WEEKLY REPORT – WASTEWATER-BASED EPIDEMIOLOGICAL SURVEILLANCE OF THE SARS-COV-2

–

RESULTS OF 08/09/2021

Authors: R. Janssens[1], H. Maloux[1], S. Hanoteaux[1], B. Verhaegen[2], K. Van Hoorde[2], K. Dierick[2], S. Quoilin[1], M. Lesenfants[1]*

[1] Sciensano, Service Epidemiology of infectious diseases
[2] Sciensano, Service Foodborne pathogens
*Project responsible and contact: marie.lesenfants@sciensano.be

In collaboration with:

TABLE OF CONTENTS

1. Summary

The circulation of the SARS-CoV-2 virus in the environment is assessed in the present work based on three indicators. The analysis of wastewater samples collected in 39 wastewater treatment plants covers 45% of the Belgian population, with increased accuracy in the urban areas. The results of the wastewater surveillance is a source of complementary information to the infection cases number as the populations represented are different. Indeed, the wastewater results do notably include all asymptomatic persons, and are independent of the testing strategy.

Here are the conclusions based on the latest results of September 08th 2021:

- At the national level: The number of treatment plants belonging to the "Fast increase" and "Increasing trend" indicators rose quickly at the beginning of July 2021 and stabilized at the same alert level since mid-July. At the same time, an increase in the number of plants for which the "High circulation" indicator is fulfilled is observed since mid-August (see Figure 3).
- At the provincial level: Antwerpen, Hainaut, Liège, Luxembourg, and Namur are the provinces positive to at least one of the three alerting indicators. The province of Liege is of particular concern as the conditions for 3 indicators are met in this province (see Table 1).
- At the catchment area level: Amongst the 39 catchment areas covered[1], the number of treatment plants with at least one alerting indicator positive is 17, 5 belonging to the "High circulation" indicator, 12 belonging to the "Fast increase" indicator, and 13 belonging to the "Increasing trend" indicator (see Table 2). Last week (results of September 01th 2021), 14 areas had at least one alerting indicator positive.
- This week, all three indicators are fulfilled in 5 treatment plants: Froyennes, Amay, Liège Sclessin, Liège Oupeye and Mornimont. It should be noted that three of these 5 plants are located in the province of Liège (see Table 2).

The wastewater situation can be followed on a weekly basis on:

- The graphics available on the public COVID-19 dashboard
- The Risk Assessment Group (RAG) updating the weekly epidemiological situation through a report validated by the Risk Management Group (RMG) and published every Friday in French and Dutch.
- Further details on the methodology applied for the wastewater surveillance can be found in Appendix.

2. Introduction

In mid-September 2020 started the SARS-CoV-2 national wastewater surveillance project. The present report is an outcome of this project aiming to assess weekly the wastewater-based epidemiological situation of Belgium.

The surveillance is based on the analysis of water samples collected twice per week from the influent of 39 WasteWater Treatment Plants (WWTPs) spread over Belgium. The evolution of the SARS-CoV-2 viral concentrations measured over time in the different treatment plants is analyzed at different levels: regional, provincial, and the catchment area covered by the individual treatment plants. Also, alerting indicators were developed to highlight areas of concern regarding the high circulation, the fast evolution, and the increasing trend of the observed viral concentrations.

In this report, the weekly wastewater-based epidemiological situation is presented and discussed at the above-mentioned levels based on the three categories of alerting indicators. Moreover, the remaining

---

[1] Due to the recent flooding events, the treatment plants from Wegnez (Verviers) and Grosses-Battes (Liège) are temporarily out of order. These two areas had therefore not been screened for SARS-CoV-2. Fortunately, Liège is still well represented within this surveillance, however Verviers is not. Furthermore, no samples were received for the treatment plant in Montignies-sur-Sambre.

sources of uncertainties are discussed together with their expected impacts on the wastewater results interpretation.

3. Methodology

3.1. SAMPLE COLLECTION AND ANALYSIS

The surveillance project, which started in mid-September 2020 covers around 45% of the Belgian population. The population covered is 40% in the Flemish region, 35% in the Walloon region, and nearly 100% in the Brussels region. Further coverage details can be found in Table 1 by province (see also Tables A4, A5 and A6) and on the Sciensano public dashboard. Figure 1 shows the areas covered by the 39 WWTPs included in the project, which are located in high population density areas.

In practice, 24-hour composite samples are collected twice a week on Mondays and Wednesdays from the influent of WWTP and are analyzed for the presence of SARS-CoV-2 RNA by three different laboratories. The resulting SARS-CoV-2 concentrations (3 targeted genes) are delivered to Sciensano within 2 days for data analysis purpose. Concretely, the results which are representative of Mondays and Wednesdays are respectively available on Wednesdays and Fridays.

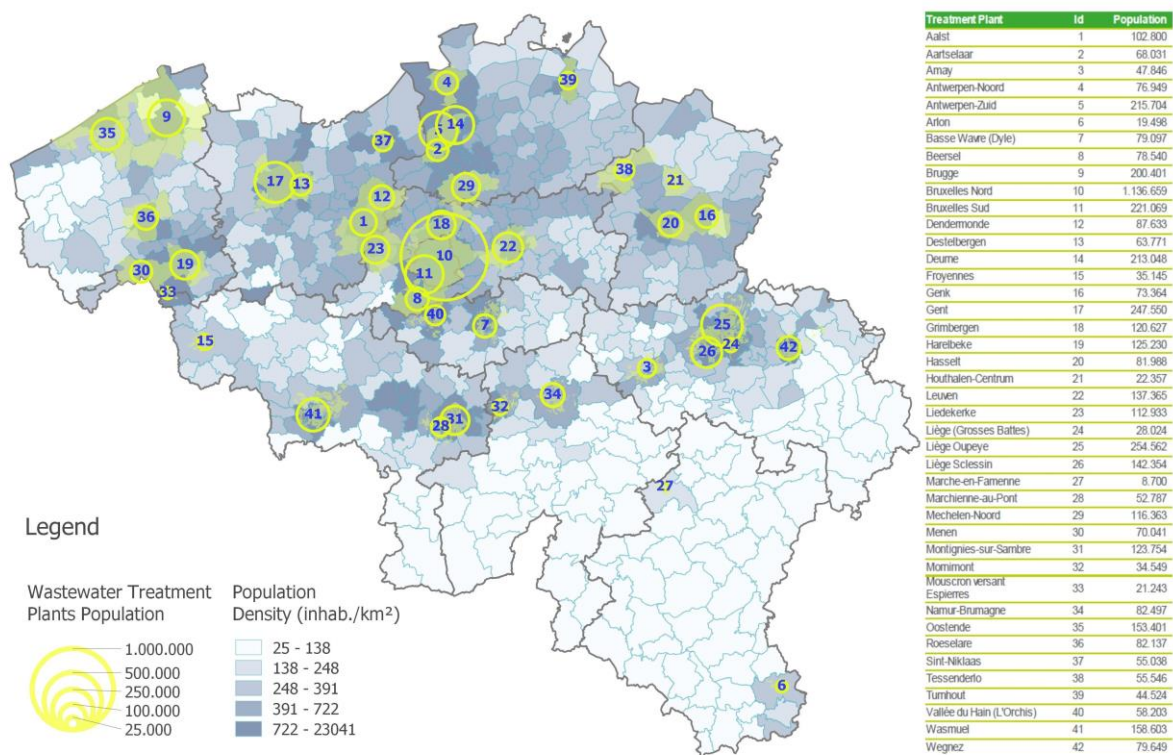| Treatment Plant	Id	Population
Aalst	1	102.800
Aartselaar	2	68.031
Amay	3	47.846
Antwerpen-Noord	4	76.949
Antwerpen-Zuid	5	215.704
Arlon	6	19.498
Basse Wavre (Dyle)	7	79.097
Beersel	8	78.540
Brugge	9	200.401
Bruxelles Nord	10	1.136.659
Bruxelles Sud	11	221.069
Dendermonde	12	87.633
Destelbergen	13	63.771
Deurne	14	213.048
Froyennes	15	35.145
Genk	16	73.364
Gent	17	247.550
Grimbergen	18	120.627
Harelbeke	19	125.230
Hasselt	20	81.988
Houthalen-Centrum	21	22.357
Leuven	22	137.365
Liedekerke	23	112.933
Liège (Grosses Battes)	24	28.024
Liège Oupeye	25	254.562
Liège Sclessin	26	142.354
Marche-en-Famenne	27	8.700
Marchienne-au-Pont	28	52.787
Mechelen-Noord	29	116.363
Menen	30	70.041
Montignies-sur-Sambre	31	123.754
Momimont	32	34.549
Mouscron versant Espierres	33	21.243
Namur-Brumagne	34	82.497
Oostende	35	153.401
Roeselare	36	82.137
Sint-Niklaas	37	55.038
Tessenderlo	38	55.546
Turnhout	39	44.524
Vallée du Hain (L'Orchix)	40	58.203
Wasmuel	41	158.603
Wegnez	42	79.649

Figure 1: The population located in the catchment areas covered by the wastewater treatment plants (highlighted in yellow) and the population density for each municipality (indicated by the grey scale).

3.2. WASTEWATER RESULTS

The quantification of SARS-CoV-2 in RNA copies/ ml (concentration) was generalized in mid-February 2021 to all the treatment plants investigated. Before this date, the quantitative values were estimated based on the retrospective application of the quantification method (see details in Appendix) from mid-

September 2020 to mid-February 2021 for Flanders and Brussels. In Wallonia, the quantitative results were available since the start.

Preliminary results allowed for estimating the limit of quantification of the analytical method at 20 copies/ml.

3.3. ALERTING INDICATORS

To highlight the areas of possible concerns, the three following types of alerting indicators are assessed twice a week, based on the viral concentration (RNA copies/ml) measured for the three targeted gene fragments (E, N1, and N2):

1. The **High circulation** indicator highlights the catchment areas where the viral concentrations are high. It corresponds to a situation where the viral concentrations exceed half of the highest value recorded since mid-February 2021.
2. The **Fast increase** indicator highlights the catchment areas where the viral concentrations have rapidly increased for the last week. It corresponds to a situation where the moving average on the past 7 days of the viral concentration has increased faster than 10% per day if being above the estimated limit of quantification. The increasing slope is normalized for each treatment plant .
3. The **Increasing trend** indicator highlights the catchment areas where the viral concentrations have been increasing for more than 6 days. It corresponds to a situation where the moving average on the past two weeks of the viral concentration has increased during more than 6 days.

3.4. CASES RESULTS

The cases number data presented in this report come from the COVID-19 laboratory-confirmed cases database centralized by Sciensano. The cases number, used to compute the 14 days-incidence, only accounts for the physical areas covered by the wastewater project (see Figure 1).

3.5. CAUTION POINTS FOR THE RESULTS INTERPRETATION

Only the trends, observed through the alerting indicators, should be assessed for the comparison of different areas. Absolute values should not be compared as the concentration values differ from an WWTP to another, notably due to the differences in population sizes covered. Additionally, the situation between the regions should be considered with caution. The degree of comparability is not yet known and depends on the comparability of the results between the different laboratories performing the analysis (see Appendices details).

Moreover, the wastewater concentrations and the cases numbers presented in this report do not originate from the same population, even though the positive cases are selected only for the municipalities covered by the wastewater surveillance. For instance, the wastewater results account for all infected persons (whatever age or symptomatic condition) while the cases include only the persons tested clinically positive. Likewise, an infected person covered by the wastewater results could be associated with another area in the clinical surveillance as the person's postal code is used for clinical statistics (mobility bias). Therefore, the correlation between the wastewater concentrations and the cases number varies according to the area considered. The wastewater results are thus complementary and independent information to the results of the cases.

The correlation between the wastewater viral concentrations and the cases numbers could also be influenced by the vaccination campaign and the circulation of variants.

Finally, all values below the limit of quantification (< 20 RNA copies/ml) should be considered as non-quantitatively reliable values. A reported value lower than 20 copies/ml only shows that SARS-CoV-2 has been detected in the sample at an undetermined concentration.

4. Results

4.1. REGIONS

Figure 2 presents the quantitative SARS-CoV-2 RNA concentration in the wastewaters and the 14 days incidence for each region in the area covered by the surveillance, the estimated viral concentrations can also be seen for the period before mid-February (see 2.2. and Appendix for more details).

The second wave peak occurring in November 2020 can be seen in the three regions in Figure 2 above. This remains true for the third wave, but to a lower extend in Wallonia (see Figure 2 below). Several hypotheses could explain this:

- The sizes of the treatment plants in Wallonia are smaller than the ones located in the two other regions, affecting the viral concentrations measured.
- The extent to which the results are comparable between the different laboratories is unknown (see Methodology for more details)

As can be seen in Figure 2, the viral concentrations in the wastewater were increasing since the beginning of July in Wallonia, Brussels and Flanders. However, this increase was much more pronounced in Wallonia and Brussels than in Flanders. In Brussels, the peak of viral concentration was recorded at the end of August and the concentration is now decreasing. In Flanders, the viral concentration seems to be stable since the end of August while in Wallonia, the concentration is still increasing.
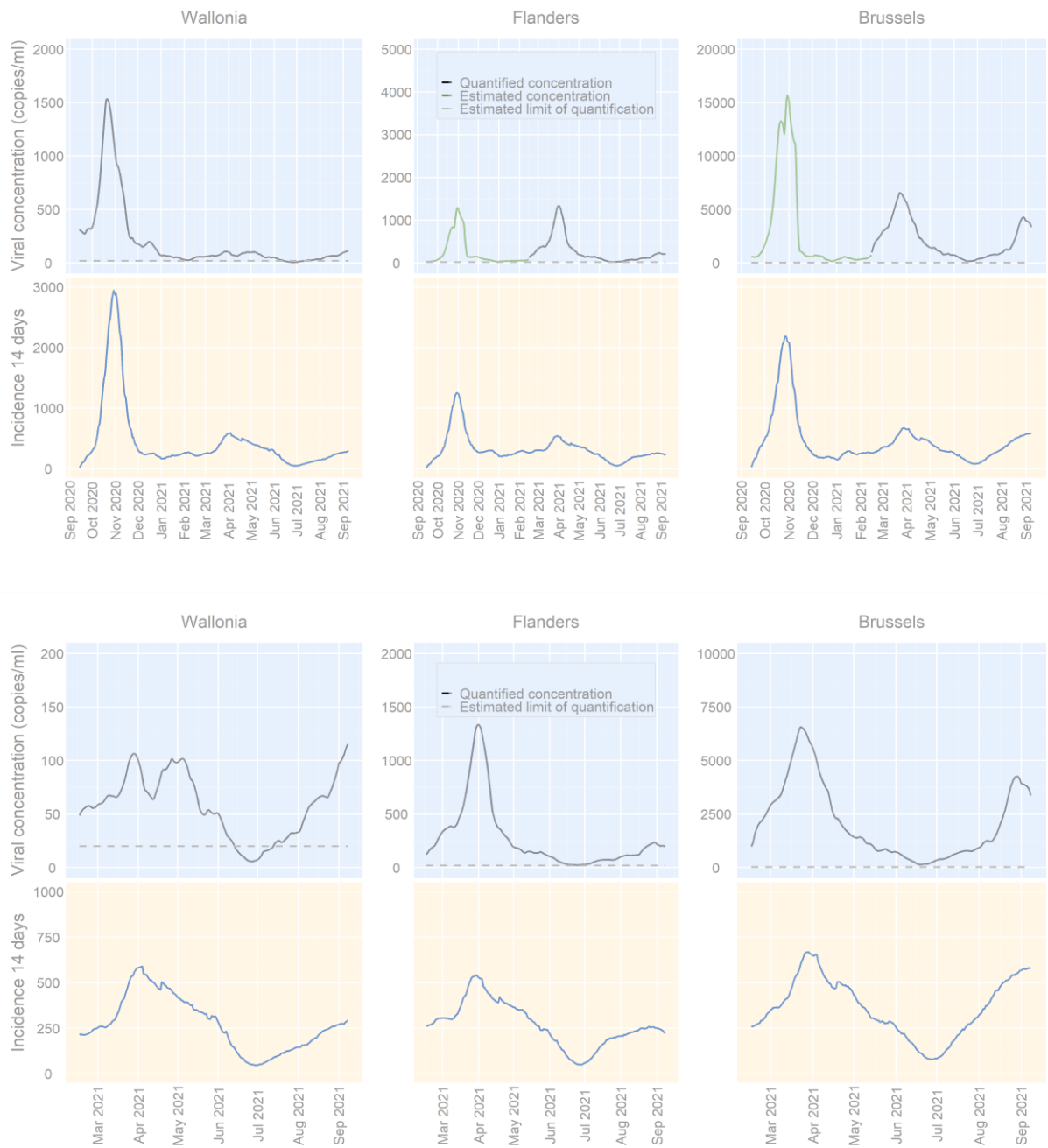
Figure 2: The SARS-CoV-2 RNA estimated and quantified concentrations expressed as copies/ml (two weeks centered moving average applied on the linear interpolation), the estimated limit of quantification of 20 SARS-CoV-2 RNA copies/ ml, and the 14 days incidence in the population covered by the wastewater surveillance since the surveillance starting date (graph set above) and mid-February 2021 (graph set below).

4.2. PROVINCES

Table 1 shows, for each Province, the results associated with the samples of Wednesday September 08th 2021, for the three alerting indicators:

- Last week on Wednesday September 01th 2021, 5 provinces had at least one alerting indicator positive.
- This week on Wednesday September 08th 2021, 5 provinces had at least one alerting indicator positive. The provinces of Liege is of particular concern as 3 indicators are fulfilled in this province.

Table 1: Alerting indicators fulfilled (1) or not (0) on September 08th 2021 and the percentage of population covered by the provinces. Columns represent the population coverage of the WWTP within the Province (Pop. coverage), the High Circulation indicator (High), the Fast increase indicator (Fast) and the Increasing trend indicator (Incr.). The specifications of the four last columns are explained in the footnotes 1-4 below the table. Missing data is indicated with a "/".

Province	Pop. coverage	High	Fast	Incr.	Norm. viral cc. (%)[1]	Mean viral. cc. (c./ml)[2]	Norm. evol. (%/day)[3]	Incr. days[4]
Antwerpen	39%	0	1	0	5	53	13	3
Brabant Wallon	34%	0	0	0	19	25	-13	4
Brussels	100%	0	0	0	19	1617	-22	0
Hainaut	29%	0	1	0	43	78	17	7
Liège	50%	1	1	1	100	320	31	20
Limburg	26%	0	0	0	14	40	8	1
Luxembourg	10%	0	1	0	23	45	15	1
Namur	24%	0	0	1	40	94	7	14
Oost-Vlaanderen	38%	0	0	0	14	260	2	0
Vlaams-Brabant	49%	0	0	0	10	304	-3	0
West-Vlaanderen	52%	0	0	0	4	147	-13	2

[1] : the viral concentration normalized with the maximum viral concentration measured in the corresponding catchment area since mid-February 2021.

[2] : the viral concentration computed on the replicate of the three targeted gene fragments.

[3] : the slope (%/day) of the past 7 days moving average of the viral concentration if being above the estimated limit of quantification.

[4] : the cumulative number of days of increase of the past 14 days moving average of the viral concentration

4.3. CATCHMENT AREAS

Table 2 shows, for each catchment area, the values of the three alerting indicators obtained based on the results of last Wednesday's sample. A catchment area is the area delimiting the population covered by a specific wastewater sample taken at the inlet of the WasteWater Treatment Plant (WWTP) (see Figure 1).

- The number of treatment plants with at least one alerting indicator positive is 17 (out of 39 areas covered).
- The indicator "High circulation" is fulfilled in 5 catchment areas: Froyennes, Amay, Liège Sclessin, Liège Oupeye, and Mornimont (see Appendix A1 for more details).
- The indicator "Fast increase" is fulfilled in 12 catchment areas. Amongst these areas, an increase of more than 50% per day of the normalized viral concentration is observed for 2 stations: Froyennes (140% increase per day) and Amay (121% increase per day) (see Appendix A2 for more details).
- The indicator "Increasing trend" is fulfilled in 13 catchment areas. Amongst these areas, the viral concentration is continually increasing since more than two weeks in 2 stations: Liège Sclessin (44 days) and Namur-Brumagne (16 days) (see Appendix A3 for more details).
- This week, all three indicators are fulfilled in 5 treatment plants: Froyennes, Amay, Liège Sclessin, Liège Oupeye and Mornimont. It should be noted that three of these 5 plants are located in the province of Liège.

The wastewater results can be accessed online for each area on the COVID-19 dashboard.
Table 2 is a snapshot of the number of areas highlighted by the indicators for the last results obtained, which correspond to the 24h-representative samples of last Wednesday September 08th 2021.

Table 2: Alerting indicators fulfilled (1) or not (0) on September 08th 2021 for the catchment areas covered by the wastewater treatment plants. Columns represent the population coverage of the WWTP within the Province (Pop. coverage), the High Circulation indicator(High), the Fast increase indicator (Fast) and the Increasing trend indicator (Incr.). The specifications of the four last columns are explained in the footnotes 1-4 below the table.

Province	WWTP	High	Fast	Incr.	Norm. viral cc (%)[1]	Mean viral cc (c./ml)[2]	Norm evol. (%/d)[3]	Incr days[4]
Antwerpen	Turnhout	0	0	1	4	18	0	7
Antwerpen	Deurne	0	1	1	2	125	31	7
Antwerpen	Antwerpen-North	0	1	1	21	66	40	7
Brabant Wallon	Vallee du Hain (L'Orchis)	0	0	1	18	22	-30	7
Hainaut	Froyennes	1	1	1	100	190	140	9
Hainaut	Marchienne-au-Pont	0	1	0	42	93	22	2
Hainaut	Wasmuel	0	0	1	34	55	3	14
Liège	Amay	1	1	1	100	151	121	7
Liège	Liège Sclessin	1	1	1	100	510	15	44
Liège	Liège Oupeye	1	1	1	100	250	20	9
Limburg	Houthalen-Centrum	0	1	1	32	68	32	7
Limburg	Tessenderlo	0	1	0	17	65	15	2
Luxembourg	Arlon	0	1	0	34	66	22	2

Province	WWTP	High	Fast	Incr.	Norm. viral cc (%)[1]	Mean viral cc (c./ml)[2]	Norm evol. (%/d)[3]	Incr days[4]
Namur	Namur-Brumagne	0	0	1	34	94	2	16
Namur	Mornimont	1	1	1	57	93	20	9
Oost-Vlaanderen	Destelbergen	0	1	0	6	298	26	0
West-Vlaanderen	Roeselare	0	0	1	3	424	8	14

[1] : the viral concentration normalized with the maximum viral concentration measured in the corresponding catchment area since mid-February 2021.

[2] : the viral concentration computed on the replicate of the three targeted gene fragments.

[3] : the slope (%/day) of the past 7 days moving average of the viral concentration if being above the estimated limit of quantification.

[4] : the cumulative number of days of increase of the past 14 days moving average of the viral concentration.

Figure 3 was developed to offer a dynamic view of the three indicators over time.

The number of treatment plants belonging to the "Fast increase" and "Increasing trend" indicators rose quickly at the beginning of July 2021 and stabilized at the same alert level since mid-July. At the same time, an increase in the number of plants for which the "High circulation" indicator is fulfilled is observed since mid-August (see Figure 3), notably in the province of Liège (see Appendix A1 for details).
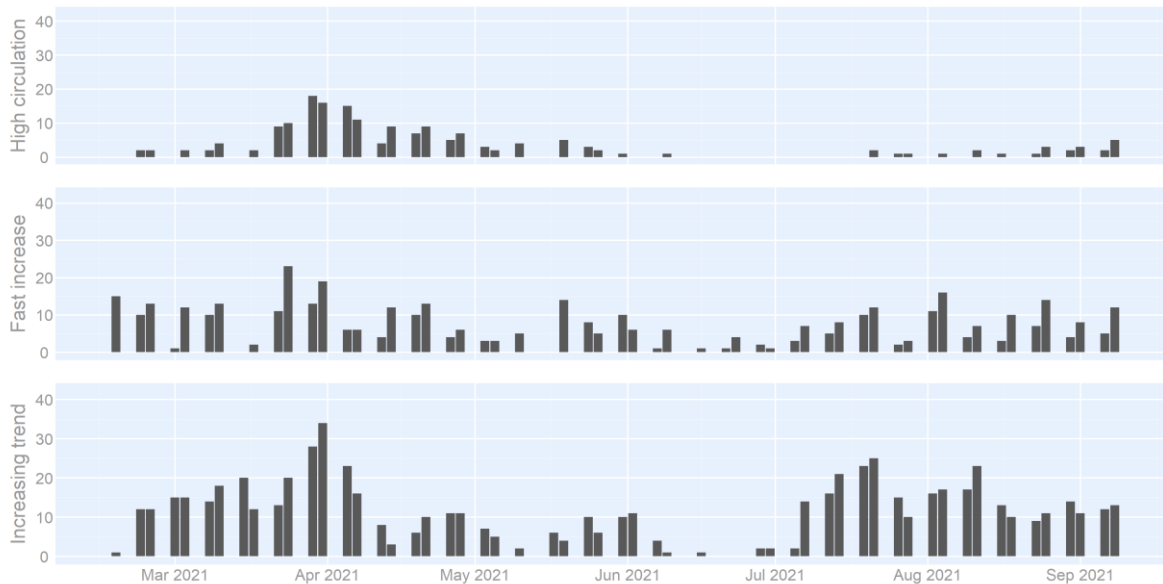

Figure 3: The number of areas, amongst the 39 areas covered by the wastewater surveillance, with positive alerting indicators (latest results on September 08th 2021).

5. Appendix – Areas classified by alerting indicator

A 1: The number of catchment areas (5 out of 39 on September 08th 2021) characterized as "High circulation" sorted in the descending order of importance.

Province	WWTP	High	Fast	Incr.	Norm. viral cc (%)[1]	Mean viral cc (c./ml)[2]	Norm evol. (%/d)[3]	Incr days[4]
Hainaut	Froyennes	1	1	1	100	190	140	9
Liège	Amay	1	1	1	100	151	121	7
Liège	Liège Sclessin	1	1	1	100	510	15	44
Liège	Liège Oupeye	1	1	1	100	250	20	9
Namur	Mornimont	1	1	1	57	93	20	9

[1] : the viral concentration normalized with the maximum viral concentration measured in the corresponding catchment area since mid-February 2021.

[2] : the viral concentration computed on the replicate of the three targeted gene fragments.

[3] : the slope (%/day) of the past 7 days moving average of the viral concentration if being above the estimated limit of quantification.

[4] : the cumulative number of days of increase of the past 14 days moving average of the viral concentration

A 2: The number of catchment areas (12 out of 39 on the September 08th 2021) characterized as "Fast increase" sorted in the descending order of importance.

Province	WWTP	High	Fast	Incr.	Norm. viral cc (%)[1]	Mean viral cc (c./ml)[2]	Norm evol. (%/d)[3]	Incr days[4]
Hainaut	Froyennes	1	1	1	100	190	140	9
Liège	Amay	1	1	1	100	151	121	7
Antwerpen	Antwerpen-North	0	1	1	21	66	40	7
Limburg	Houthalen-Centrum	0	1	1	32	68	32	7
Antwerpen	Deurne	0	1	1	2	125	31	7
Oost-Vlaanderen	Destelbergen	0	1	0	6	298	26	0
Hainaut	Marchienne-au-Pont	0	1	0	42	93	22	2
Luxembourg	Arlon	0	1	0	34	66	22	2
Liège	Liège Oupeye	1	1	1	100	250	20	9
Namur	Mornimont	1	1	1	57	93	20	9
Liège	Liège Sclessin	1	1	1	100	510	15	44
Limburg	Tessenderlo	0	1	0	17	65	15	2

A 3: The number of catchment areas (13 out of 39 on the September 08th 2021) characterized as "Increasing trend" sorted in the descending order of importance.

Province	WWTP	High	Fast	Incr.	Norm. viral cc (%)[1]	Mean viral cc (c./ml)[2]	Norm evol. (%/d)[3]	Incr days[4]
Liège	Liège Sclessin	1	1	1	100	510	15	44
Namur	Namur-Brumagne	0	0	1	34	94	2	16
Hainaut	Wasmuel	0	0	1	34	55	3	14
West-Vlaanderen	Roeselare	0	0	1	3	424	8	14
Hainaut	Froyennes	1	1	1	100	190	140	9
Liège	Liège Oupeye	1	1	1	100	250	20	9
Namur	Mornimont	1	1	1	57	93	20	9
Antwerpen	Turnhout	0	0	1	4	18	0	7
Antwerpen	Deurne	0	1	1	2	125	31	7
Antwerpen	Antwerpen-North	0	1	1	21	66	40	7
Brabant Wallon	Vallee du Hain (L'Orchis)	0	0	1	18	22	-30	7
Liège	Amay	1	1	1	100	151	121	7
Limburg	Houthalen-Centrum	0	1	1	32	68	32	7