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

LAST RESULTS OF 25/08/2021

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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 38 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 August 25th 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 (see Figure 3).
- At the provincial level: Antwerpen, Brussels, Liège, Luxembourg, Namur, Oost-Vlaanderen, Vlaams-Brabant and West-Vlaanderen are the provinces positive to at least one of the three alerting indicators. Also, the province of Brussels and Liege are of particular concern as the conditions for respectively two and three indicators are met in these two provinces (see Table 1).
- At the catchment area level: Amongst the 38 catchment areas covered¹, the number of treatment plants with at least one alerting indicator positive is 21, 4 belonging to the "High circulation" indicator, 14 belonging to the "Fast increase" indicator, and 11 belonging to the "Increasing trend" indicator (see Table 2). Last week (results of the 18th of August), 16 areas had at least one alerting indicator positive.
- Attention point for Brussels-North and Liège-Sclessin where all three indicators are fulfilled.

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 technical reasons, since the 16th of August, no results are available for the two stations located in the Brabant Wallon: Basse-Wavre and Vallée du Hain

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 38 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 <u>Sciensano public dashboard</u>. Figure 1 shows the areas covered by the 38 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.

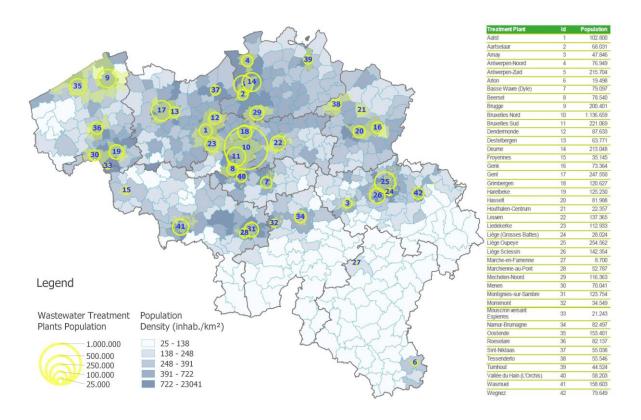


Figure 1: The population located in the catchment areas covered by the wastewater treatment plants (highlighted in yellow) and the population density for each municipality (indicated by the grey scale).

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 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 as mentioned in section 2.5.

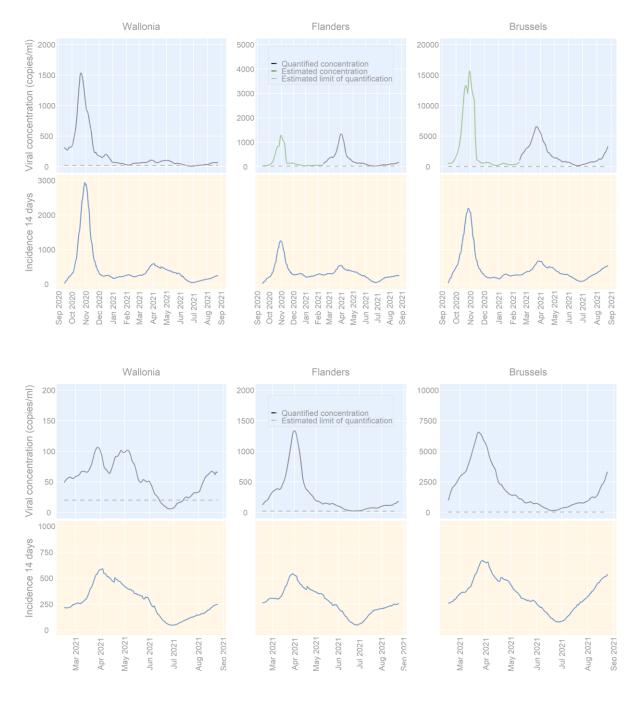


Figure 2: The SARS-CoV-2 RNA estimated and quantified concentrations expressed as copies/ml (two weeks centered moving average applied on the linear interpolation), the estimated limit of quantification of 20 SARS-CoV-2 RNA copies/ ml, and the 14 days incidence in the population covered by the wastewater 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 August 25th 2021, for the three alerting indicators:

- Last week on Wednesday 18/08/2021, 6 provinces had at least one alerting indicator positive.
- This week on Wednesday 25/08/2021, 8 provinces had at least one alerting indicator positive. The provinces of Brussels and Liège are of particular concern as respectively two and three indicators are fulfilled in these two provinces. Brabant Wallon has not been screened since the sample of Monday 16th of August.

Table 1: Alerting indicators fulfilled (1) or not (0) on August 25th 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. days⁴
Antwerpen	39%	0	1	0	10	163	31	4
Brabant Wallon	34%	/	/	/	/	/	/	/
Brussels	100%	1	1	0	51	4169	15	7
Hainaut	29%	0	0	0	14	33	-2	6
Liège	50%	1	1	1	67	164	16	13
Limburg	26%	0	0	0	24	48	-6	1
Luxembourg	10%	0	1	0	28	54	17	2
Namur	24%	0	1	0	15	40	19	3
Oost-Vlaanderen	38%	0	1	0	15	283	15	4
Vlaams-Brabant	49%	0	1	0	27	798	48	4
West-Vlaanderen	52%	0	1	0	12	338	19	3

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 21 (out of 38 areas covered), 4 belonging to "High circulation", 14 belonging to "Fast increase", and 11 belonging to "Increasing trend" (see detailed tables annexed A 1, A 2, and A 3).

- The normalized viral concentrations for Brussels-North, Liège Oupeye and Hasselt are higher than 50% of the highest recorded values. In the station of Liège Sclessin, the recorded value for the viral concentration on August 25th 2021 is the highest since mid-February 2021! (="High circulation" alerting indicator)
- Amongst the 14 areas for which the indicator "Fast increase" is fulfilled, a very fast increase of the normalized viral concentration (compared to the highest observed concentration for the same area since mid-February) is observed for three stations: Antwerpen-South (110% increase per day), Liedekerk (90% increase per day) and Leuven (80% increase per day).
- Amongst the 11 increasing area in which the indicator "Increasing trend" is fulfilled (increase for more than 6 days), the viral concentration is continually increasing since more than or almost a month in three stations: Marchienneau-Pont (42 days), Amay (30 days) and Liège Sclessin (30 days).
- Remark: all three indicators are fulfilled for the stations of Brussels-North and Liège Sclessin.
- The covered areas in Brabant Wallon have not been monitored since Monday 16th of August.

The wastewater results can be accessed online for each area on the <u>COVID-19</u> 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 August 25th 2021.

Table 2: Alerting indicators fulfilled (1) or not (0) on August 25th 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	23	469	110	9
Antwerpen	Antwerpen-North	0	0	1	10	32	-1	7
Brussels	Brussels-North	1	1	1	60	4721	18	9
Hainaut	Marchienne-au-Pont	0	0	1	11	25	-16	42
Liège	Liège Oupeye	1	1	0	54	123	16	0
Liège	Amay	0	0	1	40	58	8	30
Liège	Liège Sclessin	1	1	1	100	279	20	30
Limburg	Hasselt	1	0	0	51	75	-16	2
Luxembourg	Arlon	0	1	0	32	61	27	2
Namur	Namur-Brumagne	0	1	0	19	53	27	2
Namur	Mornimont	0	0	1	4	7	0	7
Oost-Vlaanderen	Destelbergen	0	1	0	7	384	47	0
Oost-Vlaanderen	Aalst	0	0	1	10	207	-4	14
Oost-Vlaanderen	Sint-Niklaas	0	1	0	7	81	16	2
Oost-Vlaanderen	Gent	0	1	0	26	432	20	2
Vlaams-Brabant	Liedekerke	0	1	0	28	798	90	2
Vlaams-Brabant	Leuven	0	1	0	27	812	80	2
Vlaams-Brabant	Grimbergen	0	1	1	41	1082	21	9
West-Vlaanderen	Harelbeke	0	1	1	30	725	56	7
West-Vlaanderen	Oostende	0	1	0	17	458	55	2
West-Vlaanderen	Menen	0	0	1	3	582	5	9

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

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 (see Figure 3).

 $^{^{2}}$: 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.

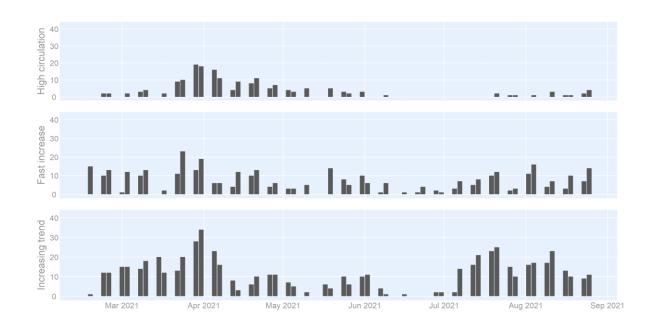


Figure 3: The number of areas, amongst the 38 areas covered by the wastewater surveillance, with positive alerting indicators (latest results on August 25th 2021).

5. Appendix - Areas classified by alerting indicator

A 1: The number of catchment areas (4 out of 38 on August 25th 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	1	1	100	279	20	30
Brussels	Brussels-North	1	1	1	60	4721	18	9
Liège	Liège Oupeye	1	1	0	54	123	16	0
Limburg	Hasselt	1	0	0	51	75	-16	2

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

A 2: The number of catchment areas (14 out of 38 on the August 25th 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
Antwerpen	Antwerpen-South	0	1	1	23	469	110	9
Vlaams-Brabant	Liedekerke	0	1	0	28	798	90	2
Vlaams-Brabant	Leuven	0	1	0	27	812	80	2
West-Vlaanderen	Harelbeke	0	1	1	30	725	56	7
West-Vlaanderen	Oostende	0	1	0	17	458	55	2
Oost-Vlaanderen	Destelbergen	0	1	0	7	384	47	0
Luxembourg	Arlon	0	1	0	32	61	27	2
Namur	Namur-Brumagne	0	1	0	19	53	27	2
Vlaams-Brabant	Grimbergen	0	1	1	41	1082	21	9
Liège	Liège Sclessin	1	1	1	100	279	20	30
Oost-Vlaanderen	Gent	0	1	0	26	432	20	2
Brussels	Brussels-North	1	1	1	60	4721	18	9

 $^{^{\}rm 2}$: 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

Province	WWTP	High	Fast	Incr.	Norm. viral cc (%) ¹	Mean viral cc (c./ml)²	Norm evol. (%/d)³	Incr days ⁴
Liège	Liège Oupeye	1	1	0	54	123	16	0
Oost-Vlaanderen	Sint-Niklaas	0	1	0	7	81	16	2

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

A 3: The number of catchment areas (11 out of 38 on the August 25th 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
Hainaut	Marchienne-au-Pont	0	0	1	11	25	-16	42
Liège	Amay	0	0	1	40	58	8	30
Liège	Liège Sclessin	1	1	1	100	279	20	30
Oost-Vlaanderen	Aalst	0	0	1	10	207	-4	14
Antwerpen	Antwerpen-South	0	1	1	23	469	110	9
Brussels	Brussels-North	1	1	1	60	4721	18	9
Vlaams-Brabant	Grimbergen	0	1	1	41	1082	21	9
West-Vlaanderen	Menen	0	0	1	3	582	5	9
Antwerpen	Antwerpen-North	0	0	1	10	32	-1	7
Namur	Mornimont	0	0	1	4	7	0	7
West-Vlaanderen	Harelbeke	0	1	1	30	725	56	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

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