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## An approach to pathogens flux simulation in a combined sewer system

### Une approche de la simulation des pathogènes dans un réseau unitaire

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## RÉSUMÉ

Les collecteurs d'assainissement unitaires transportent des matières organiques et des pathogènes issus des déjections humaines et animales. Ces matières sont mélangées avec le ruissellement lors des événements pluvieux et peuvent sur-verser dans les milieux aquatiques naturels. Les premières analyses génétiques ont été réalisées sur un petit cours d'eau expérimental et son réseau unitaire latéral (site OTHU). Les résultats indiquent que les pathogènes du milieu naturel sont une évolution des pathogènes du réseau. Toutefois la survie présumée ne doit pas être confondue avec la fréquence de leurs apports au cours d'eau. Une première étape consiste à vérifier les concentrations et les fréquences auxquelles ils sont délivrés au milieu récepteur. Les premières données sur trois espèces de pathogènes ont été échantillonnées de 2009 à 2011 dans le collecteur unitaire. Les données montrent des variations qui peuvent être reliées aux séquences de temps sec et humide. Toutefois les variations entre les espèces pathogènes ne sont pas totalement corrélées. Cela pourrait indiquer des taux de croissance et/ou des localisations différentes dans le collecteur d'assainissement. L'hypothèse principale pour expliquer l'augmentation des concentrations avec les événements de pluie est l'arrachage des bio-films organiques qui tapissent la partie immergée de la conduite d'assainissement. Cette hypothèse est à confirmer par des données complémentaires et plus ciblées sur la colonne d'eau en temps de pluie et les tapis de bio-films en temps sec.

## ABSTRACT

Combined sewer systems convey organic matters and pathogens coming from human and animal faeces. They are mixed with rainfall runoff during storm event and can overflow to natural aquatic systems. Preliminary genetic analyses of pathogens were conducted in an experimental small water course and its lateral combined sewer system (OTHU site). Results indicate that natural system pathogens are an evolution of sewer system pathogens. However their presumed survival should not be confounded with their frequent delivery to the water course. Then a first step is to check for concentrations and frequencies that are delivered to receiving waters. First data on three pathogen species concentrations were sampled from 2009 to 2011 into the combined sewer system. Data exhibit variations that can be related to dry and wet weather sequences. However variations do not fully correlate between pathogen species. This would indicate growing rate and/or differing locations in the sewer pipe. The main hypothesis to explain concentration increase with storm events is the flushing of organic biofilms lining the immersed part of sewer conduit. This hypothesis is to be confirmed with complementary data more focussed on the water column during storm events and lining biofilms during dry weather.

## KEYWORDS

Combined sewer, Hydrological indicators, Pathogens, Sewer overflow

## 1 PROBLEMATIC

From the literature (Tait *et al.*, 2003; Mohanakrishnan *et al.*, 2009; Pai *et al.*, 2010) it is known that organic compounds and biofilms can develop in the immersed part of a sewer pipe, resulting from nutrient-rich water conveyed during dry weather periods, and also from a quite stable water temperature of 12 °C all over the year long. It results in a mineral and organic layer which is partially scraped from the immersed part of pipe during storm events, when runoff enters the sewer systems, increasing flow velocity and shear stress. Combined sewer overflows (CSOs) are loaded with pollution including pathogens. A field experiment (Tait *et al.*, 2003) implemented in a combined sewer pipe showed the suspended solid concentration in waters was depending on flow magnitude and was decreasing if a same discharge flow was maintained. Only an increase in discharge was able to provide a new increase in suspended solid, indicating more cohesion of the remaining sediment. A large cohesion of the biofilm and organic matrix complex was also observed (Rocher *et al.*, 2003).

Bacteria linked to human faeces flow with sewer waters but are also known to develop in biofilm on and into organic deposits taking place on immersed part of sewer pipes (Movassagh Ghazani *et al.*, 2009; Soleimani *et al.*, 2013). The amount of bacteria can be close to that of activated sludge (Chen *et al.*, 2003). Faecal bacteria seem to be more abundant close to bottom pipe communities (Gomez-Alvarez *et al.*, 2012) when the density of the live population of *E. coli* DH5 $\alpha$  is the highest at three fourths of the biofilm depth. The structural heterogeneity in a biofilm is explained both by shear flow velocity at biofilm surface and pH at concrete surface. These results help understand why bacteria amount can vary in sewage water during storm events (Stewart *et al.*, 1995; Chen *et al.*, 2003; Leung *et al.*, 2005).

To study the fate of pathogens delivered by CSOs in a small stream, ten water sampling were collected from a combined sewer pipe between October 2009 and September 2011. Calculated intestinal enterococci, *E. coli* and *P. aeruginosa* concentrations showed large variations between samples. It appeared that each bacterial concentration could change from one order of magnitude and were not varying in the same way (Petit, 2012). This would influence notably their dissemination into receiving waters and then contribute to the understanding of their population fluctuations in the natural system.

The objective of this work is to assess if there are correlations between bacteria-concentrations and antecedent flow conditions. The following two hypotheses are tested:

H1: During dry weather periods lower pathogen concentrations should be observed in the sewage water. At the same time biofilm is expected to grow.

H2: During and just after flushing events the scouring of lining biofilm should induce a rapid increase in pathogens concentrations into the water column.

These two hypotheses can influence each other: the longer the dry period, the greater the potential to deliver larger amount of pathogens for a subsequent storm event and *vice versa*.

## 2 MATERIAL AND METHODS

- *Combined Sewer Overflow (CSO) time series*

The drained impervious area is of 0.16 km<sup>2</sup> at the CSO unit (see Fig.1). This unit overflows when discharge in the sewer is exceeding 0.010 m<sup>3</sup>/s, almost five folds the mean dry weather discharge of 0.0018 m<sup>3</sup>/s.

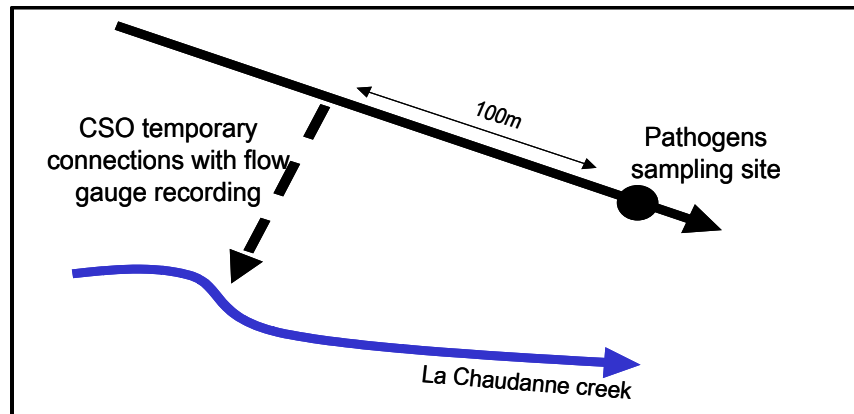


Figure 1: Monitoring site of la Chaudanne (OTHU site)

Overflow is recorded thanks to a gauge station which provides a flow time series over the period of interest (2009 to 2011) at a fine time step. CSO peaks median value is of  $0.01 \text{ m}^3/\text{s}$  with a median CSO events duration of 17 minutes. Events occur 113 days over 718, several time a day with a mean overflow frequency of 0.44 day. From these summary statistics there are clearly dominant periods without overflows, called dry periods and others concentrating overflows, called wet period. Overflow events are considered to be indicators of hydraulic stresses that would explain the observed pathogen concentration variability at the sampling site.

- *Hydrological Indicators (HIs)*

To test different aspects of hydraulic constraints like magnitude, duration and frequency, several hydrological indicators (HI) were defined. These indicators, previously tested in another experiment (Breil *et al.*, 2007), are summarized into 11 flow characteristics. Each HI is computed from the CSO time series over a preceding period-duration (PPDs) to account for hydraulic history. PPDs range from 1 to 15 days before each date of sampling. Their potential effects on pathogens concentration in the sewage water are indicated in Table 1.

Table 1. Hydrological indicators and expected effects on bacterial concentrations

Id.	Hydrological Indicators (HI)	Unit	Expected effect on pathogens concentrations when a HI increases
HI-1	Maximum CSO peak	$\text{m}^3/\text{s}$	Increase due to scoring of biofilm
HI-2	Maximum CSO duration	day	Possible decrease due to rinse effect by rain water
HI-3	Maximum CSO water amount	$\text{Mm}^3$	Increase if HI-1 is large enough to scrap biofilm
HI-4	Dry time duration just before HI-1	day	Increase the available amount of biofilm, hence of pathogens
HI-5	Duration from HI-1 to date of sampling	day	Decrease if no intermediate large CSO occur
HI-6	Number of CSOs		Decrease because there is less time for biofilm to grow
HI-7	Total CSO duration	day	Possible decrease due to rinse effect by rain water
HI-8	Total CSOs water amount	$\text{Mm}^3$	Decrease because there is less time for biofilm to grow
HI-9	Duration of longest dry period	day	Decrease in the absence of CSOs
HI-10	Total duration of dry periods	day	Decrease in the absence of CSOs
HI-11	Number of dry periods		Complement to number of CSOs- same effect

- *Microbiological data*

Samples were collected from the sewer water column (Petit S., 2012). Analyses were performed to detect intestinal enterococci, *E. coli* and *P. aeruginosa*. Some statistics are presented in Table 2.

Table2. Minimum and maximum values of analysed pathogens into collected CSS waters.

	<i>E. coli</i> (CFU/100mL)	intestinal enterococci (CFU/100mL)	<i>P.aeruginosa</i> (MPN/100mL)
number of samples	10	10	9
minimum	6.51E+05	2.68E+05	7.23E+02
mean	1.14E+07	2.20E+06	2.89E+04
maximum	4.17E+07	6.63E+06	9.30E+04
range	4.10E+07	6.36E+06	9.23E+04
relative dispersion	1.21E+00	8.30E-01	1.09E+00

*E.Coli* is the most abundant followed by intestinal enterococci and then *P.aeruginosa*. There are three orders of magnitude between first and last pathogen species mean values. It can indicate the pathogen developments are different in the sewer. A Wilcoxon non-parametric test to account for deviance from normal distributions was performed on rank-data. It confirmed the pathogens exhibit very different distributions (two-sided probabilities less than 0.007).

The lowest relative dispersion is observed for intestinal enterococci with a mean value of 0.8. Dispersion is not dependant on mean pathogen concentration. Other factors linked to each pathogen species would explain these differences. Not limited to, there are the population rates of growth, their preference for specific conditions and then locations in the sewer network and their strength of attachment to the biofilm.

The three species positively but only partially correlate (see Table 3) indicating they do not fully fluctuate in the same way. *P. aeruginosa* (Pae) is most connected to *Escherichia coli* (EC). This would indicate a closer behaviour than with intestinal enterococci (IE), which seems independent.

Table 3. Rank correlation values between concentrations of *E. coli* (EC), intestinal enterococci (IE) and *P. aeruginosa* (Pae)

	EC	IE	Pae
EC	1.000		
IE	0.050	1.000	
Pae	0.460	0.075	1.000

- *Relationships between bacterial concentrations and hydrological indicators.*

Considering the limited number of biotic data, the observed non-normal distribution of variables, in particular for hydrological data containing tied data, the Kendall's Tau test was used. Only significant Tau values ( $U_{0.975}$ ) are discussed in the results item.

- *Hierarchical clustering of each HI contribution to biotic indicators variability*

Multi-linear regression analyses were performed. The eleven hydrological indicators were computed (see Table 3) meaning 1 more than the number of data. It means resulting model would not be efficient at all. Then a stepwise regression procedure was used to keep only more relevant hydrological indicators, controlling for co-linearity effects, rejecting on necessary already entered variables. As the method works with normal distributions, biotic and hydrological indicators were log transformed with (x+1) to account for zero values. Overall most of the hydrological indicators were not really adapted even using for example the sophisticated Box-Cox transformation. This was due to tied data inherent to the sampling process and definition of most of the HIs. Only PPDs of 1, 2, 3, 5, 10 and 15 were used. All adjusted R2 values were near 1 and greater than any individual correlation. All coefficients were checked to be significant with a p-value of less than 0.05. No cross correlations between selected independent variables was greater than 0.6 when a threshold of 0.8 (Klein rule) is recommended. Also signs of contributions to the model were compared to signs of direct correlations with bacteria concentration, as to detect "compensation effects". It happens at least for the third variable with *P. aeruginosa* and forth variables for the two others bacteria. It is also considered that by

definition of the multi-linear regression model, each following variable entering the model is correlated to the remaining residual to the model. Then it is difficult to establish causal interpretation since the third variable.

### 3 RESULTS AND DISCUSSION

#### 3.1 Relationships between *P.aeruginosa*, intestinal enterococci, *E.coli* and hydrological indicators (HI)

Figures 2, 3 and 4 represent on the horizontal axis the PPD from date of sampling. In each figure large light circles indicate significant Tau values (bilateral test with  $U_{0.975}$ ).

- *P.aeruginosa*

Seven hydrological indicators exhibit significant correlation with *P. aeruginosa* concentrations in sewage waters. However, only three are not redundant and are reported in Figure 2.

The maximum overflow duration (HI-2) recorded in the 4-5 days prior dates of sampling correlates negatively with *P.aeruginosa* concentration in the sewage water. This seems to result from the cleaning effect of long rain events that rinse the sewer network as it can be observed on CSOs electrical conductivity for such hydraulic events.

Positive Tau values are observed for total time without CSOs (HI-10) occurring in the 4-5 days before date of sampling. Long dry sequences allow growing of biofilm with the delivering of more *P.aeruginosa* bacteria in the sewage water when a hydraulic perturbation occurs. A PPD of 4-5 days tends to indicate this bacteria species gets a fast growing rate.

Finally, for a PPD of 13-14 days, the longer the time-duration between maximum CSO peak (HI-5) and date of sampling, the higher the concentration of *P.aeruginosa* at this date. This result does not contradict hypothesis H1 because smaller hydraulic events can occur between maximum CSO peak and date of sampling. This result probably indicates large hydraulic events have the ability to weaken the biofilm cohesion for subsequent smaller events. Large events seem to occur on a two weeks basis in this case.

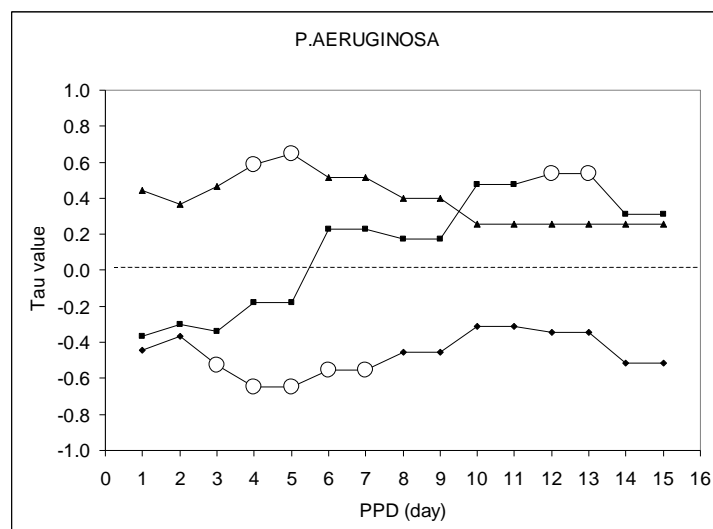


Figure 2. Kendall's Tau rank correlation values with Preceding Period Duration. Triangles: total time without CSOs (HI-10); Squares: Duration between sampling date and max-CSO (HI-5); Diamonds: Maximum overflow duration (HI-2).

All selected HIs with their correlation signs well support the formulated hypotheses H1 and H2.

- *Intestinal enterococci*

Only two HIs have significant Tau values with intestinal enterococci concentrations (Fig.3). They are negative and concern hydraulic conditions that occurred in the day prior date of sampling. This result clearly indicates independence of intestinal enterococci concentration from hydraulic conditions that occurred in previous days. No interpretation can be derived from HI-5 and HI-10 in this case.

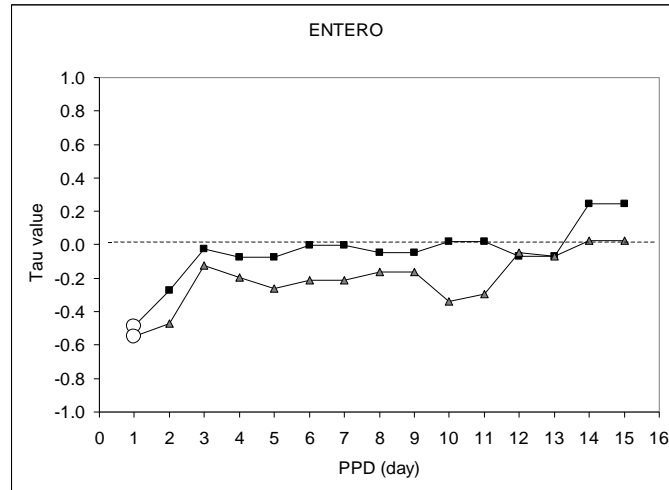


Figure 3. Kendall's Tau rank correlation values with Preceding Period Duration. Triangles: number of dry sequences (HI-11); Squares: Duration between sampling date and max-CSO (H-5).

Intestinal enterococci pathogens exhibit an intermediate abundance with the smaller dispersion around its mean value (see table 1). This would confirm that there is no specific storage location for this pathogen in the sewer system other than in the water column.

- *Escherichia coli*

In that case, six HIs have significant correlation with *E.coli* concentrations in sewage waters. Only two really differ and are presented in Figure 4. They both exhibit a correlation peak at 9 days but with opposite signs.

With duration of maximum CSO (HI-2), the negative correlation can be interpreted as the remnant effect of sewer flushing by a flood event in the sewer. This is the same effect than for *P.aeruginosa* but it requires a longer PPD of 9 days against 5 to be detected. It means the delivery of *E.coli* is more persistent in time after a flushing event. It can be due to a larger quantity of this pathogen species in the biofilm considering its mean concentration is the highest in the sewage water (see table 1). The long duration of 9 days could also indicate more resistance to hydraulic scraping of this species, meaning it not located on biofilm surface but in the deeper and oxygen most depleted layers. This possibility is in agreement with observations done by several authors (Rocher *et al.*, 2003; Gomez-Alvarez *et al.*, 2012).

Concerning duration of the longest dry period (HI-9), it has more or less the same effect than HI-10 on *P.aeruginosa* concentrations. The positive correlation would indicate the release of *E.coli* from developed biofilms after suitable conditions for its growing and with small hydraulic events.

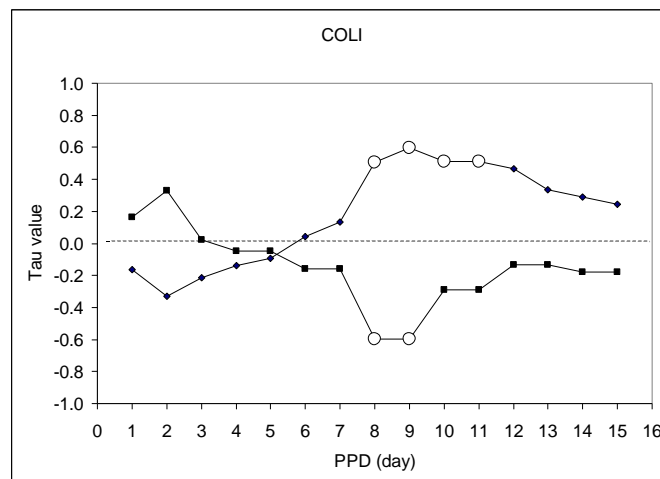


Figure 4. Kendall's Tau rank correlation with Preceding Period Duration. Squares: Maximum overflow duration (HI-2); Diamonds: Duration of longest dry period (HI-9).

- *Intermediate conclusion*

Overall, what is noticeable are the time durations to detect significant correlations of bacteria concentrations with HIs, or the absence of correlations with given HIs. On one side, there are negative correlations that can be compared to a remnant effect of perturbations which is to decrease bacteria concentrations because of the repeated biofilm reduction and possibly not enough time for regeneration. On the other side, there are positive correlations that can correspond to periods without perturbations and then to an increase in concentrations when a hydraulic event occurs. So, depending on the antecedent conditions, we could get either increasing or decreasing of bacteria concentrations.

Intestinal enterococci seems to decrease rapidly, on a short term, with no perturbation event, which tends to demonstrate it mainly stands in the water column with no other sources than sewage waters coming to the sewer. This could explain the fact that there were no significant positive correlations and only two hydrological indicators in overall correlations.

*P. aeruginosa* and *E. coli*, seems to follow a same behaviour which is confirmed in table 2. Relevant hydrological indicators are quite the same with same explanations. However, they differ in term of PPD to detect correlations. The difference from 5 to 9 days between these two pathogen species would indicate *E.coli* is more resistant to flushing because it develops at closer to the pipe, in the more anoxic layers as it was observed by some authors (e.g. Soleimani *et al.*, 2013).

### 3.2 Multi-linear Regression Analysis

As a result of the combination of observed positive and negative effects of HIs on the pathogen concentrations in the sewage water, there are possibly some indirect effects with hidden influences not detectable using single correlation approaches. To check for these potential effects, multi-linear regressions were performed. That kind of model helps to identify contribution and hierarchical rank of each HI in variability of biotic indicators. Then some causal analysis can be conducted from the models if it well represent the observed variability, meaning an adjusted R2 greater than 0.9.

Results are listed in table 4 with selected independent variables, given in order of contribution with their sign to multi-linear models (first row is the most contributing), with their period (PPD) of detection, and their meaning for interpretation.

Seven over eleven HIs were selected by the stepwise regression process. Among them, dry time with different PPDs and sign is the first. Also noticeable are CSO related HIs (HI-1, HI-7, HI-8) in the selected independent variables.

Table 4. Contribution of hydrological indicators (HI) to the pathogens concentrations variability.

<i>Entero</i>	<i>HI meaning</i>	<i>E. coli</i>	<i>HI meaning</i>	<i>P.aeruginosa</i>	<i>HI meaning</i>
(-) HI4 - 1day	Dry time duration just before max. CSO peak	(+) HI4 - 3 days	Dry time duration just before max. CSO peak	(-) HI4 - 3 days	Dry time duration just before max. CSO peak
(-) HI1 - 10 days	Maximum CSO peak	(+) HI8 - 2 days	Number of CSOs	(+) HI5 - 10 days	Duration from max. CSO peak to date of sampling
(+) HI7- 5 days	Total CSOs duration	(-) HI12 - 1 day	Percent of sewage water	(+) HI1 - 2 days	Maximum CSO peak
(-) HI4 - 10 days	Dry time duration just before max. CSO peak	(+), HI1 - 10 days	Maximum CSO peak	(-) HI11 - 15 days	Number of dry sequences
(-) HI5 - 10 days	Duration from max. CSO peak to date of sampling	(+) HI5 - 5 days	Duration from max. CSO peak to date of sampling	(-) HI12 - 1 day	Percent of sewage water
(-) HI12 - 3 days	Percent of sewage water				
(-) HI1 - 3 days	Maximum CSO peak				

- *Intestinal enterococci*

First HI is the duration time without runoff in the sewer system just before the maximum observed CSO in the period of calculation. It correlates negatively, considering here the day before each sample date (10 samples were performed). Previously, it was displayed that this bacteria was linked to short term HIs like time to maximum (H-I5) and number of dry sequences (HI-11) (Figure 2). These



observations can indicate that intestinal enterococci are mainly present in the sewer water and then react to short term modifications. For example on a 1 day duration, longer is the duration before maximum observed CSO (in the day) before sampling, closer from sampling is the runoff event that induces dilution.

Second HI selected is maximum CSO in the preceding 10 days. Again it has a negative effect on bacterial concentration. The concentration decreasing would then be attributed to a large flow occurring in the sewer with somewhat like a cleaning effect.

Third HI is the total CSO duration over a PPD of 5 days. It is the only HI having a positive effect on the intestinal enterococci concentration. Longer is the duration, longer is the dilution of water by rainfall-runoff and we then do not expect an increase in bacterial concentration living mainly in the water column.

This result is not consistent with the expected role of the two first variables in the model, which is questioning and would indicate a mathematical compensation effect.

- *E. Coli*

First HI is the same than for the two others bacteria but it correlates positively with *E. coli*, for a PPD of 3 days. Referring to our initial assumption, *E.coli* is expected to develop in priority in the attached biofilm lining the pipe. So, the longer the dry sequence, the more we get bacteria in the water after a flushing event.

Second is number of CSOs in the preceding two days. Again, the explanation can be: the more flushing occurs, the more biofilm provides bacteria.

Third (not presented in table 1) is per cent of sewage water with a negative contribution to the model. This result is in agreement with the assumption *E.coli* mainly develops in the attached biofilm because for less dilution we have, less bacteria are in the sewer water.

- *P. aeruginosa*

First HI is the same for the two others bacteria, but with a negative correlation for a PPD of 3 days. Referring to our initial assumption, it seems this bacterium has the same behaviour of intestinal enterococci, meaning it develops in the water column rather than in the attached biofilm. However global correlations between bacterial species (Table 2) do not indicate such a link.

Second HI is the duration from date of sampling to maximum CSO that occurred in the PPD, here of 10 days. The contribution to the model is positive, indicating that: the longer the time from the maximum CSO, the more bacteria are present in the sewage water. It tends to confirm the first HI interpretation.

Third HI is maximum CSO peak in the preceding 2 days. The positive contribution tends to indicate scouring of biofilm, which is not in agreement with the two first HIs interpretation. This again leads to question a mathematical compensation effect.

Overall, third variables seem difficult to interpret in such multi-linear models. Referring to the two first variables in each model, we can observed that intestinal enterococci has a short and long term responses (1 and 10 days), *E.coli* remains on the short term (3 and 2 days) and *P.aeruginosa* has also short (3) and long (10) terms responses.

Model characteristics are then considered keeping no more than 4 HIs with coefficient probability less than 0.05 (two side test). They are provided in table 5. We can note the adjusted  $R^2$  values are acceptable and would be used to get a prediction of pathogens release from CSOs. It can be generated calculating for each time step the corresponding HIs and then affecting them with the given coefficients.

Table 5. Multi-linear regression coefficients and their probability level

Enterobacteria / R2: 0.952			E. Coli bacteria / R2: 0.819			P.Aeruginosa / R2: 0.953		
Effect	Coeff.	P(2 Tail)	Effect	Coeff.	P(2 Tail)	Effect	Coeff.	P(2 Tail)
CONSTANT	6.421	0	CONSTANT	7.955	0	CONSTANT	3.723	0
HI4-1 day	-0.826	0.002	HI4- 3 days	-1.085	0.001	HI4- 3 days	-0.865	0
HI1-10 days	-3.128	0.002	HI8- 2 days	0.867	0.006	HI5- 10 days	0.464	0.001
HI7-5 days	0.428	0	HI12- 1 day	-0.339	0.04	HI1- 2 days	11.039	0.018
HI4-10 days	-0.304	0.001						

## 4 CONCLUSION

These are preliminary results combining pathogens concentration and hydraulic past conditions. It seems that intestinal enterococci do not have the behaviour of *E.coli* and *P.aeruginosa*. This was not expected and assumptions on its location in the sewer pipe have been proposed to explain it.

The final objective is to build a model able to simulated pathogen fluxes that are delivered to a natural system during storm events. To confirm first assumptions stated from this analysis on a limited set of observations, complementary data will be collected in the frame of a running PhD work and in collaboration with Australian specialists. Sampling design is to collect water samples from the water column just before and during flushing events as to catch dynamics of pathogen delivery to the sewer water.

Also dry weather samplings will be performed in the water column in the following days after a flushing event. This will allow checking of the assumption that there is a biological releasing process from biofilms or water-column bio-production in the absence of flushing event.

Grab sampling will also be performed both in the water column and biofilm during wet weather periods to confirm biofilm growth and location of pathogens. Other investigation techniques are foreseen to directly assess biofilm growth rate.

Simulation model will combine growth functions of pathogens as a free process and hydrological indicators as forcing constraints that can positively or negatively influence pathogen concentrations in the sewer water. It will use flow time series to generate a continuous pathogen flux production. Such a model was built to modelize the growth and ageing of macrophyte populations along the years in a large French river (Breil P. *et al.*, 2007). Also water overflows can already be simulated. Pathogens model delivery is expected to run in the coming years.

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