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

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RESULTS OF 22/09/2021

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In collaboration with:

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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 42 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 22th 2021:

- At the national level: The number of areas covered, belonging to the "Increasing trend" indicators, rose quickly at the beginning of July 2021 and stabilized at the same alert level since mid-July.
- At the provincial level: Antwerpen, Brabant Wallon, Vlaams-Brabant, and West-Vlaanderen are the provinces positive to at least one of the three alerting indicators. Also, the province of Antwerpen is of particular concern as the conditions for 2 indicators are met in this province.
- At the covered areas level: Amongst the 39 areas covered[1], the number of treatment plants with at least one alerting indicator positive is 20, 1 belonging to the "High circulation" indicator, 17 belonging to the "Fast increase" indicator, and 16 belonging to the "Increasing trend" indicator. Last week (results of September 15th 2021), 9 areas had at least one alerting indicator positive.
- Attention point for Liège Sclessin, area for which all three indicators are fulfilled and where the level of circulation has reached more than 80% of the maximum level observed since the 3rd wave period. Not only Liège Sclessin presents a high circulation of the virus. Indeed, in total, at least 6 of the areas covered have a viral circulation level above 40% of the highest level measured since the 3rd wave, for these specific areas (see Table 2 – column "Norm. viral cc - %").

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 online).

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 viral 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, alerting

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[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. No samples were received for the area of Ghent.

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. 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 1 by province (see also Tables A4, A5 and A6) 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.

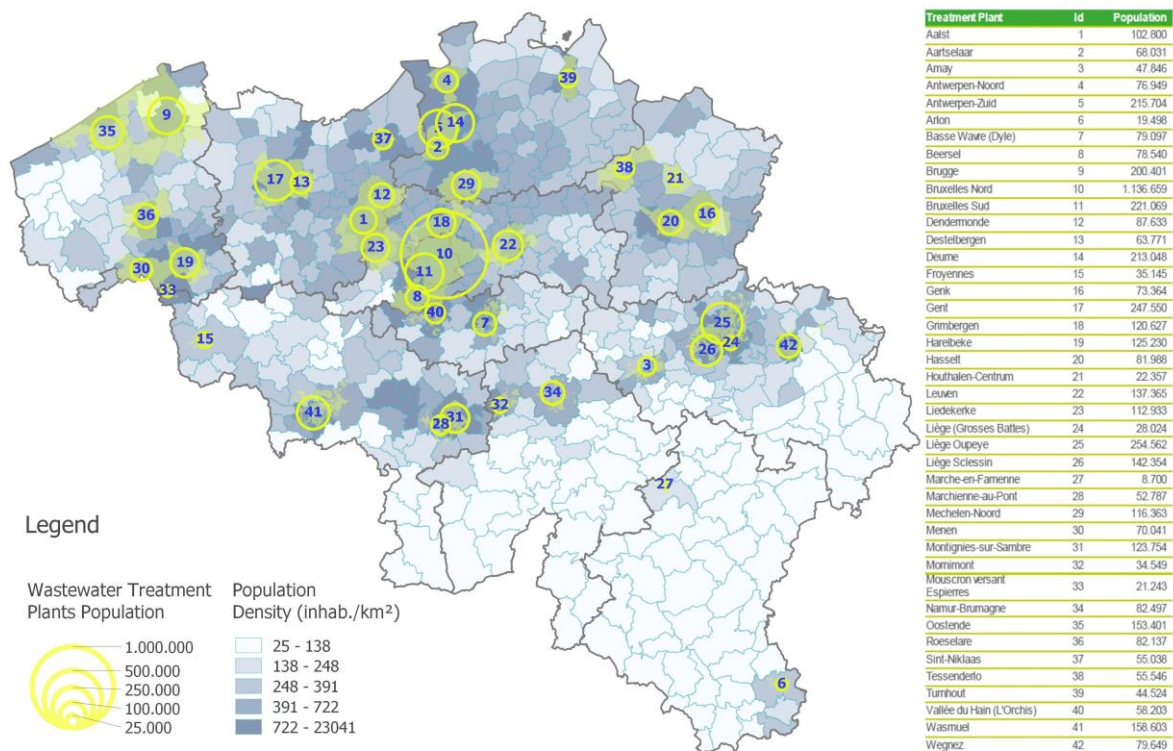| Treatment Plant	Id	Population
Aalst	1	102.800
Aartselaar	2	68.031
Amay	3	47.846
Antwerpen-Noord	4	76.949
Antwerpen-Zuid	5	215.704
Arlon	6	19.498
Basse Wavre (Dyle)	7	79.097
Beersel	8	78.540
Brugge	9	200.401
Bruxelles Nord	10	1.136.659
Bruxelles Sud	11	221.069
Dendermonde	12	87.633
Destelbergen	13	63.771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

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

3.2. WASTEWATER RESULTS

The quantification of SARS-CoV-2 in RNA copies/ ml (concentration) was generalized in mid-February 2021 to all the treatment plants investigated. Before this date, the quantitative values were estimated based on the retrospective application of the quantification method (see details in Appendix) from mid-September 2020 to mid-February 2021 for Flanders and Brussels. In Wallonia, the quantitative results were available since the start.

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

3.3. ALERTING INDICATORS

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

1. The **High circulation** indicator highlights the catchment areas where the viral concentrations are high. It corresponds to a situation where the viral concentrations exceed half of the highest value recorded since mid-February 2021.

2. The **Fast increase** indicator highlights the catchment areas where the viral concentrations have rapidly increased for the last week. It corresponds to a situation where the moving average on the past 7 days of the viral concentration has increased faster than 10% per day if being above the estimated limit of quantification. The increasing slope is normalized for each treatment plant .

3. The **Increasing trend** indicator highlights the catchment areas where the viral concentrations have been increasing for more than 6 days. It corresponds to a situation where the moving average on the past two weeks of the viral concentration has increased during more than 6 days.

3.4. CASES RESULTS

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

3.5. CAUTION POINTS FOR THE RESULTS INTERPRETATION

Only the trends, observed through the alerting indicators, should be assessed for the comparison of different areas. Absolute values should not be compared as the concentration values differ from an WWTP to another, notably due to the differences in population sizes covered. Additionally, the situation 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 analysis (see Appendix Methodology document).

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, compiling the incidence data of the area covered by the wastewater surveillance. The estimated viral concentrations can also be seen for the period before mid-February (see 2.2. and Appendix Methodology document for more details).

The second wave peak occurring in November 2020 can be seen in the three regions in Figure 2 below. This remains true for the third wave, but to a lower extend in Wallonia. 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 concentration in the wastewater was increasing since the beginning of July in Wallonia, Brussels and Flanders. However, this increase was much more pronounced in Wallonia and Brussels than in Flanders, if we compare it to the highest level of circulation in wastewater since the 3rd wave. In Brussels and Flanders, a peak of viral concentration was recorded at the end of August, while at the beginning of September for Wallonia. The concentrations are now increasing again.
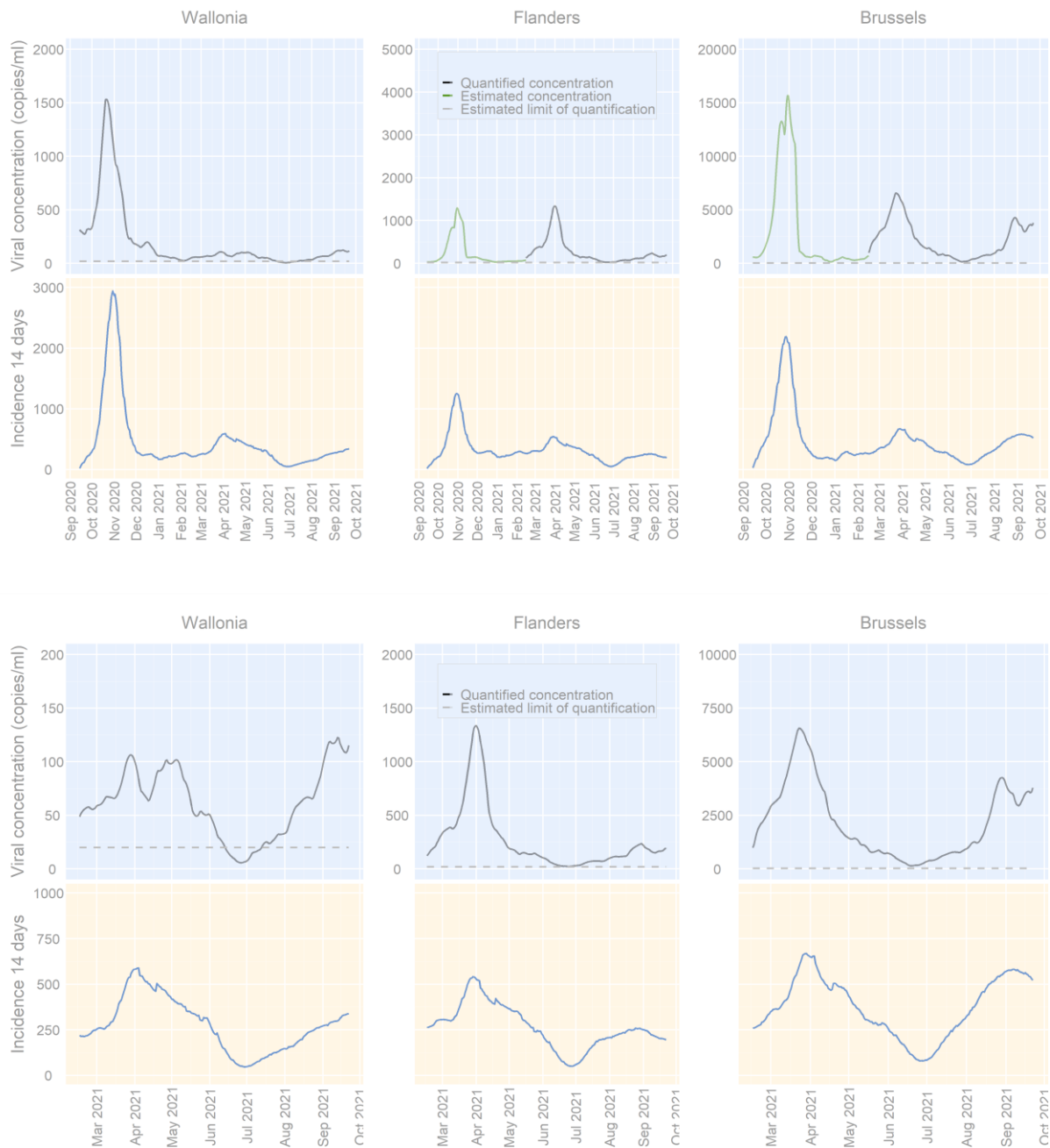
Figure 2: The SARS-CoV-2 RNA estimated and quantified concentrations expressed as copies/ml (two weeks centered moving average applied on the linear interpolation), the estimated limit of quantification of 20 SARS-CoV-2 RNA copies/ ml, and the 14 days incidence in the population covered by the wastewater 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 22th 2021, for the three alerting indicators:

- Last week on Wednesday September 15th 2021, no province had one alerting indicator positive. To note however that Luxembourg and Namur provinces were not included in the surveillance last week.
- This week on Wednesday September 22th 2021, 4 provinces have at least one alerting indicator positive: Antwerpen, Brabant Wallon, Vlaams-Brabant, and West-Vlaanderen. The province of Antwerpen is of particular concern as 2 indicators are fulfilled in this province.
- In the provinces of Liège and Brussels, even if the threshold of 50% of normalized viral concentration is not reached, the normalized concentrations are high in both provinces (43 and 46% respectively for Brussels and Liège).

Table 1: Alerting indicators fulfilled (1) or not (0) on September 22th 2021 and the percentage of population covered by the provinces. Columns represent the population coverage of the WWTP within the Province (Pop. coverage), the High Circulation indicator (High), the Fast increase indicator (Fast) and the Increasing trend indicator (Incr.). The specifications of the four last columns are explained in the footnotes 1-4 below the table. Missing data is indicated with a "/".

Province	Pop. coverage	High	Fast	Incr.	Norm. viral cc. (%)[1]	Mean viral. cc. (c./ml)[2]	Norm. evol. (%/day)[3]	Incr. days[4]
Antwerpen	39%	0	1	1	17	186	84	7
Brabant Wallon	34%	0	0	1	15	18	2	9
Brussels	100%	0	0	0	43	3651	10	6
Hainaut	29%	0	0	0	24	44	-2	3
Liège	50%	0	0	0	46	210	3	3
Limburg	26%	0	0	0	5	12	2	1
Luxembourg	10%	0	0	0	8	15	-22	0
Namur	24%	0	0	0	16	41	-16	0
Oost-Vlaanderen	38%	0	0	0	8	112	6	1
Vlaams-Brabant	49%	0	1	0	17	517	19	5
West-Vlaanderen	52%	0	1	0	9	274	43	4

[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. The number of treatment plants with at least one alerting indicator positive is 20 (out of 39 areas covered).

- The indicator "High circulation" is fulfilled in 1 covered area: Liège Sclessin , where the viral circulation has reached 84% of the maximum circulation observed since the 3rd wave (see Appendix A1). Not only Liège Sclessin presents a high circulation of the virus. Indeed, in total, at least 6 of the areas covered have a viral circulation level above 40% of the highest level measured during the 3rd wave, for these specific areas (see Table 2 – column "Norm. viral cc - %").
- The indicator "Fast increase" is fulfilled in 17 covered areas. Amongst these covered areas, an increase of more than 50% per day of the normalized viral concentration is observed for 5 areas: Antwerpen-North (126% increase per day), Mechelen-Noord (110% increase per day), Deurne (108% increase per day), Oostende (105% increase per day), and Antwerpen-South (64% increase per day) (see Appendix A2 for more details).
- The indicator "Increasing trend" is fulfilled in 16 covered areas. Amongst these areas, the viral concentration is continually increasing since more than two weeks in 2 stations: Turnhout (21 days) and Vallée du Hain (L'Orchis) (21 days) (see Appendix A3 for more details).
- Attention point for Liège Sclessin, area for which all three indicators are fulfilled.

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 22th 2021.

Table 2: Alerting indicators fulfilled (1) or not (0) on September 22th 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-North	0	1	1	36	114	126	7
Antwerpen	Deurne	0	1	1	6	350	108	7
Antwerpen	Mechelen-Noord	0	1	1	40	144	110	7
Antwerpen	Antwerpen-South	0	1	1	6	131	64	7
Antwerpen	Turnhout	0	1	1	44	172	49	21
Brabant Wallon	Vallée du Hain (L'Orchis)	0	0	1	32	40	5	21
Brussels	Brussels-North	0	0	1	43	3391	9	7
Brussels	Brussel-South	0	1	1	43	4521	13	7
Hainaut	Wasmuel	0	1	1	42	68	14	7
Liège	Liège Sclessin	1	1	1	84	518	12	9
Limburg	Houthalen-Centrum	0	1	1	42	89	26	7
Oost-Vlaanderen	Aalst	0	1	0	8	163	17	0

Province	WWTP	High	Fast	Incr.	Norm. viral cc (%)[1]	Mean viral cc (c./ml)[2]	Norm evol. (%/d)[3]	Incr days[4]
Oost-Vlaanderen	Dendermonde	0	1	1	15	52	36	7
Vlaams-Brabant	Leuven	0	1	1	17	508	37	7
Vlaams-Brabant	Grimbergen	0	0	1	34	876	7	7
Vlaams-Brabant	Beersel	0	1	0	6	360	34	2
West-Vlaanderen	Brugge	0	1	1	11	102	41	7
West-Vlaanderen	Harelbeke	0	1	1	11	264	11	7
West-Vlaanderen	Oostende	0	1	0	12	321	105	2
West-Vlaanderen	Menen	0	1	0	5	997	18	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.

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

The number of treatment plants belonging to the "Increasing trend" indicators rose quickly at the beginning of July 2021 and stabilized at the same alert level since mid-July.
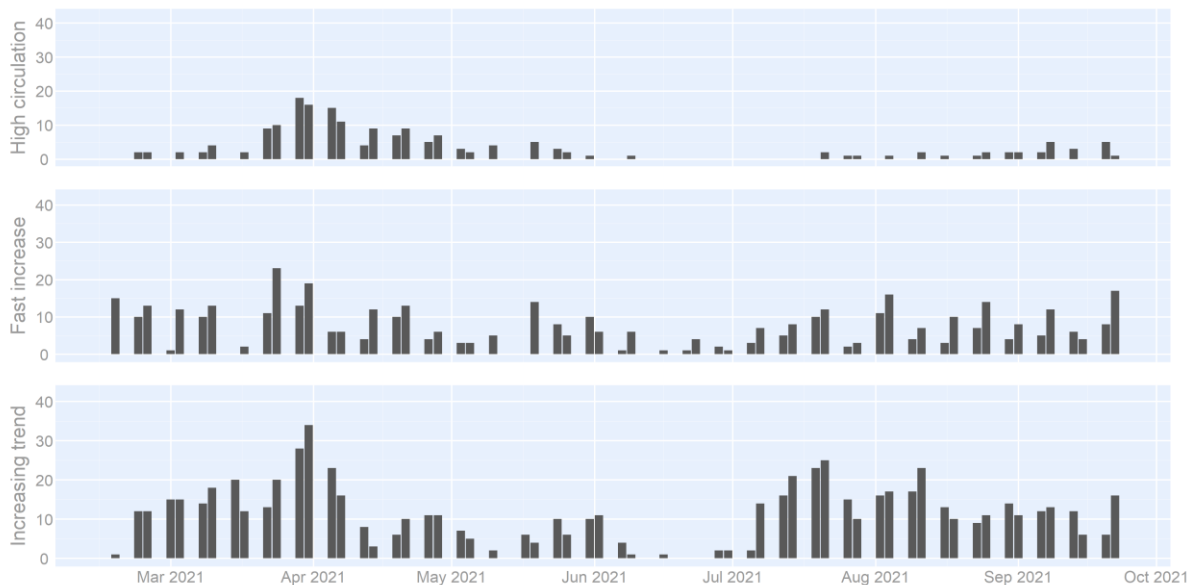
Figure 3: The number of areas (amongst the 39 covered by the wastewater surveillance this week and the 42 normally considered), with positive alerting indicators (latest results on September 22th 2021).

5. Appendix – Areas classified by alerting indicator

A 1: The number of catchment areas (1 out of 39 on September 22th 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	84	518	12	9

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

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

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

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

A 2: The number of catchment areas (17 out of 39 on the September 22th 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-North	0	1	1	36	114	126	7
Antwerpen	Mechelen-Noord	0	1	1	40	144	110	7
Antwerpen	Deurne	0	1	1	6	350	108	7
West-Vlaanderen	Oostende	0	1	0	12	321	105	2
Antwerpen	Antwerpen-South	0	1	1	6	131	64	7
Antwerpen	Turnhout	0	1	1	44	172	49	21
West-Vlaanderen	Brugge	0	1	1	11	102	41	7
Vlaams-Brabant	Leuven	0	1	1	17	508	37	7
Oost-Vlaanderen	Dendermonde	0	1	1	15	52	36	7
Vlaams-Brabant	Beersel	0	1	0	6	360	34	2
Limburg	Houthalen-Centrum	0	1	1	42	89	26	7
West-Vlaanderen	Menen	0	1	0	5	997	18	2
Oost-Vlaanderen	Aalst	0	1	0	8	163	17	0
Hainaut	Wasmuel	0	1	1	42	68	14	7
Brussels	Brussel-South	0	1	1	43	4521	13	7

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	84	518	12	9
West-Vlaanderen	Harelbeke	0	1	1	11	264	11	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

A 3: The number of catchment areas (16 out of 39 on the September 22th 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]
Antwerpen	Turnhout	0	1	1	44	172	49	21
Brabant Wallon	Vallee du Hain (L'Orchis)	0	0	1	32	40	5	21
Liège	Liège Sclessin	1	1	1	84	518	12	9
Antwerpen	Antwerpen-North	0	1	1	36	114	126	7
Antwerpen	Deurne	0	1	1	6	350	108	7
Antwerpen	Mechelen-Noord	0	1	1	40	144	110	7
Antwerpen	Antwerpen-South	0	1	1	6	131	64	7
Brussels	Brussels-North	0	0	1	43	3391	9	7
Brussels	Brussel-South	0	1	1	43	4521	13	7
Hainaut	Wasmuel	0	1	1	42	68	14	7
Limburg	Houthalen-Centrum	0	1	1	42	89	26	7
Oost-Vlaanderen	Dendermonde	0	1	1	15	52	36	7
Vlaams-Brabant	Leuven	0	1	1	17	508	37	7
Vlaams-Brabant	Grimbergen	0	0	1	34	876	7	7
West-Vlaanderen	Brugge	0	1	1	11	102	41	7
West-Vlaanderen	Harelbeke	0	1	1	11	264	11	7

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

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