

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

## — RESULTS OF 21/09/2022

**Authors:** R. Janssens<sup>1</sup>, H. Maloux<sup>1</sup>, S. Hanoteaux<sup>1</sup>, B. Verhaegen<sup>2</sup>, K. Van Hoorde<sup>2</sup>, K. Dierick<sup>2</sup>, S. Quoilin<sup>1</sup>, K. Blot<sup>1</sup>, M. Lesenfants<sup>1\*</sup>

<sup>1</sup> Sciensano, Service Epidemiology of infectious diseases

<sup>2</sup> Sciensano, Service Foodborne pathogens

\*Project leader and contact: [marie.lesenfants@sciensano.be](mailto:marie.lesenfants@sciensano.be)

In collaboration with:



# TABLE OF CONTENTS

<b>TABLE OF CONTENTS</b> .....	<b>2</b>
<b>1. Summary</b> .....	<b>3</b>
<b>2. Introduction</b> .....	<b>4</b>
<b>3. Methodology</b> .....	<b>4</b>
3.1. SAMPLE COLLECTION AND ANALYSIS .....	4
3.2. WASTEWATER RESULTS .....	5
3.3. ALERTING INDICATORS .....	5
3.4. CASES RESULTS.....	6
3.5. CAUTION POINTS FOR THE RESULTS INTERPRETATION.....	6
<b>4. Results</b> .....	<b>7</b>
4.1. REGIONS.....	7
4.2. PROVINCES .....	8
4.3. CATCHMENT AREAS.....	9
<b>5. Appendix – Areas classified by alerting indicator</b> .....	<b>14</b>

# 1. Summary

In the present work, the circulation of the SARS-CoV-2 virus in the environment is assessed based on three indicators. The analysis of wastewater samples collected in 42 wastewater treatment plants covers 45% of the Belgian population, with increased accuracy in the urban areas. The results of the wastewater surveillance are a source of complementary information to the infection cases number. 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 21<sup>th</sup> 2022:

- At the national level: Since 30<sup>th</sup> August 2022, the number of areas in Increasing Trend is rising with half the areas fulfilled. The High Circulation trend indicator remains at a low level with less than 5 areas fulfilled. Overall, the viral loads have steadily increased since 4 weeks.
  - At the regional level: The Increasing Trend indicator is fulfilled in Brussels.
  - At the provincial level: The Increasing Trend indicator is fulfilled in 3 provinces: Brussels, Luxembourg and Oost-Vlaanderen, and was in 3 last week. The Fast Increase indicator is fulfilled in 3 provinces: Hainaut, Luxembourg and Namur, and was in none last week. The High Circulation indicator is not fulfilled in any province, and was in none last week.
  - At the covered areas level: among the 42 areas covered<sup>1</sup>, the number of treatment plants belonging to the different indicators are 21 for the Increasing Trend indicator, 11 for the Fast Increase indicator, and 0 for the High Circulation indicator.
- 

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 [the Appendix Methodology document \(access available online\)](#). The methodology was updated on June 20<sup>th</sup> 2022.
- 

<sup>1</sup> Due to the 2021 flooding events, the treatment plants from Wegnez (Verviers) is temporarily out of order. This area is therefore not screened for SARS-CoV-2. Liège is still well represented within this surveillance, however Verviers is not. Since the 13<sup>th</sup> of October, the covered area of Soumagne is added to the surveillance.

## 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 42 WasteWater Treatment Plants (WWTPs) spread over Belgium. The evolution of the SARS-CoV-2 wastewater concentrations measured over time in the different treatment plants is analysed at different levels: regional, provincial, and the catchment area covered by the individual treatment plants. Also, three alerting indicators were developed to highlight areas of concern regarding the high circulation, the fast evolution, and the increasing trend of the observed wastewater 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. Figure 1 shows the catchment areas covered by the 42 WWTPs included in the project, which are located in high population density areas. A catchment area is defined by the area delimiting the population covered by a specific wastewater sample, collected at the inlet of the WWTPs. Further coverage details can be found in Table 2 by province (see also Table A1 in [the Appendix Methodology document \(access available online\)](#) ) and on the [Sciensano public dashboard](#). In practice, 24-hour composite samples are collected twice a week on Mondays and Wednesdays from the influent of WWTP and are analysed 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 areas covered by the wastewater treatment plants (highlighted in yellow) and the population density for each municipality (indicated by the blue scale). Note that due to the recent flooding, the treatment plants of Wegnez is out of order.

### 3.2. WASTEWATER RESULTS

Between mid-September 2020 and mid-February 2021, the concentration were not quantified for all treatment plant and were expressed in Ct values. In mid-February 2021, the quantification of SARS-CoV-2 viral concentration (RNA copies/ml) was generalized to all the treatment plants investigated. Until 10th June 2022 the NIBSC standard was used to quantify the SARS-CoV-2 and after this date, the EURM standard was used.

In order to account for possible dilution by rainy events and for the number of people persons living in the catchment of each WWTP, a correction is applied on the viral concentration: the concentration is multiplied by the inlet flow and divided by the number of inhabitant equivalent of its respective WWTP and expressed by 100k inhabitant. The units of viral loads are copy/day/100k inhabitants equivalent.

Preliminary results estimated the limit of quantification of the analytical method at 20 copies/ml or 2 1012 copy/day/100k inhab.

### 3.3. ALERTING INDICATORS

To highlight the areas of possible concern, the three alerting indicators are assessed twice a week, based on viral loads (RNA copies/day/100k inhabitants) measured for the three targeted gene fragments (E, N1, and N2):

1. The **Increasing Trend** indicator highlights the catchment areas where the viral loads have been increasing for more than 6 days. The indicator is computed based on the moving average on the past two weeks of the viral load.
2. The **Fast Increase** indicator highlights the catchment areas where the viral loads have rapidly increased for the last week. It corresponds to a situation where the moving average on the past

7 days of the viral load has increased faster than 70% per week if being above the estimated limit of quantification. The increasing slope is normalized for each treatment plant.

3. The **High Circulation** indicator highlights the catchment areas where the viral loads are high. It corresponds to a situation where the viral loads exceed half of the highest value recorded during the fifth wave (i.e. from first of January 2022 till first of March 2022).

The indicators were developed in order to be able to track the different phases of an outbreak. Typically, when the viral loads in wastewaters in an area start to increase, the indicator Increasing Trend will be the first fulfilled. If the viral loads increase quickly, the Fast Increase indicator will be fulfilled. Finally, after the initial increasing phase, the viral loads in an area may be sufficiently high to result in the High Circulation to be fulfilled.

### 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. As the viral load values differ from a WWTP to another, notably due to the differences in population sizes covered, absolute values should not be compared. Additionally, the situation comparison 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 analyses (see [the Appendix Methodology document \(access online\)](#)).

Moreover, the wastewater viral loads 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 numbers include only the persons clinically tested 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, i.e. mobility bias. Therefore, the correlation between the wastewater viral load 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 loads and the cases numbers could also be influenced by the vaccination campaign and the circulation of variants.

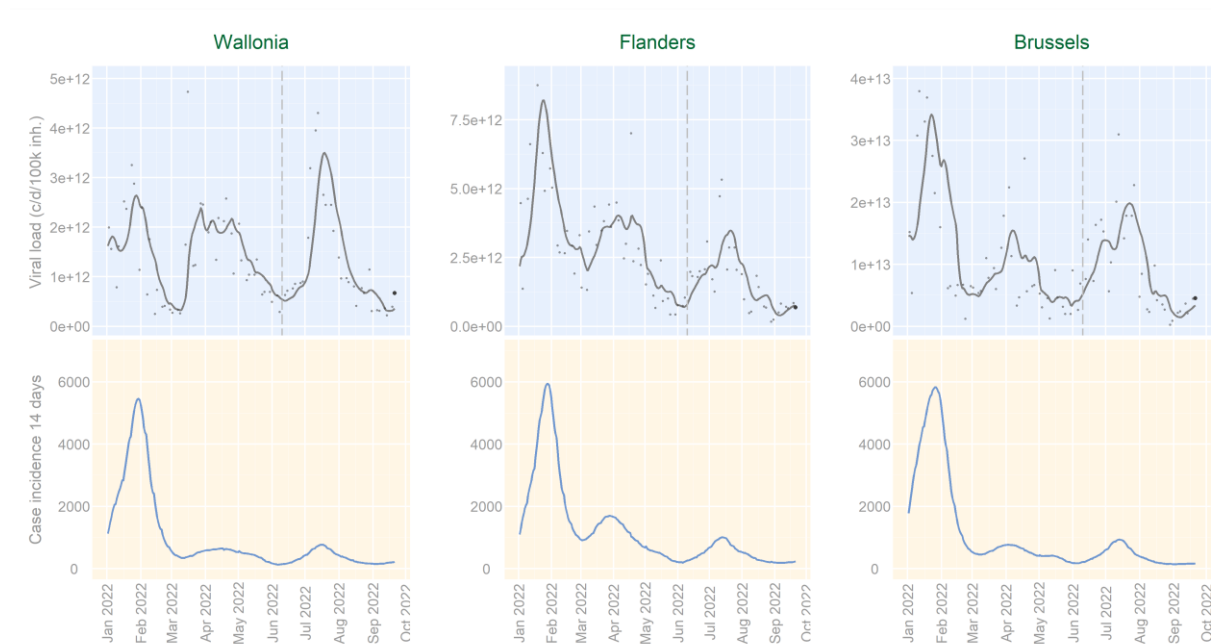
## 4. Results

### 4.1. REGIONS

Figure 2 represents the quantitative SARS-CoV-2 RNA viral loads in the wastewaters and the 14 days incidence for each region, compiling the incidence data of the area covered by the wastewater surveillance.

Several waves can be seen in Figure 2:

- The 5th wave occurring starting on 27th December 2022.
- The 6th wave starting on 28th February 2022.
- The 7th wave starting on 30th May 2022.



**Figure 2: The SARS-CoV-2 RNA viral loads expressed as copies/days/100k habitants (based on the past two weeks moving average applied on the linear interpolation) (Top), and the 14 days incidence in the population covered by the wastewater surveillance since mid-February 2021 (bottom). Before the date marked by the vertical dashed line, the NIBSC standard was used to quantify the SARS-CoV-2 and after this date, the EURM standard was used.**

Table 1 shows, for each region, the results associated with the samples of Wednesday September 21<sup>th</sup> 2022. It allows to track the changes between the situation as of today (September 21<sup>th</sup> 2022) and the situation as of last week (September 14<sup>th</sup> 2022). Hereby, two distinct cases are taken into account:

1. If a region has at least one indicator fulfilled this week and it was not the case last week, its **name is displayed in bold** in the table.
2. If a region has at least one indicator fulfilled this week and if it also was the case last week, any change in indicator fulfilment (i.e. if the value for any indicator has changed from 0 to 1 or from 1 to 0) is indicated in **coloured bold text**.

The Increasing Trend indicator is fulfilled in Brussels and the Fast Increase indicator is fulfilled in Wallonia this week. No indicator is fulfilled in Flanders.

**Table 1: Alerting indicators fulfilled (1) or not (0) on September 21<sup>th</sup> 2022. Columns represent the population coverage of the regions (Pop. coverage) and the three alerting indicators High Circulation (High), Fast Increase (Fast) and Increasing Trend (Incr.). The specifications of the four last columns are explained in the footnotes 1-4 below the table. Missing data is indicated with a “/”.**

Region	Pop. coverage	High	Fast	Incr.	Norm. viral load (%) <sup>1</sup>	Mean viral load <sup>2</sup>	Norm. evol. (%/week) <sup>3</sup>	Incr. days <sup>4</sup>
<b>Brussels</b>	100%	0	0	1	9	4.50	52	11
Flanders	41%	0	0	0	7	0.68	14	5
<b>Wallonia</b>	31%	0	1	0	16	0.67	77	5

<sup>1</sup> : the viral load normalized with the maximum viral load measured in the corresponding catchment area during the fifth wave (i.e. from first of January 2022 till the first of march 2022).

<sup>2</sup> : the viral load computed on the mean of the replicate of the three targeted gene fragments as explained in section “3.2 Wastewater results”. The mean viral load is expressed in 10<sup>12</sup> copies/day/100k inhabitants.

<sup>3</sup> : the slope (%/week) of the past 7 days moving average of the viral load if the corresponding concentration is above the estimated limit of quantification.

<sup>4</sup> : the cumulative number of days of increase of the past 14 days moving average of the viral load.

## 4.2. PROVINCES

Table 2 shows, for each Province, the results associated with the samples of Wednesday September 21<sup>th</sup> 2022. It allows to track the changes between the situation as of today (September 21<sup>th</sup> 2022) and the situation as of last week (September 14<sup>th</sup> 2022). Hereby, two distinct cases are taken into account:

1. If a province has at least one indicator fulfilled this week and it was not the case last week, its **name is displayed in bold** in the table.
2. If a province has at least one indicator fulfilled this week and if it also was the case last week, any change in indicator fulfilment (i.e. if the value for any indicator has changed from 0 to 1 or from 1 to 0) is indicated in **coloured bold text**.

Table 2 shows, for each Province, the results associated with the samples of Wednesday September 21<sup>th</sup> 2022, for the three alerting indicators:

- The Increasing Trend indicator is fulfilled in 3 provinces: Brussels, Luxembourg and Oost-Vlaanderen, and was in 3 last week.
- The Fast Increase indicator is fulfilled in 3 provinces: Hainaut, Luxembourg and Namur, and was in none last week.
- The High Circulation indicator is not fulfilled in any province, and was in none last week.
- It should be noted that although the 50% threshold of viral circulation is not exceeded (in comparison to the highest level observed during the 5th wave), the viral load in the province of Namur remains high (with 42%).



**Table 2: Alerting indicators fulfilled (1) or not (0) on September 21<sup>th</sup> 2022. Columns represent the population coverage of the WWTPs within the Province (Pop. coverage) and the three alerting indicators High Circulation (High), Fast Increase (Fast) and Increasing Trend (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 load (%) <sup>1</sup>	Mean viral load <sup>2</sup>	Norm. evol. (%/w) <sup>3</sup>	Incr. days <sup>4</sup>
Antwerpen	41%	0	0	0	1	0.17	0	2
Brabant Wallon	33%	0	0	0	9	0.25	50	3
<b>Brussels</b>	100%	0	0	1	9	4.50	52	11
<b>Hainaut</b>	29%	0	1	0	20	0.76	100	5
Liège	50%	0	0	0	10	0.57	51	4
Limburg	26%	0	0	0	1	0.19	0	1
<b>Luxembourg</b>	10%	0	1	1	17	1.70	126	12
<b>Namur</b>	23%	0	1	0	36	1.00	131	6
Oost-Vlaanderen	38%	0	0	1	10	0.76	48	11
Vlaams-Brabant	61%	0	0	<b>0</b>	8	1.10	-10	6
West-Vlaanderen	52%	0	0	<b>0</b>	12	1.10	21	4

<sup>1</sup> : the viral load normalized with the maximum viral load measured in the corresponding catchment area during the fifth wave (i.e. from first of January 2022 till the first of march 2022).

<sup>2</sup> : the viral load computed on the mean of the replicates of the three targeted gene fragments as explained in section “3.2 Wastewater results”. The mean viral load is expressed in 10<sup>12</sup> copies/day/100k inhabitants.

<sup>3</sup> : the slope (%/week) of the past 7 days moving average of the viral load if the corresponding concentration is above the estimated limit of quantification.

<sup>4</sup> : the cumulative number of days of increase of the past 14 days moving average of the viral load.

### 4.3. CATCHMENT AREAS

Table 3 shows, for each catchment area, the values of the three alerting indicators obtained based on the results of last Wednesday’s sample. Table 3 is a snapshot of the number of areas highlighted by the indicators for the last results obtained. It allows to track the changes between the situation as of today (September 21<sup>th</sup> 2022) and the situation as of last week (September 14<sup>th</sup> 2022). Hereby, three distinct cases are taken into account:

1. If an area has at least one indicator fulfilled this week and it was not the case last week, its **name is displayed in bold** in the table.
2. If an area has at least one indicator fulfilled this week and if it also was the case last week, any change in indicator fulfilment (i.e. if the value for any indicator has changed from 0 to 1 or from 1 to 0) is indicated in **coloured bold text**.
3. Any area which had at least one indicator fulfilled last week but not this week is listed below Table 3.

Here are the results associated with the samples of September 21<sup>th</sup> 2022:

- The Increasing Trend indicator is fulfilled in 21 covered areas. Amongst these areas, the viral load is continually increasing since more than two weeks in 8 areas: Grimbergen (21 days), Aalst (16 days), Gent (16 days), Arlon (14 days), Brussels-North (14 days), Harelbeke (14 days), Houthalen-Centrum (14 days) and Menen (14 days). Further details can be found in Appendix A3. Last week, this indicator was fulfilled in 14 covered areas.
- The Fast Increase indicator is fulfilled in 11 covered areas. Among these covered areas, an increase of more than 300% per week of the normalized viral load (i.e. the measured loads have more than quadrupled during last week) is observed for 1 area: Marchienne-au-Pont (314% increase per week). Further details can be found in Appendix A2. Last week, this indicator was fulfilled in 1 covered area.
- The High Circulation indicator is not fulfilled in any covered area. Last week, this indicator was not fulfilled in any covered area.

The wastewater results at the level of the local covered areas can be accessed online for each area on the [COVID-19 dashboard](#).

**Table 3: Alerting indicators fulfilled (1) or not (0) on September 21<sup>th</sup> 2022. Columns represent the provinces, different WWTPs within the Provinces and the three alerting indicators High Circulation (High), Fast Increase (Fast) and Increasing Trend (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	WWTP	High	Fast	Incr.	Norm. viral load (%) <sup>1</sup>	Mean viral load <sup>2</sup>	Norm evol. (%/week) <sup>3</sup>	Incr. days <sup>4</sup>
Oost-Vlaanderen	Aalst	0	1	1	26	1.49	85	16
Liège	Amay	0	1	0	9	0.52	127	2
Antwerpen	Antwerpen-North	0	0	1	2	0.19	0	7
Luxembourg	Arlon	0	1	1	18	2.13	182	14
Brussels	Brussels-North	0	0	1	10	5.32	69	14
Hainaut	Froyennes	0	1	1	15	0.34	75	9
Oost-Vlaanderen	Gent	0	0	1	10	0.82	56	16
Vlaams-Brabant	Grimbergen	0	0	1	7	1.44	6	21
West-Vlaanderen	Harelbeke	0	0	1	8	1.01	38	14
Limburg	Houthalen-Centrum	0	0	1	3	0.19	0	14
Liège	Liège (Grosses Battes)	0	0	1	9	0.86	33	7
Liège	Liège Sclessin	0	1	1	7	0.80	108	9
Luxembourg	Marche-en-Famenne	0	0	1	14	0.65	0	7
Hainaut	Marchienne-au-Pont	0	1	1	29	1.59	314	7
Antwerpen	Mechelen-Noord	0	0	1	2	0.18	0	7
West-Vlaanderen	Menen	0	0	1	16	2.03	28	14
Hainaut	Montignies-sur-Sambre	0	0	1	22	0.96	4	7
Hainaut	Mouscron versant Espierres	0	0	1	17	1.41	47	9
Namur	Namur-Brumagne	0	1	1	42	1.13	173	7
West-Vlaanderen	Oostende	0	1	0	9	0.85	77	0
Oost-Vlaanderen	Sint-Niklaas	0	0	1	1	0.14	0	9
Liège	Soumagne	0	1	0	22	2.86	263	0
Antwerpen	Turnhout	0	0	1	2	0.37	0	7
Brabant Wallon	Vallée du Hain (L'Orchis)	0	1	1	16	0.40	119	7
Hainaut	Wasmuel	0	1	0	16	0.33	118	2

<sup>1</sup> : the viral load normalized with the maximum viral load measured in the corresponding catchment area during the fifth wave (i.e. from first of January 2022 till the first of march 2022).

<sup>2</sup> : the viral load computed on the mean of the replicates of the three targeted gene fragments as explained in section “3.2 Wastewater results”; The mean viral load is expressed in 10<sup>12</sup> copies/day/100k inhabitants.

<sup>3</sup> : the slope (%/week) of the past 7 days moving average of the viral load if the corresponding concentration is above the estimated limit of quantification.

<sup>4</sup> : the cumulative number of days of increase of the past 14 days moving average of the viral load.

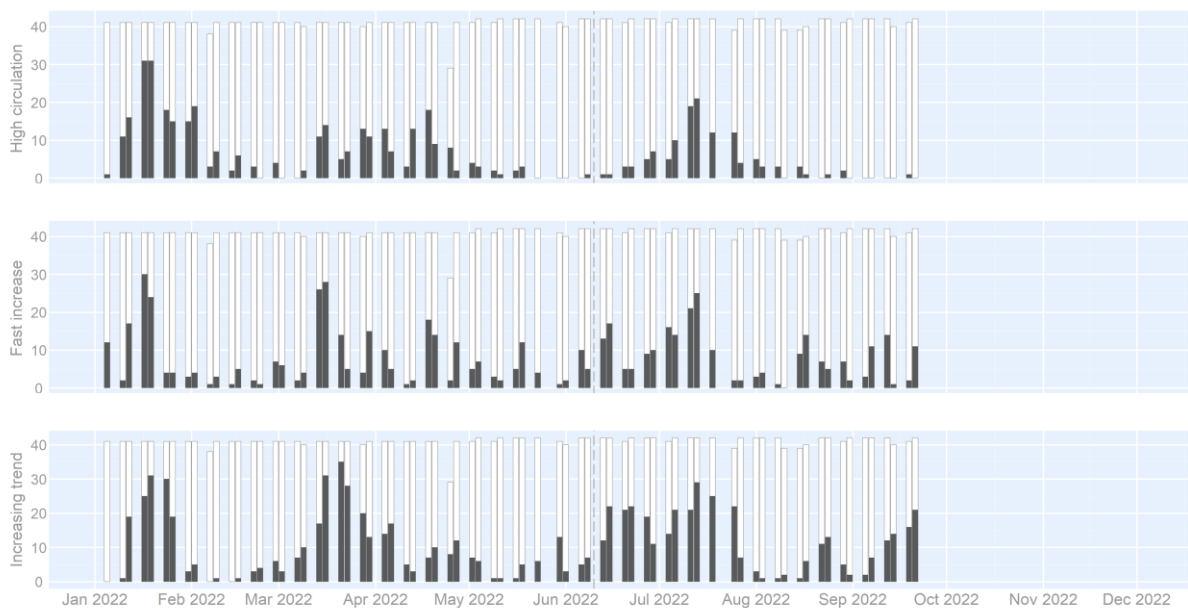
The following areas had the corresponding indicator fulfilled last week but not this week:

- Increasing Trend indicator: Basse Wavre (Dyle), Brugge, Destelbergen, Deurne, Leuven and Liedekerke.
- Fast Increase indicator: Basse Wavre (Dyle) and Liedekerke.
- High Circulation indicator: Basse Wavre (Dyle).

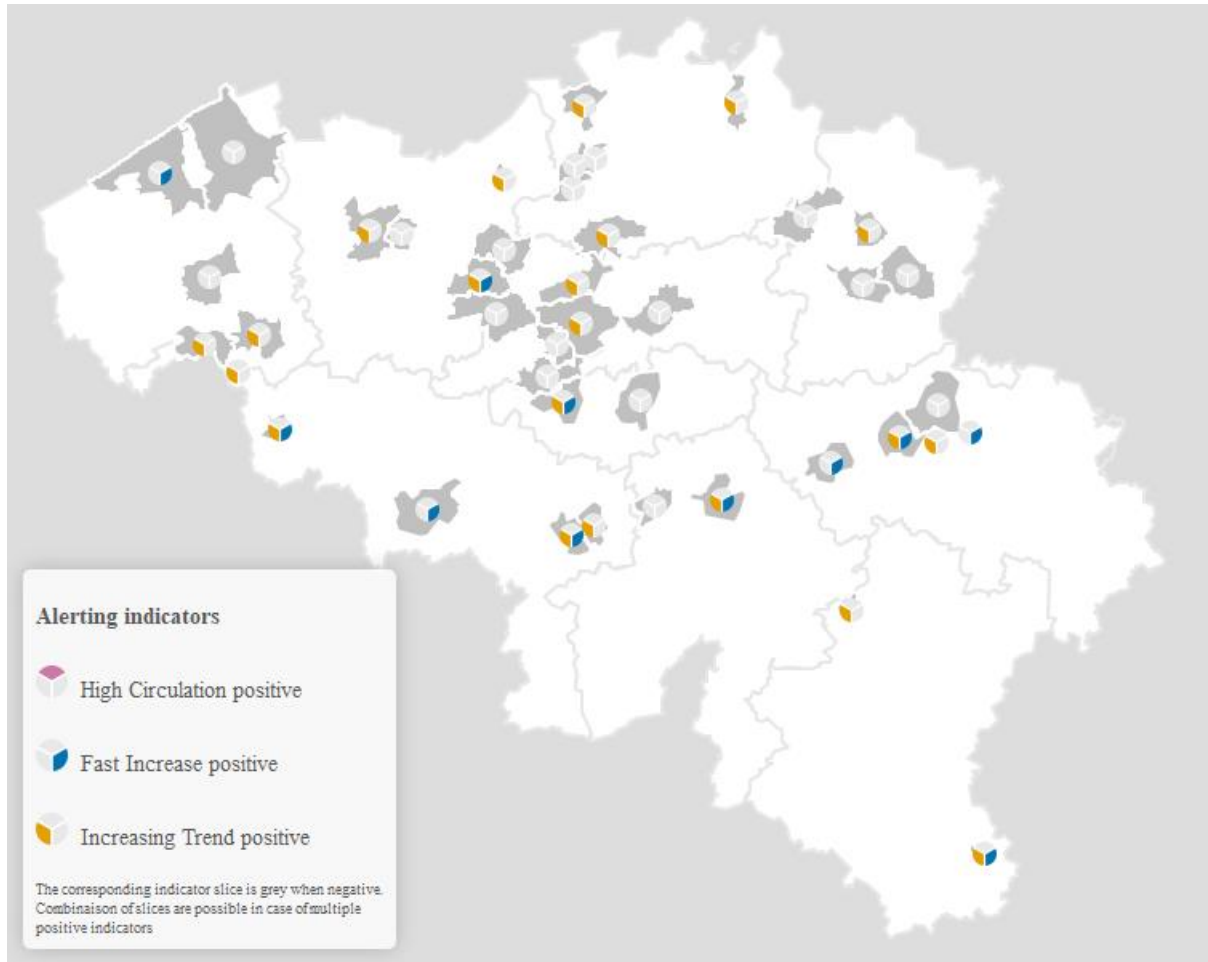
Further details on covered area without fulfilled indicators can be found in Table A4.

Figure 3 was developed to offer a dynamic view of the three alerting indicators over time. Figure 4 was developed to show the localization of the covered areas by the wastewater surveillance in Belgium and the state of the three alerting indicators in each of them. For further insights on the dynamic of the different indicators, see Section 3.3.

Since 30th August 2022, the number of areas in Increasing Trend is rising, with now half the areas fulfilled. Also the Fast Increase indicator has risen with more than 10 areas fulfilled. The High Circulation trend indicator remains at a low level, with less than 5 areas fulfilled. Overall, the viral loads have increased since last week.



**Figure 3: The number of areas (among the 42 covered by the wastewater surveillance this week and the 42 normally considered), with positive alerting indicators (black) compared to the total number of areas considered every week in the wastewater surveillance (white). Latest results (last bar) are from September 21<sup>st</sup> 2022. The white bars represent the total number of areas considered every week in the wastewater surveillance. Before the date marked by the vertical dashed line, the NIBSC standard was used to quantify the SARS-CoV-2 and after this date, the EURM standard was used.**



**Figure 4: Localisation of the covered areas (grey polygons) by the wastewater surveillance in Belgium. In each area, the fulfillment of the High Circulation, Fast Increase and Increasing Trend indicator is indicated by a pink, blue and yellow slice, respectively. The names of the covered areas with respect of their localization can be found in Figure 1.**

## 5. Appendix – Areas classified by alerting indicator

**A 1: Covered areas (0 out of 42 on September 21<sup>th</sup> 2022) characterized as High Circulation sorted in the descending order of importance.**

No area was characterized as High circulation this week.

**A 2: Covered areas (11 out of 42 on the September 21<sup>th</sup> 2022) characterized as Fast Increase sorted in the descending order of importance.**

Province	WWTP	High	Fast	Incr.	Norm. viral load (%) <sup>1</sup>	Mean viral load <sup>2</sup>	Norm. evol. (%/week) <sup>3</sup>	Incr. days <sup>4</sup>	Date Max cc <sup>5</sup>
Hainaut	Marchienne-au-Pont	0	1	1	29	1.59	314	7	27/07/2022
Liège	Soumagne	0	1	0	22	2.86	263	0	28/03/2022
Luxembourg	Arlon	0	1	1	18	2.13	182	14	5 <sup>th</sup> wave
Namur	Namur-Brumagne	0	1	1	42	1.13	173	7	11/07/2022
Liège	Amay	0	1	0	9	0.52	127	2	5 <sup>th</sup> wave
Brabant Wallon	Vallée du Hain (L'Orchis)	0	1	1	16	0.40	119	7	11/07/2022
Hainaut	Wasmuel	0	1	0	16	0.33	118	2	13/07/2022
Liège	Liège Sclessin	0	1	1	7	0.80	108	9	16/03/2022
Oost-Vlaanderen	Aalst	0	1	1	26	1.49	85	16	18/04/2022
West-Vlaanderen	Oostende	0	1	0	9	0.85	77	0	13/07/2022
Hainaut	Froyennes	0	1	1	15	0.34	75	9	11/07/2022

<sup>1</sup> : the viral load normalized with the maximum viral load measured in the corresponding catchment area during the fifth wave (i.e. from first of January 2022 till the first of March 2022).

<sup>2</sup> : the viral load computed on the mean of the replicates of the three targeted gene fragments as explained in section "3.2 Wastewater results". The mean viral load is expressed in 10<sup>12</sup> copies/day/100k inhabitants.

<sup>3</sup> : the slope (%/week) of the past 7 days moving average of the viral load if the corresponding concentration is above the estimated limit of quantification.

<sup>4</sup> : the cumulative number of days of increase of the past 14 days moving average of the viral load.

<sup>5</sup> : date at which the measured viral load was the highest since the beginning of 2022. If the date was between the first of January 2022 and the first of March 2022, the date is considered to be during the fifth wave and mentioned as such.

**A 3: Covered areas (21 out of 42 on the September 21<sup>th</sup> 2022) characterized as Increasing Trend sorted in the descending order of importance.**

Province	WWTP	High	Fast	Incr.	Norm. viral load (%) <sup>1</sup>	Mean viral load <sup>2</sup>	Norm. evol. (%/week) <sup>3</sup>	Incr. days <sup>4</sup>	Date Max cc <sup>5</sup>
Vlaams-Brabant	Grimbergen	0	0	1	7	1.44	6	21	5 <sup>th</sup> wave
Oost-Vlaanderen	Aalst	0	1	1	26	1.49	85	16	18/04/2022
Oost-Vlaanderen	Gent	0	0	1	10	0.82	56	16	11/07/2022
Luxembourg	Arlon	0	1	1	18	2.13	182	14	5 <sup>th</sup> wave
Brussels	Brussels-North	0	0	1	10	5.32	69	14	5 <sup>th</sup> wave
West-Vlaanderen	Harelbeke	0	0	1	8	1.01	38	14	13/07/2022
Limburg	Houthalen-Centrum	0	0	1	3	0.19	0	14	5 <sup>th</sup> wave
West-Vlaanderen	Menen	0	0	1	16	2.03	28	14	13/07/2022
Hainaut	Froyennes	0	1	1	15	0.34	75	9	11/07/2022
Liège	Liège Sclessin	0	1	1	7	0.80	108	9	16/03/2022
Hainaut	Mouscron versant Espierres	0	0	1	17	1.41	47	9	27/04/2022
Oost-Vlaanderen	Sint-Niklaas	0	0	1	1	0.14	0	9	5 <sup>th</sup> wave
Antwerpen	Antwerpen-North	0	0	1	2	0.19	0	7	5 <sup>th</sup> wave
Liège	Liège (Grosses Battes)	0	0	1	9	0.86	33	7	5 <sup>th</sup> wave
Luxembourg	Marche-en-Famenne	0	0	1	14	0.65	0	7	23/03/2022
Hainaut	Marchienne-au-Pont	0	1	1	29	1.59	314	7	27/07/2022
Antwerpen	Mechelen-Noord	0	0	1	2	0.18	0	7	5 <sup>th</sup> wave
Hainaut	Montignies-sur-Sambre	0	0	1	22	0.96	4	7	13/07/2022
Namur	Namur-Brumagne	0	1	1	42	1.13	173	7	11/07/2022
Antwerpen	Turnhout	0	0	1	2	0.37	0	7	16/05/2022
Brabant Wallon	Vallée du Hain (L'Orchis)	0	1	1	16	0.40	119	7	11/07/2022

<sup>1</sup> : the viral load normalized with the maximum viral load measured in the corresponding catchment area during the fifth wave (i.e. from first of January 2022 till the first of March 2022).

<sup>2</sup> : the viral load computed on the mean of the replicates of the three targeted gene fragments as explained in section "3.2 Wastewater results". The mean viral load is expressed in 10<sup>12</sup> copies/day/100k inhabitants.

<sup>3</sup> : the slope (%/week) of the past 7 days moving average of the viral load if the corresponding concentration is above the estimated limit of quantification.

<sup>4</sup> : the cumulative number of days of increase of the past 14 days moving average of the viral load.

<sup>5</sup> : date at which the measured viral load was the highest since the beginning of 2022. If the date was between the first of January 2022 and the first of March 2022, the date is considered to be during the fifth wave and mentioned as such.

**A 4: Covered areas (17 out of 42 on the September 21<sup>th</sup> 2022) in which no alerting indicator are fulfilled.**

Province	WWTP	High	Fast	Incr.	Norm. viral load (%) <sup>1</sup>	Mean viral load <sup>2</sup>	Norm evol. (%/week) <sup>3</sup>	Incr. days <sup>4</sup>	Date Max cc <sup>5</sup>
Antwerpen	Aartselaar	0	0	0	0	0.05	0	0	5 <sup>th</sup> wave
Antwerpen	Antwerpen-South	0	0	0	2	0.19	0	0	5 <sup>th</sup> wave
Brabant Wallon	Basse Wavre (Dyle)	0	0	0	4	0.14	0	0	16/03/2022
Vlaams-Brabant	Beersel	0	0	0	5	1.15	-29	0	5 <sup>th</sup> wave
West-Vlaanderen	Brugge	0	0	0	14	0.74	-33	0	18/04/2022
Brussels	Brussel-South	0	0	0	4	1.89	-2	0	5 <sup>th</sup> wave
Oost-Vlaanderen	Dendermonde	0	0	0	0	0.04	0	0	5 <sup>th</sup> wave
Oost-Vlaanderen	Destelbergen	0	0	0	7	0.85	63	0	18/04/2022
Antwerpen	Deurne	0	0	0	0	0.14	0	0	5 <sup>th</sup> wave
Limburg	Genk	0	0	0	2	0.27	0	0	09/03/2022
Limburg	Hasselt	0	0	0	1	0.15	0	0	5 <sup>th</sup> wave
Vlaams-Brabant	Leuven	0	0	0	6	0.9	26	0	18/04/2022
Vlaams-Brabant	Liedekerke	0	0	0	15	1.13	-57	0	18/04/2022
Liège	Liège Oupeye	0	0	0	11	0.35	0	2	06/07/2022
Namur	Mornimont	0	0	0	20	0.80	29	2	13/07/2022
West-Vlaanderen	Roeselare	0	0	0	18	1.45	15	0	5 <sup>th</sup> wave
Limburg	Tessenderlo	0	0	0	1	0.14	0	0	5 <sup>th</sup> wave

<sup>1</sup> : the viral load normalized with the maximum viral load measured in the corresponding catchment area during the fifth wave (i.e. from first of January 2022 till the first of March 2022).

<sup>2</sup> : the viral load computed on the mean of the replicates of the three targeted gene fragments as explained in section "3.2 Wastewater results". The mean viral load is expressed in 10<sup>12</sup> copies/day/100k inhabitants.

<sup>3</sup> : the slope (%/week) of the past 7 days moving average of the viral load if the corresponding concentration is above the estimated limit of quantification.

<sup>4</sup> : the cumulative number of days of increase of the past 14 days moving average of the viral load.

<sup>5</sup> : date at which the measured viral load was the highest since the beginning of 2022. If the date was between the first of January 2022 and the first of March 2022, the date is considered to be during the fifth wave and mentioned as such.