

# Wastewater-based genomic epidemiology for SARS-CoV-2 surveillance in South Africa

#### 19 April 2024

Sample collection dates up to 12 April 2024 (Epidemiological week 15)

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## Summary: SARS-CoV-2 transmission and genomics based on evaluation of wastewater at sentinel sites across RSA

### Wastewater <u>levels</u> Epidemiological weeks 48 (2023) –15 (2024)

- From weeks 48-13 the cumulative SARS-CoV-2 levels measured at wastewater treatment works (WWTW) has remained around one log genome copies/ml of wastewater. This has followed on from the increases observed in weeks 31-41, when the cumulative SARS-CoV-2 levels in wastewater at sentinel sites in South Africa showed increases to levels above 2 log (100) genome copies/ml of wastewater, up from below one log copy/ml in epidemiological week 22 (first week in June 2023). From week 45, levels measured below 2 log genome copies/ml of wastewater.
- In weeks 1-6 lower levels were observed nationally, with an increase seen in week 7 and a subsequent decrease in week 8. Levels increase to moderate in Epi week 10. Levels increased to high (above 2 log (100) genome copies/ml) in Epi week 14 and decreased to moderate in Epi week 15.
- Correlation with syndromic surveillance for influenza-like illness (ILI) and severe
  acute respiratory infection findings (SARI) is required to determine the clinical and
  public health significance of ongoing transmission.
  <a href="https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/weekly-respiratory-pathogens-surveillance-report-week/">https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/weekly-respiratory-pathogens-surveillance-report-week/</a>

### Wastewater <u>genomics</u> Epidemiological weeks 48 (2023) – 15 (2024)

- Omicron lineage BA.2.86.X, and JN.1.X sub-lineages were the dominant lineages circulating in wastewater samples between January 2024 March 2024 (Epiweek 1-10)
- In clinical samples, **BA.2.86** was also the dominant lineage circulating throughout November 2023 and January 2024, followed by **XBB.1.5\***, **XBB.1.19\*** and **JN.1\***.
- The Omicron lineage BA.2.86, JN.1\* and Recombinant lineages are circulating in KwaZulu-Natal in eThekwini (in the catchments of Northern and Central WWTWs). In Gauteng, in the City of Johannesburg (in catchments of Northern and Goudkoppies WWTWs), in Ekurhuleni (in the catchments of Olifantsfontein, Vlakplaats, and Hartebeesfontein WWTWs), and the City of Tshwane (in the catchment of Daspoort WWTP) BA.2.86.X and JN.1.X are the dominantly circulating. It is also circulating in Eastern Cape in Buffalo City (in the catchment of Mdantsane WWTW), in Western Cape, in the City of Cape Town (in the catchment of Borches Quarry WWTW), Free State, in Mangaung (catchments Bloemspruit and Sterkwater WWTWs) and North West (Boitekong). In Limpopo (Musina) and Mpumalanga (Kingstonvale) BA.2.86.X lineage was the main lineage detected.

**Interpretation:** Ongoing transmission of SARS-CoV-2 due to Omicron lineages including the new BA.2.86 and JN.1\* lineages.



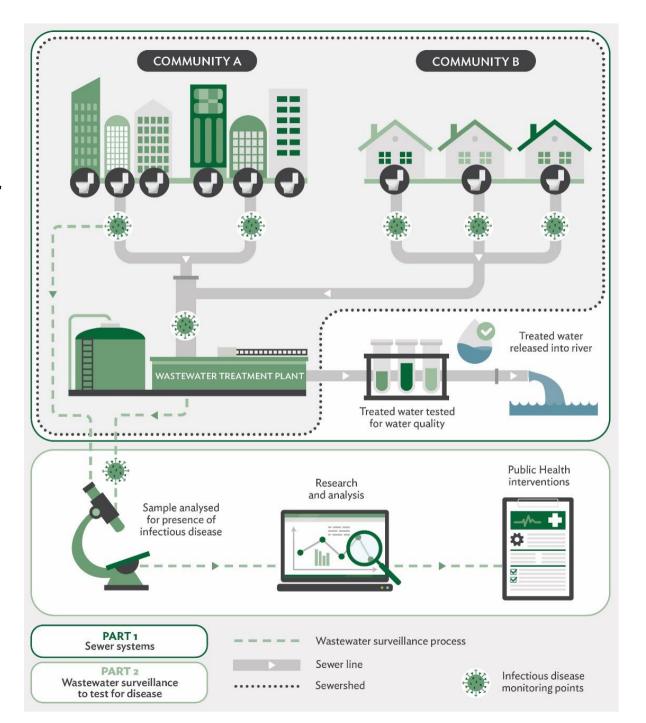
# Wastewater-based Epidemiology for COVID-19 How is wastewater tested for SARS-CoV-2?

For a full description of this process, see the photo essay developed in collaboration with the Gauteng City Region Observatory <a href="https://www.gcro.ac.za/outputs/photo-essay-sewersheds-what-can-wastewater-tell-us-about-community-health/">https://www.gcro.ac.za/outputs/photo-essay-sewersheds-what-can-wastewater-tell-us-about-community-health/</a>

For a technical description and analysis of wastewater levels and results see

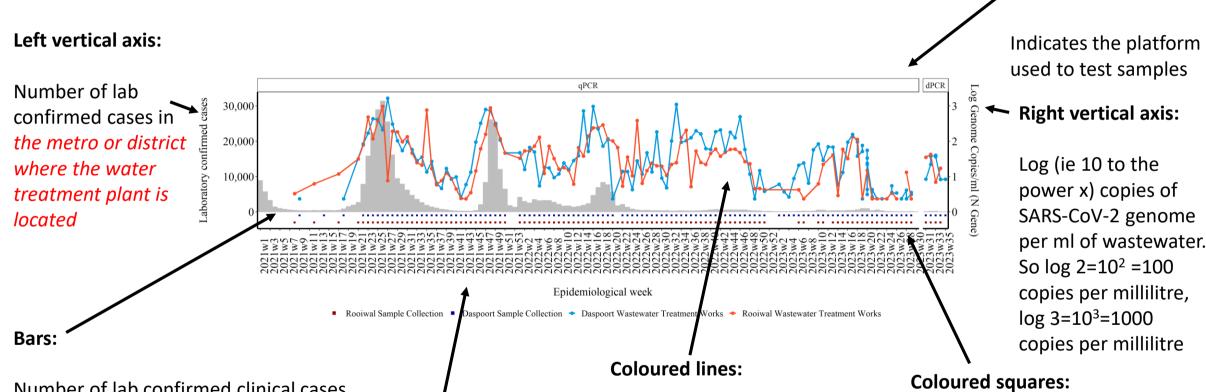
https://pubmed.ncbi.nlm.nih.gov/37506905/ https://www.medrxiv.org/content/10.1101/2022.12.15.22283506v1 (accepted by Nature Communications, publication pending)

SARS-CoV-2 is not transmitted by faeco-oral route. Wastewater with SARS-CoV-2 is not infectious



### Wastewater-based Epidemiology for COVID-19

What does wastewater testing for SARS-CoV-2 mean?



Number of lab confirmed clinical cases in specimens submitted to NICD from persons in the metro/subdistrict where the plant is located

#### **Horizontal axis:**

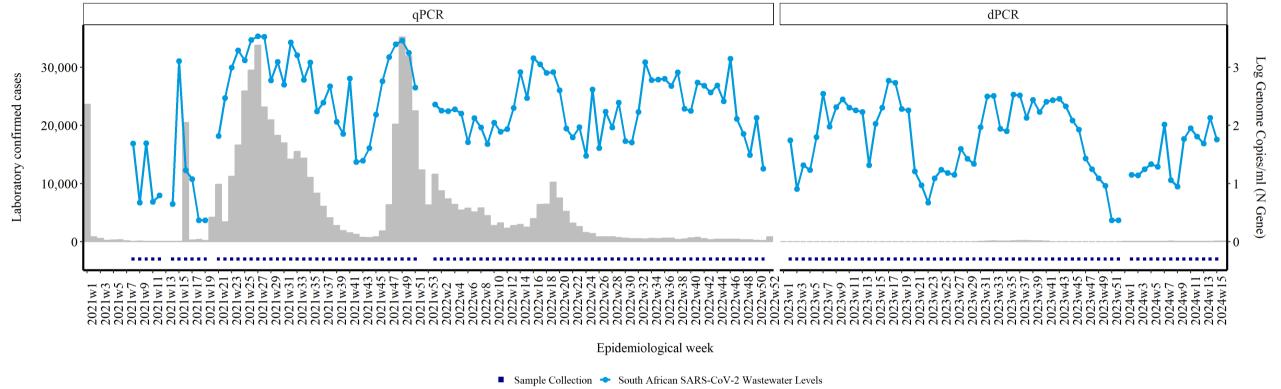
Epidemiological weeks from 2021 to 2023

Changes in wastewater SARS-CoV-2 results over time for different treatment facilities

Epi weeks during which samples were collected

**Facets:** 

### South Africa at a glance: Summed total of clinical and genome copies



Changes in levels of SARS-Cov-2 in log-transformed genome copies/ml (right vertical axis, line graph) in in-flowing untreated wastewater from plants tested by NICD, compared with <u>laboratory-confirmed cases from Tshwane, Johannesburg, Ekurhuleni, eThekwini, Mangaung, Nelson Mandela, Buffalo City, City of Cape Town, Vhembe, Ehlanzeni and Bonjanala Platinum (left vertical axis, grey bars)</u>, by epidemiological week, 2021-2023. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2 wastewater level data points are only joined if samples on sequential weeks register positive values. The top bar indicates when the change from qPCR to dPCR occurred for SARS-CoV-2 testing.

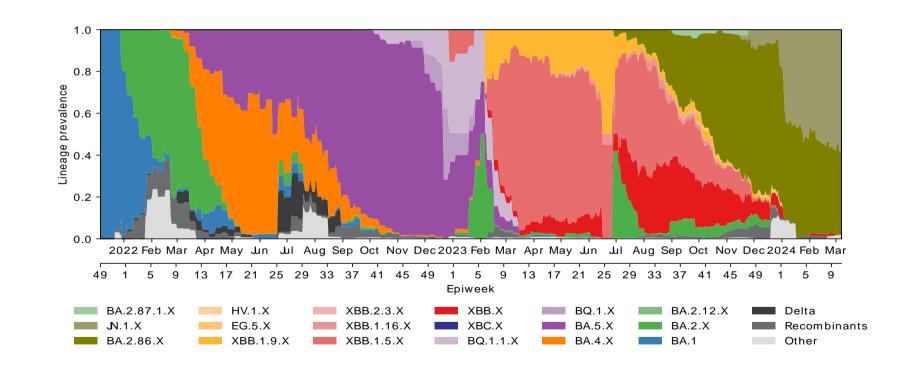




### South Africa at a glance: Circulating variants as determined by Freyja deconvolution of sequence data

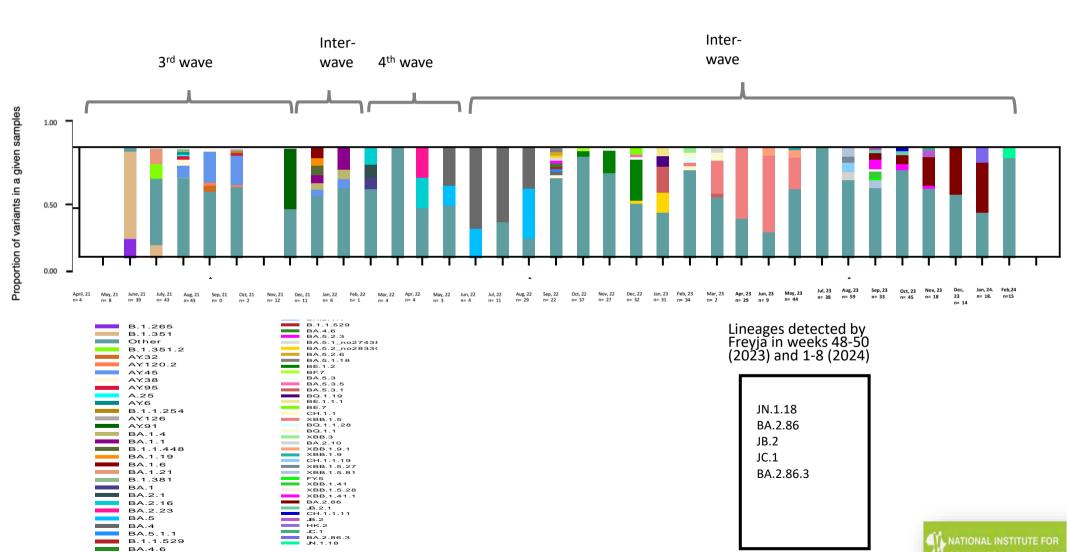
- SARS-CoV-2 variants in wastewater as determined by the 'Freyja' tool (Scripps Institute)
  - Allows determination of variants in each wastewater sample
- Results from sequencing data ending in epi week 9 (March 2024)
  - Omicron lineages
     BA.2.86.X and JN.1.X
     were circulating
     from December to
     March, with
     Recombinants and
     XBB.X sub-lineages
     circulating at low
     proportion.

Inferred variants in wastewater samples from South African wastewater treatment plants by month, between April 2021- March 2024

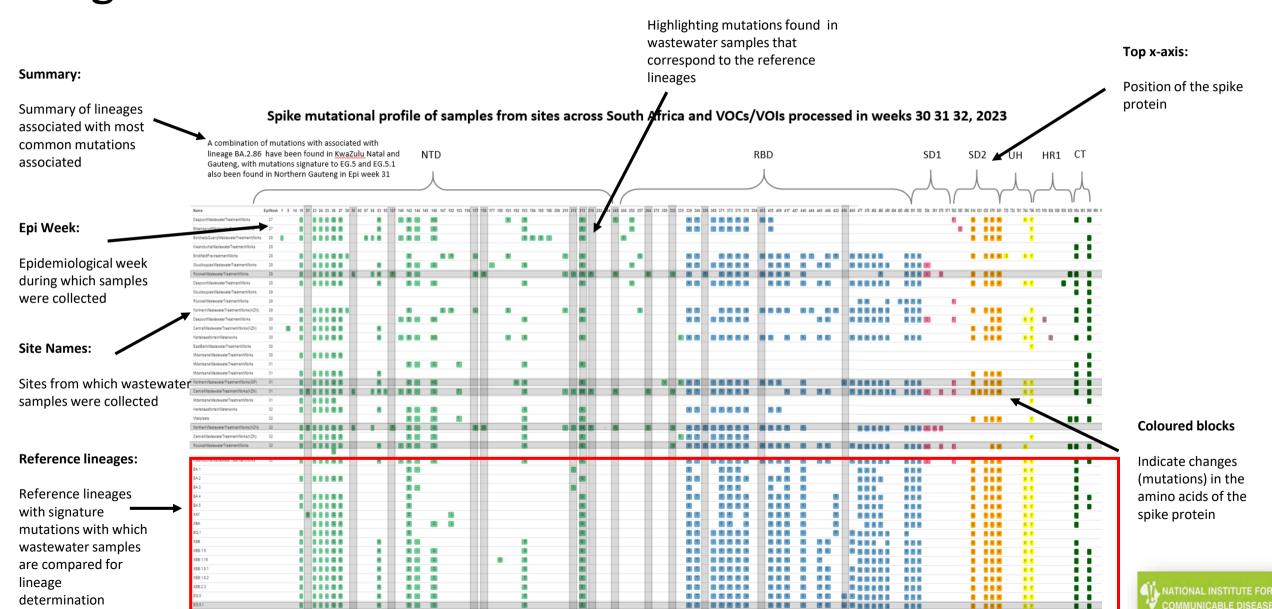


# South Africa at a glance: Circulating <u>lineages</u> as determined by Freyja deconvolution of sequence data

- Results from sequencing data ending in epi week 9 (March 2024)
  - Omicron lineages BA.2.86 and JN.1 were circulating from January to March with BA.2.86 dominance.
  - Omicron XBB sublineages were in circulation throughout.
  - The predominant lineage circulating in clinical samples in the recent week is BA.2.86 and JN.1 sublineages.



# What mutations in the spike protein tell us about the circulating lineages



#### Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 6 - 10, 2024

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A. combination of mutations associated with lineage BA.2.86, JN.1 and XBB sublineages have been found in sites across all South African provinces during Epi weeks 6-10



#### Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 42 - 46, 2023

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A. combination of mutations associated with lineage BA.2.86, JN.1 and XBB sublineages have been found in sites across all South African provinces during Epi weeks 6-10



#### Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 42 - 46, 2023

Site Name	Epi Weel	p570	p571	p572	р573 р	610 p6	611 p6	512 p61	3 p61	4 p61	L5 p61	6 p617	p650	) p651	p652	p653	3 p654	4 p655	p678	p679	p680	p681	p760	p761	p762	p763	p764	p765	p7	15	p796	p797	p79	98 r	p824	p9: 8	p939	p94	40 n	941 เ	942	p954
aspoortWastewaterTreatmentWorks	6					·			1										i i				1				i i	l '	μ,	,,,	,,,,,	pror	p, 2	,,	1	ps	F P30.	S	т Т	Δ,	, , , , , , , , , , , , , , , , , , ,	1
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orchedsQuarryWastewaterTreatmentWorks	7	٧	) Т	Т	V	L	Υ	Q	G	V	N	С	L	1	G	Α	Ε	Υ	Т	K	S	R	С	Т	Q	L	К	R	ĸ	Ý		F.	G	N	ī		F	S	Ť	Α		
ickfieldPre-treatmentWorks	7	V	) Т	Т	V	L	Y	Q	G	V	N	С	L	1	G	Α	E	Y	Т	K	S	R	С	T	Q	L	K	R	ĸ	v		F	G	N	ī		F	S	T .	Δ		. /
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orchedsQuarryWastewaterTreatmentWorks	8	٧	) Т	Т	V	L	Υ	Q	G	V	N	С	L	1	G	Α	E	Υ	Т	K	S	R	С	T	Q	L	K	R	K	Y		F	G	N	ī		F	S	Ť	A		. /
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orthernWastewaterTreatmentWorks(GP)	9	٧	) Т	Т	V	L	Υ	Q	G	V	N	С	L	1	G	Α	E	Υ	Т	K	S	R	С	T	Q	L	K	R	K	Y		F	G	N	Ī.		F	S	T	A		4
entralWastewaterTreatmentWorks(KZN)	9	٧	) Т	T	V	L	Υ	Q	G	V	N	С	L	1	G	Α	E	Υ	Т	K	S	R							K	Y		F	G	N				· -				
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ndvleitWastewaterTreatmentWorks	9	٧	) Т	T	V	L	Υ	Q	G	V	N	C	L	1	G	Α	E	Υ	T	K	s	R	С	T	Q	L	K	R	K	Y		F	G	N	ī		F	S	Ť	A		
usinaWWTW(intown)	9	٧	) Т	Т	V	L	Υ	Q	G	V	N	С	L	1	G	Α	Ε	Υ	Т	K	s	R							K	Y		F	G	N				_				· /
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oudkoppiesWastewaterTreatmentWorks	10	٧	) Т	T	V	L	Υ	Q	G	V	N	C	L	1	G	Α	E	Υ	Т	K	s	R	С	T	Q	L	K	R	K	Y		F	G	N	L		F	s	т	А		4
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entralWastewaterTreatmentWorks(KZN)	10	٧	) Т	Т	V	L	Y	Q	G	V	N	С	L	1	G	Α	E	Y	Т	K	S	R	С	T	Q	L	K	R	K	Υ		F	G	N	L		F	s	т	A		4
spoortWastewaterTreatmentWorks	10	٧	) Т	Т	V	L	Υ	Q	G	V	N	C	L	1	G	Α	E	Υ	Т	K	S	R	С	Т	Q	L	K	R	K	Υ		F	G	N	Ĺ		F	s	т	A		4
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erkwaterWastewaterTreatmentWorks	10	Α	) Т	1	V	L	Υ	R	G	Α	N	C	L	1	G	Α	Ε	н	Т	N	s	R	С	Т	Q	L	N	R	K	D		F	G	N	L		s	s	т	A		۱
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A. combination of mutations associated with lineage BA.2.86, JN.1 and XBB sublineages have been found in sites across all South African provinces during Epi weeks 6-10



### Amino acid mutations and frequency - Spike protein

XBB\* is a recombinant of BA.2.10.1 and BA.2.75 that is characterised by one or more of the following mutations in the spike protein: V83A, Y144-, H146Q, Q183E, V213E,G252V, G339H, R346T, L368I, V445P, G446S, N460K, F486S, F490S

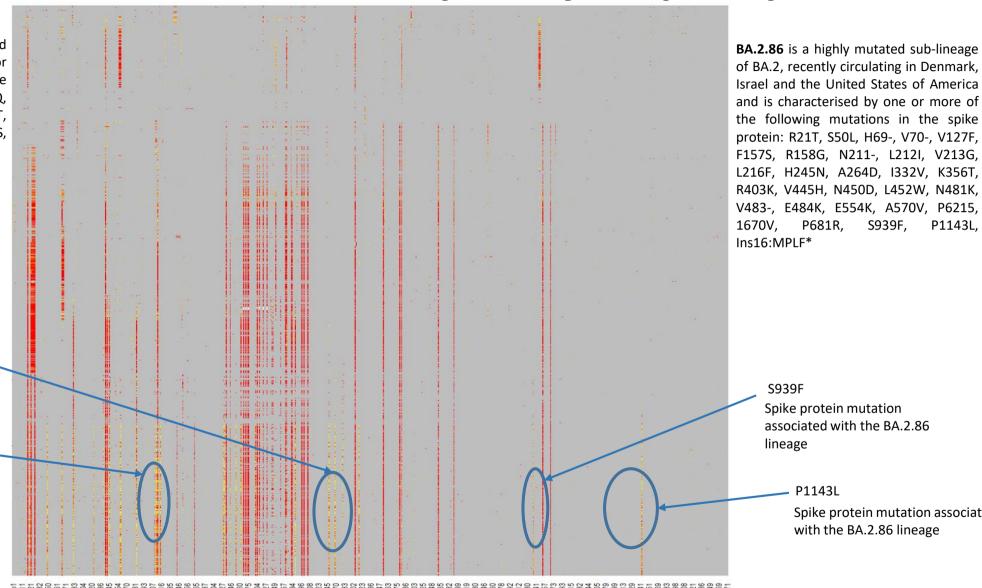
List of variants and sub-lineages of interest and

(https://www.who.int/en/activities/tracking-SARS-CoV-2-variants)

> E554K Mutations in spike protein associated with BA.2.86

> > V213E, R346T

Mutations in spike protein associated with XBB\* sublineages



Heatmap showing patterns of emerging mutations in the spike region of SARS-CoV-2, collected from April, 2021 - November, 2023. Mutations appearing in yellow have a low read frequency, those appearing in orange have a medium read frequency and those appearing in red have a high read frequency. Mutations are included and updated weekly.



P681R.

Spike protein mutation

P1143L

associated with the BA.2.86

S939F

lineage

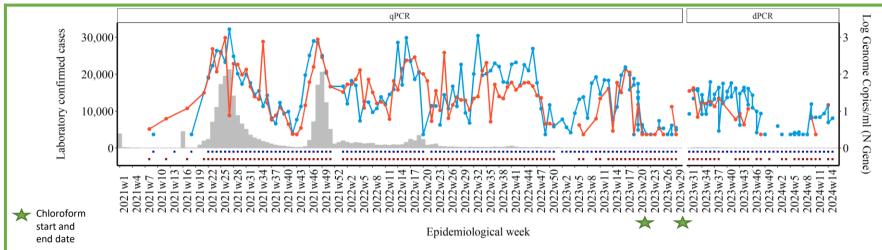
S939F.

Spike protein mutation associated

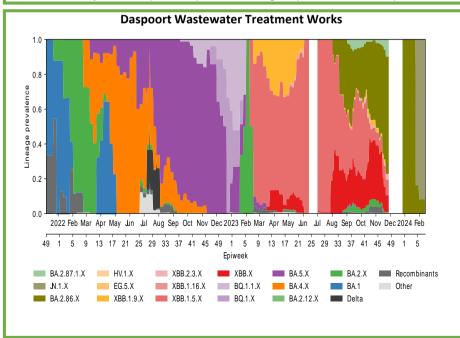
with the BA.2.86 lineage

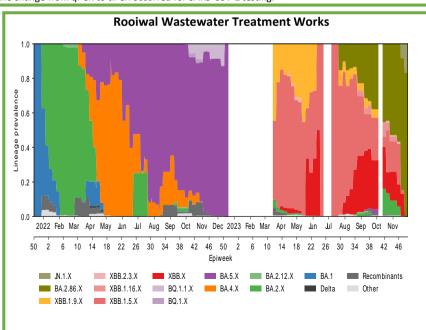
P1143L.

### Gauteng - Tshwane



■ Rooiwal Sample Collection ■ Daspoort Sample Collection ● Daspoort Wastewater Treatment Works ◆ Rooiwal Wastewater Treatment Works Wastewater levels of SARS-CoV-2 in log-transformed genome copies/ml (right vertical axis, line graph) and laboratory confirmed cases of SARS-CoV-2 for Tshwane (left vertical axis, grey bars) by epidemiological week. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2 wastewater level data points are only joined if samples on sequential weeks register positive values. The top bar indicates when the change from qPCR to dPCR occurred for SARS-CoV-2 testing.





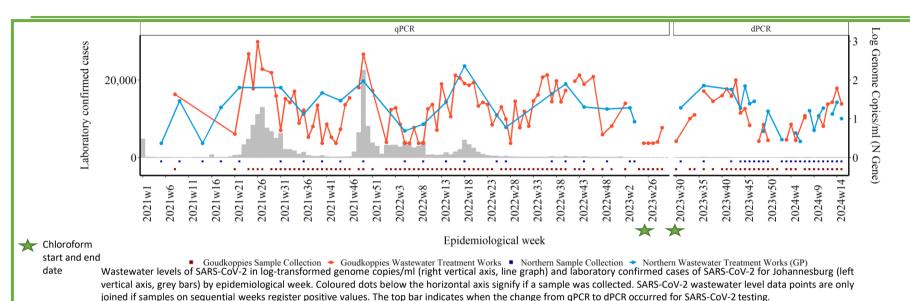
SARS-CoV-2 levels and Genomic Results in Epi week 15:

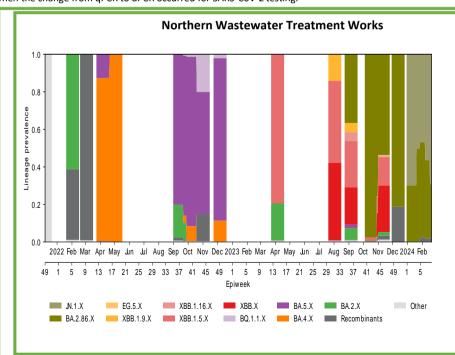
- SARS-CoV-2 levels in Daspoort WWTW decreased to low in Epi week 4, rose to moderate in Epi week 10 and dropped back to low in Epi week 11. Levels remain low in Epi week 13 and increase to moderate in Epi week 14 and decrease to low again in Epi week 15.
- SARS-CoV-2 levels in Rooiwal WWTW have decreased in Epi week 47. Levels remain low in Epi week 11 and increase to moderate in Epi week 14. No new results for Epi week 15 are available.
- \* Sequencing data ending in Epi week 5 in Daspoort and 46 in Rooiwal.
  - Omicron lineages JN.1.X and BA.2.86.X, XBB.1.19.X and JN.1.X were circulating in Daspoort during Epi week 5.
  - BA.2.86.X, XBB.1.5.X, XBB.2.3.X and JN.1.X was also the dominant lineage detected in Rooiwal during Epi week 42-46

#### **SNP Analysis:**

 A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 and JN.1 were found in both Daspoort and Rooiwal.

### Gauteng - Johannesburg





SARS-CoV-2 levels and Genomic Results in Epi week 15:

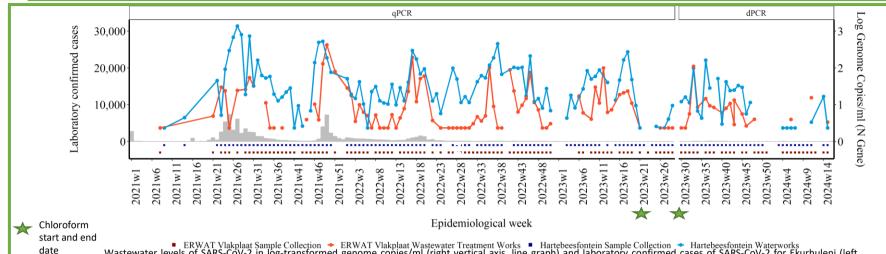
- SARS-CoV-2 levels in Goudkoppies WWTW increased from Epi week 5 to Epi week 7, but levels remain low. Levels increase to moderate in Epi week 12. Levels decreased in Epi week 15 but remain moderate.
- In Northern WWTW, SARS-CoV-2 levels increased to moderate in week 8 from low levels seen in week 6. Levels decreased to low in Epi week 9, followed by an increase to moderate in Epi week 10. Levels remain moderate in Epi week 14. Levels decrease to low in Epi week 15.
- \* Sequencing data ending in Epi week 9 in Goudkoppies and 5 in Northern Wastewater Treatment Works.
  - JN.1.X and BA.2.8.X lineages were dominating during epiweek 1 - 9 in Goudkoppies.
  - During Epi week 1 5 BA.2.86.X, JN.1.X lineages were circulating in Northern (GP). In Epi week 5, Recombinants were also observed.

#### SNP Analysis:

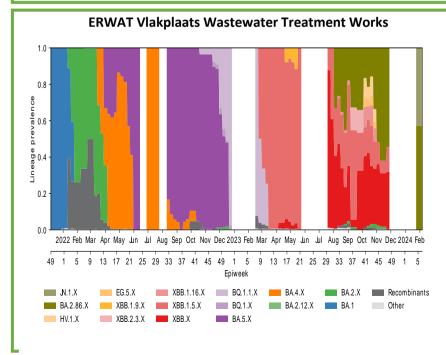
- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in Goudkoppies.
- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 and JN.1were found in Northern Johannesburg.

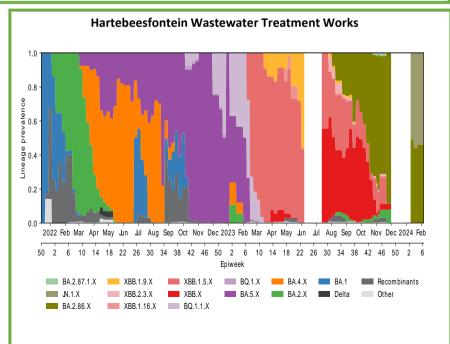


### Gauteng - Ekurhuleni



ERWAT Vlakplaat Sample Collection ERWAT Vlakplaat Wastewater Treatment Works Hartebeesfontein Sample Collection Hartebeesfontein Waterworks Wastewater levels of SARS-CoV-2 in log-transformed genome copies/ml (right vertical axis, line graph) and laboratory confirmed cases of SARS-CoV-2 for Ekurhuleni (left vertical axis, grey bars) by epidemiological week. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2 wastewater level data points are only joined if samples on sequential weeks register positive values. The top bar indicates when the change from qPCR to dPCR occurred for SARS-CoV-2 testing.





SARS-CoV-2 levels and Genomic Results in Epi week 15:

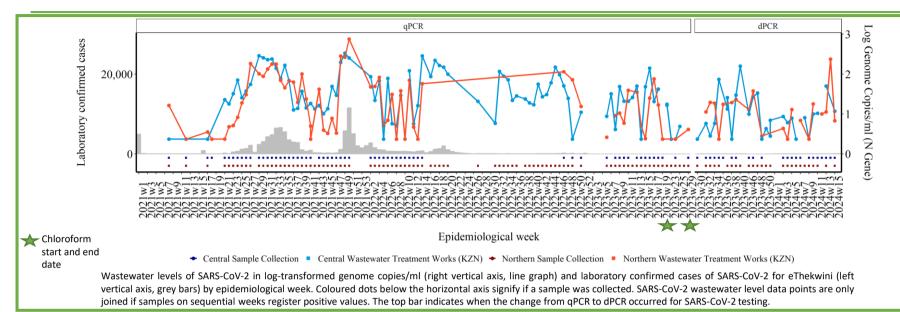
- The SARS-CoV-2 levels in Hartebeesfontein WWTW decreased in Epi weeks 4 and remain low in Epi week 7. Levels remain low in Epi week 11. Levels increase to moderate in Epi week 14. Levels decrease to low in Epi week 15.
- As of Epi week 43, there was a slight increase in SARS-CoV-2 levels in Vlakplaats WWTW, after a decrease from Epi week 35. Levels remain low in Epi week 6. Levels rise to moderate in Epi week 11. Levels decrease to low in Epi week 15.
- \* Sequencing data ending in Epi week 5 in Vlakplaats and 6 in Hartebeesfontein.
  - Omicron lineages BA.2.86.X and JN.1.X were circulating in Vlakplaats during Epi week 5.
  - Lineages BA.2.86 and JN.1.X were predominantly circulating during Epi week 6 at the Hartebeesfontein water treatment plant.

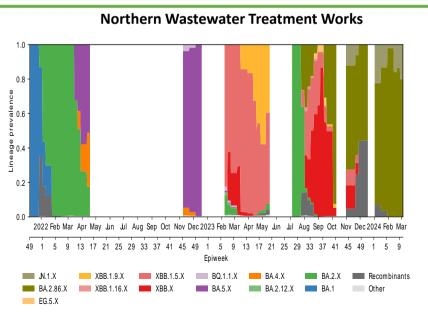
#### SNP Analysis:

 A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 and JN.1were found in both Ekurhuleni treatment plants.



### KwaZulu-Natal - eThekwini





SARS-CoV-2 levels and Genomic Results in Epi week 15:

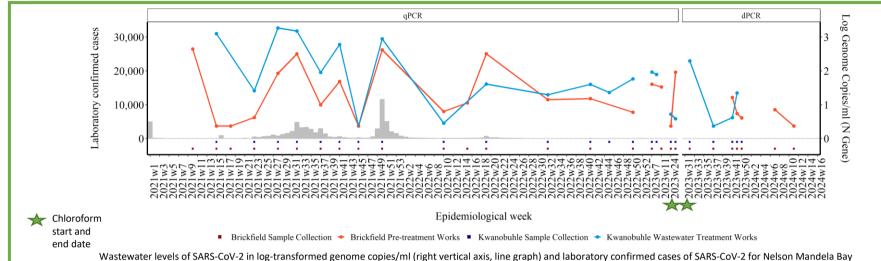
- SARS-CoV-2 levels in Central WWTW in Epi week 9 decrease from Epi week 8, as levels remain low. Levels increase in Epi week 10 but remain low. Levels increase to moderate in Epi week 13. Levels decreased to low in Epi week 15.
- SARS-CoV-2 levels increased in Epi week 5 in Northern WWTW. Levels decrease in Epi week 7, as remain low in Epi week 9. Levels increase to moderate in Epi week 10 and remain moderate in Epi week 13. Levels increase to high in Epi week 14. Levels decrease to low in Epi week 15.
- \* Sequencing data ending in Epi week 9 in Central eThekwini and in eThekwini North.
  - During Epiweek 1 9 Lineage BA.2.86.X, JN.1.X were dominantly circulating in eThekwini Central. XBB.X, XBB.1.9.X and Recombinants were also circulating in the recent weeks.
  - In eThekwini North, BA.2.86.X lineages were dominating. In circulation there were also lineages JN.1.X and Recombinant lineages during Epi week 1 - 9.

#### SNP Analysis:

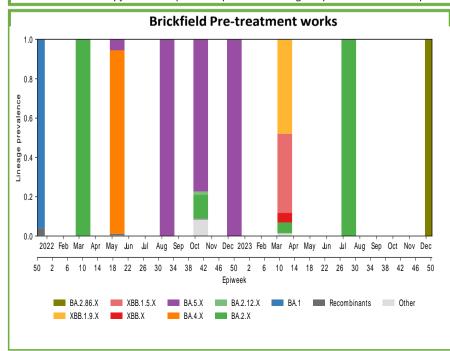
 A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 and JN.1were found in both eThekwini wastewater treatment plants.

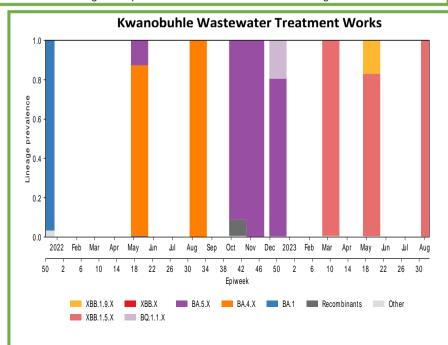


### Eastern Cape – Nelson Mandela



Wastewater levels of SARS-CoV-2 in log-transformed genome copies/ml (right vertical axis, line graph) and laboratory confirmed cases of SARS-CoV-2 for Nelson Mandela Bay (left vertical axis, grey bars) by epidemiological week. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2 wastewater level data points are only joined if samples on sequential weeks register positive values. The top bar indicates when the change from gPCR to dPCR occurred for SARS-CoV-2 testing.





SARS-CoV-2 levels and Genomic Results in Epi week 15:

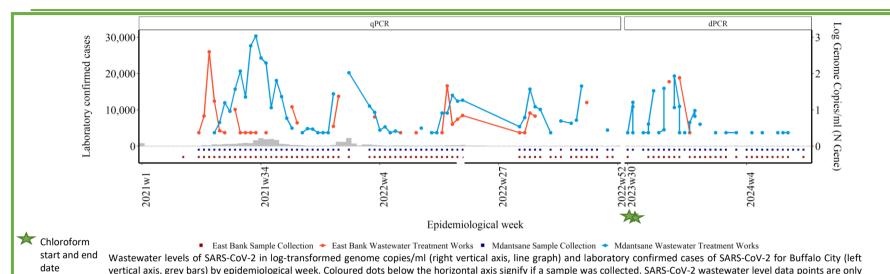
- As of Epi week 46, SARS-CoV-2 levels are moderate after an increase was observed after Epi week 36 in the Kwanobuhle WWTW. No new results are available for Epi week 15.
- SARS-CoV-2 levels decreased from moderate to low from Epi week 43 to Epi week 46 in Brickfield Pre-treatment works. There was a slight increase in level in Epi week 7, but levels remain low. Levels decrease and remain low in Epi week 11. No new results are available for Epi week 15.
- \* Sequencing data ending in Epi week 50 in Brickfield and 30 in Kwanobuhle.
  - BA.2.86.X lineage was dominating during Epi week 50 in Mdantsane
  - Omicron lineages XBB.1.5.X sub-lineages were circulating in Kwanobuhle during Epi week 30.

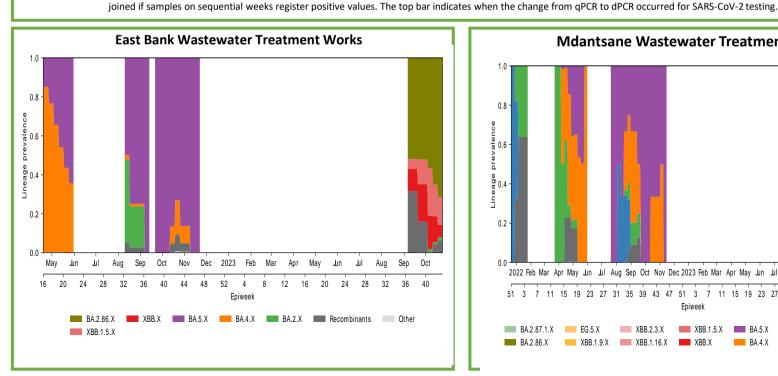
#### SNP Analysis:

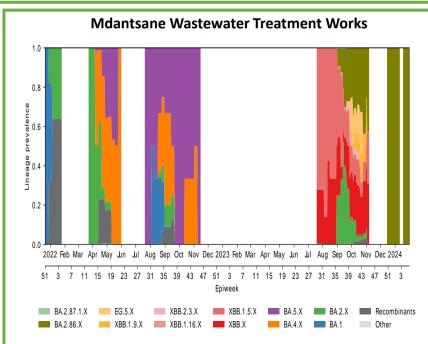
- SNP analysis could not be performed as the SARS-CoV-2 sequencing coverage in the Brickfield samples collected during Epi weeks 30-39 were too low for meaningful interpretation.
- A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in the Kwanobuhle wastewater treatment plants.



### Eastern Cape – Buffalo City







SARS-CoV-2 levels and Genomic Results in Epi week 15:

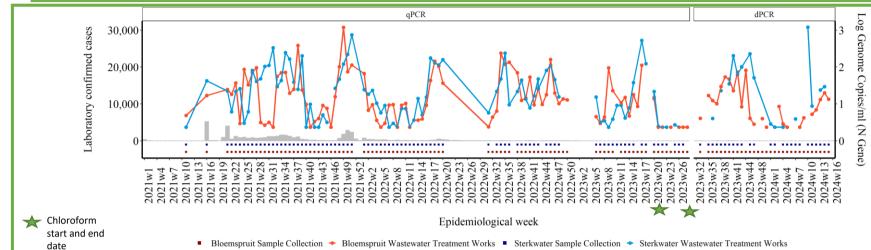
- In Epi week 46, SARS-CoV-2 levels in Mdantsane WWTW decreased and levels are low. Levels in Epi week 12 remain low. No new results for Epi week 15 are available.
- SARS-CoV-2 levels in East Bank WWTW in Epi week 44 remain low after decrease was observed from Epi week 38. No new results for Epi week 15 are available.
- \* Sequencing data ending in Epi week 40 in Eastbank and Epiweek 3 in Mdantsane.
  - Omicron lineages BA.2.86.X, XBB.1.5.X, XBB.X and Recombinantswere circulating in Eastbank during Epi week 40.
  - Lineage BA.2.86.X was dominating in Mdantsane during Epi week 3.

#### SNP Analysis:

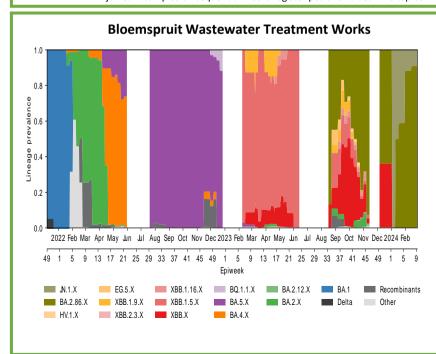
A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Eastbank and Mdantsane.

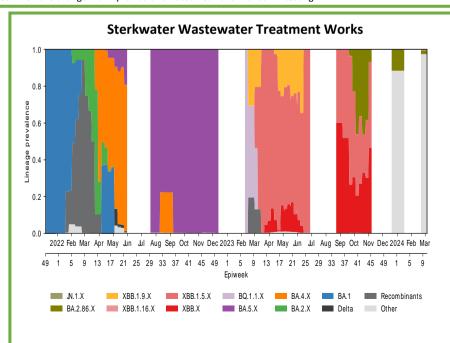


### Free State – Mangaung



■ Bloemspruit Sample Collection ◆ Bloemspruit Wastewater Treatment Works ■ Sterkwater Sample Collection ◆ Sterkwater Wastewater Treatment Works Wastewater levels of SARS-CoV-2 in log-transformed genome copies/ml (right vertical axis, line graph) and laboratory confirmed cases of SARS-CoV-2 for Manguang (left vertical axis, grey bars) by epidemiological week. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2 wastewater level data points are only joined if samples on sequential weeks register positive values. The top bar indicates when the change from gPCR to dPCR occurred for SARS-CoV-2 testing.





SARS-CoV-2 levels and Genomic Results in Epi week 15:

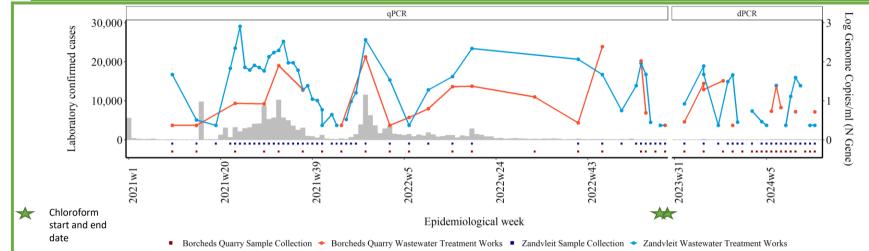
- In Bloemspruit WWTW, a 2-fold increase in SARS-CoV-2 levels were seen in Epi week 44. In Epi week 4 levels decreased and remain low in Epi week 9. Levels increase to moderate in Epi week 13 and remain moderate in Epi week 15.
- A decrease in SARS-CoV-2 levels was seen in Sterkwater WWTW in Epi week 52 and levels remain low in Epi week 4. There is a slight increase in levels in Epi week 7 but levels remain low. Levels in Epi week 10 increase to high (above 3 genome copies/ml). Levels drop to low in Epi week 11 and increase to moderate in Epi week 13 and remain moderate in Epi week 14. No new results for Epi week 15 are available.
- \* Sequencing data ending in Epi week 9 in Bloemspruit and Sterkwater Treatment Works.
  - BA.2.86.X, JN..1.X and XBB.X sub-lineages were the dominant lineage circulating in Bloemspruit during Epi week 1 to 9.
  - JN.1.X and BA.2.86.X lineages were predominantly circulating in Epi week 9 in Bloemspruit
  - BA.2.86.X (at low proportions) and Other Omicron lineages were circulating in Sterkwater in epiweek 9.

#### SNP Analysis:

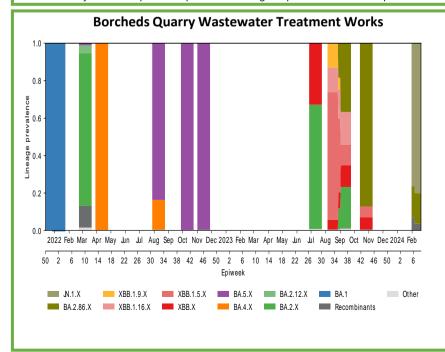
A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 were found in both Bloemspruit and Sterkwater.

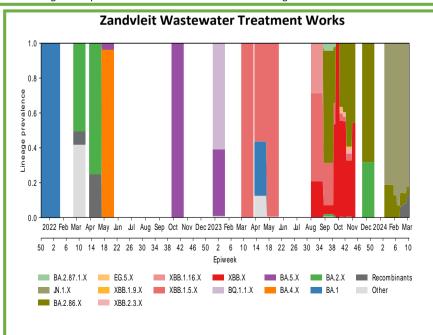


### Western Cape – City of Cape Town



Wastewater levels of SARS-CoV-2 in log-transformed genome copies/ml (right vertical axis, line graph) and laboratory confirmed cases of SARS-CoV-2 for Cape Town (left vertical axis, grey bars) by epidemiological week. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2 wastewater level data points are only joined if samples on sequential weeks register positive values. The top bar indicates when the change from gPCR to dPCR occurred for SARS-CoV-2 testing.





SARS-CoV-2 levels and Genomic Results in Epi week 15:

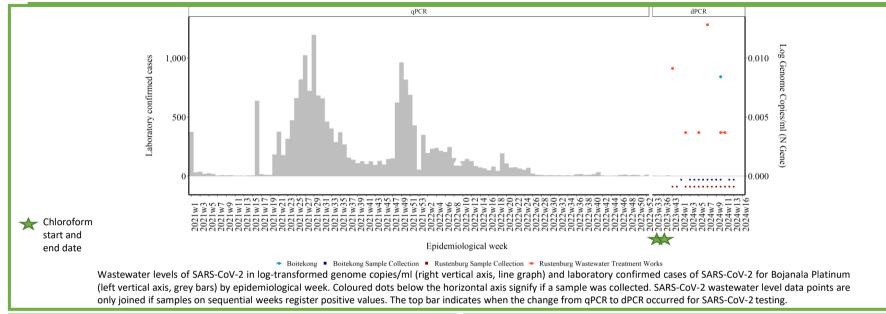
- After a sharp increase in SARS-CoV-2 levels was seen in Epi week 37, a subsequent decrease in SARS-CoV-2 levels in Borcheds Quarry WWTW was seen. Levels are low in Epi week 6 and increase to moderate in Epi week 7. Levels decrease in Epi week 8 to low and remain low in Epi week 15.
- In Epi week 4, SARS-CoV-2 levels were low at Zandvleit WWTW. Levels decrease slightly in Epi week 5. In Epi week 7 levels increase to moderate. Levels drop to low in Epi week 9 and increase to moderate in Epi week 11. Levels remain moderate in Epi week 12 and decrease to low in Epi week 14 and remain low in Epi week 15.
- \* Sequencing data ending in Epi week 6 in Borcheds Quarry and Epi week 10 in Zandvleit.
  - During Epi week 6, JN.1.X sub-lineages were dominantly circulating in Borcheds. BA.2.86.X and Recombinants were also detected.
  - During Epi week 2 6, lineages BA.2.86.X and JN.1.X were circulating in Zandvlet, with JN.1.X sub-lineages being dominant.
  - In epi week 10 BA.2.86,X, JN.1.X and Recombinants lineages were circulating in Zandvlet, with JN.1.X lineages at highest proportion

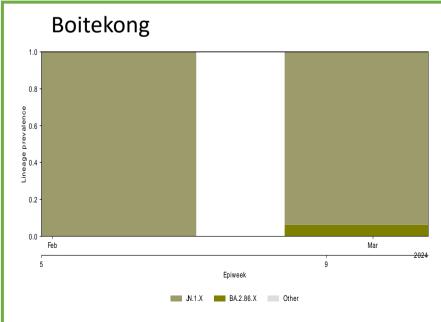
#### **SNP Analysis:**

 A combination of mutations (V127F, L212I, V213G, L216F, H245N, A264D, I332V, K356T) associated with lineage BA.2.86 and JN.1 were found in both Borcheds Quarry and Zandevleit.



### North West – Bojanala Platinum





#### Rustenburg

\*No sequencing data currently available

- \* SARS-CoV-2 wastewater sample collection and testing at Rustenburg WWTW began in Epi week 43, 2023.
- \* SARS-CoV-2 wastewater sample collection and testing at Boitekong began in Epi week 1, 2024.

SARS-CoV-2 levels and Genomic Results in Epi week 15:

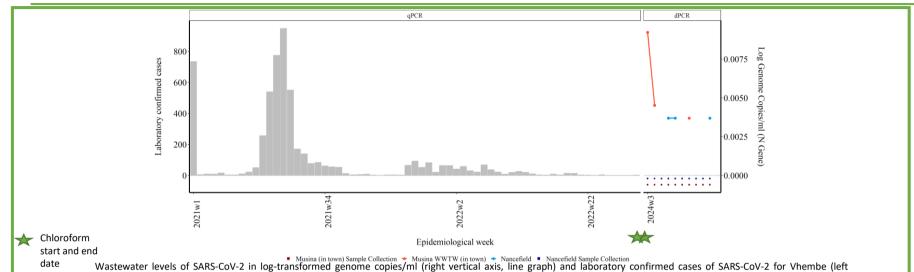
- At Rustenburg WWTW levels from Epi week 43 remain below 0.015 log genome copies/ml and remain low up until Epi week 11, 2024. No new results available for Epi week 15.
- At Boitekong wastewater results for SARS-CoV-2 were low in Epi week 10, 2024. No new results available for Epi week 15.
- \* Sequencing data ending in Epi week 9 in Boitekong

SARS-CoV-2 levels and Genomic Results in Epi week 13:

 At Boitekong, JN.1.X sub-lineages were dominantly circulating in Epi week 5 and 9. In Epi week 9, BA.2.86.X was also circulating at low proportions.



### Limpopo – Vhembe



- vertical axis, grey bars) by epidemiological week. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2 wastewater level data points are only joined if samples on sequential weeks register positive values. The top bar indicates when the change from qPCR to dPCR occurred for SARS-CoV-2 testing.

### Nancefield

\*No sequencing data currently available

- \* SARS-CoV-2 wastewater sample collection and testing at Musina WWTW began in Epi week 3, 2024.
- \* SARS-CoV-2 wastewater sample collection and testing at Nancefield began in Epi week 3, 2024.

SARS-CoV-2 levels and Genomic Results in Epi week 15:

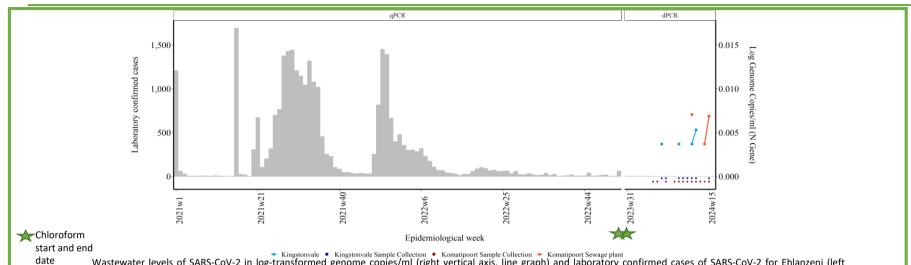
- At Musina WWTW levels from Epi week 3 remain below 0.01 log genome copies/ml and remain low up until Epi week 4, 2024. Levels remain low in Epi week 9. No new results for Epi week 15 are available.
- At Nancefield levels were low in Epi weeks 6 and 7, below 0.005 log genome copies/ml. They remain low in Epi week 12. No new results for Epi week 15 are available.
- \* Sequencing data ending in Epiweek 13 in Musina

SARS-CoV-2 levels and Genomic Results in Epi week 13:

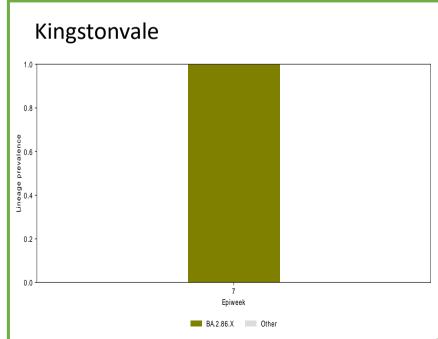
 Sequencing in Musina began in Epiweek 3, and BA.2.86.X sub-lineages were found to be the main circulating lineages. In Epi week 9 BA.2.86.X sub-lineages were still the dominant lineages detected in Musina WWTW



### Mpumalanga – Ehlanzeni



\* Kingstonvale \* Kingstonvale Sample Collection \* Komatipoort Sample Collection \* Komatipoort Sewage plant
Wastewater levels of SARS-CoV-2 in log-transformed genome copies/ml (right vertical axis, line graph) and laboratory confirmed cases of SARS-CoV-2 for Ehlanzeni (left vertical axis, grey bars) by epidemiological week. Coloured dots below the horizontal axis signify if a sample was collected. SARS-CoV-2 wastewater level data points are only joined if samples on sequential weeks register positive values. The top bar indicates when the change from qPCR to dPCR occurred for SARS-CoV-2 testing.



#### Komatipoort

\*No sequencing data currently available

- \* SARS-CoV-2 wastewater sample collection and testing at Komatipoort WWTW began in Epi week 2, 2024.
- \* SARS-CoV-2 wastewater sample collection and testing at Kingstonvale began in Epi week 4, 2024.

SARS-CoV-2 levels and Genomic Results in Epi week 15:

- At Komatipoort samples collected between Epi week 2 and Epi week 10 results were negative for SARS-CoV-2. Levels were low in Epi week 11 and remain low in Epi week 15.
- At Kingstonvale SARS-CoV-2 levels remain below 0.01 log genome copies/ml from Epi week 4 to Epi week 8. Levels remain low in Epi week 12. No new results for Epi week 15 are available.
- \* Sequencing data ending in Epi week 13 in Kingstonvale
- No lineages detected in Kingstonvale during Epiweek 2 6 due to low coverage and negative results. In Epiweek 7, BA.2.86.X was detected.



COLLABORATORS TEAM

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#### **FUNDERS**





BILL & MELINDA GATES foundation



