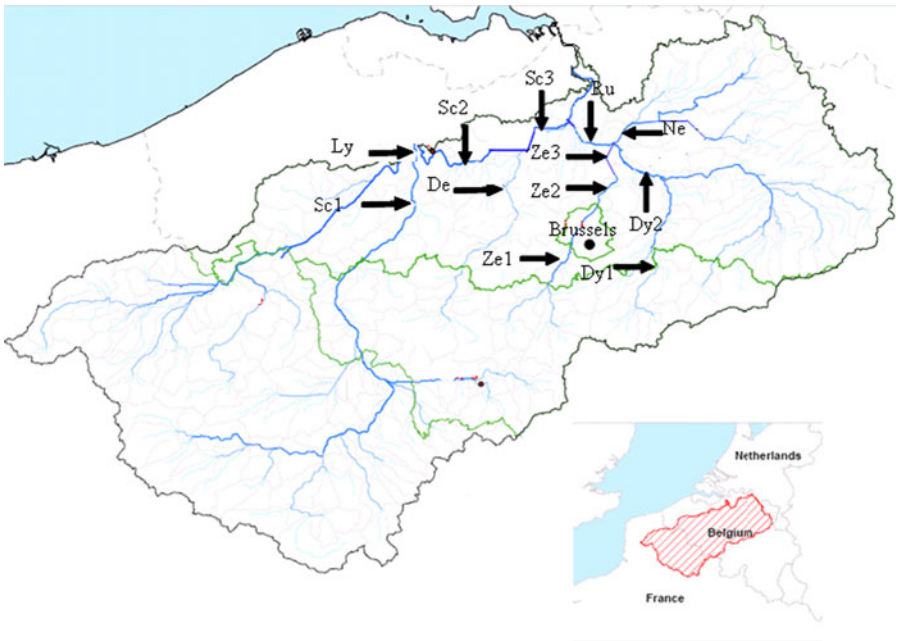

Fig. 1 Location of sampling sites in the downstream part of the main rivers of the Scheldt watershed. *Ly* Lys River at St-Martens-Leerne, *De* Dendre River at Gijzegem, *Ne* Nethe River at Duffel, *Dy1* Dyle River at Gastuche, *Dy2* Dyle River at Rijmenam, *Ze1* Zenne River at Lot, *Ze2* Zenne River at Eppegem, *Ze3* Zenne River at Leest, *Ru* Rupel River at Boom, *Sc1* Scheldt River at Gavere, *Sc2* Scheldt River at Uitbergen, *Sc3* Scheldt River at Temse

Strahler (1957)) located in rural areas sampled upstream from any wastewater outfall so that FIB present in the samples resulted only from soil leaching and surface runoff. These small streams were classified on the basis of their watershed land use: forested areas, cultivated areas and pastured areas.

Enumeration of faecal indicator bacteria in water and sediments

E. coli and IE were enumerated in water samples by standard 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. Prats et al. (2008) and Miranda et al. (2005) reported high percentages of specificity, respectively, for CCA enumeration of *E. coli* (96%) for CEA enumeration of enterococci (98%) in water samples. CCA and CEA plates were incubated at 36°C for, respectively, 24 and 48 h. Plate counts were expressed as colony-forming units (CFU) per 100 mL of sample.

Enumeration of faecal indicators by plate counts in sediment samples required first to detach the bacteria from the sediment particles. In the scope of the present study, extraction of FIB from sediments by sonication was optimised with the objective of detaching a maximum of bacteria from the particles without impairing their cultivability. Sonication using probes (Labsonic L Braun, 220 V, 50/60 Hz) and a sonication bath (47 kHz; 60 W) were tested. Treatments with two probes (4 and 9 mm diameter), three sonication times (1, 2 and 3 min) for each probe and two intensity levels were tested (60 and 100 W). Three sonication times (1, 5 and 10 min) were tested using the sonication bath. For each treatment type, 0.5 g of wet sediment was placed in 50 mL of sterile Ringer solution. The higher recovery of FIB from sediments was obtained for both indicators using 4 mm sonication probe at 60 W for 2 min; this sonication procedure was used during the whole study. We verified that this procedure of sonication did not affect the culturability of the FIB. In order to avoid, a too high load of particles on the plate, samples were let to settle for 10 min after sonication as proposed by Anderson et al. (2005), and aliquots of the supernatant were collected with a sterile syringe (50 mL) and plated onto CCA and CEA. *E. coli* and IE counts were expressed as CFU per gram dry weight sediment. The sediment was weighted before and after drying at 105°C for 24 h in order to determine the dry weight-to-wet weight ratio.

β−D-Glucuronidase activity measurement

The measurement of β-D-glucuronidase activity (an enzymatic activity specific to *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 β-D-glucuronidase (GLUase) activity in river water using the substrate 4-methylumbelliferyl-β-D-glucuronide (MUG) in a period as short as 30 min. This method was tested in this study in parallel with *E. coli* plate counts. The protocol used for GLUase activity measurements was as follows (Servais et al. 2005): Briefly, the water sample (10 or 100 mL) was filtered through a 0.2-μm pore-size 47-mm diameter polycarbonate filter (Nuclepore). The filter was placed in a 100-mL sterile DURAN flask containing 17 mL of sterile phosphate buffer (pH 6.9) and 3 mL of a MUG solution (55 mg of MUG (Biosynth, Switzerland), and 20 μL of Triton X-100 in 50 mL of sterile water) was added to the flask (final MUG saturating concentration of 165 mg L^{-1}). The flask was 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 μL of a 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 an 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 a linear least squares regression of MUF concentration on incubation time.

Suspended matter and potential contribution of sediment resuspension to the faecal contamination in water column

Suspended matter (SM) of water samples was estimated as the weight of material remaining on a Whatman GF/F membrane per volume unit after drying of the filter for 24 h at 105°C. SM data are expressed in milligrams per litre.

In order to calculate the maximum potential concentration of FIB due to sediment resuspension, we considered that, at each sampling site, the maximum resuspended sediment in the water column corresponds to the highest SM concentration measured during the survey. By this approach that considers that all SM in the river originated from sediment resuspension and that fixes the SM concentration to its highest observed value, the hypothesis made about the concentration of resuspended sediment in the water column was probably on the upper range of what could be actually observed. The concentration of potential resuspended sediment (the maximum SM concentration observed at each sampling site) was then multiplied with the geometric mean of the abundance of each FIB in the sediment to determine the potential abundance of resuspended FIB. Percentage of FIB potentially originating from sediment resuspension in the water column was calculated as the ratio of potentially resuspended FIB to the geometric mean abundance of FIB in the water column.

Flow rate data

Daily average flow rates data of the different studied rivers were obtained from the Flemish Environment Agency (Vlaamse Milieumaatschappij).

Statistical analysis

The probability test (p value) associated to linear regression was performed using Student test, and significance was determined at 95% confidence level. A Wilcoxon–Mann–Whitney test was also performed to compare the abundances of faecal bacteria from different non-point sources. These analyses were performed using statistical software R.

Results

Monitoring of faecal contamination in the main rivers of the Scheldt drainage network

Faecal contamination in the river water column

The faecal contamination level of the main rivers of the watershed was monitored for 16 months by monthly measurements at 12 locations. *E. coli* and IE abundances as well as glucuronidase activity presented similar trends from one sampling site to another (Fig. 2). The contamination level of the sampling locations covered a wide range. The less contaminated site (Sc3) had median abundances of 1.4×10^3 *E. coli* and 3.4×10^2 IE per 100 mL while the most contaminated site (Ze3) had median abundances of 4.0×10^5 *E. coli* and 7.6×10^4 IE per 100 mL, representing a range of 2.5 logs for *E. coli* and 2.4 logs for IE. The range between the lowest and the highest abundances measured during the monitoring reached 4 orders of magnitude (e.g. from 3.7×10^2 to 3.8×10^6 CFU/100 mL for *E. coli*). Sites from the Lys River (Ly), the Nethe River (Ne) and from the Scheldt River (Sc1, 2 and 3) were the less contaminated, while the sites from the Zenne River downstream from Brussels (Ze2 and 3) and from the Rupel (Ru), which receives the water from the Zenne River, were the most contaminated.

During the monitoring, a high variability was observed in the contamination level of several sites (Fig. 2). This abundance variability was more pronounced for IE than for *E. coli*. The most contaminated sites were among the most variable (sites from the Zenne River, the Dyle River and the Rupel River) but the most variable site was De, with 2.4 and 3.7 logs between the minimal and maximal abundances of *E. coli* and IE, respectively. Comparatively, the less contaminations sites (Sc2 and Sc3) were also the less variable, with differences between minimal and maximal abundances of 0.7 and 1.5 logs for *E. coli* and IE, respectively. The river flow rate is often reported in the literature as a factor affecting the level of faecal contamination (Schilling et al. 2009). We investigated if variations in the flow rate of the rivers measured on the sampling date could explain the observed fluctuations in the contamination level

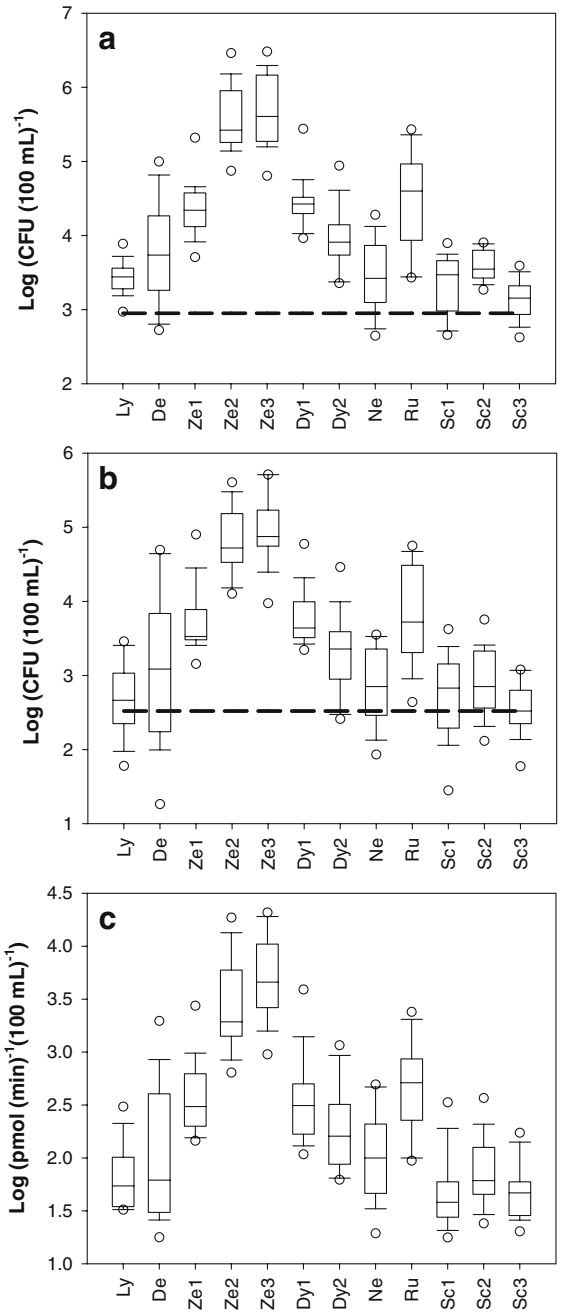

Fig. 2 Box plots in log units of the abundance of *E. coli* (**a**), intestinal enterococci (**b**), and the GLUase activity (**c**) measured in the main rivers of the Scheldt drainage network during a monthly monitoring survey ($n = 16$). *Box plots* represent the median (*horizontal line in the box*), the lower and upper quartiles (*bottom and top box lines*), the 10th and 90th percentiles (*bottom and top whiskers*) and the outliers (*circles*). *Dashed lines* represent the maximum admissible value of the 90th percentile for a sufficient quality of bathing waters according to the EU Directive 2006/7/EC

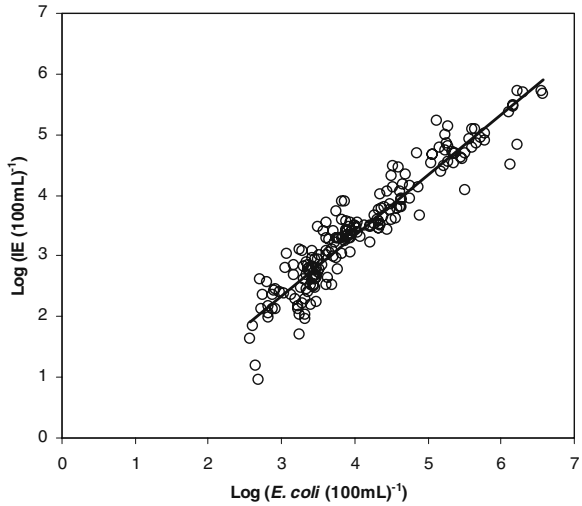

Fig. 3 Log–log linear regression between IE and *E. coli* abundances in the main rivers of the Scheldt watershed. Correlation: log (IE/100 mL) = 0.99 log (*E. coli*/100 mL) − 0.62 ($r^2 = 0.89$; $n = 192$; $p < 0.01$)

of the sites. The *E. coli* and IE abundances (in log units) were positively correlated at three sites (De, $p < 0.05$; Dy2 and Sc2, $p < 0.01$) with the flow rate of the river. Flow rate variations explained between 32% and 46% of the variability of the log abundances ($0.32 < r^2 < 0.46$). No significant

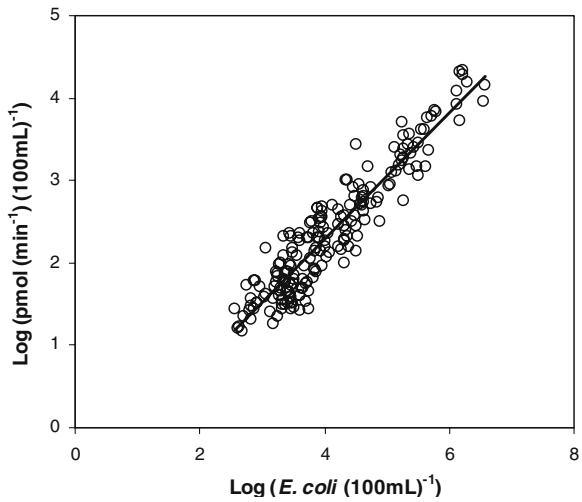

Fig. 4 Log–log linear regression between GLUase activities and *E. coli* abundances in the main rivers of the Scheldt watershed. Correlation: log (GLUase Act (picomoles per minute per 100 mL) = 0.76 log (*E. coli*/100 mL) − 0.79 ($r^2 = 0.87$; $n = 191$, $p < 0.01$))

Table 1 FIB abundances measured in the sediment and in the water column of the rivers of the Scheldt watershed and estimation of the potential contribution of sediment resuspension to the FIB load of the water column

River	Sampling site	SM (min–max)[a] (mg L^{-1})	*E. coli*				Intestinal enterococci			
			Sediment[b] (CFU g^{-1})	Water column[c] (CFU (100 mL)$^{-1}$)	Potentially resuspended[d] (CFU (100 mL)$^{-1}$)	Potential contribution[e] (%)	Sediment (CFU g^{-1})	Water column (CFU (100 mL)$^{-1}$)	Potentially resuspended (CFU (100 mL)$^{-1}$)	Potential contribution (%)
Lys	Ly	4–56	2.1×10^2	2.7×10^3	0.1×10^1	<0.1	1.0×10^2	4.8×10^2	<1	0.1
Dendre	De	8–324	1.3×10^4	5.4×10^3	4.2×10^2	7.8	6.6×10^3	1.1×10^3	2.1×10^2	19.1
Zenne	Ze1	10–393	6.1×10^3	2.3×10^4	2.4×10^2	1.0	3.3×10^3	5.7×10^3	1.3×10^2	2.3
	Ze2	12–98	3.0×10^4	3.7×10^5	3.0×10^2	0.1	3.8×10^5	6.7×10^4	3.7×10^3	5.6
	Ze3	19–109	2.2×10^5	4.6×10^5	2.4×10^3	0.5	1.4×10^5	8.8×10^4	1.6×10^3	1.8
Dyle	Dy1	12–192	7.7×10^2	2.9×10^4	1.5×10^1	0.1	1.6×10^3	6.2×10^3	3.0×10^1	0.5
	Dy2	9–169	3.6×10^3	9.1×10^3	6.2×10^1	0.7	2.0×10^3	2.3×10^3	3.5×10^1	1.5
Nethe	Ne	20–113	3.3×10^5	2.9×10^3	1.5×10^3	51.8	1.7×10^5	6.8×10^2	7.5×10^2	109.6
Rupel	Ru	12–81	1.9×10^4	2.9×10^4	1.6×10^2	0.5	2.4×10^4	6.4×10^3	2.0×10^2	3.1
Scheldt	Sc1	19–125	7.1×10^3	2.3×10^3	8.9×10^1	4.0	7.8×10^3	5.2×10^2	9.8×10^1	18.9
	Sc2	12–113	4.0×10^3	3.8×10^3	4.5×10^1	1.2	2.2×10^3	8.3×10^2	2.4×10^1	3.0
	Sc3	22–124	3.5×10^4	1.4×10^3	4.4×10^2	32.0	1.6×10^4	3.5×10^2	2.0×10^2	58.2

[a]Minimal and maximal SM concentrations measured during the monitoring survey ($n = 16$)
[b]Geometric mean of the abundances measured in the sediments during the monitoring survey ($n = 2$)
[c]Geometric mean of the abundances measured in the water column during the monitoring survey ($n = 16$)
[d]Potential abundance of resuspended FIB as calculated by multiplying the FIB abundance in the sediments with the maximum SM concentration observed at each sampling site
[e]Percentage of FIB potentially originating from sediment resuspension in the water column, calculated as the ratio of potentially resuspended FIB (d) to the geometric mean abundance of FIB in the water column (c)

correlation was found between the flow rate and the log abundances of FIB for the other sites ($0.08 < r^2 < 0.23$; $p > 0.05$).

Comparison of Fig. 2a, b showed that the abundances of both FIB followed very similar trends. Accordingly, when IE numbers were plotted against *E. coli* numbers in a log–log scale, a significant correlation ($r^2 = 0.89$, $p < 0.01$) was found (Fig. 3). The GLUase activity, tested here as a surrogate for *E. coli* plate counts, followed a trend between sampling sites very similar to the one observed for *E. coli* abundance (Fig. 2a, c). When GLUase activities were plotted against *E. coli* counts in a log–log scale, a significant correlation ($r^2 = 0.87$, $p < 0.01$) was found (Fig. 4).

Faecal contamination in the river sediments

The geometric means of *E. coli* and IE concentrations measured in the sediments of the main rivers of the watershed during two sampling campaigns are presented in Table 1. These values ranged, respectively, between 2.1×10^2 and 2.2×10^5 CFU/g dry weight sediment for *E. coli* and between 1.0×10^2 and 3.8×10^5 CFU/g dry weight sediment for IE. The higher levels of faecal contamination were observed in the Zenne River downstream from Brussels area and in the Nethe River.

In this study, comparison of the abundances of both FIB observed in the water column and in the sediments of rivers was performed. The abundances of *E. coli* and IE in the sediments did not follow a similar pattern of variation between sampling as observed in the water column. The abundances of FIB in the sediments in log units (geometric means for two sampling campaigns) were not significantly correlated with the abundances in the water samples in log units (geometric means for 16 sampling campaigns).

Sources of faecal contamination in the rivers

Point sources of faecal indicator bacteria

In order to quantify the contribution of treated wastewater to FIB loading and to investigate the efficiency of different wastewater treatments on the removal of FIB, samples collected at the entrance and the outlet of various WWTPs were analysed. In raw waters, the abundances ranged between 9.6×10^5 and 2.0×10^7 CFU/100 mL for *E. coli* (geometric mean 1.0×10^7 CFU/100 mL) and between 2.3×10^5 and 4.1×10^6 CFU/100 mL for IE (geometric mean 1.7×10^6 CFU/100 mL; Fig. 5). In the treated effluents of the WWTPs, the abundances of both FIB were significantly reduced. The log removal of FIB due to the different types of treatment was calculated as the difference between the log of the concentration before and after treatment. When a primary treatment was applied alone, average log removals of 0.40 and 0.38 were estimated for *E. coli* and IE, respectively; the removal is due to settling of FIB attached to suspended matter. When PT was followed by an activated sludge process for carbon removal, average log removals were 1.8 and 1.6 for *E. coli* and IE, respectively. As shown in Fig. 5, when the residence time of the water in biological processes is increased to allow nitrification and denitrification, the efficiency of faecal pollution removal is also increased. In the present study, the lowest concentrations after an intensive wastewater treatment were observed when a complete treatment (primary treatment followed by AS for carbon and N removal and dephosphatation) was

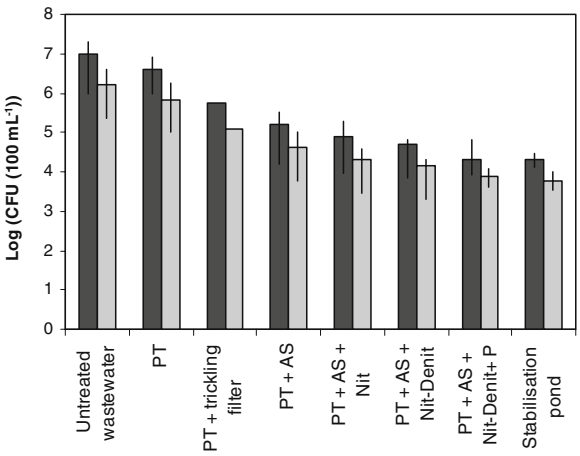

Fig. 5 *E. coli* (*black*) and IE (*grey*) abundances (log units) in raw and differently treated wastewaters (see text in "Materials and methods" section for the symbol definitions of the different wastewater treatments). Data are expressed as geometric mean values, and *vertical bars* represent the range between minimal and maximal values

Table 2 Budget for the whole Scheldt River watershed of the point and non-point sources of faecal contamination to the rivers

Contribution of point sources

Point sources	WWTP type	Number of WWTPs	Volume (m^3)/year	*E. coli*		Intestinal enterococci	
				CFU (100 mL)$^{-1}$	Flux (CFU s^{-1})	CFU (100 mL)$^{-1}$	Flux (CFU s^{-1})
	PT	6	4.0×10^6	4.0×10^6	5.0×10^9	6.9×10^5	8.8×10^8
	PT + AS	165	2.4×10^8	1.6×10^5	1.2×10^{10}	4.2×10^4	3.2×10^9
	PT + AS + Nit	21	3.5×10^6	7.9×10^4	8.9×10^7	2.0×10^4	2.2×10^7
	PT + AS + Nit-Denit	44	1.9×10^7	5.0×10^4	3.0×10^8	1.4×10^4	8.6×10^7
	PT + AS + Nit-Denit + P	148	6.3×10^8	2.0×10^4	4.0×10^9	7.4×10^3	1.5×10^9
	PT + trickling filter	1	2.0×10^6	5.4×10^5	3.4×10^8	1.2×10^5	7.6×10^7
	Stabilisation pond	35	6.4×10^6	2.1×10^4	4.3×10^7	5.6×10^3	1.1×10^7
	Total	420	9.0×10^8		2.2×10^{10}		5.7×10^9

Contribution of non-point sources

Non-point sources	Land use	Surface (km^2)	*E. coli*		Intestinal enterococci	
			CFU (100 mL)$^{-1}$	Flux (CFU s^{-1})	CFU (100 mL)$^{-1}$	Flux (CFU s^{-1})
	Forested areas	1.5×10^3	4.8×10^1	5.4×10^6	3.0×10^1	3.4×10^6
	Cultivated areas	8.4×10^3	2.2×10^2	1.4×10^8	1.9×10^2	1.2×10^8
	Pastured areas	4.9×10^3	1.1×10^3	4.7×10^8	5.7×10^2	2.4×10^8
	Total	1.5×10^4		6.2×10^8		3.6×10^8

used (log removals of 2.7 and 2.4 for *E. coli* and IE, respectively). Finally, the average efficiency of the stabilisation ponds investigated in this study was in the same order of magnitude than for the latter treatment (log removals of 2.67 and 2.47 for *E. coli* and IE, respectively). Whatever the treatment applied, the waters at the outlet of the WWTPs still contained high abundances of FIB (concentrations higher than 2×10^4 and 5×10^3 for *E. coli* and IE, respectively) leading to a major impact on the receiving waters if the dilution factor is not sufficient (see "Discussion" section). Of course, lower values in the treated effluents can be obtained if a disinfection stage (UV irradiation, for example) is added at the end of the wastewater treatment line (George et al. 2002), but this was not investigated in the present study as there is presently no WWTP with a disinfection unit in the Scheldt watershed.

Non-point sources of faecal indicator bacteria

In this study, FIB abundances were measured in samples collected in small streams located in rural areas. The small streams were classified on the basis of the land use of their watershed: forested areas, cultivated areas and pastured areas. Table 2 presents the level of faecal contamination due to surface runoff and soil leaching for both FIB. Data showed that small streams flowing through pastures were, on average, more contaminated than those flowing through cultivated areas, which were more contaminated than those flowing in forested areas. However, a high variability was observed between the minimal and the maximal values for each type of land use. A Wilcoxon and Mann–Whitney test performed to compare the level of *E. coli* from different land uses showed that there was a significant difference between pastured and cultivated areas ($p < 0.05$) and also between pastured and forested areas ($p < 0.01$). However, no significant difference was found between cultivated and forested areas ($p > 0.05$). This analysis demonstrates that the land use of the watershed has a significant impact on the quantity of faecal microorganisms brought to rivers by surface runoff and soil leaching and thus on the microbiological quality of the small streams flowing in rural areas.

Discussion

Relationship between GLUase activity and *E. coli* abundance

In the present study, GLUase activity was tested as a surrogate for a rapid evaluation of *E. coli* abundance in river waters. A significant correlation was found (in log–log units) between the enzymatic activities and the plate counts of *E. coli*. 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 these previous studies (Garcia-Armisen et al. 2005). 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 cell in less contaminated natural waters may be due to an underestimation of the number of FIB when enumerated by culture-based methods. This underestimation may be explained by a higher proportion of viable but non-culturable cells (VBNC; cells presenting a detectable GLUase activity but unable to multiply in or on the specific media used in culture-based methods) in low contaminated waters. The higher proportion of VBNC faecal indicator bacteria in less polluted environments could be the result of more severe or longer environmental stress factors such as nutrient limitation and enhanced solar radiation effects due to deeper light penetration (Servais et al. 2009). The quality of the correlation presented in Fig. 4 showed that GLUase activity measurement can be considered as a valid alternative for monitoring *E. coli* in the rivers of the Scheldt watershed. This method offers the advantage to give a result in less than 1 h while the culture-based method requires a 24-h incubation.

Relationship between *E. coli* and IE abundances

E. coli and IE are now usually enumerated to evaluate the microbiological quality of recreational

waters (EPA 1999; EU 2006). In this study, the abundances of both indicators were measured in parallel in river water samples (Fig. 2) and in sediments (Table 1). For the water samples, significant correlation was found between *E. coli* and IE numbers in log–log units ($r^2 = 0.89$, $p < 0.01$; Fig. 3). Significant correlations between these two FIB were already reported in the literature (Kinzelman et al. 2003; Roslev et al. 2008; Kirschner et al. 2009). Additionally, in the present study, a significant correlation was also found between *E. coli* and IE numbers in log–log units for the sediment samples ($r^2 = 0.86$, $p < 0.01$; data not shown). A similar relationship was already reported by Roslev et al. (2008) for stream and estuarine sediment samples.

Several studies have shown that the composition of the faecal flora was different in animal and human faeces; for example, the proportion of faecal coliforms compared to faecal streptococci and the proportion of *E. coli* to IE were higher in human faeces than in animal faeces (Feachem 1975; Geldreich 1976). Thus, some authors (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 *E. coli*/IE ratios were calculated for runoff and soil leaching waters (small streams sampled upstream from any point source), main rivers and WWTP effluents. The average of the *E. coli*/IE ratios estimated for WWTPs effluents (5.24) was the highest, followed by the ratio for the main rivers (5.12); it was much lower for the small streams (1.63). The Wilcoxon and Mann–Whitney test was performed to compare the *E. coli*/IE ratios observed in rivers, WWTP effluents and stream waters. The ratios of *E. coli* to IE observed in WWTP effluents were significantly higher ($p < 0.05$) than those observed in the small streams. This result seems in agreement with the origin of the faecal contamination. Soil leaching and runoff brought to small streams FIB mainly from animal origin while in WWTP effluents FIB are predominantly from human origin. However, the ratio of *E. coli* to IE values can also be influenced by the residence time of faeces in the environment as IE survive longer than *E. coli* (Craig et al. 2002b). As we can assume that the residence time is longer in small streams than in wastewaters, this could partly explain the lower ratio values in small streams waters with regards to ratio values for WWTP effluents. The *E. coli*/IE ratios observed in river waters and in WWTP effluents were not significantly different ($p > 0.05$). This could suggest that the FIB in the main rivers were mainly from human origin and brought to rivers by wastewaters. The average *E. coli*/IE ratio found in the main rivers of the Scheldt watershed was quite close to the mean value reported by Kirschner et al. (2009) for the rivers of the Danube watershed (5.8).

The average ratio of *E. coli* to IE was also calculated for the sediment samples collected in the main rivers of the watershed; the value (1.89) was significantly lower than the one estimated for the corresponding overlying water columns. A longer survival of IE in the sediments in comparison to *E. coli* (Craig et al. 2002b) could explain this observation. Roslev et al. (2008) also reported lower *E. coli*/IE ratios in sediments than in overlying river and estuarine samples.

Faecal contamination of the rivers of the Scheldt drainage network

In order to qualify the microbiological water quality of the main rivers of the watershed observed during our monitoring, we compared the measured *E. coli* and IE abundances with the requirements of the new EU Directive for bathing water quality (EU 2006). We considered the 90th percentiles of the *E. coli* and IE abundances at each location and compared them to the 'sufficient' values set out in the Directive (i.e. the minimal quality requirement)—9.0×10^2 and 3.3×10^2 CFU/100 mL for *E. coli* and IE, respectively. They were higher at every location since the less contaminated site Sc3 displayed 90th percentiles of 3.0×10^3 and 1.1×10^3 CFU/100 mL for *E. coli* and IE, respectively (Fig. 2a, b). This indicated that none of the sampling sites had a microbiological water quality sufficient for bathing activities and that the main rivers of the watershed have globally a poor microbiological water quality.

The sites from the Zenne River downstream from Brussels (Se2 and 3) were heavily contaminated: The abundances of *E. coli* and IE were in

the same order of magnitude than those usually measured in treated wastewaters (Fig. 5). The Zenne River, a tributary of the Dyle River, has a watershed of 1,011 km^2 characterized by agriculture activities in its upstream part and an important urbanization in its downstream part. The population density in the watershed is very high (on average 1,259 inhabitants km^{-2}) and most of the inhabitants live in the city of Brussels. The Zenne River crosses this city from south to north over a distance of about 20 km and receives the sewage from two WWTPs: the Brussels South WWTP (360,000 inhabitant equivalents) and the Brussels North WWTP (1.1 millions inhabitant equivalents). The annual average discharge of the Zenne River upstream from Brussels is 3.93 m^3 s^{-1} (average for the 2007–2008 period) while the flow released by the two WWTPs of Brussels is of the same order of magnitude (average for the 2007–2008 period—3.85 m^3 s^{-1}). This means that, on average, the Zenne River water downstream from Brussels is roughly half composed of treated wastewaters, this proportion being higher during the low flow periods of the river. In addition, during rain events, combined sewer overflows (CSOs) occur in the Brussels area and release in the river a mixture of untreated wastewater and surface runoff water.

Similar degradations of river microbiological quality due to the discharge of treated urban wastewater effluents from large cities were already reported in the literature. For example, a large increase of faecal indicator bacteria concentration was observed in the Thames River downstream from London (Tryland et al. 2002) where *E. coli* concentration can reach up to 10^5 *E. coli*/100 mL. An important increase of faecal coliforms was reported by Servais et al. (2007) in the Seine River downstream from the Parisian area. A moderate increase in coliform counts was observed in the Danube River downstream from Vienna (Hoch et al. 1996) and large increases downstream from Budapest and Bucarest (Kirschner et al. 2009). The importance of such quality decrease is directly related to the ratio of the effluents and the river flow rates and to the FIB concentration in the treated effluent depending on the type of treatment; this ratio is particularly high in the case of the impact of Brussels treated wastewaters on the Zenne River.

Impact of sediment resuspension on the microbiological water quality of the rivers

Sediments have been recognised as in-stream store of faecal contamination that can be mobilised during hydrological events (rapid increase of the flow rate, floods) or other sediment-disturbing events (Muirhead et al. 2004). Several studies have shown that resuspension of faecally contaminated sediments can deteriorate the microbiological quality of the overlying water column (Crabill et al. 1999). During this study, sediments of the main rivers of the watershed were shown to contain between 10^2 and 10^5 FIB/g dry weight. In order to determine the possible contribution of sediment resuspension to the faecal contamination in the rivers of the watershed, we compared the FIB abundance that could result from sediment resuspension with the FIB abundance measured in the water column during the monitoring survey. FIB that could have originated from sediment resuspension according to this calculation represented in general a low proportion of the FIB found in the water column (Table 1). Because the ratio of *E. coli* to IE was lower in the sediment than in the water, the contribution of sediment resuspension to the FIB load of the water was higher for IE than for *E. coli*. Potential resuspended *E. coli* represented less than 1% of the *E. coli* in the water column at six sites out of 12 and between 1% and 10% at four sites. For IE, these contribution levels were observed at two and six sites, respectively. The contribution of sediment resuspension to the water contamination was potentially high in only two sites: Sc3 (32% and 58% of the total *E. coli* and IE, respectively) and Ne (52% and 110% of the total *E. coli* and IE, respectively; Table 1). Therefore, except for these two sites, sediment resuspension was not estimated to be a major cause of water quality degradation in the rivers of the Scheldt watershed, unlike what was observed in other studies in which the water column was far less contaminated (Crabill et al. 1999; Nagels et al. 2002).

Respective contribution of point and non-point sources to river faecal contamination

In order to compare the global contributions of point and non-point sources to the faecal contamination of the Scheldt drainage network, we estimated the fluxes of FIB emitted at the scale of the whole watershed by both types of source. For point sources, we estimated the flux of FIB discharged by the WWTPs located in the watershed. For year 2008, 420 WWTPs were inventoried in the watershed along with their treatment type and their annual treated volume. For each WWTP, the annual discharge was then multiplied by an average FIB concentration in function of its treatment type. The geometric means of the abundances measured in this study at the outlet of WWTPs for different types of treatment (Fig. 5) were used for this calculation. The FIB fluxes of all WWTPs were then summed to estimate the global contribution of point sources; it represented a flux of 2.2×10^{10} *E. coli*/s and 5.7×10^{9} IE/s (Table 2).

For non-point sources, the surface occupied by the different land uses in the watershed was first evaluated. These surfaces were derived from the CORINE Land Cover database (Bossard et al. 2000): forested, pastured, cultivated and urban areas represented, respectively, 7%, 26%, 39% and 25% of the total watershed surface (21,863 km^2). As almost all urban areas in the watershed are equipped with combined sewers, runoff on urban areas was assumed to be collected by sewers and treated in WWTPs; it was thus included in our estimation of the FIB flux from point sources. For the other types of surface, the flux of runoff or soil leaching water emitted to the watercourses at the scale of the whole watershed was estimated by multiplying the specific discharge of the Scheldt watershed (7.4 L s^{-1} km^{-2}) with the total surface of each land use in the watershed. These water fluxes were multiplied by a FIB concentration specific to each land use (average abundances measured in this study in the small streams from forested, pastured or cultivated areas (Table 2)). The FIB fluxes obtained for the three types of land use were finally summed to get the global contribution of non-point sources; it was estimated at 6.2×10^{8} *E. coli*/s and 3.6×10^{8} IE/s (Table 2).

At the scale of the whole watershed, point sources (WWTPs effluents) appeared therefore to contribute 35 and 15 times more than non-point sources to the flux of *E. coli* and IE to the rivers. Moreover, discharges of untreated wastewater were neglected in this budget. This could result in an underestimation of the point source contribution since a small part of the population in the watershed is connected to a sewer system but not to a WWTP. In addition, during strong rain events CSOs can occur, releasing in rivers a mixture of urban runoff water and wastewater. Due to the absence of data on discharged volumes of untreated wastewater and CSOs, our estimate of the flux of FIB emitted by point sources should be considered as a minimum. If release of untreated wastewater and CSOs would be taken into account, this would reinforce the predominance of point sources over non-point sources.

Finally, it should be kept in mind that this global budget does not give any information on the local impact that non-point sources can have on the microbiological quality of small rivers.

Conclusions

The microbial pollution of the main rivers of Scheldt basin was assessed by monitoring faecal indicator bacteria (*E. coli* and enterococci) contamination in water and sediments. This study demonstrated the poor microbiological quality of the main rivers of the Scheldt watershed. Sediments of studied rivers contained high levels of FIB; however, the FIB concentrations in sediments were not sufficient to contribute significantly to river water contamination during resuspension events excepted for the Scheldt and the Nethe. The quantification of point and non-point sources in the Scheldt watershed allowed the comparison of their respective contribution to the faecal contamination of the rivers of the Scheldt drainage network. This comparison showed that, at the scale of the Scheldt watershed, point sources were largely predominant in comparison to non-point sources. This finding indicates that to improve the microbiological water quality of the rivers, the faecal contaminants released by

the WWTPs should be reduced. This can be done by adding, at the end of the treatment line in WWTPs, a treatment stage specifically devoted to disinfection. UV treatment can be used for this purpose; it has been shown that UV treatment as final stage of wastewater treatment allowed increasing the Log removal by 2 to 3 U (Servais et al. 2007).

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