

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

— LAST RESULTS OF 01/09/2021

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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 37 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 01th 2021:

- At the national level: Beginning of July 2021, an increase of the viral concentrations was observed which is stabilized at the same level of alert since mid-July 2021 (Figure 3).
- At the provincial level: Brussels, Hainaut, Liège, Namur and West-Vlaanderen are the provinces positive to at least one of the three alerting indicators. Especially the provinces of Liège, Namur and Brussels are of particular concern as the conditions for respectively two, two and three indicators are met in these three provinces (Table 1).
- At the catchment area level: Amongst the 37 catchment areas covered¹, the number of treatment plants with at least one alerting indicator positive is 14, 3 belonging to the “High circulation” indicator, 8 belonging to the “Fast increase” indicator, and 11 belonging to the “Increasing trend” indicator (Table 2). Last week (results of the 25th of August), 21 areas had at least one alerting indicator positive.
- Particular attention should be drawn to Liège Sclessin where the measured viral concentration is higher than during the third wave and keeps increasing since more than a month.

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](#).

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. Further, due to a lack of samples, the results for the stations of Marchienne-au-Pont, Montignies-sur-Sambre and Mornimont could not be analysed.

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 37 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 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 37 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.

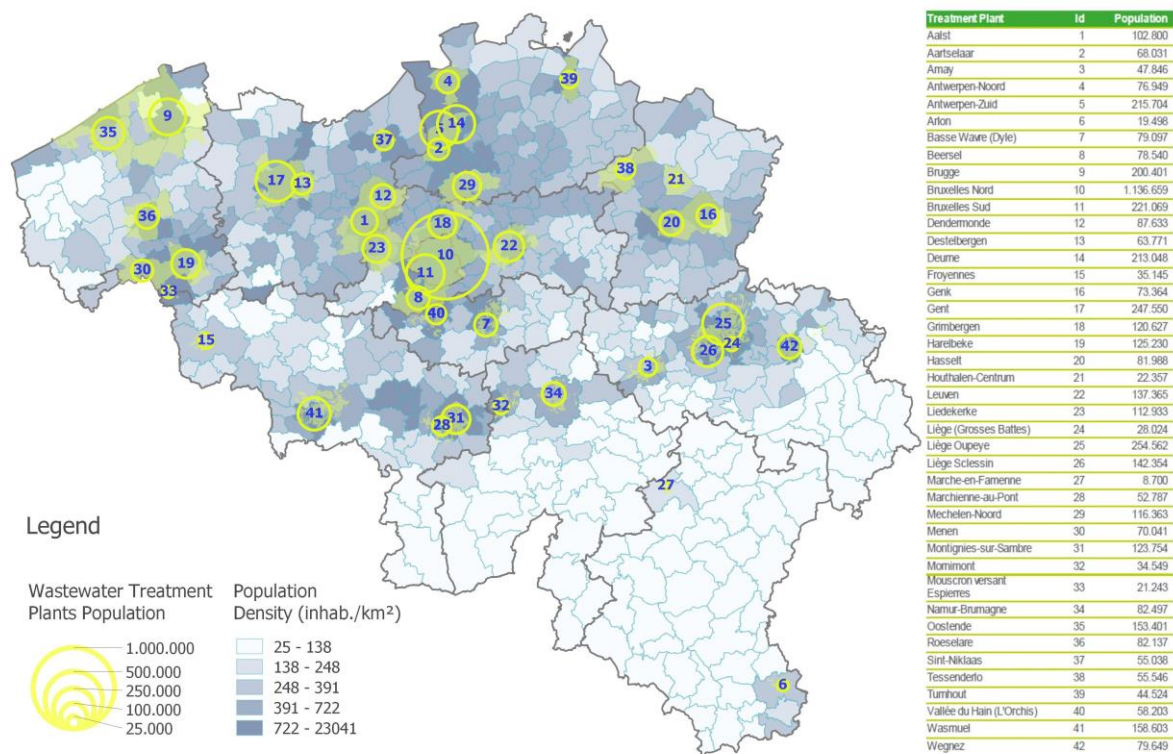


Figure 1: The population located in the catchment areas covered by the wastewater treatment plants (highlighted in yellow) and the population density for each municipality (indicated by the grey scale).

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 extent 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 are increasing since beginning of July. However, this increase is much more pronounced in Wallonia and Brussels than in Flanders.

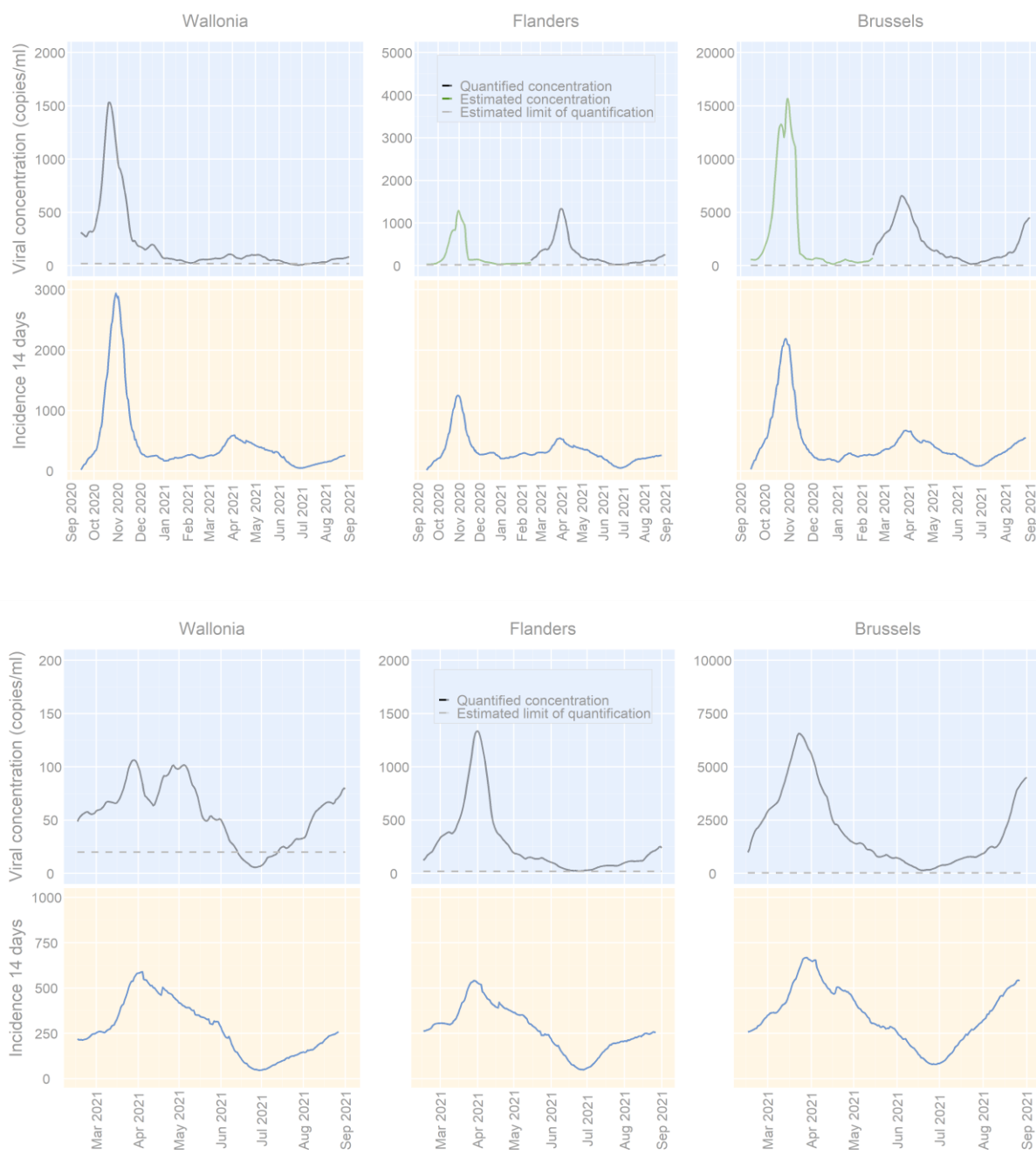


Figure 2: The SARS-CoV-2 RNA estimated and quantified concentrations expressed as copies/ml (two weeks centered moving average applied on the linear interpolation), the estimated limit of quantification of 20 SARS-CoV-2 RNA copies/ ml, and the 14 days incidence in the population covered by the wastewater 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 01th 2021, for the three alerting indicators:

- Last week on Wednesday 25/08/2021, 8 provinces had at least one alerting indicator positive.
- This week on Wednesday 01/09/2021, 5 provinces had at least one alerting indicator positive. The provinces of Namur, Liège and Brussels are of particular concern as respectively two, two and three indicators are fulfilled in these two provinces.

Table 1: Alerting indicators fulfilled (1) or not (0) on September 01th 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	0	0	14	256	5	5
Brabant Wallon	34%	0	0	0	11	15	-8	0
Brussels	100%	1	1	1	64	5275	13	14
Hainaut	29%	0	1	0	31	48	17	3
Liège	50%	1	0	1	70	176	2	13
Limburg	26%	0	0	0	15	23	-5	1
Luxembourg	10%	0	0	0	17	34	-6	2
Namur	24%	0	1	1	31	87	10	7
Oost-Vlaanderen	38%	0	0	0	13	212	-3	5
Vlaams-Brabant	49%	0	0	0	12	370	-16	2
West-Vlaanderen	52%	0	1	0	11	298	40	6

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 (n) of treatment plants with at least one alerting indicator positive is 14 (out of 37 areas covered).
- The indicator “High circulation” is fulfilled in 3 catchment areas: Brussels-North, Liège Oupeye and Liège Sclessin (see Appendix A1 for more details).
- The indicator “Fast increase” is fulfilled in 8 catchment areas. Amongst these areas, an increase of more than 50% per day of the normalized viral concentration is observed for one station: Roeselare (288% increase per day) (see Appendix A2 for more details).
- The indicator “Increasing trend” is fulfilled in 11 catchment areas. Amongst these areas, the viral concentration is continually increasing since more than two weeks in four stations: Liège Sclessin (37 days), Aalst (21 days), Antwerpen-South (16 days) and Brussels-North (16 days) (see Appendix A3 for more details).

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 01st 2021.

Table 2: Alerting indicators fulfilled (1) or not (0) on September 01th 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	Antwerpen-South	0	1	1	39	810	27	16
Brussels	Brussel-South	0	1	1	41	4337	44	7
Brussels	Brussels-North	1	0	1	71	5555	5	16
Hainaut	Wasmuel	0	1	1	31	49	29	7
Hainaut	Froyennes	0	1	0	34	44	48	2
Liège	Liège Sclessin	1	0	1	99	277	-0	37
Liège	Liège Oupeye	1	0	0	68	154	8	2
Namur	Namur-Brumagne	0	1	1	31	87	14	9

Province	WWTP	High	Fast	Incr.	Norm. viral cc (%) ¹	Mean viral cc (c./ml) ²	Norm evol. (%/d) ³	Incr days ⁴
Oost-Vlaanderen	Aalst	0	0	1	13	260	8	21
Oost-Vlaanderen	Sint-Niklaas	0	1	0	11	122	26	2
Vlaams-Brabant	Liedekerke	0	0	1	11	309	-22	9
West-Vlaanderen	Harelbeke	0	0	1	34	835	5	14
West-Vlaanderen	Brugge	0	1	1	9	85	33	7
West-Vlaanderen	Roeselare	0	1	1	3	343	288	7

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.

Beginning of July 2021, an increase of the viral concentrations was observed which is stabilized at the same level of alert since mid-July 2021 (Figure 3).

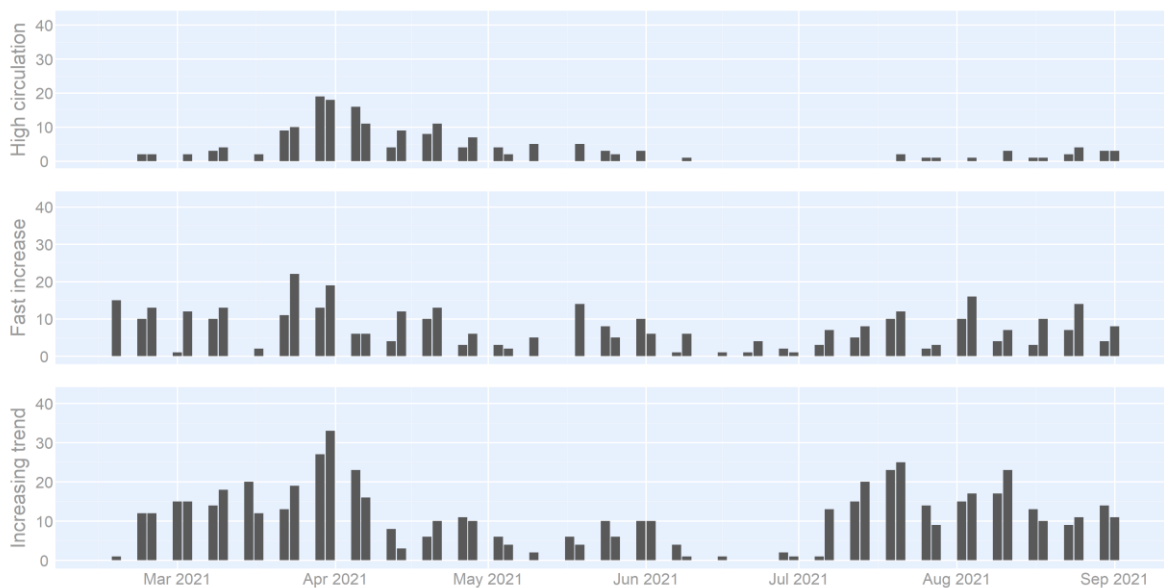


Figure 3: The number of areas, amongst the 37 areas covered by the wastewater surveillance, with positive alerting indicators (latest results on September 01th 2021).

5. Appendix – Areas classified by alerting indicator

A 1: The number of catchment areas (3 out of 37 on September 01th 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
Liège	Liège Sclessin	1	0	1	99	277	-0	37
Brussels	Brussels-North	1	0	1	71	5555	5	16
Liège	Liège Oupeye	1	0	0	68	154	8	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

A 2: The number of catchment areas (8 out of 37 on the September 01th 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
West-Vlaanderen	Roeselare	0	1	1	3	343	288	7
Hainaut	Froyennes	0	1	0	34	44	48	2
Brussels	Brussel-South	0	1	1	41	4337	44	7
West-Vlaanderen	Brugge	0	1	1	9	85	33	7
Hainaut	Wasmuel	0	1	1	31	49	29	7
Antwerpen	Antwerpen-South	0	1	1	39	810	27	16
Oost-Vlaanderen	Sint-Niklaas	0	1	0	11	122	26	2
Namur	Namur-Brumagne	0	1	1	31	87	14	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 3: The number of catchment areas (11 out of 37 on the September 01th 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	0	1	99	277	-0	37
Oost-Vlaanderen	Aalst	0	0	1	13	260	8	21
Antwerpen	Antwerpen-South	0	1	1	39	810	27	16
Brussels	Brussels-North	1	0	1	71	5555	5	16
West-Vlaanderen	Harelbeke	0	0	1	34	835	5	14
Namur	Namur-Brumagne	0	1	1	31	87	14	9
Vlaams-Brabant	Liedekerke	0	0	1	11	309	-22	9
Brussels	Brussel-South	0	1	1	41	4337	44	7
Hainaut	Wasmuel	0	1	1	31	49	29	7
West-Vlaanderen	Brugge	0	1	1	9	85	33	7
West-Vlaanderen	Roeselare	0	1	1	3	343	288	7

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