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

RESULTS OF 06/04/2022

Authors: R. Janssens¹, H. Maloux¹, S. Hanoteaux¹, B. Verhaegen², K. Van Hoorde², K. Dierick², S. Quoilin¹, K. Blot¹, M. Lesenfants^{1*}

¹ Sciensano, Service Epidemiology of infectious diseases
 ² Sciensano, Service Foodborne pathogens
 *Project leader and contact: <u>marie.lesenfants@sciensano.be</u>

In collaboration with:

Société Publique de Gestion de l'Eau



Universiteit Antwerpen









TABLE OF CONTENTS

TABLE OF CONTENTS	2
1. Summary	3
2. Introduction	4
3. Methodology	4
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
4. Results	7
4.1. REGIONS	7
4.2. PROVINCES	8
4.3. CATCHMENT AREAS	9
4.4. VARIANTS	12
5. Appendix – Areas classified by alerting indicator	13

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 April 06th 2022:

- At the national level: a short term rapid increase is now only observed in a limited amount of areas, as the number of Fast increase areas reached its lowest level in 7 weeks. In the coming weeks, this tendency may induce a reduction of the areas positive to the longer trend indicator. This Increasing trend indicator is currently fulfilled for almost half of the areas covered by the wastewater surveillance.
- At the regional level: only Brussels is still showing a steady Increasing trend, for more than 2 weeks.
- At the provincial level: the Fast increase indicator is not fulfilled in any province. The Increasing trend indicator is fulfilled in 5 provinces: Brussels, Liège, Namur, Vlaams-Brabant, and West-Vlaanderen. In Brussels, the viral loads are steadily increasing since 18 days. The High circulation indicator is fulfilled in 3 provinces: Liège, Luxembourg, and West-Vlaanderen.
- At the covered areas level: among the 40 areas covered1, the number of treatment plants belonging to the different indicators are 8 for the High circulation indicator, 4 for the "Fast increase" indicator, and 18 for the "Increasing trend" indicator.
- An alerting situation is evidenced for the covered areas of Menen, Mouscron versant Espierres, and Turnhout as all three indicators are fulfilled.
- The risk of underestimating the Omicron variant in wastewater was assessed to be negligible.

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 <u>French</u> and <u>Dutch</u>.
- Further details on the methodology applied for the wastewater surveillance can be found in <u>the</u> <u>Appendix Methodology document (access available online)</u>. The methodology was updated on 29/04/2022.

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. Liège is still well represented within this surveillance, however Verviers is not. Since the 13th of October, the covered area of Soumagne is added to the surveillance. The situation in the area of Namur-Brumagne was not assessed this week as flow rate data were not available.

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.

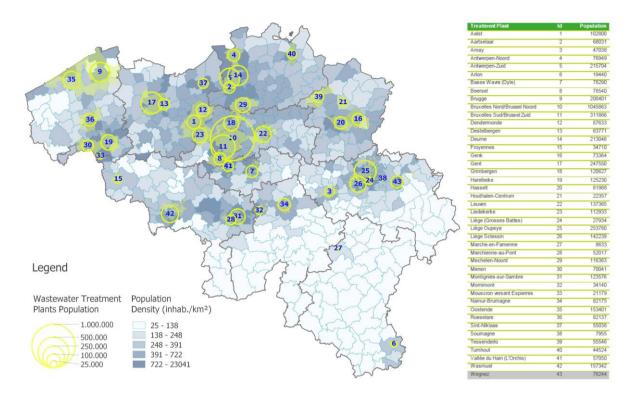


Figure 1: The population located in the areas covered by the wastewater treatment plants (highlighted in yellow) and the population density for each municipality (indicated by the blue scale). Note that due to the recent flooding, the treatment plants of Wegnez and Liège (Grosses Battes) are out of order. These areas are, hence, not considered anymore in the wastewater surveillance and has been partially replaced by the treatment plant of Soumagne.

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.

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 divided by the inlet flow and by the number of inhabitant equivalent of its respective WWTP and expressed by 100k inhabitant. This correction changes the reporting units from copy/ml into copy/day/100k inhabitants equivalent. We will refer this new signal as viral load instead of viral concentration.

3.3. ALERTING INDICATORS

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

1. 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).

- 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 **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

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.

It should be noted that in several areas, the viral load exceeded the maximal viral load registered during the 3rd wave. The date at which the maximal viral load has been reached for each area is indicated in the tables A1 to A4 in the appendices.

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 <u>the Appendix Methodology document (access online)</u>).

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 3rd wave occurring in March 2021.
- The 4th wave occurring in December 2021.
- The 5th wave occurring in January 2022.

Table 1 shows, for each region, the results associated with the samples of Wednesday January 26th 2022.

Table 1 allows to track the changes between the situation as of today (April 06th 2022) and the situation as of last week (March 30th 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 last trends were analysed based on the alerting indicators presented in Table 1. Amongst the three regions, only one indicator is fulfilled in Brussels: the Increasing trend. This indicates that the viral loads are steadily increasing since 18 days in Brussels.

Table 1: Alerting indicators fulfilled (1) or not (0) on April 06th 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 (%) ¹	Mean viral load²	Norm. evol. (%/wee k) ³	lncr. days⁴
Brussels	100%	0	0	1	22	130	3	18
Flanders	41%	0	0	0	34	35	13	6
Wallonia	31%	0	0	0	45	13	-9	5

¹: 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).

² : 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 10^12 copies/day/100k inhabitants.

³ : 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.

⁴: the cumulative number of days of increase of the past 14 days moving average of the viral load.



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).

4.2. PROVINCES

Table 2 shows, for each Province, the results associated with the samples of Wednesday April 06th 2022.

Table 2 allows to track the changes between the situation as of today (April 06th 2022) and the situation as of last week (March 30th 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 April 06th 2022, for the three alerting indicators:

- The High circulation indicator is fulfilled in 3 provinces: Liège, Luxembourg, and West-Vlaanderen.
- The Fast increase indicator is not fulfilled in any province.
- The Increasing trend indicator is fulfilled in 5 provinces: Brussels, Liège, Namur, Vlaams-Brabant, and West-Vlaanderen.
- 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 loads in the provinces of Namur and Vlaams-Brabant remain high (with 47% and 48%).

Table 2: Alerting indicators fulfilled (1) or not (0) on April 06th 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 (%) ¹	Mean viral load²	Norm. evol. (%/w) ³	Incr. days⁴
Antwerpen	41%	0	0	0	15	6.7	17	1
Brabant Wallon	33%	0	0	0	35	8.1	-21	1
Brussels	100%	0	0	1	22	130	3	18
Hainaut	29%	0	0	0	35	7.6	-7	3
Liège	50%	1	0	1	52	16	-9	7
Limburg	26%	0	0	0	32	11	-11	1
Luxembourg	10%	1	0	0	108	40	-23	1
Namur	23%	0	0	1	47	12	22	7
Oost-Vlaanderen	38%	0	0	0	29	26	22	6
Vlaams-Brabant	61%	0	0	1	48	81	-22	10
West-Vlaanderen	52%	1	0	1	50	53	34	11

¹: 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).

²: 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 10^12 copies/day/100k inhabitants.

³: 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.

⁴: 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.

Table 3 allows to track the changes between the situation as of today (April 06th 2022) and the situation as of last week (March 30th 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 April 06th 2022:

- The High circulation indicator is fulfilled in 8 covered areas. The full list of these areas can be found in Appendix A1. Last week, this indicator was fulfilled in 12 covered areas.
- The Fast increase is fulfilled in 4 covered areas: Dendermonde (175% increase per week), Menen (141% increase per week), Turnhout (131% increase per week), and Mouscron versant Espierres (107% increase per week) (see Appendix A2 for more details). Last week, this indicator was fulfilled in 15 covered areas.
- The Increasing trend indicator is fulfilled in 18 covered areas. Amongst these areas, the viral load is continually increasing since more than two weeks in 6 areas: Brussel-South (30 days), Grimbergen (28 days), Brugge (23 days), Beersel (14 days), Brussels-North (14 days), and Dendermonde (14 days). Further details can be found in Appendix A3. Last week, this indicator was fulfilled in 14 covered areas.
- An alerting situation is evidenced for the covered areas of Menen, Mouscron versant Espierres, and Turnhout as all three indicators are fulfilled.

The wastewater results at the level of the local covered areas can be accessed online for each area on the <u>COVID-19</u> dashboard.

Table 3: Alerting indicators fulfilled (1) or not (0) on April 06th 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 (%) ¹	Mean viral load²	Norm evol. (%/wee k) ³	Incr. days⁴
Liège	Amay	0	0	1	14	4.8	32	7
Vlaams-Brabant	Beersel	0	0	1	29	81	18	14
West-Vlaanderen	Brugge	0	0	1	44	28	-18	23
Brussels	Brussels-North	0	0	1	21	140	14	14
Brussels	Brussel-South	0	0	1	24	120	-33	30
Oost-Vlaanderen	Dendermonde	0	1	1	25	5.9	175	14
Oost-Vlaanderen	Gent	0	0	1	36	37	-8	9
Vlaams-Brabant	Grimbergen	0	0	1	48	120	-20	28
West-Vlaanderen	Harelbeke	0	0	1	37	53	66	9
Limburg	Houthalen-Centrum	1	0	0	92	11	69	2
Vlaams-Brabant	Liedekerke	1	0	0	83	74	-17	0
Liège	Liège Oupeye	1	0	1	65	12	-29	7
Liège	Liège Sclessin	0	0	1	41	27	12	7
Luxembourg	Marche-en-Famenne	1	0	0	282	83	-7	0
West-Vlaanderen	Menen	1	1	1	91	140	141	7
Hainaut	Montignies-sur-Sambre	0	0	1	42	11	-7	7
Namur	Mornimont	0	0	1	47	12	22	7
Hainaut	Mouscron versant Espierres	1	1	1	54	26	107	7

Province	WWTP	High	Fast	incr.	Norm. viral load (%) ¹	Mean viral load²	Norm evol. (%/wee k) ³	lncr. days⁴
West-Vlaanderen	Roeselare	1	0	1	64	57	-18	9
Liège	Soumagne	0	0	1	49	39	-19	7
Antwerpen	Turnhout	1	1	1	68	32	131	9

¹: 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).

²: 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 10^12 copies/day/100k inhabitants.

³ : 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.

⁴: 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:

- High circulation indicator: Basse Wavre (Dyle), Froyennes, Genk, Leuven, Marchienne-au-Pont and Oostende.
- Fast increase indicator fulfilled: Basse Wavre (Dyle), Froyennes, Marchienne-au-Pont, Oostende, and Tessenderlo.
- Increasing trend indicator: Aartselaar, Antwerpen-North, Hasselt, Leuven, Marchienne-au-Pont, Oostende, and Tessenderlo.

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. For further insights on the dynamic of the different indicators, see Section 3.3. A short term rapid increase is now only observed in a limited amount of areas, as the number of Fast increase areas reached its lowest level in 7 weeks. In the coming weeks, this tendency may induce a reduction of the areas positive to the longer trend indicator. This Increasing trend indicator is currently fulfilled for almost half of the areas covered by the wastewater surveillance.

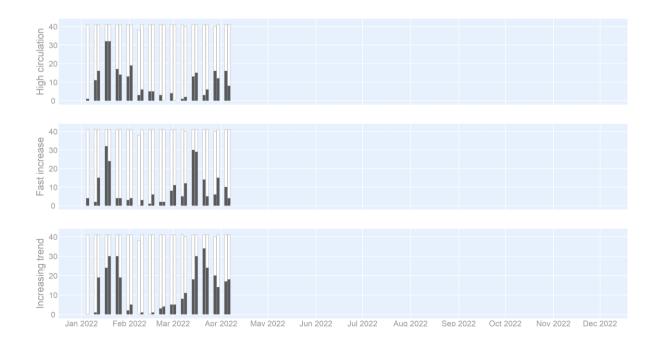


Figure 3: The number of areas (among the 40 covered by the wastewater surveillance this week and the 42 normally considered), with positive alerting indicators (black) compared to the total number of areas considered every week in the wastewater surveillance (white). Latest results (last bar) are from April 06th 2022. The white bars represent the total number of areas considered every week in the wastewater surveillance.

4.4. VARIANTS

The Omicron variant represents a high proportion of all the reported cases in Belgium². The impact of the Omicron variant was assessed to be negligible for the following reason: mutations present in the Omicron variant on the N1 and E genes could have potentially led to a drop out of those two genes leading to an underestimation of the viral loads in wastewaters. Preliminary experimental lab results indicate that no drop out of the genes should be expected. Hence, the sensitivity of the analytical lab methods used was not impacted. Therefore, the circulation of the Omicron variant is not underestimated in wastewaters.

However, the Omicron variant might have an impact on the faecal shedding load if infected people would excrete less in their faeces than for Delta. That stays hypothetical as we do not have strong evidence on it yet.

A regular investigation of the possible impact of variants of concern is done in order to screen their evolution in time.

² Source :<u>Molecular surveillance of SARS-CoV-2</u>

5. Appendix – Areas classified by alerting indicator

A 1: Covered areas (8 out of 40 on April 06th 2022) characterized as High circulation sorted in the descending order of importance.

Province	WWTP	High	Fast	Incr.	Norm . viral load (%) ¹	Mean viral load²	Norm evol. (%/we ek) ³	lncr. days⁴	Date Max cc ⁵
Luxembourg	Marche-en-Famenne	1	0	0	282	83	-7	0	23/03/2022
Limburg	Houthalen-Centrum	1	0	0	92	11	69	2	5 th wave
West-Vlaanderen	Menen	1	1	1	91	140	141	7	04/04/2022
Vlaams-Brabant	Liedekerke	1	0	0	83	74	-17	0	5 th wave
Antwerpen	Turnhout	1	1	1	68	32	131	9	5 th wave
Liège	Liège Oupeye	1	0	1	65	12	-29	7	28/03/2022
West-Vlaanderen	Roeselare	1	0	1	64	57	-18	9	5 th wave
Hainaut	Mouscron versant Espierres	1	1	1	54	26	107	7	14/03/2022

¹: 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).

²: 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 10^12 copies/day/100k inhabitants.

³ : 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.

⁴: the cumulative number of days of increase of the past 14 days moving average of the viral load.

⁵: date at which the measured viral load was the highest since the beginning of the measurements. 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.

Province	WWTP	High	Fast	Incr.	Norm . viral load (%) ¹	Mean viral load²	Norm evol. (%/we ek) ³	lncr. days⁴	Date Max cc ⁵
Oost-Vlaanderen	Dendermonde	0	1	1	25	5.9	175	14	5 th wave
West-Vlaanderen	Menen	1	1	1	91	140	141	7	04/04/2022
Antwerpen	Turnhout	1	1	1	68	32	131	9	5 th wave
Hainaut	Mouscron versant Espierres	1	1	1	54	26	107	7	14/03/2022

A 2: Covered areas (4 out of 40 on the April 06th 2022) characterized as Fast increase sorted in the descending order of importance.

¹: 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).

²: 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 10^12 copies/day/100k inhabitants.

³: 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.

⁴: the cumulative number of days of increase of the past 14 days moving average of the viral load.

⁵: date at which the measured viral load was the highest since the beginning of the measurements. 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.

Province	WWTP	High	Fast	Incr.	Norm . viral load (%) ¹	Mean viral load²	Norm evol. (%/we ek) ³	lncr. days⁴	Date Max cc⁵
Brussels	Brussel-South	0	0	1	24	120	-33	30	5 th wave
Vlaams-Brabant	Grimbergen	0	0	1	48	120	-20	28	5 th wave
West-Vlaanderen	Brugge	0	0	1	44	28	-18	23	5 th wave
Vlaams-Brabant	Beersel	0	0	1	29	81	18	14	5 th wave
Brussels	Brussels-North	0	0	1	21	140	14	14	5 th wave
Oost-Vlaanderen	Dendermonde	0	1	1	25	5.9	175	14	5 th wave
Oost-Vlaanderen	Gent	0	0	1	36	37	-8	9	5 th wave
West-Vlaanderen	Harelbeke	0	0	1	37	53	66	9	5 th wave
West-Vlaanderen	Roeselare	1	0	1	64	57	-18	9	5 th wave
Antwerpen	Turnhout	1	1	1	68	32	131	9	5 th wave
Liège	Amay	0	0	1	14	4.8	32	7	5 th wave
Liège	Liège Oupeye	1	0	1	65	12	-29	7	28/03/2022
Liège	Liège Sclessin	0	0	1	41	27	12	7	16/03/2022
West-Vlaanderen	Menen	1	1	1	91	140	141	7	04/04/2022
Hainaut	Montignies-sur-Sambre	0	0	1	42	11	-7	7	5 th wave
Namur	Mornimont	0	0	1	47	12	22	7	5 th wave
Hainaut	Mouscron versant Espierres	1	1	1	54	26	107	7	14/03/2022
Liège	Soumagne	0	0	1	49	39	-19	7	28/03/2022

A 3: Covered areas (18 out of 40 on the April 06th 2022) characterized as Increasing trend sorted in the descending order of importance.

¹: 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).

²: 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 10^12 copies/day/100k inhabitants.

³ : 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.

⁴: the cumulative number of days of increase of the past 14 days moving average of the viral load.

⁵: date at which the measured viral load was the highest since the beginning of the measurements. 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 (20 out of 40 on the April 06th 2022) in which no alerting indicator are fullfilled.

Province	WWTP	High	Fast	Incr.	Norm . viral load (%) ¹	Mean viral load²	Norm evol. (%/we ek) ³	lncr. days⁴	Date Max cc ⁵
Oost-Vlaanderen	Aalst	0	0	0	21	13	-28	0	5 th wave
Antwerpen	Aartselaar	0	0	0	23	4.8	11	0	5 th wave
Antwerpen	Antwerpen-North	0	0	0	9	2	-21	0	5 th wave
Antwerpen	Antwerpen-South	0	0	0	11	5	59	0	5 th wave
Luxembourg	Arlon	0	0	0	30	21	-30	2	5 th wave
Brabant Wallon	Basse Wavre (Dyle)	0	0	0	48	12	-15	2	16/03/2022
Oost-Vlaanderen	Destelbergen	0	0	0	28	40	18	0	5 th wave
Antwerpen	Deurne	0	0	0	9	7.2	-27	0	5 th wave
Hainaut	Froyennes	0	0	0	31	4.2	-66	0	16/03/2022
Limburg	Genk	0	0	0	27	11	-53	0	09/03/2022
Limburg	Hasselt	0	0	0	27	13	30	0	5 th wave
Vlaams-Brabant	Leuven	0	0	0	31	54	-51	0	23/03/2022
Hainaut	Marchienne-au-Pont	0	0	0	13	4.4	-38	0	5 th wave
Antwerpen	Mechelen-Noord	0	0	0	12	3.6	4	0	5 th wave
West-Vlaanderen	Oostende	0	0	0	43	45	56	0	04/04/2022
Oost-Vlaanderen	Sint-Niklaas	0	0	0	22	13	9	0	5 th wave
Limburg	Tessenderlo	0	0	0	23	8.1	-48	2	5 th wave
Brabant Wallon	Vallée du Hain (L'Orchis)	0	0	0	16	2.4	-31	0	5 th wave
Hainaut	Wasmuel	0	0	0	35	4.3	1	0	16/03/2022

¹: 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).

²: 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 10^12 copies/day/100k inhabitants.

³: 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.

⁴: the cumulative number of days of increase of the past 14 days moving average of the viral load.

⁵: date at which the measured viral load was the highest since the beginning of the measurements. 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.