



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

22 January 2024

Sample collection dates up to 15 December 2023
(Epidemiological week 50)

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

Wastewater levels

Epidemiological weeks 33-50

- From weeks 48-50 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 33-50 **increases and/or higher levels have been seen in Gauteng (Hartebeesfontein WWTW and Northern WWTW) and Free State (Sterkwater WWTW).**
- 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.
<https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/weekly-respiratory-pathogens-surveillance-report-week/>

Wastewater genomics

Epidemiological weeks 39-45

- No new sequencing results are available
- Genomics results were obtained for weeks 39-45 for the heat map and mutational profile.
- **Omicron lineage XBB.1.41.1 followed by BA.2.86, JB.2, JB.2.1 and XBB sub-lineages** were the dominant lineages circulating in wastewater samples between October and November 2023
- In clinical samples, **BA.2.86** was also the dominant lineage circulating throughout October 2023, followed by **XBB.1.5, XBB.1.16** and **XBB.1.5.81**.
- The **Omicron lineage BA.2.86** is circulating in KwaZulu-Natal in eThekweni (in the catchments of Northern and Central WWTWs), and 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). 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), and Free State, in Mangaung (catchments Bloemspruit and Sterkwater WWTWs).

Interpretation: Ongoing transmission of SARS-CoV-2 due to Omicron lineages including the new lineage BA.2.86.

Wastewater-based Epidemiology for COVID-19

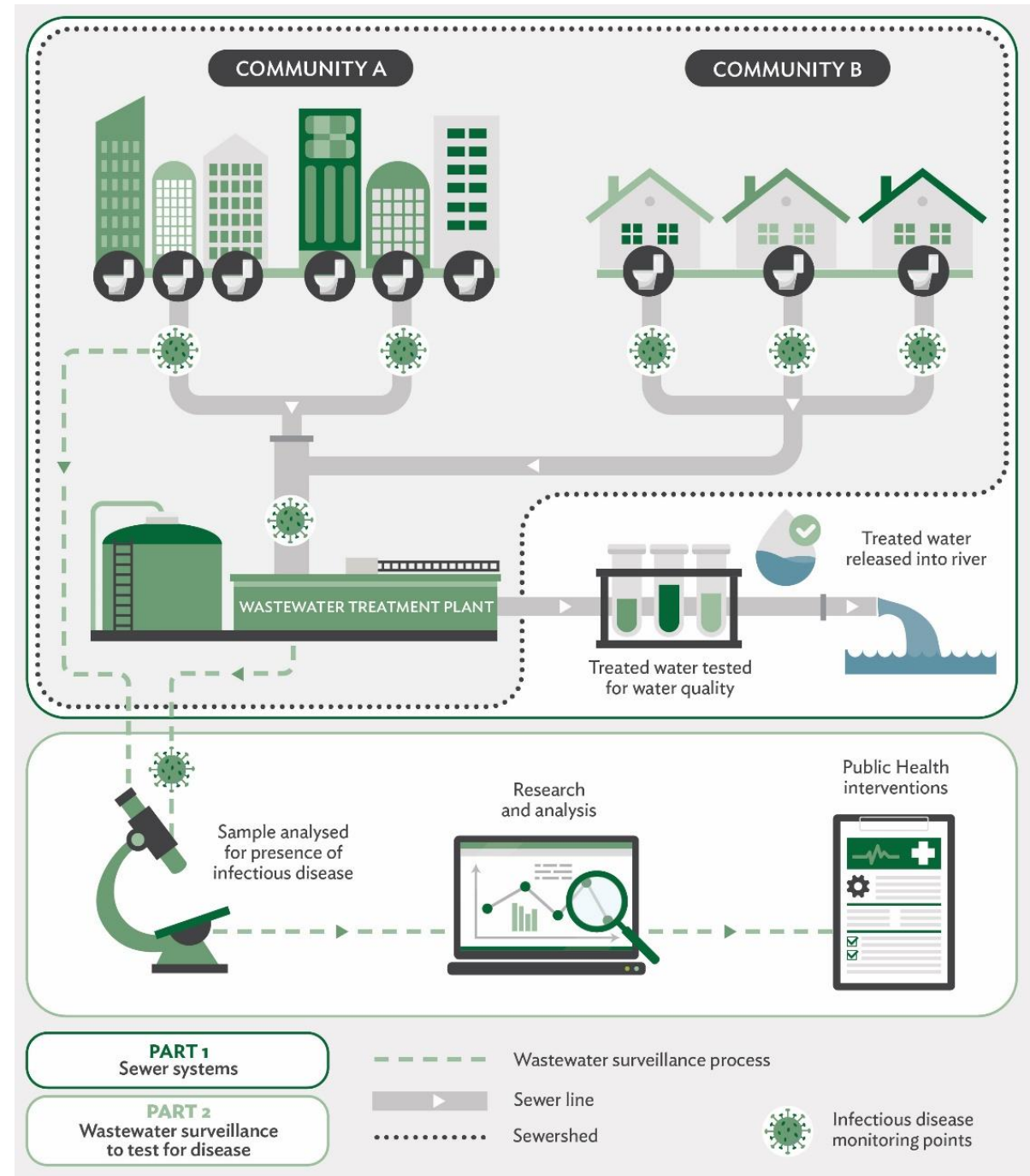
How is wastewater tested for SARS-CoV-2?

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

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?

Left vertical axis:

Number of lab confirmed cases in *the metro or district where the water treatment plant is located*

Bars:

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

Coloured lines:

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

Coloured squares:

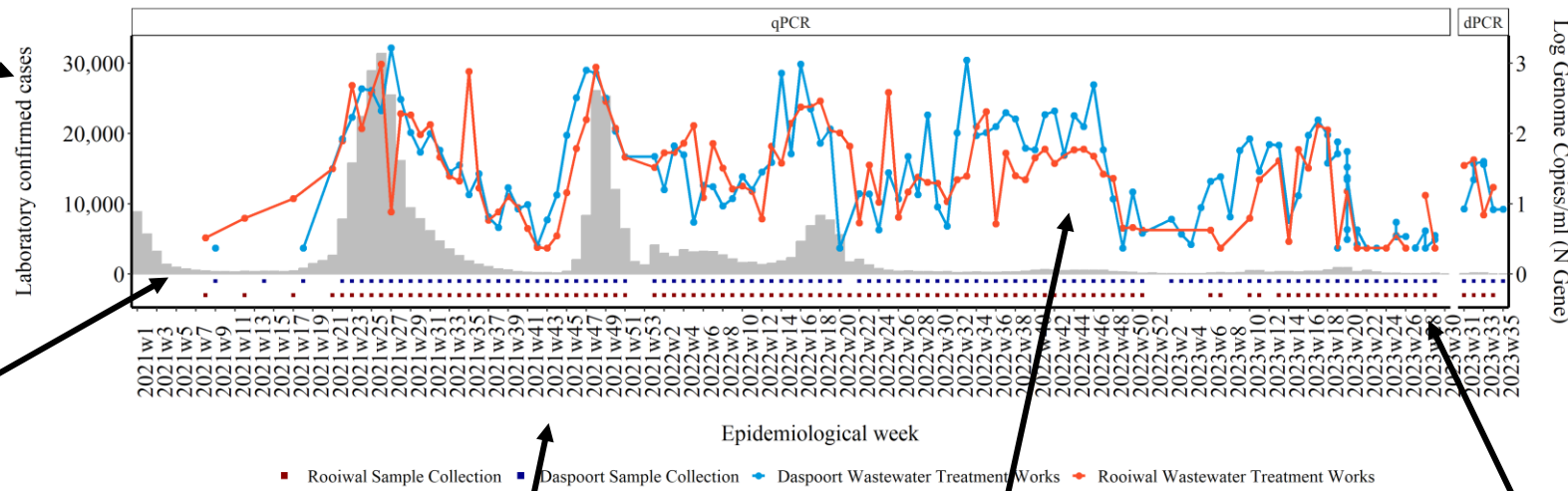
Epi weeks during which samples were collected

Facets:

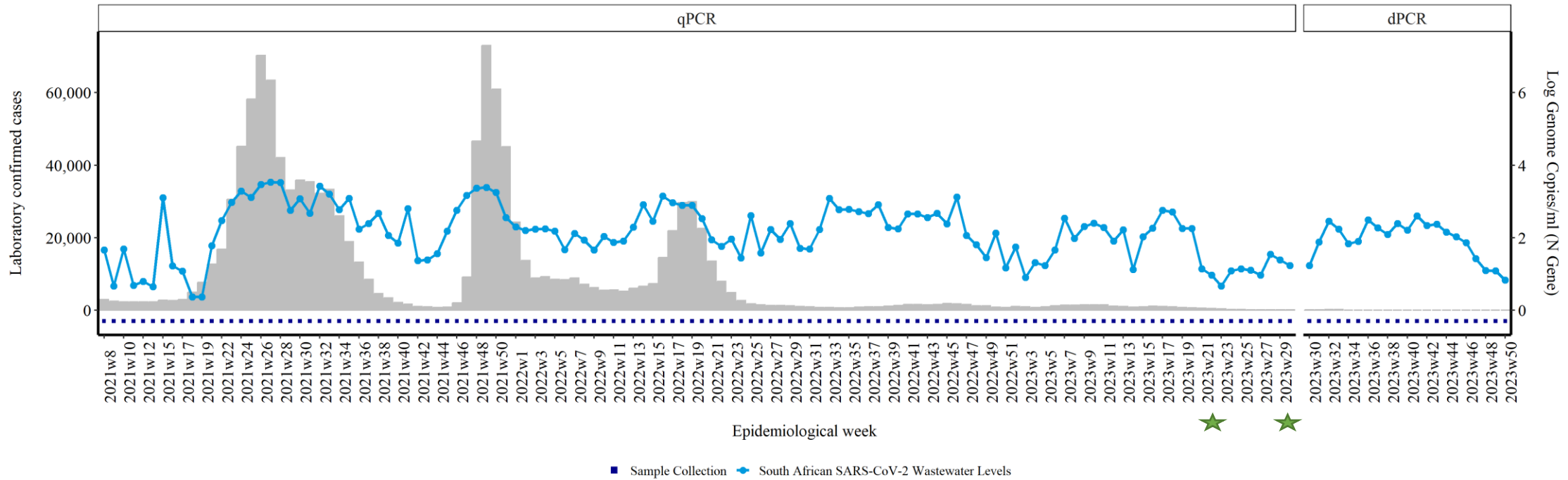
Indicates the platform used to test samples

Right vertical axis:

Log (ie 10 to the power x) copies of SARS-CoV-2 genome per ml of wastewater. So $\log 2=10^2=100$ copies per millilitre, $\log 3=10^3=1000$ copies per millilitre



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



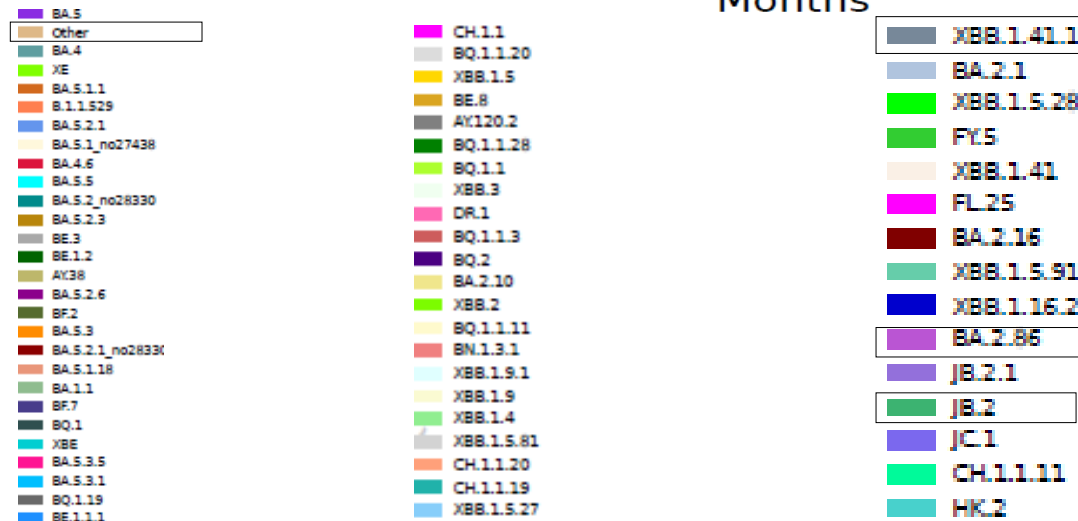
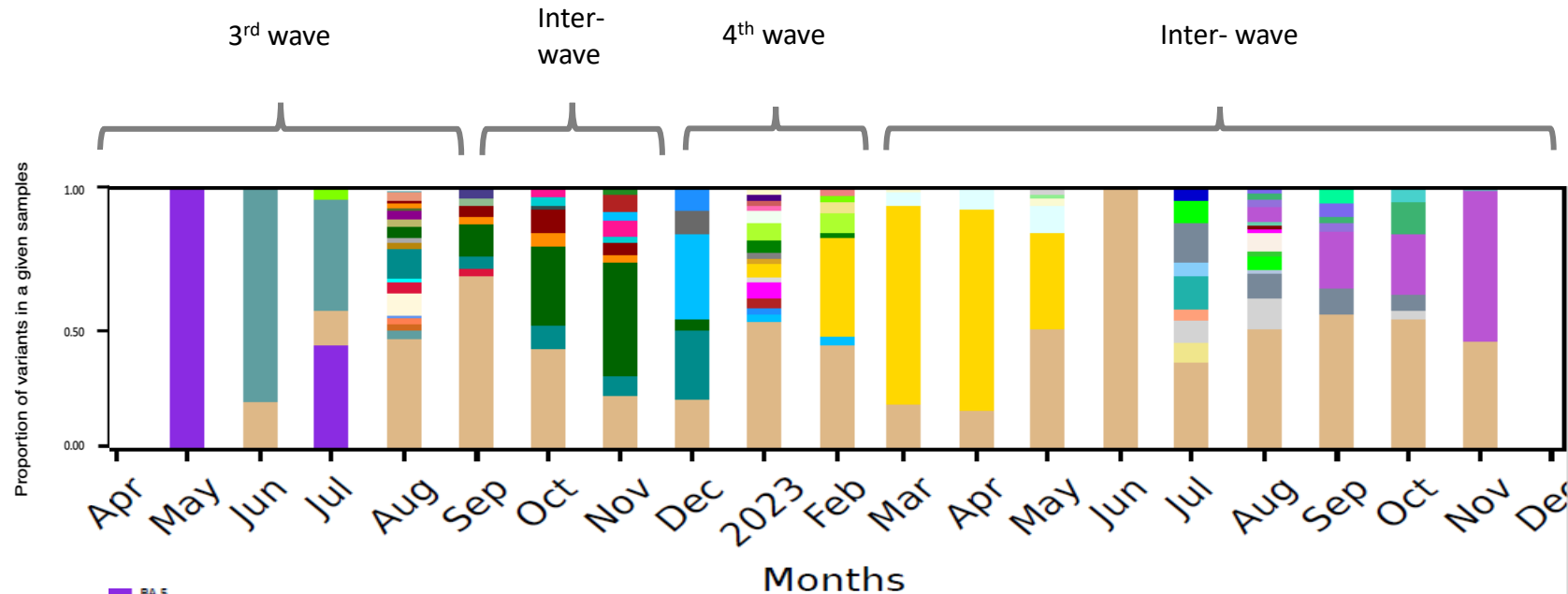
★ Chloroform start and end date

Changes in levels of SARS-Cov-2 (line graph) in in-flowing untreated wastewater from plants tested by NICD, compared with laboratory-confirmed cases from Tshwane, Johannesburg, Ekurhuleni, eThekweni, Mangaung, Nelson Mandela, Buffalo City, and City of Cape Town (grey bars), by epidemiological week, 2021-2023.

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

- Results from sequencing data ending in epi week 45 (10 November 2023)

- Omicron lineages XBB.1.41.1 and BA.2.86 were circulating from October to November with BA.2.86 dominance in November.
- Omicron XBB sub-lineages were in circulation throughout October.
- The predominant lineage circulating in clinical samples in the recent week is BA.2.86.



Lineages detected by Freyja in weeks 40-45:

XBB.1.41.1
JB.2
BA.2.86

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

Summary:

Summary of lineages associated with most common mutations associated

Epi Week:

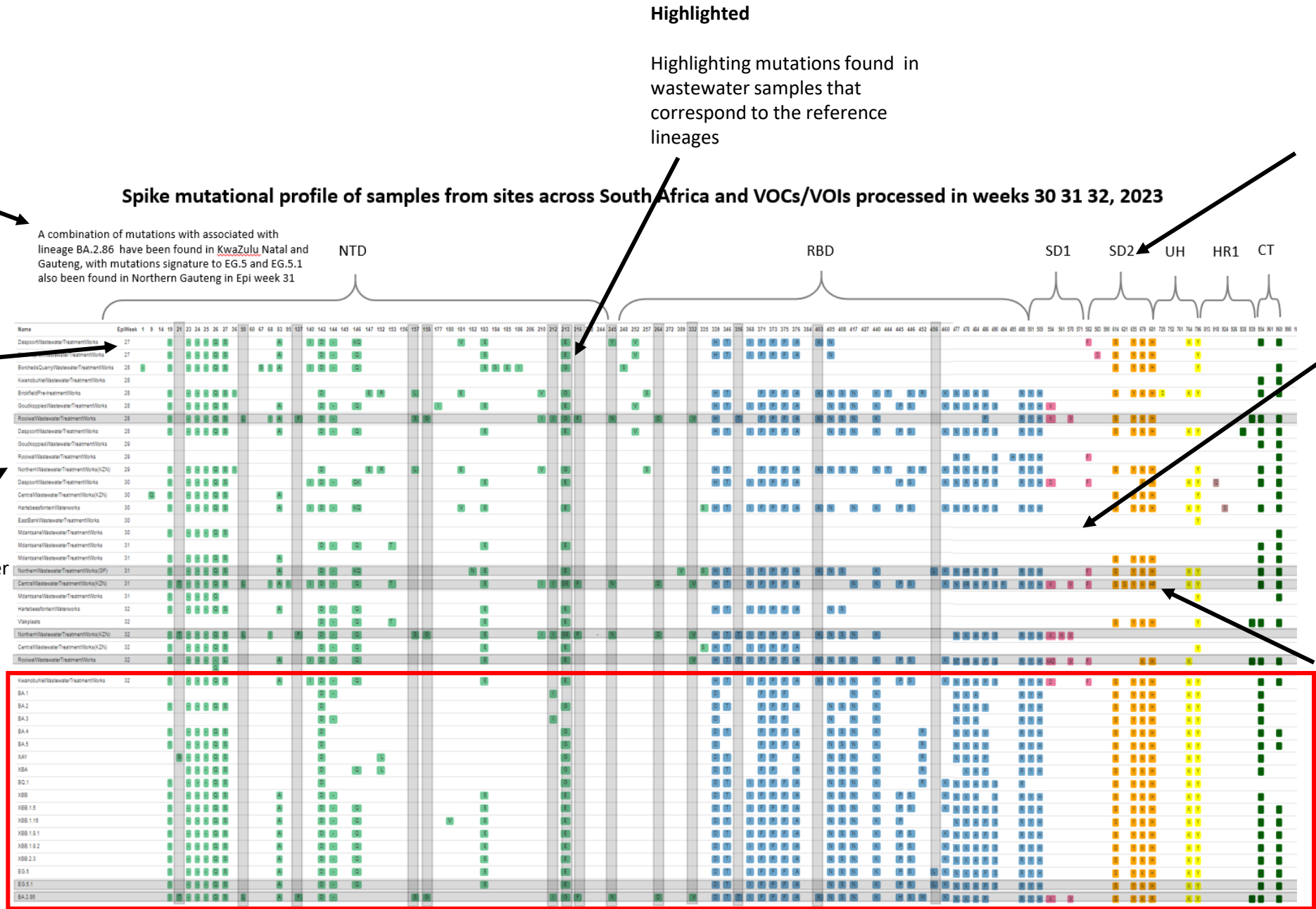
Epidemiological week during which samples were collected

Site Names:

Sites from which wastewater samples were collected

Reference lineages:

Reference lineages with signature mutations with which wastewater samples are compared for lineage determination



Spike mutational profile of samples from sites across South Africa and VOCs/VOIs processed in weeks 33 - 39, 2023

A combination of mutations associated with lineage BA.2.86 and XBB sublineages have been found in sites across all South African provinces during Epi weeks 37-45.

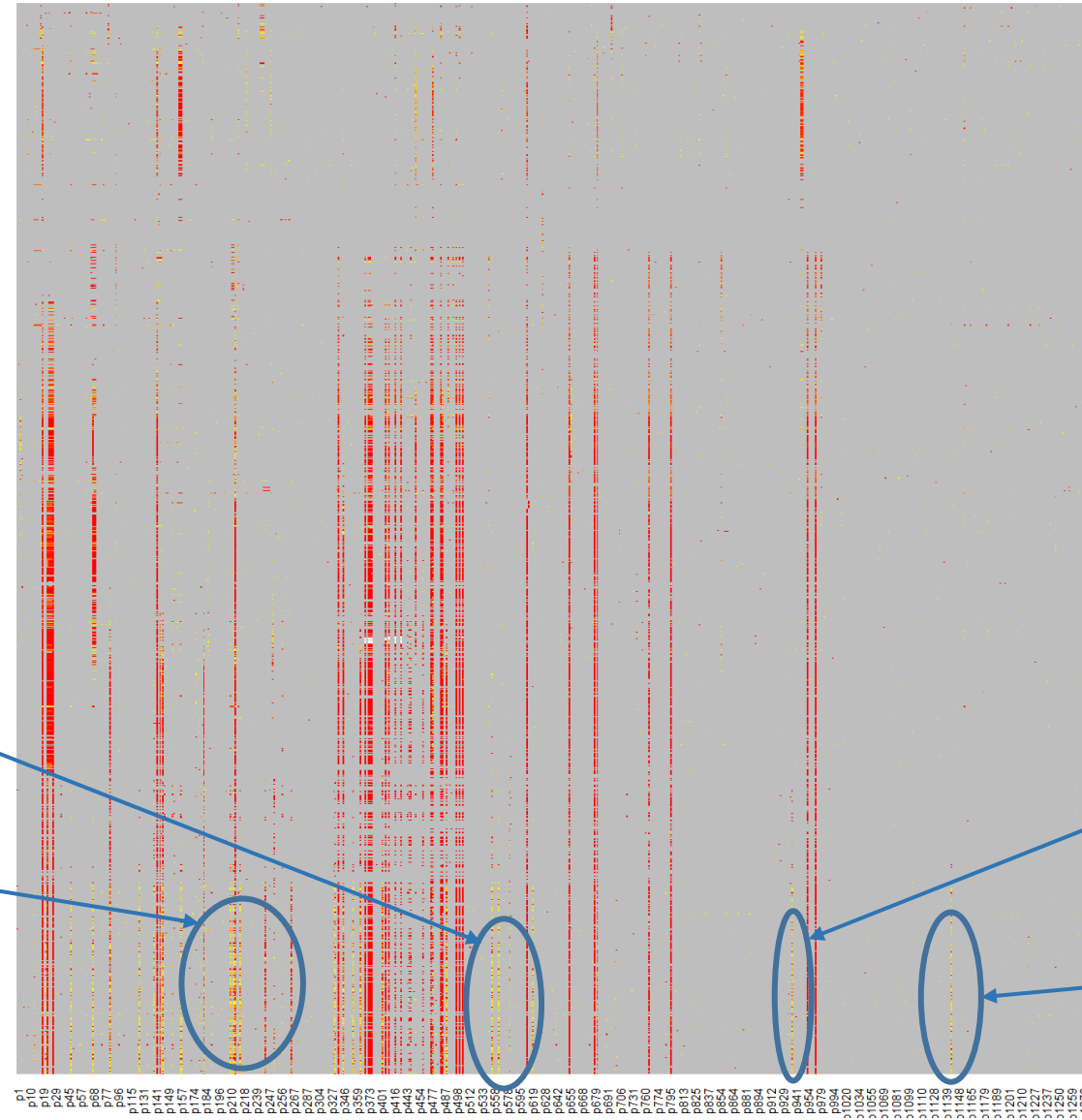


Amino acid mutations and frequency – Spike protein

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 concern
<https://www.who.int/en/activities/tracking-SARS-CoV-2-variants>

BA.2.86 is a highly mutated sub-lineage of BA.2, recently circulating in Denmark, Israel and the United States of America and is characterised by one or more of the following mutations in the spike protein: R21T, S50L, H69-, V70-, V127F, F157S, R158G, N211-, L212I, V213G, L216F, H245N, A264D, I332V, K356T, R403K, V445H, N450D, L452W, N481K, V483-, E484K, E554K, A570V, P621S, 1670V, P681R, S939F, P1143L, Ins16:MPLF*



E554K
 Mutations in spike protein associated with BA.2.86

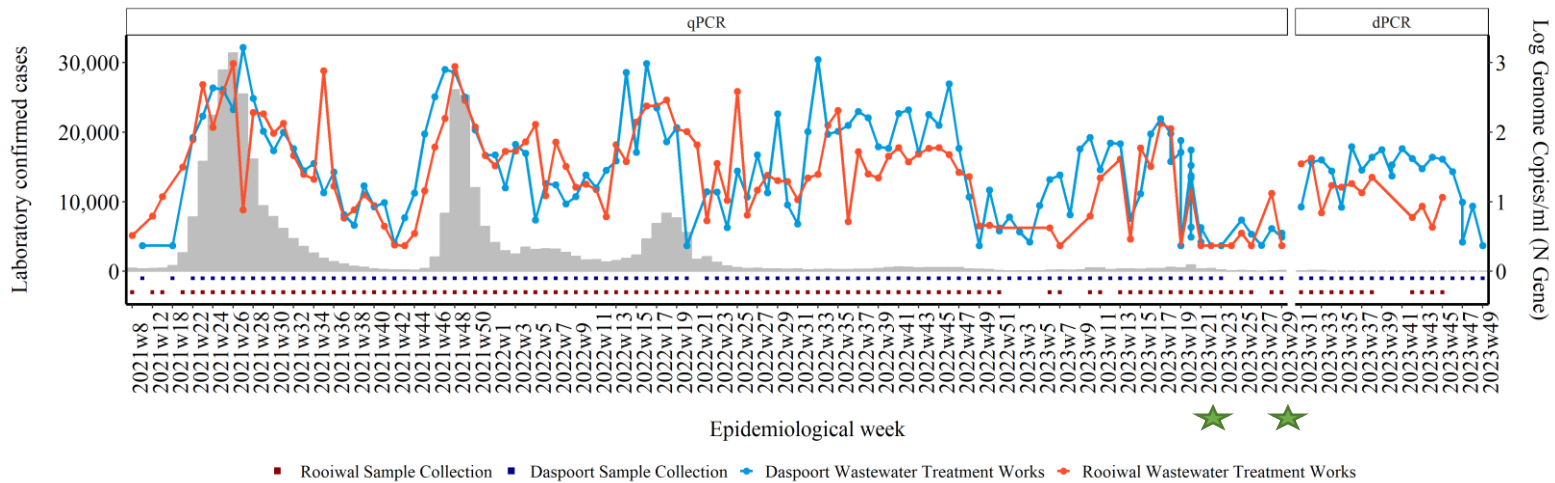
V213E, R346T
 Mutations in spike protein associated with XBB* sub-lineages

S939F
 Spike protein mutation associated with the BA.2.86 lineage

P143L
 Spike protein mutation associated with the BA.2.86 lineage

Heatmap showing patterns of emerging mutations in the spike region of SARS-CoV-2, collected from April, 2021 - August, 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.

Gauteng - Tshwane



★ Chloroform start and end date

SARS-CoV-2 levels and Genomic Results in Epi week 50:

- SARS-CoV-2 levels in Daspoort WWTW decreased to low in Epi week 49. Even though there was an increase in levels in Epi week 48, levels remain low. No new results for Epi week 50 are available.
- SARS-CoV-2 levels in Rooiwal WWTW have increased slightly in Epi week 44. Levels remain low. No new results for Epi week 50 are available.

** Sequencing data ending in Epi week 41 in Daspoort and 38 in Rooiwal.*

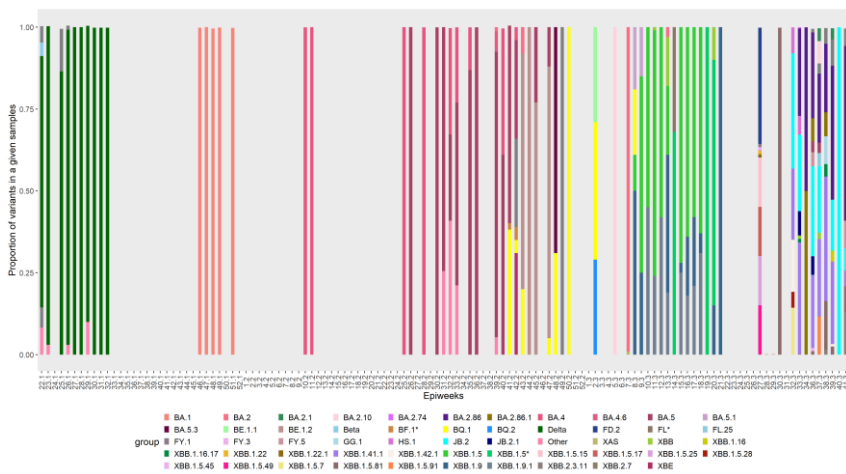
- Omicron lineages XBB.2.3.11, BE.1.2, XBB.1.41.1 and JB.2, were circulating in Daspoort during Epi week 41, with BA.2.86 dominating.

- BA.2.86 was also the dominant lineage detected in Rooiwal during epiweek 38. Other lineages including JB.2, XBB.3 and FY.5 were also in circulation.

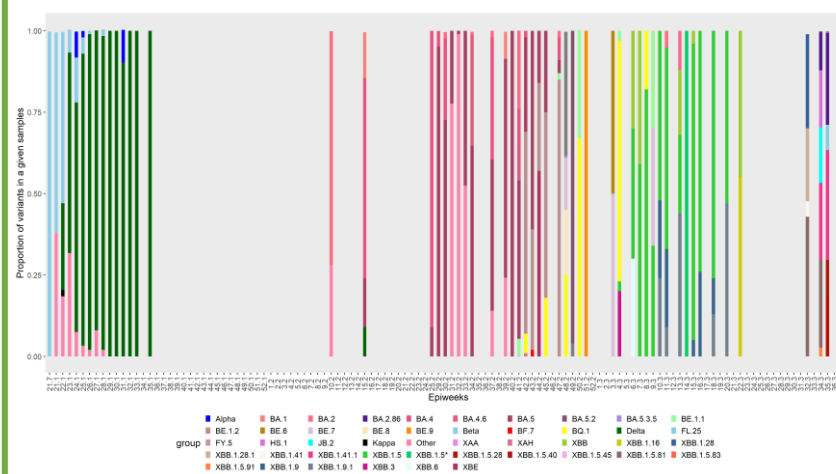
SNP Analysis:

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

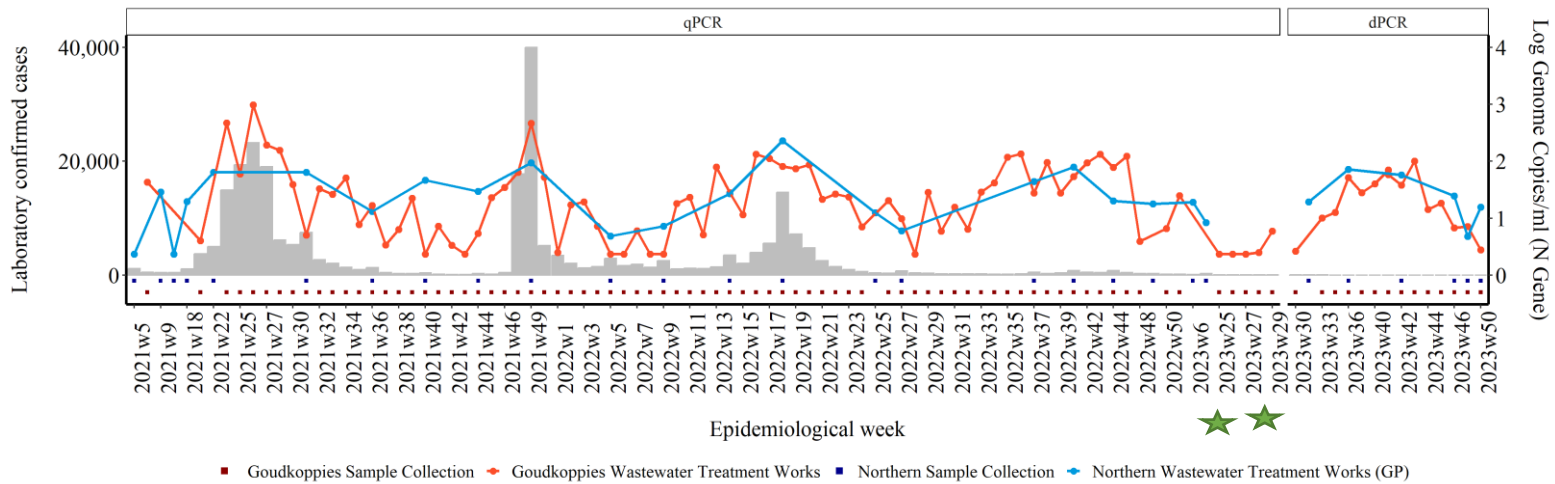
Daspoort Wastewater Treatment Works



Rooiwal Wastewater Treatment Works



Gauteng - Johannesburg



SARS-CoV-2 levels and Genomic Results in Epi week 50:

- As of Epi week 50, SARS-CoV-2 levels in Goudkoppies WWTW have decreased. Levels remain low.
- In Northern WWTW, SARS-CoV-2 levels increased slightly, but remain moderate in Epi week 50.

** Sequencing data ending in Epi week 40 in Goudkoppies and 45 in Northern.*

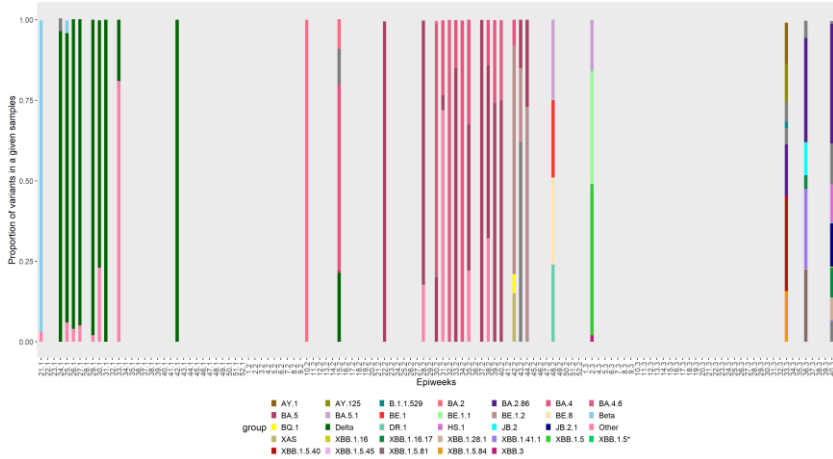
- During epiweek 40, Omicron lineage BA.2.86 was dominating in epiweek 40. Other lineages in circulation included XBB.1.5.45, XBB.1.5*, JB.2, JB.2.1 as well as XBB.1.28.1

- Omicron lineages BA.2.86, XBB.1.41.1, XBB.1.5.45, XBB.1.5.28 and XBB.1.27, JB.2 were circulating during Epi week 45 in Northern Gauteng.

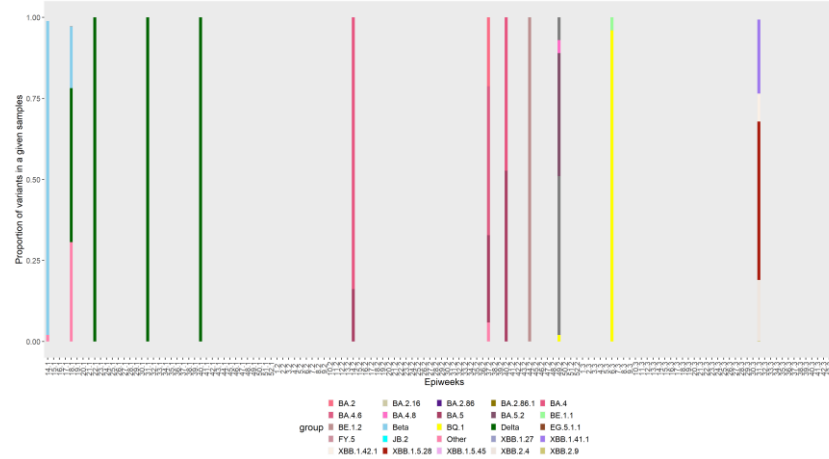
SNP Analysis:

- 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 were found in Northern Johannesburg.

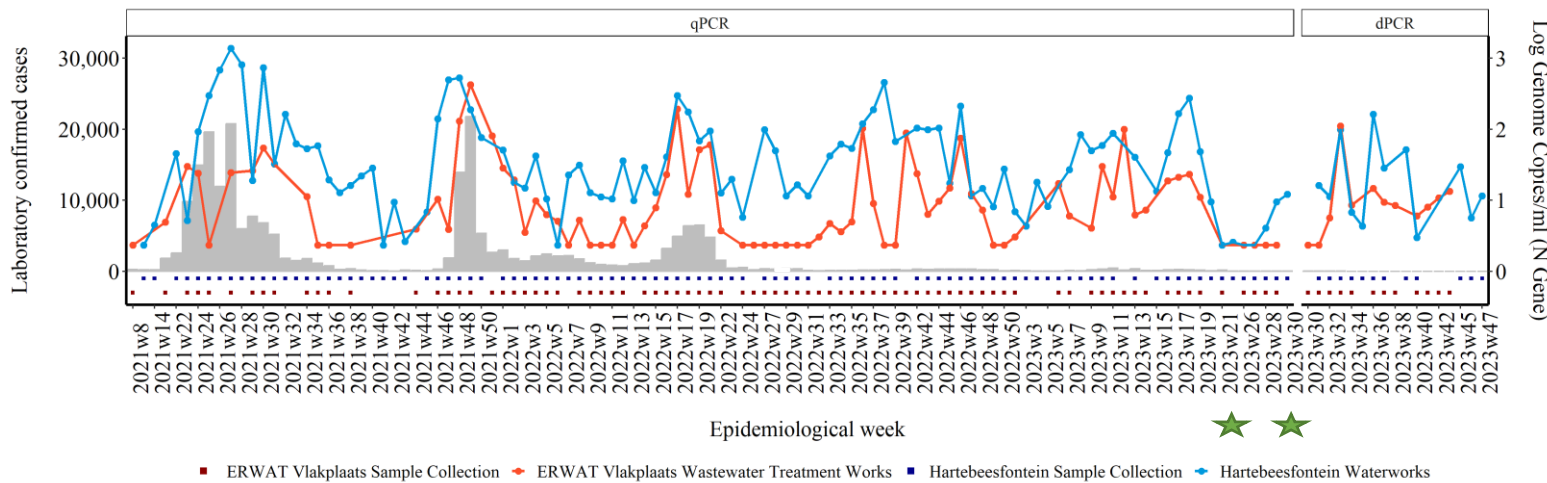
Goudkoppies Wastewater Treatment Works



Northern Wastewater Treatment Works



Gauteng - Ekurhuleni



★ Chloroform start and end date

SARS-CoV-2 levels and Genomic Results in Epi week 50:

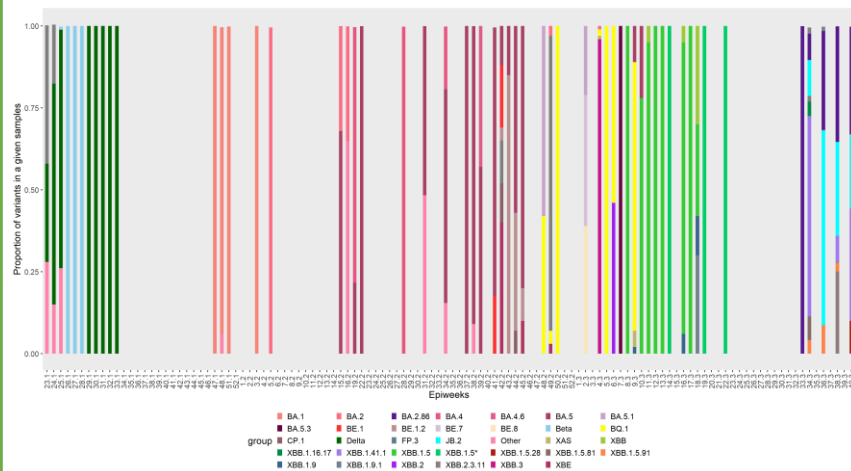
- The SARS-CoV-2 levels in Hartebeesfontein WWTW increased and levels remain low in Epi week 47, No new results for Epi week 50 are available.
- As of Epi week 43, there was a slight increase in SARS-CoV-2 levels in Vlakplaats WWTW, after a decrease from Epi week 35. No results for Epi week 50 are available.

** Sequencing data ending in Epi week 40 in Vlakplaats and 41 in Hartebeesfontein.*

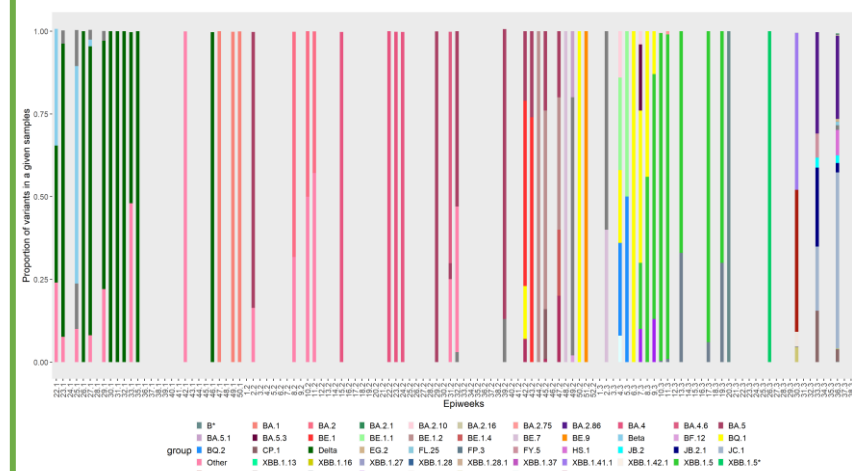
- Omicron lineages BA.2.86, JB.2, XBB.1.41.1 and XBB.1.5.28 were circulating in Vlakplaats during epiweek 40

- Lineages JB.2, XBB.1.41.1 and XBB.1.42.1 were circulating during Epi week 41 at the Hartebeesfontein water treatment plant, with BA.2.86 dominating during week 41.

ERWAT Vlakplaats Wastewater Treatment Works



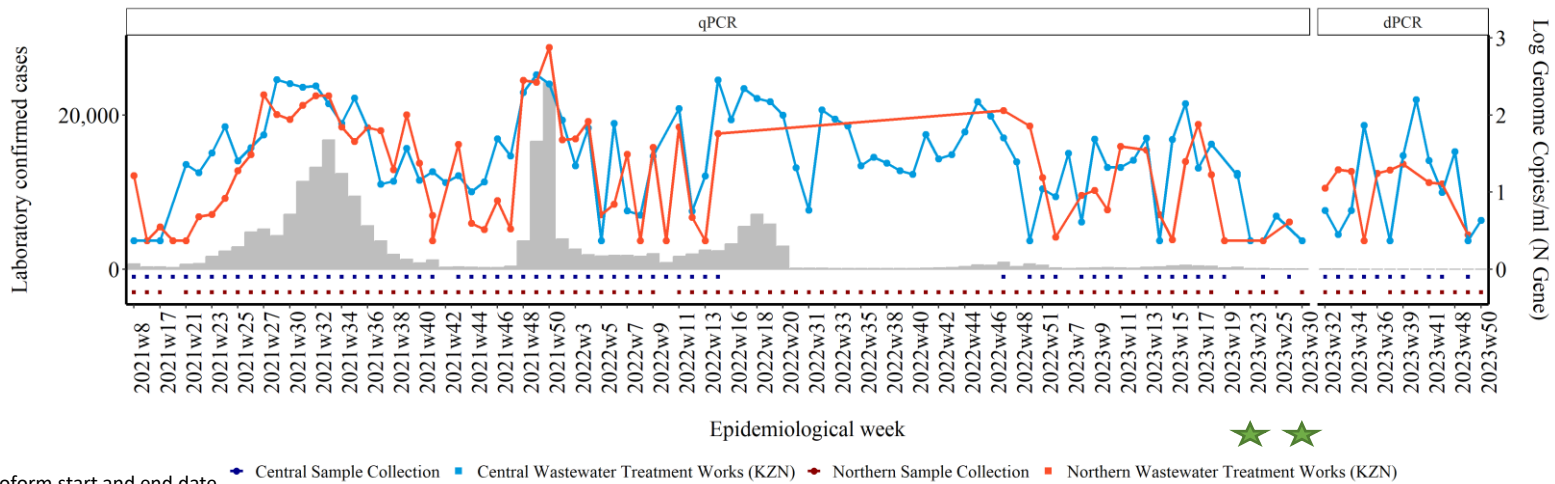
Hartebeesfontein Wastewater Treatment Works



SNP Analysis:

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

KwaZulu-Natal - eThekweni



SARS-CoV-2 levels and Genomic Results in Epi week 50:

- SARS-CoV-2 levels in Central WWTW in Epi week 39 showed a sharp increase from low levels in Epi week 38 (1 log genome copy/ml) to moderate levels (2 log genome copies/ml), followed by a decrease. Levels remain low in Epi week 50.
- SARS-CoV-2 levels increased from low to moderate in week Epi 36 in Northern WWTW, after which there was a decrease. As of Epi week 48, levels are low. No new results for Epi week 50 are available.

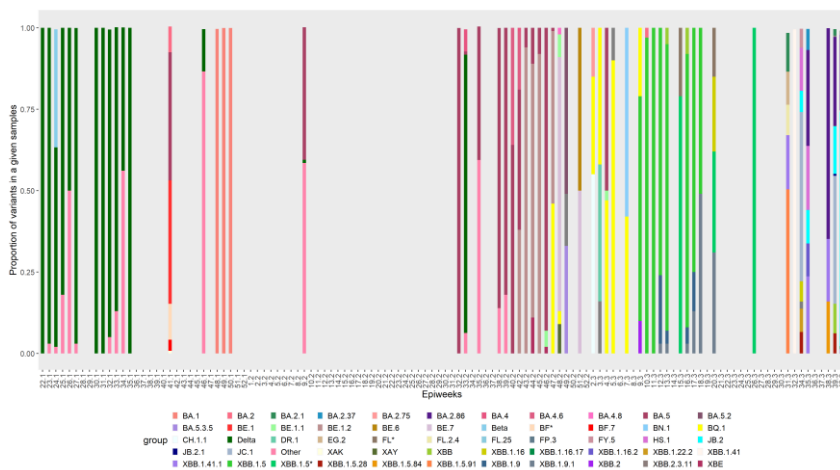
*** Sequencing data ending in Epi week 40 in Central eThekweni and 39 in eThekweni North.**

- BA.2.86, Delta and BA.5.3.5 were circulating in Epi week 40, with a JB.2 dominance at the Central eThekweni water treatment plant.
- In eThekweni North, FL.25, XBB.1.41, and BA.2.86 were circulating during Epi week 39

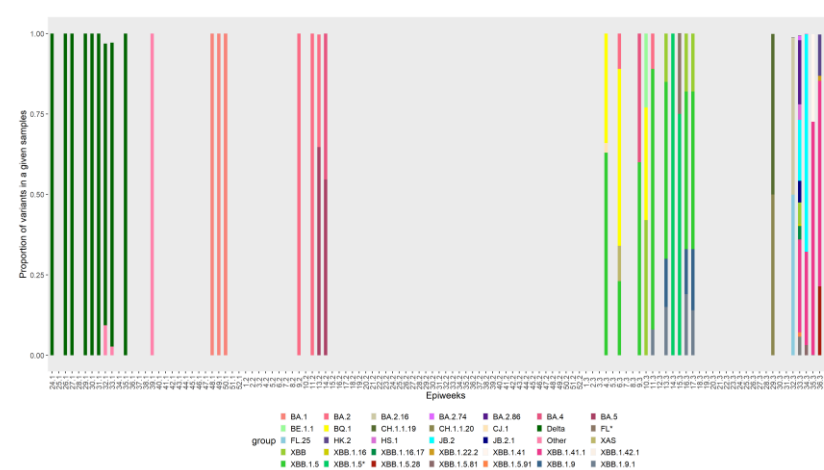
SNP Analysis:

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

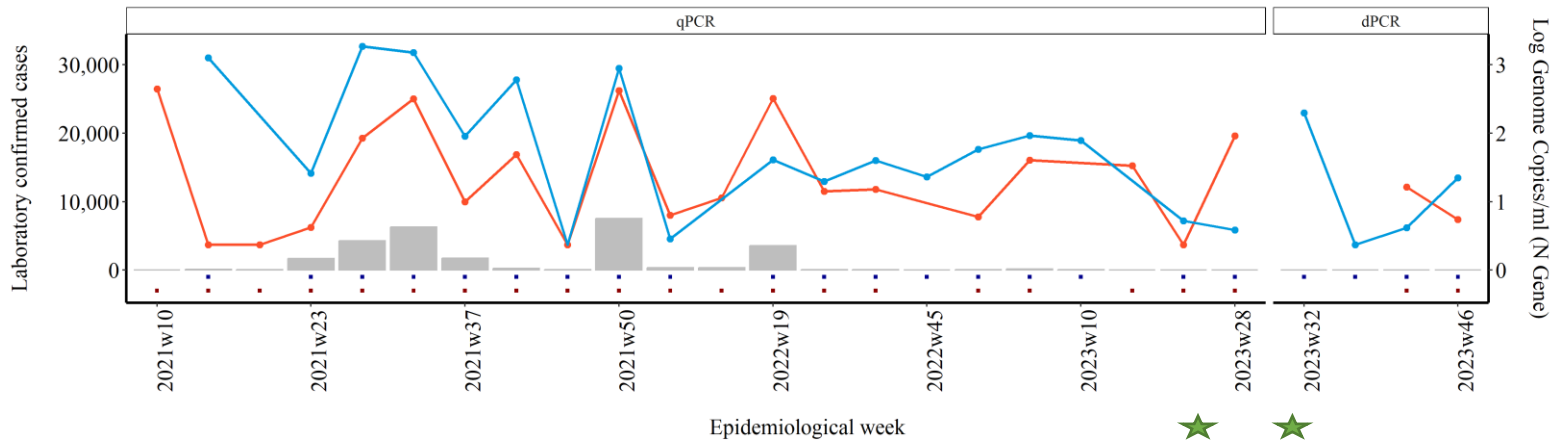
Central Wastewater Treatment Works



Northern Wastewater Treatment Works



Eastern Cape – Nelson Mandela



★ Chloroform start and end date ■ Brickfield Sample Collection ◆ Brickfield Pre-treatment Works ■ Kwanobuhle Sample Collection ◆ Kwanobuhle Wastewater Treatment Works

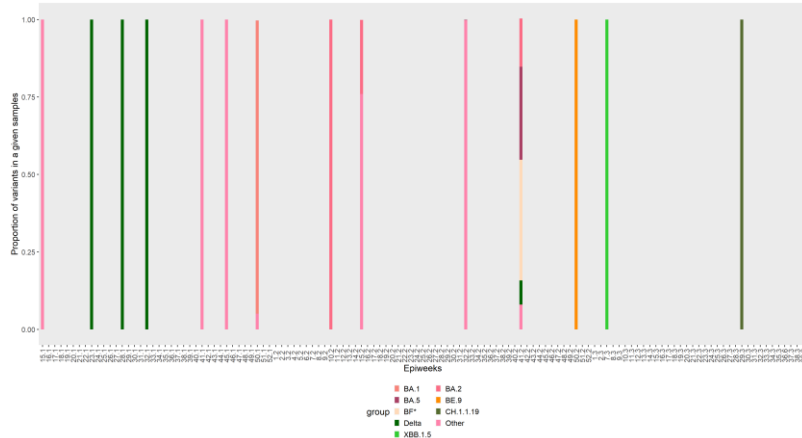
SARS-CoV-2 levels and Genomic Results in Epi week 50:

- 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 for Epi week 50 are available.
- SARS-CoV-2 levels decreased from moderate to low from Epi week 43 to Epi week 46 in Brickfield Pre-treatment works. No new results for Epi week 50 are available.

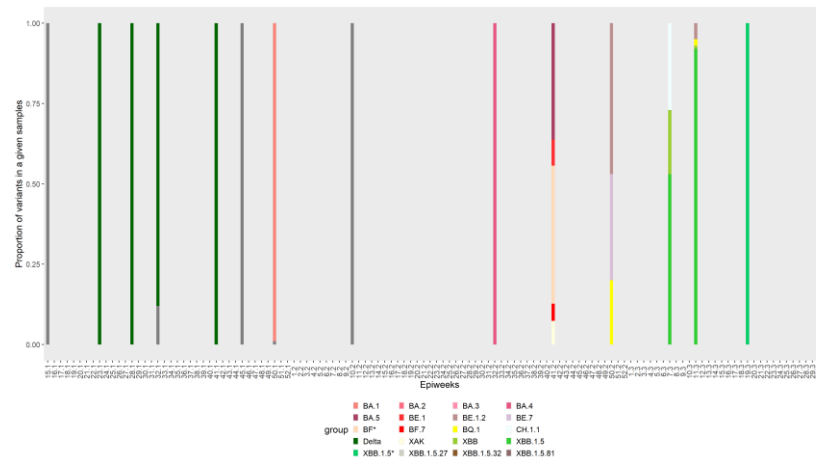
*** Sequencing data ending in Epi week 39 in Brickfield and 32 in Kwanobuhle. No new sequencing data available.**

- SARS-CoV-2 sequencing coverage in the Brickfield samples collected during Epi weeks 30-39 are too low for meaningful interpretation
- Omicron lineages XBB.1.5.81, XBB.1.5.32 and XBB.1.5.27 were circulating in Kwanobuhle during epi week 32.

Brickfield Pre-treatment works



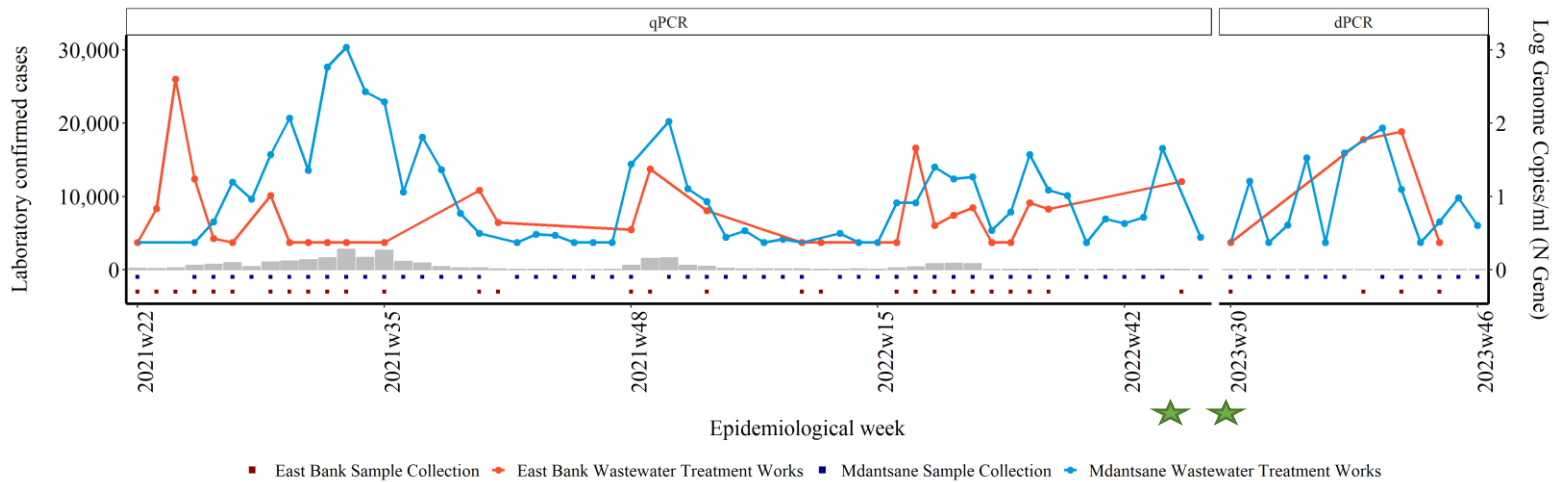
Kwanobuhle Wastewater Treatment Works



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



★ Chloroform start and end date

■ East Bank Sample Collection ◆ East Bank Wastewater Treatment Works ■ Mdantsane Sample Collection ◆ Mdantsane Wastewater Treatment Works

SARS-CoV-2 levels and Genomic Results in Epi week 50:

- In Epi week 46, SARS-CoV-2 levels in Mdantsane WWTW decreased and levels are low. No new results for Epi week 50 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 50 are available.

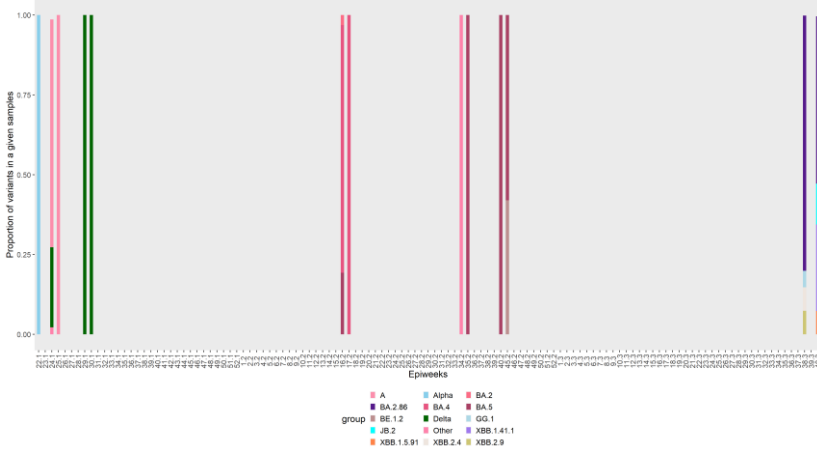
*** Sequencing data ending in Epi week 40 in Eastbank and 41 in Mdantsane.**

- Omicron lineage BA.2.86, JB.2, XBB.1.41.1 and XBB.1.5.91 were circulating in Eastbank during Epi week 40.
- Lineages GG.1, CH.1.1.11, XBB.1.41.1, XBB.2.3.11, BA.2.86 and JB.2 were circulating in Mdantsane during Epi weeks 39-41.

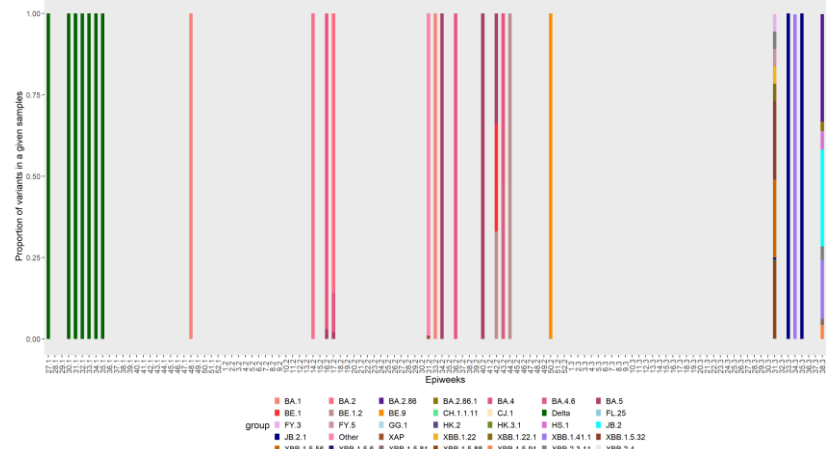
SNP Analysis:

- 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.

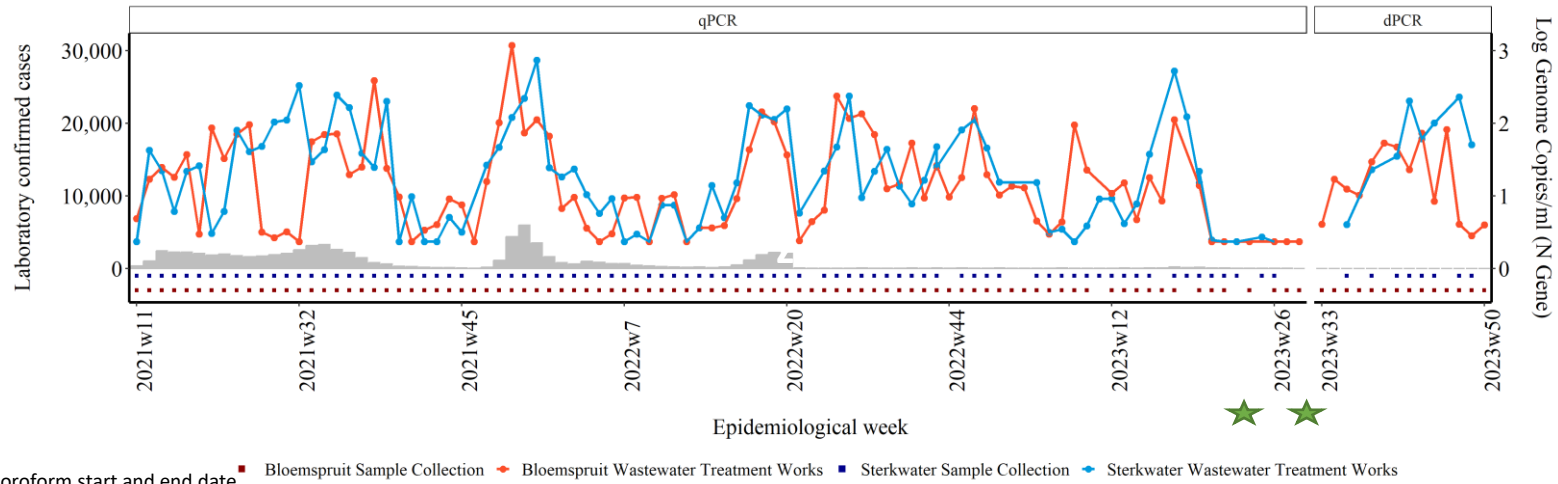
East Bank Wastewater Treatment Works



Mdantsane Wastewater Treatment Works



Free State – Mangaung



SARS-CoV-2 levels and Genomic Results in Epi week 48:

- In Bloemspruit WWTW, a 2-fold increase in SARS-CoV-2 levels were seen in Epi week 44. In Epi week 50, levels decreased and are low.
- A decrease in SARS-CoV-2 levels were seen in Sterkwater WWTW in Epi week 48 and levels are moderate. No new results for Epi week 50 are available.

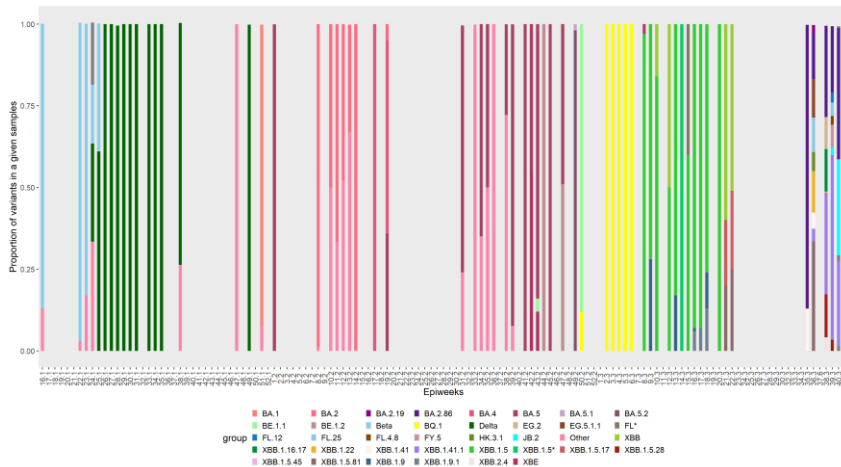
*** Sequencing data ending in Epi week 41 in Bloemspruit and in Sterkwater.**

- Lineage XBB.1.41.1 was the dominant lineage circulating in Bloemspruit during weeks 39-41, with JB.2, XBB.1.9.1, EG.2, and XBB.1.5.45 also circulating in the area.
- BA.2.86 was the dominant lineage circulating in Sterkwater during week 41. JB.2, XAS, XBB.1.5.6 and JB.2.1 were also circulating during week

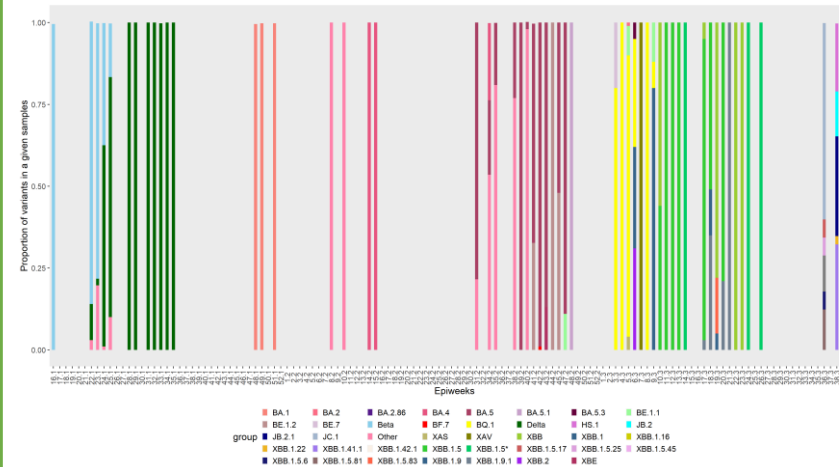
SNP Analysis:

- 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.

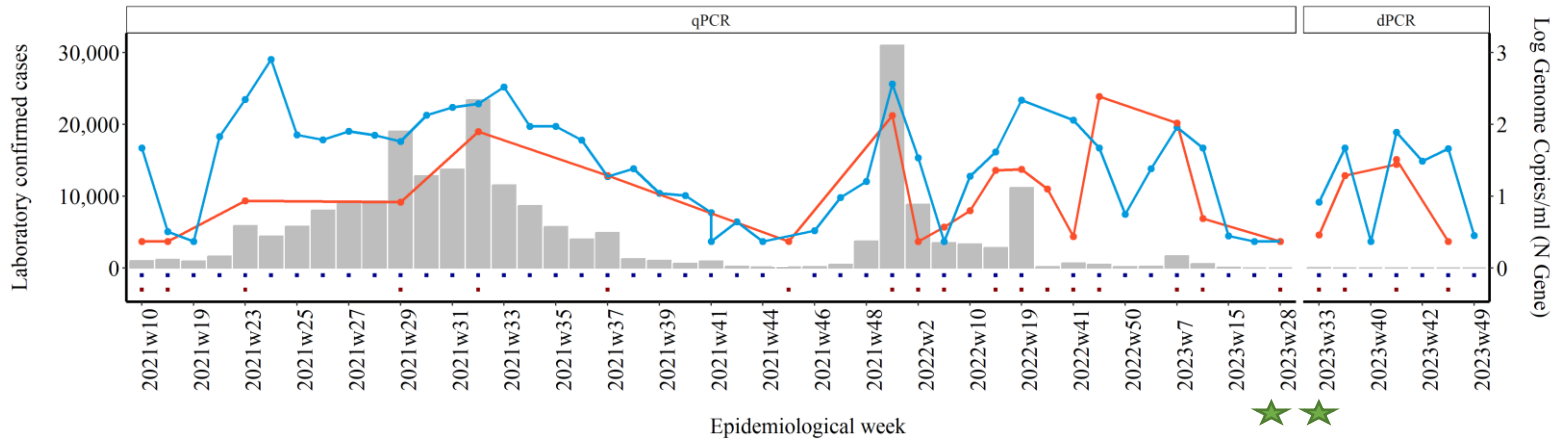
Bloemspruit Wastewater Treatment Works



Sterkwater Wastewater Treatment Works



Western Cape – City of Cape Town



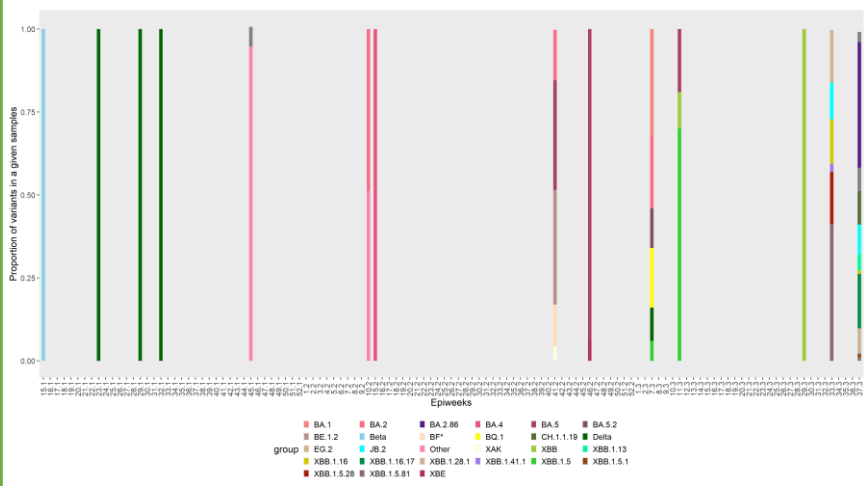
★ Chloroform start and end date ■ Borcheds Quarry Sample Collection ◆ Borcheds Quarry Wastewater Treatment Works ■ Zandvleit Sample Collection ◆ Zandvleit Wastewater Treatment Works

SARS-CoV-2 levels and Genomic Results in Epi week 50:

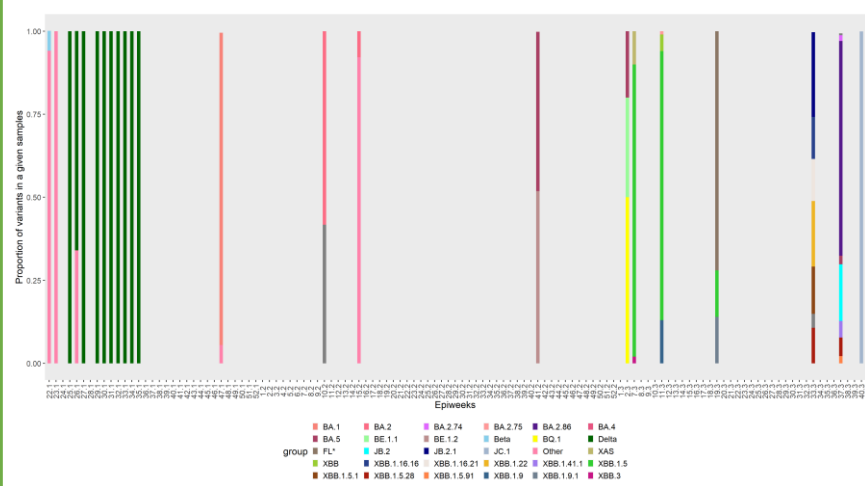
- After a sharp increase in SARS-CoV-2 levels was seen in Epi week 37, a subsequent decrease in SARS-CoV-2 levels in Borcheds Quarry WWTW was seen. Levels remain low in Epi week 49. No new results are available in Epi week 50.
- In Epi week 41, a 2-fold increase in SARS-CoV-2 levels were observed in Zandvleit WWTW. In Epi week 49, SARS-CoV-2 levels decreased and are low. No new results for Epi week 50 are available.

*** Sequencing data ending in Epi week 37 in Borcheds Quarry and Epiweek 40 in Zandvleit.**

Borcheds Quarry Wastewater Treatment Works



Zandvleit Wastewater Treatment Works



- During epiweek 37, Omicron lineage BA.2.86, CH.1.1.19, XBB.1.28.1, XBB.1.16, JB.2, and Delta were detected in Borcheds Quarry.
- During Epi week 40, lineage JC.1 was dominant in Zandvleit

SNP Analysis:

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

COLLABORATORS



FUNDERS



TEAM

