

WASTEWATER-BASED EPIDEMIOLOGY FOR SARS-COV-2 SURVEILLANCE IN SOUTH AFRICA



**NATIONAL INSTITUTE FOR
COMMUNICABLE DISEASES**

Division of the National Health Laboratory Service

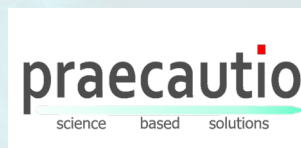
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OVERVIEW

This report summarises and interprets findings from detection and quantification of SARS-CoV-2 levels by the NICD Centre for Vaccines and Immunology in influent (untreated) wastewater in 17 wastewater treatment plants (WWTP) across five provinces tested by the NICD, and 70 additional plants tested by SACCESS partners including the National Institute for Occupational Health, Lumegen Laboratories, GreenHill Laboratories and Praecautio to cover all provinces. Levels of SARS-CoV-2 in wastewater correlate with population levels of SARS-CoV-2 over time and indicate the geographic distribution of disease. SARS-CoV-2 is shed from symptomatic and asymptomatic persons in stool but is not transmitted by faecal-oral route nor via wastewater. This report is based on data collected from June 2021 until 11th February 2022 (epidemiological week 6). Generally, levels of SARS-CoV-2 continue to decrease across the country corresponding to the decrease in clinical cases and the exit from the 4th wave. Detailed analyses are described below.

HIGHLIGHTS

- The SARS-CoV-2 levels continue to decrease or remain at very low levels across most wastewater treatment plants in the country, corresponding to the decline in clinical cases following the 4th omicron-dominated wave.
- In Gauteng, the 'wave-within-wave' (possibly due to omicron subvariant BA.2) identified in last week appears to be subsiding, with levels at all major Gauteng plants decreasing.
- After a transient decrease following the 4th omicron-dominated wave, the levels may be increasing in two areas
 - ◊ eThekweni (Hillcrest, Central and Northern) in KwaZulu-Natal Province.
 - ◊ Mangaung (Bloemspuit and Sterkwater) in Free State
- These increases may be due to the transmission of the BA.2 subvariant in the southern areas of the country.
- Levels will be continually monitored and authorities should continue to strengthen surveillance for clinical cases, promote vaccination and non-pharmaceutical interventions in all areas



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DETECTION AND QUANTITATION OF SARS-COV-2 AT SENTINEL WASTEWATER TREATMENT SITES IN SOUTH AFRICAN URBAN AREAS, MARCH 2021- JANUARY 2022

CO-FUNDED BY THE WATER RESEARCH COMMISSION AND THE NICD

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8. Lumegen Laboratories, (Pty) Ltd, Potchefstroom
9. Greenhill Laboratories
10. Praecautio
11. Tuberculosis Platform, South African Medical Research Council, Pretoria.
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13. City of Cape Town Health Department
14. Water Research Commission, Pretoria
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BACKGROUND

The detection and monitoring of SARS-CoV-2 through wastewater was first proposed in April 2020. Initial reports describing the feasibility and practical usefulness of this approach emerged simultaneously from several countries during August 2020. Recent evidence has shown that SARS-CoV-2 can be detected in wastewater prior to the appearance of clinical cases, and longitudinal tracking of SARS-CoV-2 viral load in wastewater correlates with the burden of clinically diagnosed cases. Furthermore, the sequencing of SARS-CoV-2 RNA fragments in wastewater has identified variants of concern as well as mutations not detected in clinical cases.

In South Africa, SARS-CoV-2 epidemiology is monitored through laboratory testing of clinical cases using reverse-transcriptase polymerase chain reaction (RT-PCR) tests and rapid antigen tests, COVID-19 hospital admissions and COVID-19 - related deaths. Laboratory testing data is relayed by testing laboratories to the National Institute for Communicable Diseases (NICD) via the DATCOV system. From these data sources,

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epidemiological indicators including incidence rates of testing and case detection, hospitalisation and death rates are made available to key stakeholders and the general public.

Clinical epidemiology based on reporting of laboratory-confirmed cases of SARS-CoV-2 has limitations. Household transmission studies in South African urban and rural settings have demonstrated that a large proportion of cases are asymptomatic or so mild as not to elicit health-seeking, and that laboratory-confirmed cases likely represent less than 10% of SARS-CoV-2 cases prevalent in a community at any given time. Secondly, there is increasing use of rapid antigen detection tests in clinical settings. Results of these tests may not be reported to surveillance networks. Consequently, laboratory diagnosis is increasingly less representative of the burden of disease.

In November 2020, a network of testing laboratories, which became known as the South African Collaborative COVID-19 Environmental Surveillance System (SACCESS) network, was established in order to support the development of a common testing methodology, identify and address challenges, and share best practices related to qualitative, quantitative and RNA sequencing of SARS-CoV-2 in wastewater. Treatment of wastewater in South Africa is the responsibility of local government. Approximately 1050 wastewater treatment works (WWTPs) are administered by metropolitan councils and local government and treat industrial and domestic waste. SACCESS partners and the NICD have engaged with local government to support sample collection, interpretation and utilisation of the results for public health purposes.

The SACCESS network aims to detect and quantify SARS-CoV-2 in wastewater in urban settings in South Africa, to compare trends, temporal and geographic distribution of SARS-CoV-2 levels in wastewater with trends in clinical epidemiology so as to support the use of wastewater-based epidemiology for COVID-19 outbreak prevention and response activities.

METHODS

Outbreak context and clinical case epidemiology

Since the first case of SARS-CoV-2 in South Africa was detected on 3rd March 2020, laboratories in the country have conducted over 22 million RT-PCR and antigen tests. Four distinct waves of SARS-CoV-2 infection occurred, peaking in June 2020, December 2020, July 2021, and December 2021 respectively. The current de-duplicated and geospatially allocated national line list of laboratory-confirmed cases of SARS-CoV-2 (identified by RT-PCR or antigen test) is provided by the NICD for comparison with results from SARS-CoV-2 testing of wastewater.

Establishment of the laboratory testing network

Commencing in 2018, the NICD had been conducting testing of wastewater for poliovirus as part of the National Department of Health's polio surveillance programme. In 2020, the NICD commenced testing of influent wastewater samples from these 18 sites, including eight in Gauteng Province, two in the City of Cape Town (Western Cape Province), two in Mangaung (Free State Province), two in eThekweni (KwaZulu-Natal Province) and four in Eastern Cape Province (two in Buffalo City Metro and two in Nelson Mandela Metro). Quantitative testing results for these sites are available from week 8 of 2021, onwards.

Additional plants across all metropolitan areas as well as sentinel site plants in smaller provinces were included from February 2021. From August 2021, quantitative testing was conducted on all specimens submitted to partner laboratories for testing. Presently, samples from 87 WWTPs are being tested for SARS-CoV-2. The supplementary Table 1 at the bottom of the page shows all the data for these plants, including their geographical location, the surrounding suburbs, water service authority, the testing laboratory, and dates testing began in these sites.

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SARS-CoV-2 detection and quantitation methodology

The general approach of SARS-CoV-2 detection in wastewater used at all network laboratories is virus concentration, followed by nucleic acid extraction and molecular detection. At the identified wastewater treatment facilities grab or passive samples of influent are collected and transported at <5°C to the testing facility. Table 1 summarises the sample collection, processing and detection methodology used by laboratories involved in the surveillance project. The levels of SARS-CoV-2 in wastewater are reported in copies/mL of wastewater. These values are log-transformed before constructing the graphs.

Table 1. Sampling and methodology used by laboratories involved in the NICD-WRC led COVID-19 wastewater surveillance project.

| Name of laboratory | Sampling | Virus concentration | Nucleic acid extraction | Molecular analysis | Molecular analysis platform |
|---|----------|--|--|--|---|
| National Institute for Communicable Diseases (NICD) | Grab | Ultrafiltration (Centricon® Plus-70 centrifugal ultra-filter device) | QIAamp® viral RNA mini kit | RT-qPCR ^a using the Allplex™ 2019-nCoV Assay and the EDX SARS-CoV-2 standard | 7500 Real-Time PCR System (Applied Biosystems) |
| GreenHill Laboratories / Praecautio | Grab | Ultrafiltration (Amicon® Ultra-15 Centrifugal Filter Unit) | Omega Bio-Tek Mag-Bind® Viral DNA/RNA 96 Kit | RT-qPCR using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel | Rotor-Gene Q (Qiagen) |
| National Institute for Occupational Health (NIOH) | Grab | Skim milk flocculation | MagMAX Viral and Pathogen Nucleic Acid Isolation Kit | RT-qPCR using the TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher) | QuantStudio™ 5 Real-Time PCR System 96-well, 0.1 mL, desktop (Applied Biosystems) |
| Waterlab/University of Pretoria | Grab | Skim milk flocculation | QIAamp® Ultrasens® Virus kit | RT-qPCR using the Allplex™ 2019-nCoV Assay and the using the 2019-nCoV_N positive control plasmid (Integrated DNA Technologies, Inc, Coralville, IA) | QuantStudio™ 5 Real-Time PCR System (Applied Biosystems) |
| South African Medical Research Council – Tuberculosis platform (SAMRC-TB) | Grab | None – sample is centrifuged then supernatant analysed | ZymoBiomix RNA Extraction Kit | RT-qPCR ^a using the Allplex™ 2019-nCoV Assay and the EDX SARS-CoV-2 standard | QuantStudio 5 (Applied Biosystems) |
| Lumegen | Passive | Passive sampler and resuspension in phosphate buffered saline | MN DNA/RNA pathogen extraction Kit | RT-qPCR using the TaqPath COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher) | QuantStudio 5 (Applied Biosystems) |
| Council for Scientific and Industrial Research (CSIR) | Grab | Polyethylene Glycol precipitation | Omega Bio-tek ENZA total RNA Kit II | RT-qPCR using the 2019-nCoV CDC EUA Kit | Qiagen Rotor-Gene 6000 (5-plex) (Qiagen) |
| Durban University of Technology – Institute of Wastewater Management | Grab | Ultrafiltration (Centricon® Plus-70 centrifugal ultra-filter device) | QIAamp® viral RNA mini kit | RT-ddPCR ^b using CDC 2019-nCoV_N2 Primers, Fam Labelled, double quenched probes | QX200 AutoDG Droplet Digital PCR System (Bio-rad) |

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Interpretation of SARS-CoV-2 levels in wastewater

Interpretation of SARS-CoV-2 wastewater levels is evolving. We have elected to use interpretive principles outlined in Table 2 to support public health preparedness and response activities. In general, increasing or decreasing trends in levels are reported based on two or more results, as a single sample that increases or decreases compared with the result from the previous week may represent an outlier. Small changes (up to 0.5log copies/ml) are not regarded as significant changes unless they form part of a general upward or downward trend. Comparison of results over time when quantification is done by the same laboratory using the same quantitative methodology is meaningful. The use of different methodologies by different laboratories precludes comparison of quantitative results across laboratories.

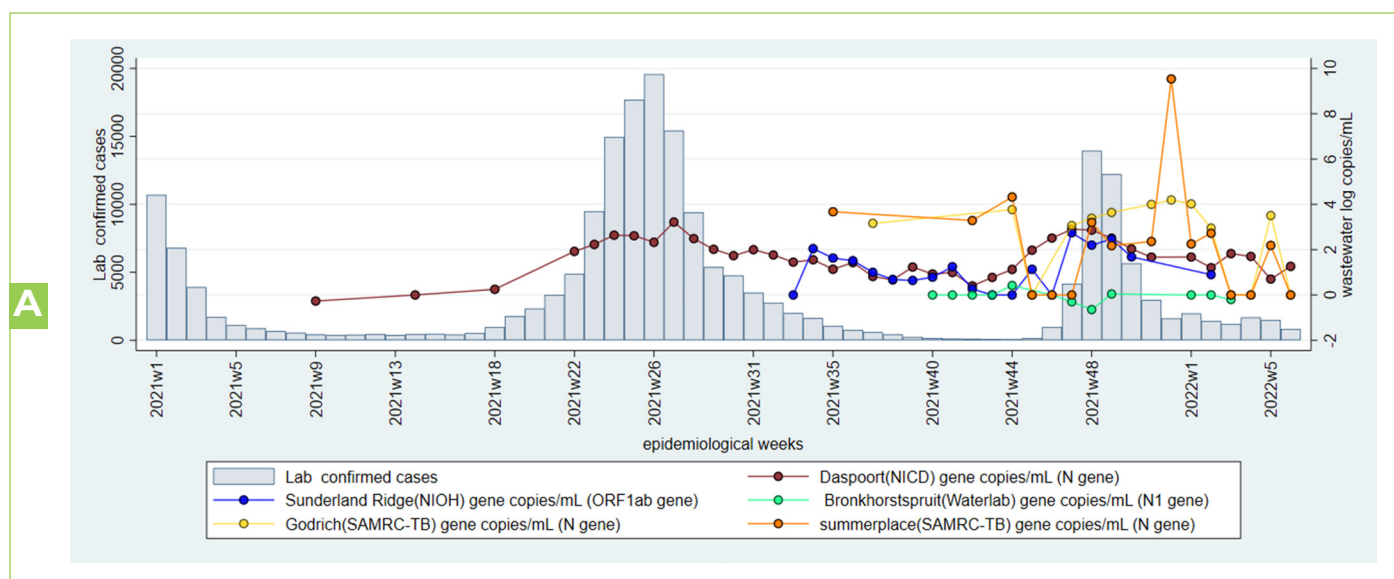
Table 2. Principles of SARS-CoV-2 detection and quantification on influent samples from wastewater treatment plants and interpretive principles to guide application of test results to support COVID-19 public health responses, South Africa.

| Testing modality | Interpretive principles to support public health responses |
|------------------------------|---|
| | When a test result changes from |
| Detection of SARS-CoV-2 | <ul style="list-style-type: none"> positive to negative, this signifies fewer/no cases in population negative to positive, this indicates the need for increased population awareness and action Qualitative results (presence or absence) are comparable between laboratories |
| Quantification of SARS-CoV-2 | <ul style="list-style-type: none"> *The concentration of SARS-CoV-2 at a particular facility may be used to infer the burden of SARS-CoV-2 in the population served by the wastewater treatment facility. *Changes in the concentration of SARS-CoV-2 give an indication of whether the burden of disease is increasing or decreasing *Quantitative results between laboratories are not comparable. *Quantitative results should be interpreted for a single wastewater treatment plant tested by the same laboratory using the same methodology over time |

RESULTS

Gauteng Province

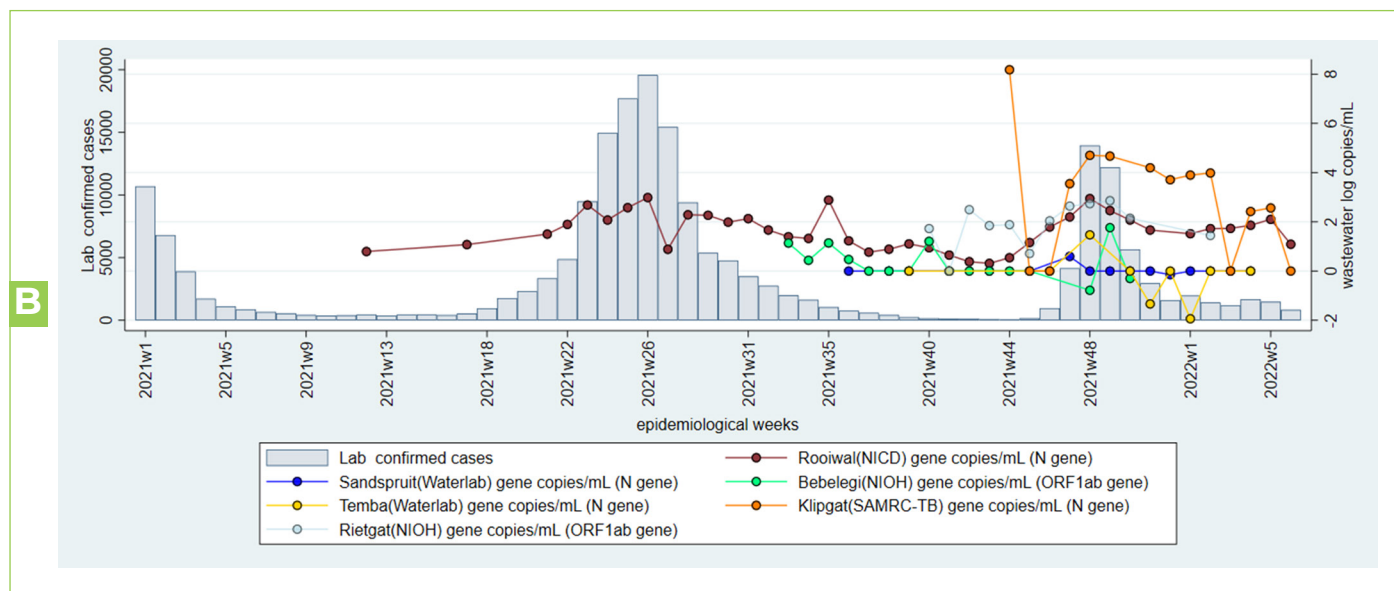
A: City of Tshwane South (sub-districts 3, 4, 6, and 7)



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B: City of Tshwane North (sub-districts 1 & 2)



C: City of Tshwane North (sub-district 5)

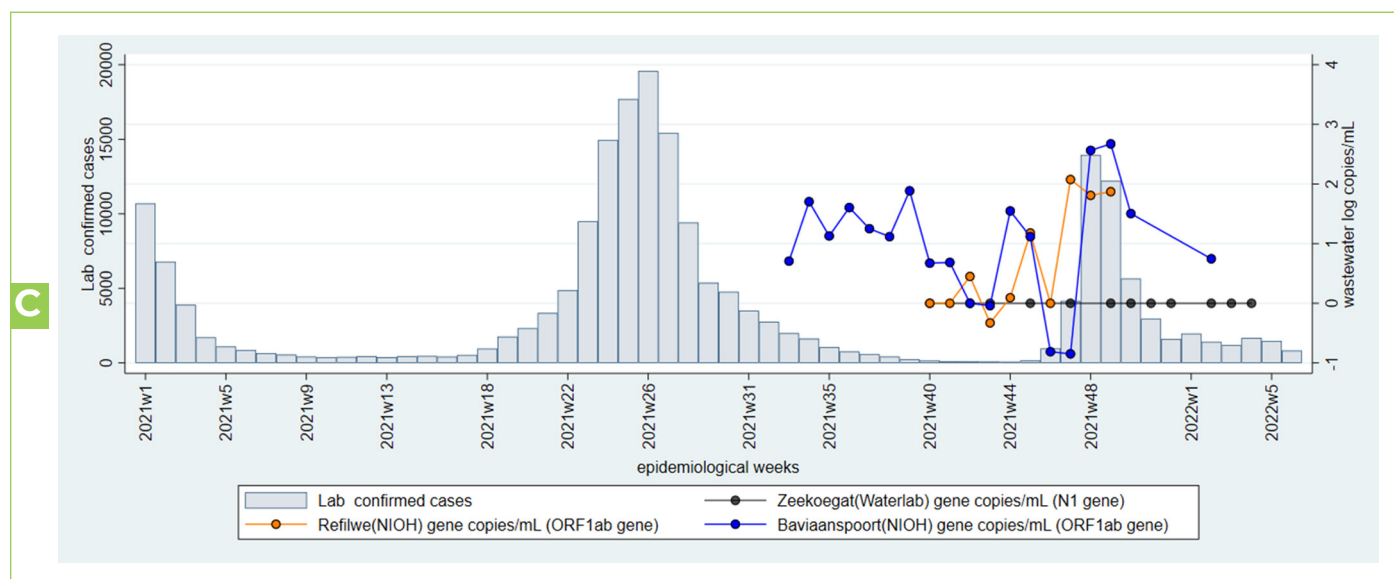


Figure 1 A-C. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) and metropolitan areas in Tshwane District Municipality (Tshwane South and North), Gauteng Province during epidemiological weeks 1 of 2021 to week 6 of 2022. The testing laboratory and quantified SARS-CoV-2 gene is named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be done for specimens tested in the same laboratory.

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D: City of Johannesburg Metropolitan Municipality

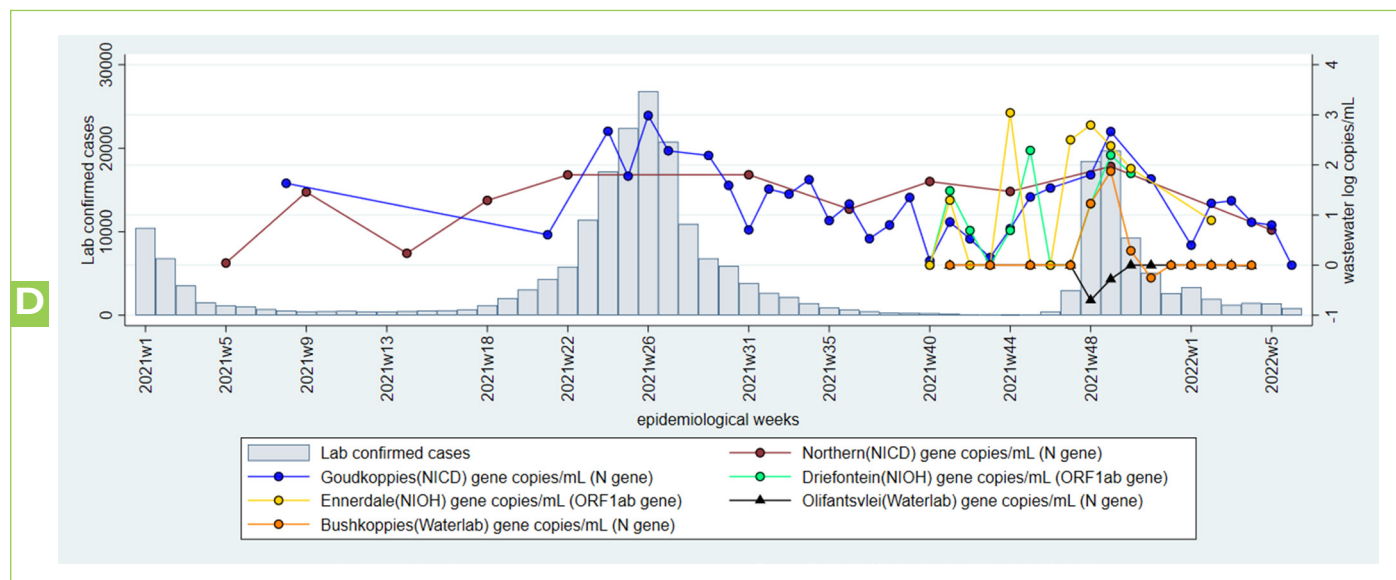
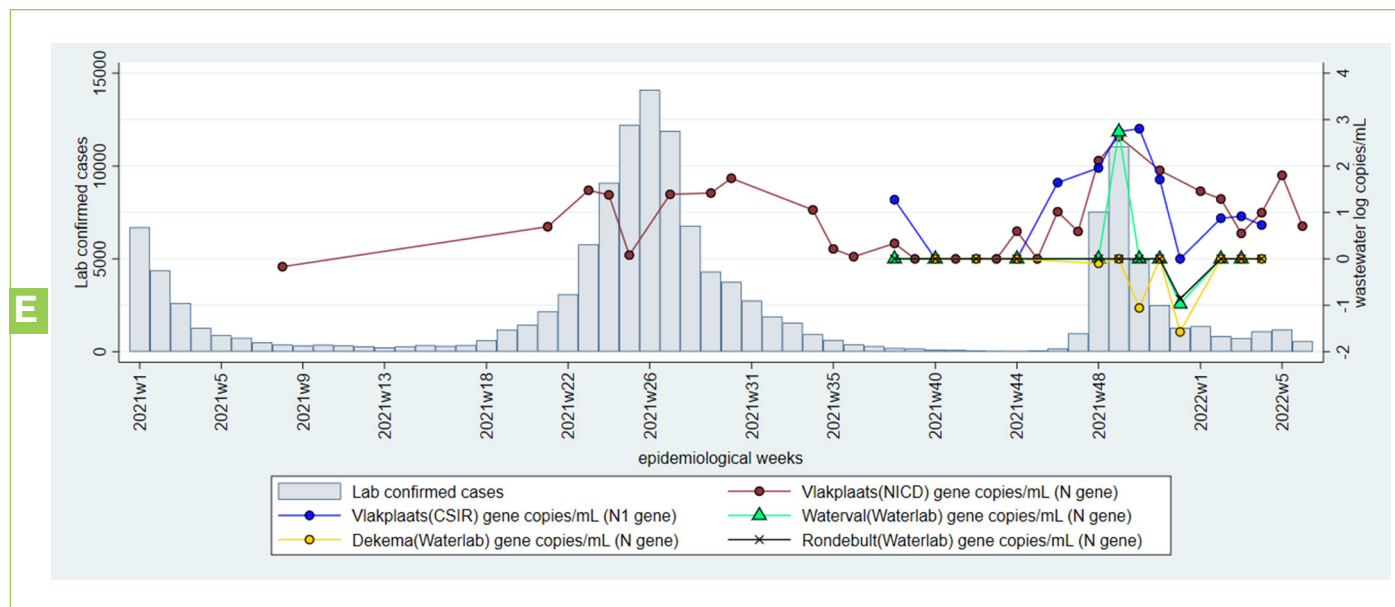


Figure 1 D. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/mL of wastewater (coloured lines) for selected wastewater treatment plants (WWTPs) in the City of Johannesburg Metropolitan Municipality, Gauteng Province during epidemiological weeks 1 of 2021 to week 6 of 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels overtime should only be made for specimens tested in the same laboratory.

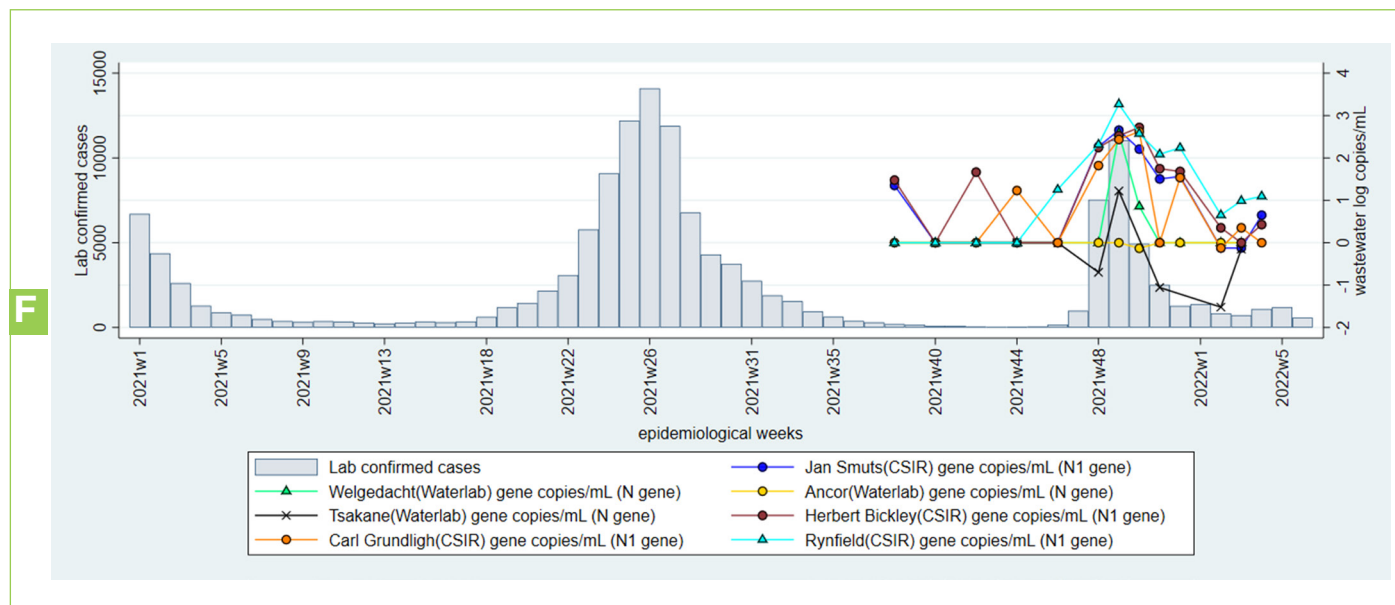
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E: Ekurhuleni East (sub-districts D, E or E1, E2)



F: Ekurhuleni South (sub-districts A, F or S1, S2)



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G: Ekurhuleni North (sub-districts B, C or N1,N2)

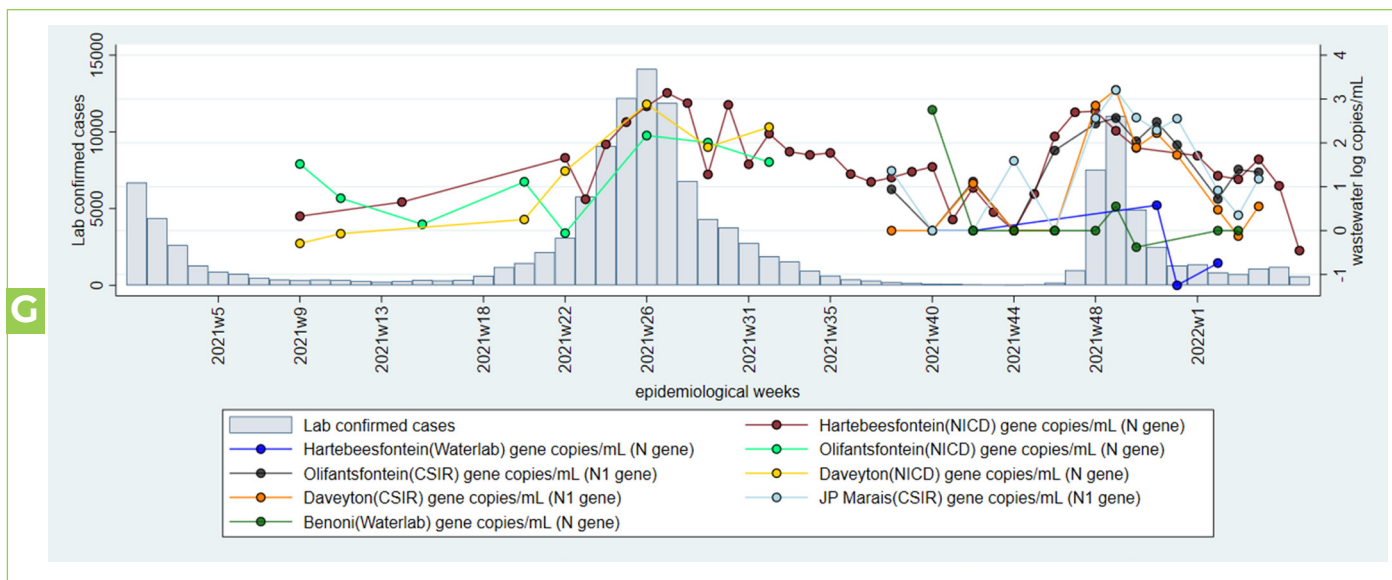


Figure 1 E-G. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/mL of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) in Ekurhuleni Metropolitan Municipality, Gauteng Province during epidemiological weeks 1 of 2021 to week 6 of 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

H: West Rand District Municipality

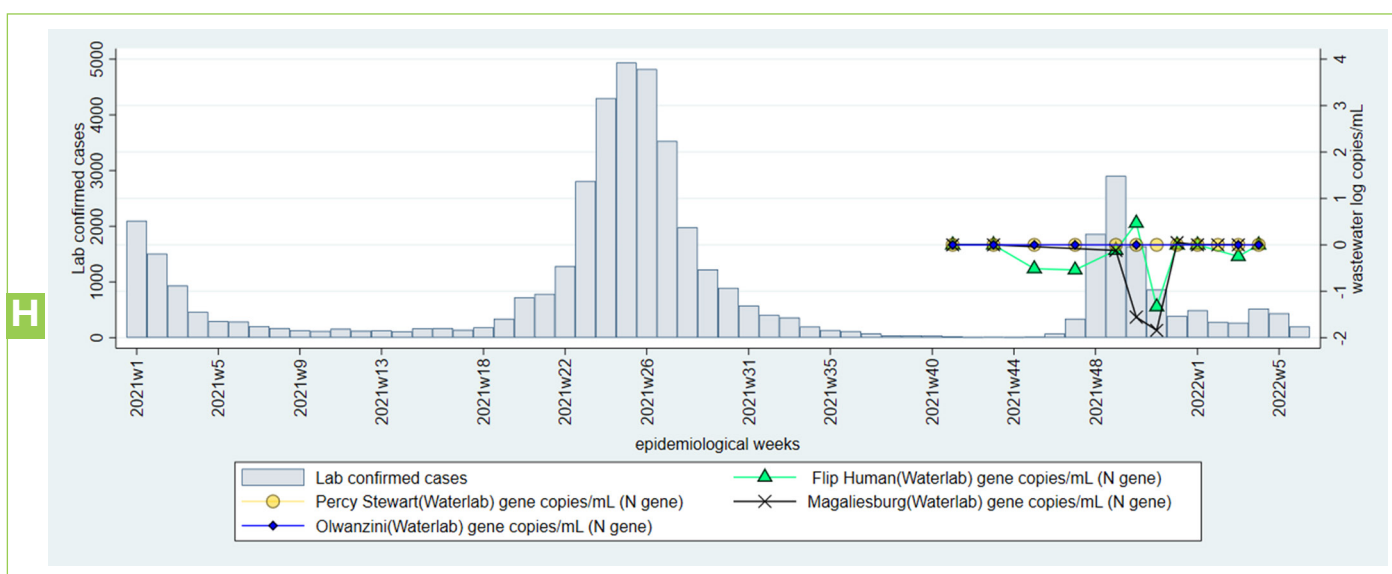


Figure 1 H. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/mL of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) in West Rand District Municipality, Gauteng Province during epidemiological weeks 1 of 2021 to week 4 of 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

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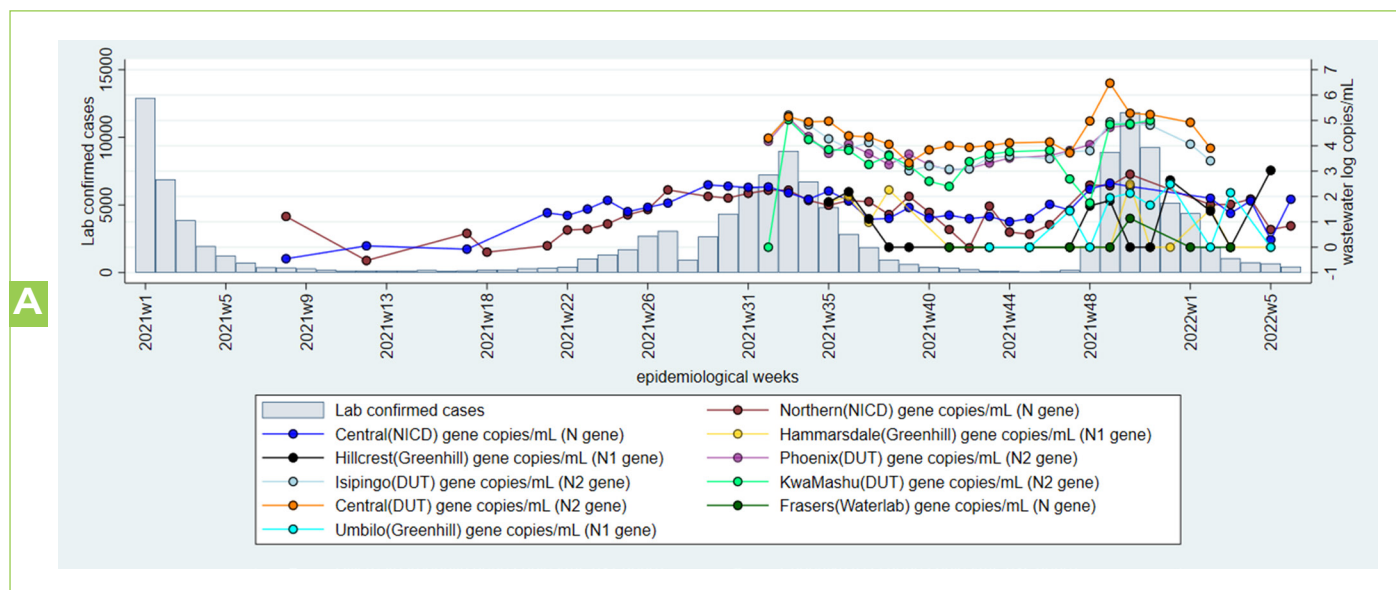
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The SARS-CoV-2 levels in Tshwane, City of Johannesburg, and Ekurhuleni continue to decrease to below 1 log copy/ml of wastewater, corresponding to the decline in caseload.

The public health authorities should continue to promote vaccination and non-pharmaceutical interventions in all areas.

KwaZulu-Natal Province

2A: eThekweni Metropolitan Municipality



B: uMgungundlovu District Municipality

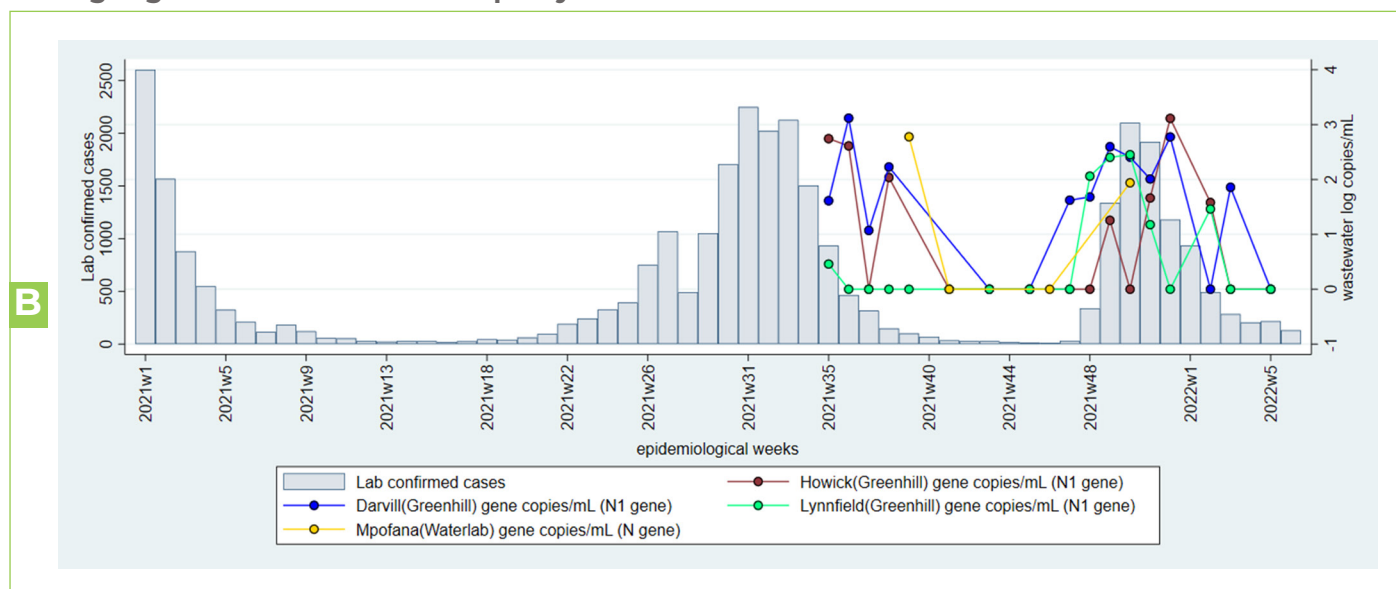


Figure 2A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTP) in Ethekeeni, (A-B) and uMgungundlovu Metro (C), KwaZulu Natal Province during epidemiological weeks 1-51, 2021 and week 6, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

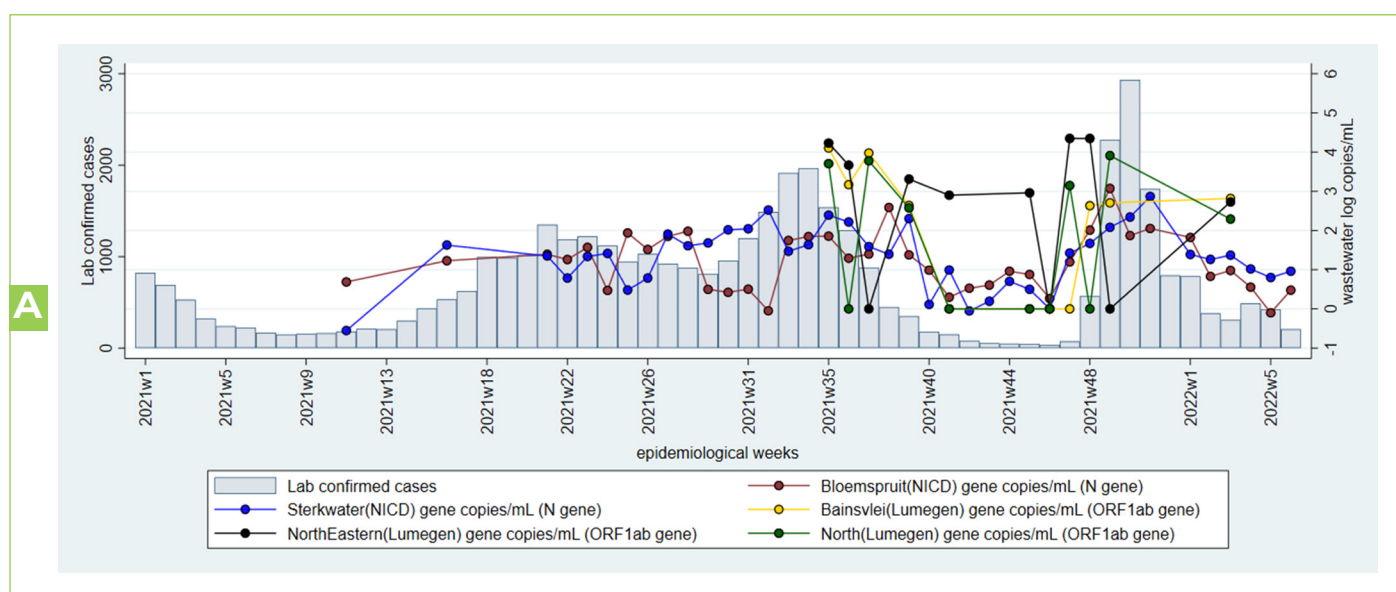
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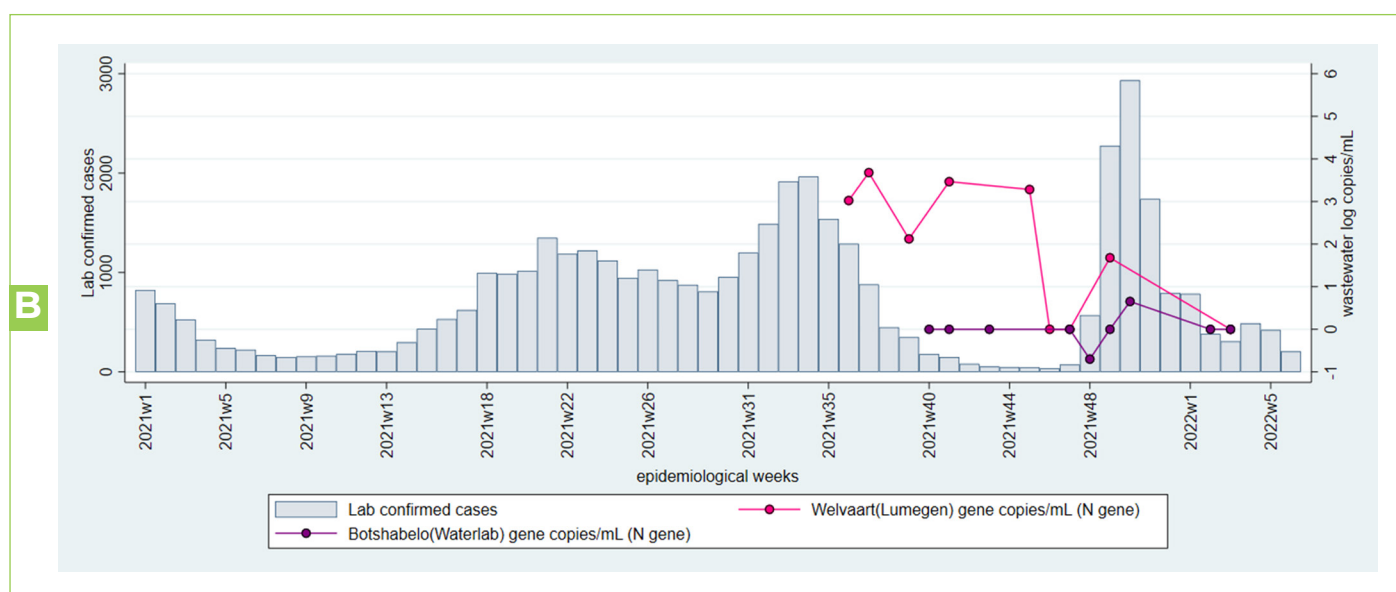
While the SARS-CoV-2 levels may be decreasing, there may still be ongoing transmission in some areas around eThekweni as the levels in Hillcrest, Central and Northern WWTPs are still high. These SARS-CoV-2 levels may be attributed to the Omicron subvariant. Therefore, the authorities should continue to strengthen surveillance for clinical cases in this area and promote vaccination and non-pharmaceutical interventions in all areas.

Free State Province- Mangaung

A: Bloemfontein sub-district



B. Botshabelo sub-district



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C. Neledi & ThabaNchu sub-districts

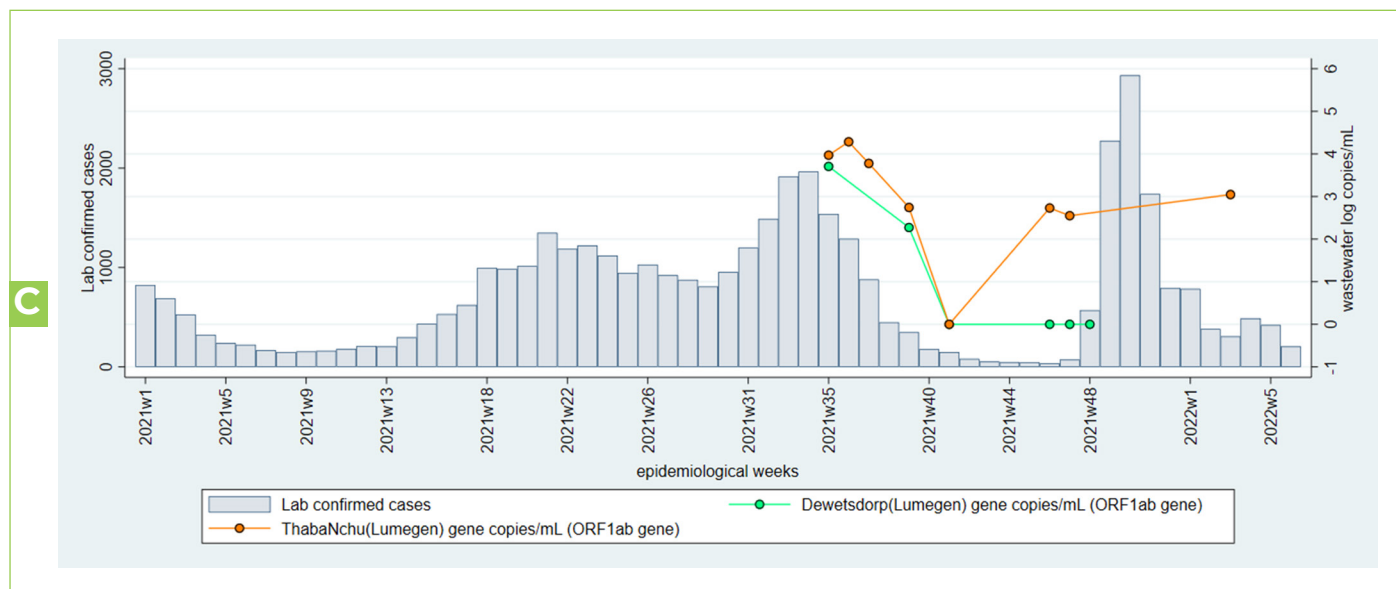


Figure 3 A-C. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Mangaung, Free State Province (Bloemfontein, Botshabelo, Naledi and ThabaNchu) during epidemiological weeks 1, 2021 to 6, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

The recent SARS-CoV-2 levels in all the plants in the three sub-districts in Free State continue to decline or remain stable at low levels, corresponding to the decreases in clinical cases. However, the SARS-CoV-2, the levels in two plants (Bloemspruit and Sterwater) in Mangaung, may be increasing, requiring close monitoring. The public health authorities should continue surveillance for cases, promote vaccination and non-pharmaceutical interventions.

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Eastern Cape Province

A: Nelson Mandela Metropolitan Municipality

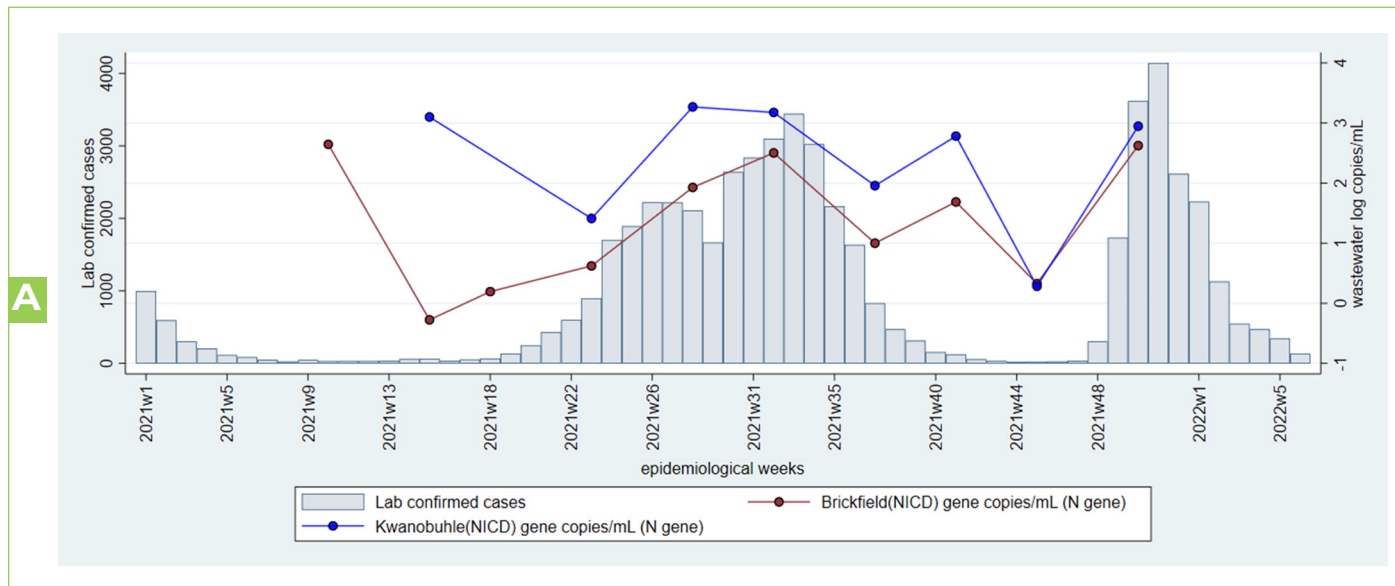


Figure 4A. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Nelson Mandela Metro, Eastern Cape Province during epidemiological weeks 1-45, 2021. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory

B: Buffalo City Metropolitan Municipality

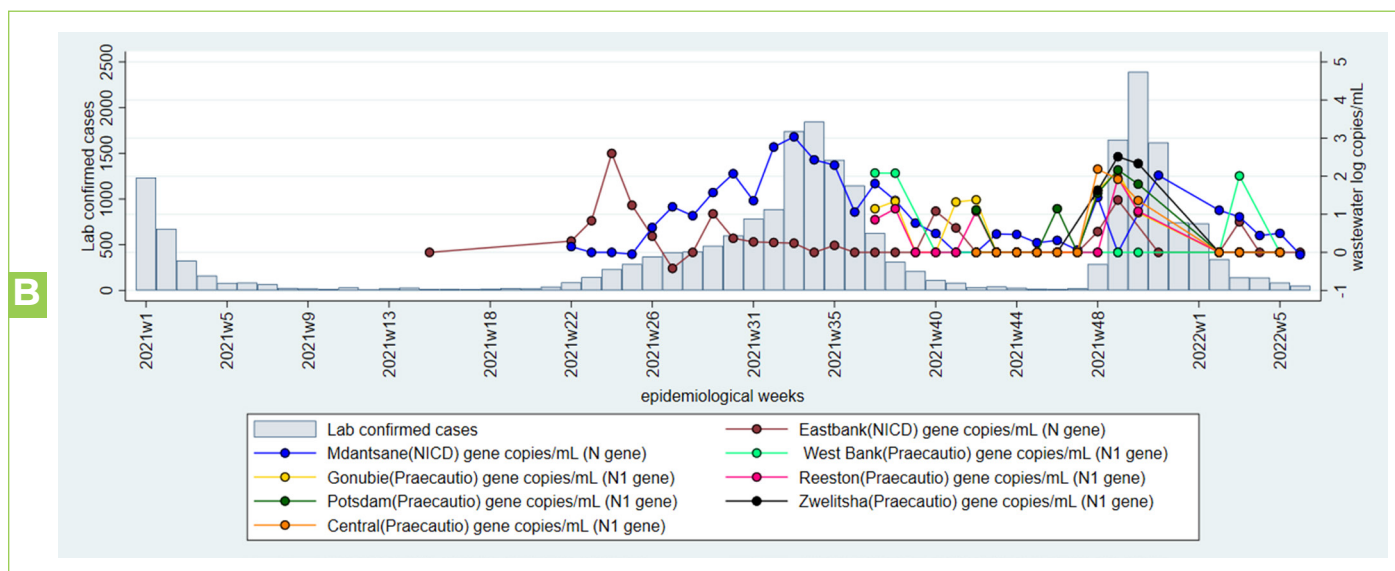


Figure 4B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Nelson Mandela Metro, Eastern Cape Province during epidemiological weeks 1, 2021 to 6, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory

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In Buffalo City Metro, results show that SARS-CoV-2 levels in all WWTPs have steadily decreased and remained at zero levels, corresponding to the decline in clinical cases. The public health authorities should continue to promote vaccination and non-pharmaceutical interventions. In Nelson Mandela Metro, assessment cannot be made due to absence of recent results. Readers are referred to the SA MRC wastewater dashboard for more in-depth data regarding levels of SARS-CoV-2 in wastewater plants in Nelson Mandela Metro (<https://www.samrc.ac.za/wbe/>).

Western Cape Province

City of Cape Town:

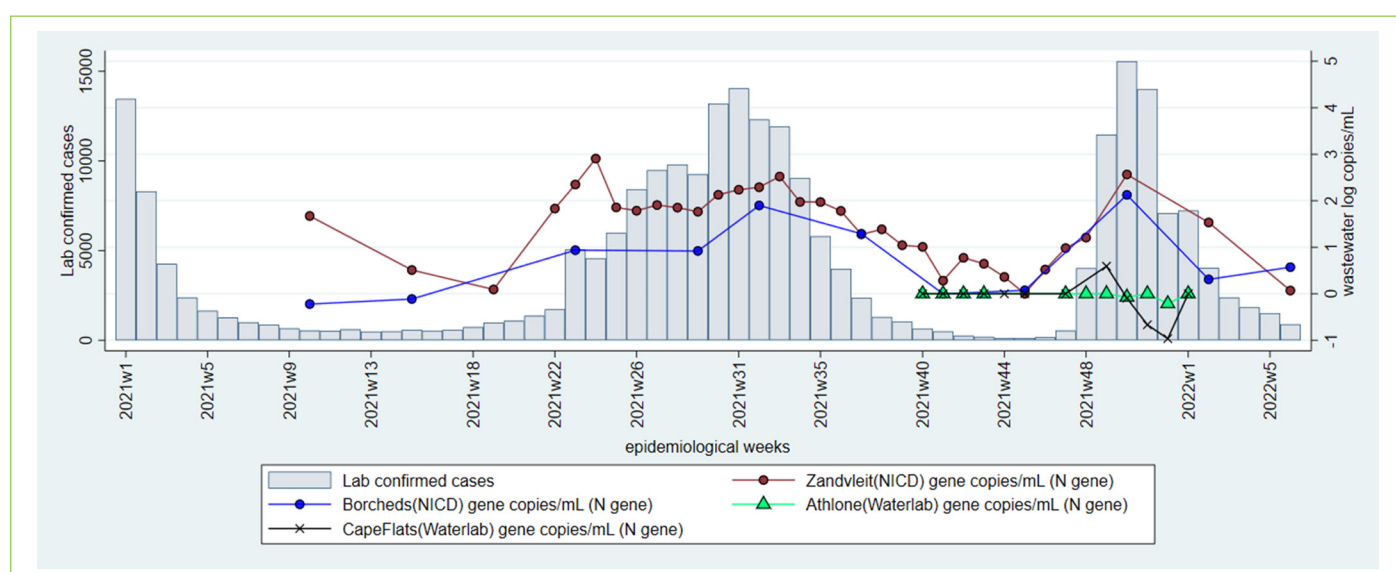


Figure 5. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in the City of Cape Town, Western Cape Province during epidemiological weeks 1, 2021 to 6, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP.

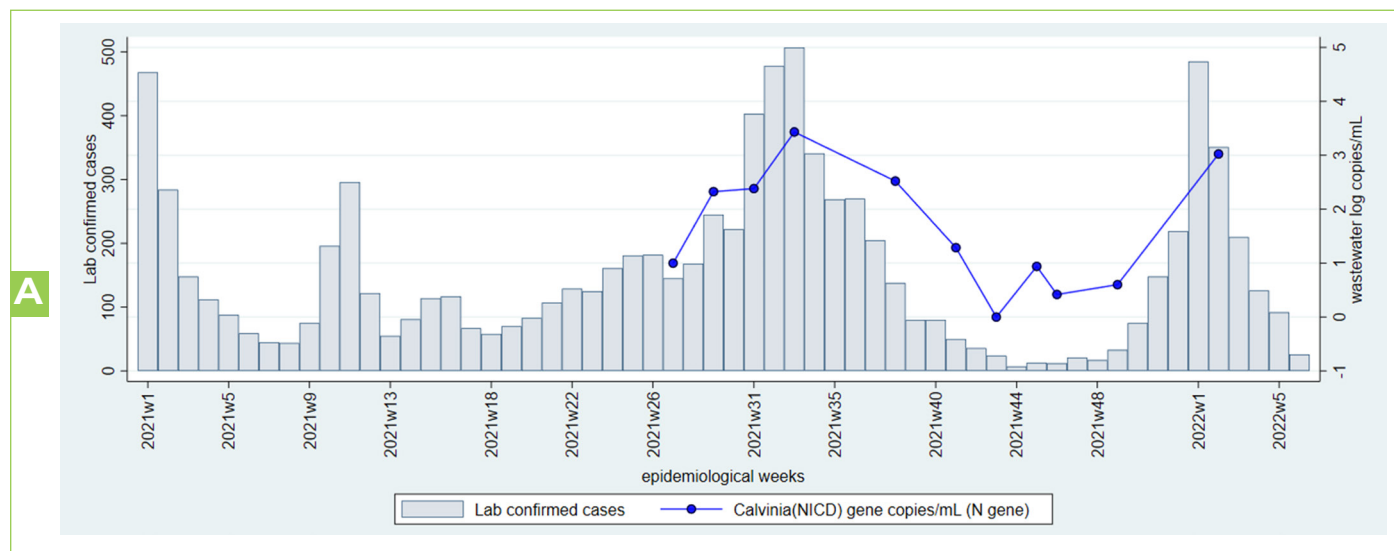
The SARS-CoV-2 levels in Borchers and Zandvleit have also declined to below 1 gene copy/ml of wastewater, corresponding to the decline in clinical case load. Readers are referred to the MRC website, which provides data from additional wastewater treatment plants in the City of Cape Town and other Western Cape districts (<https://www.samrc.ac.za/wbe/>) to contextualise the results. The public health authorities should continue surveillance for cases, promote vaccination and non-pharmaceutical interventions.

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Northern Cape Province

A: Namakwa District Municipality



B: Frances Baard District Municipality

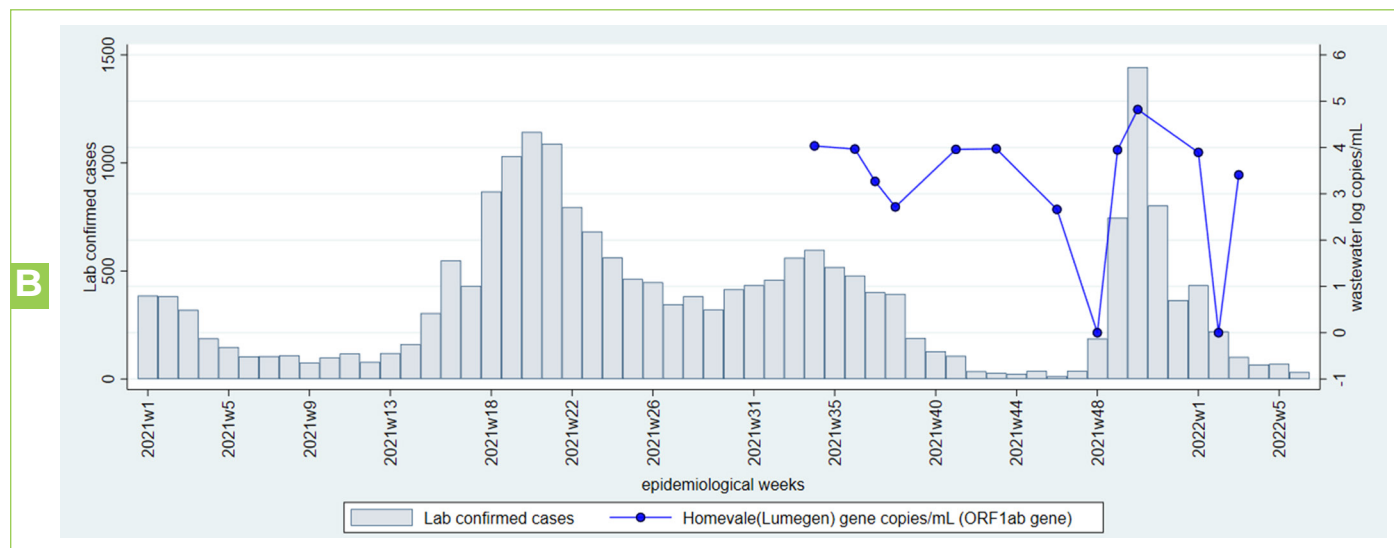


Figure 6A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Calvinia in Namakwa Metro (a) and Kimberly in Frances Baard District (b), Northern Cape Province during epidemiological weeks 1, 2021 to week 2, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

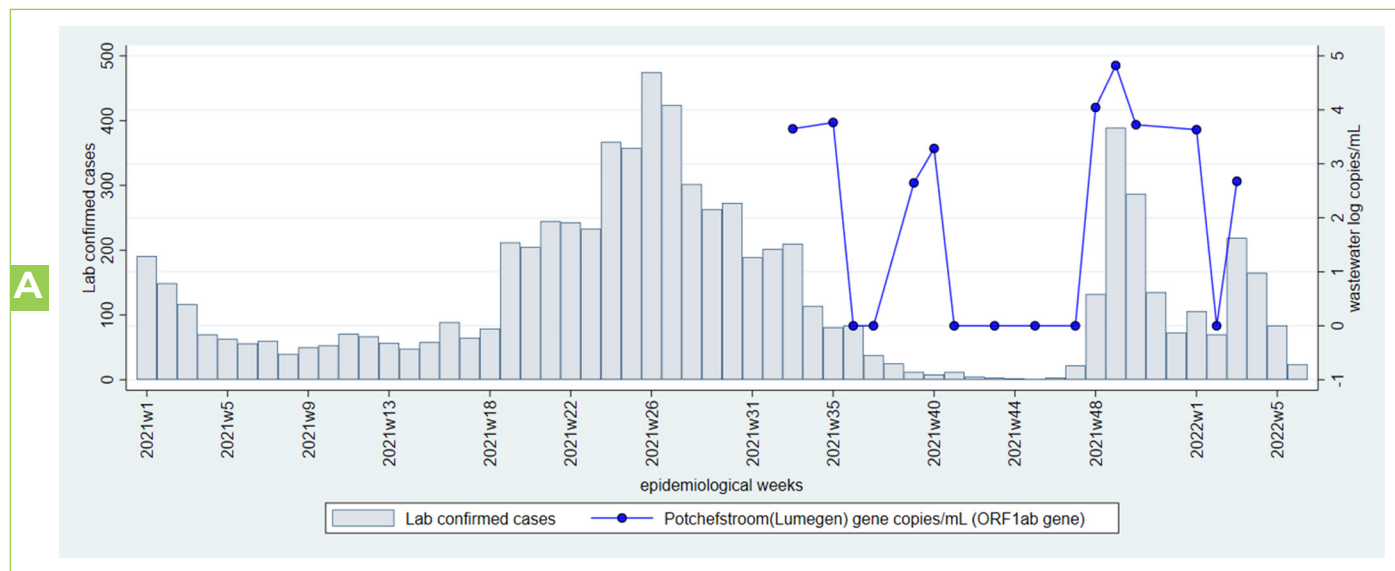
Up until week 49, 2021, SARS-CoV-2 levels were rising or high and suggested ongoing transmission. In Namakwa (Calvinia), the levels were as high as 3 log copies/ml in week 2, 2022. More results are required to compare with the latest number of clinical cases. On the other hand, a current assessment cannot be made for France Baard due to the absence of recent results. The public health authorities should continue surveillance for cases, promote vaccination and non-pharmaceutical interventions.

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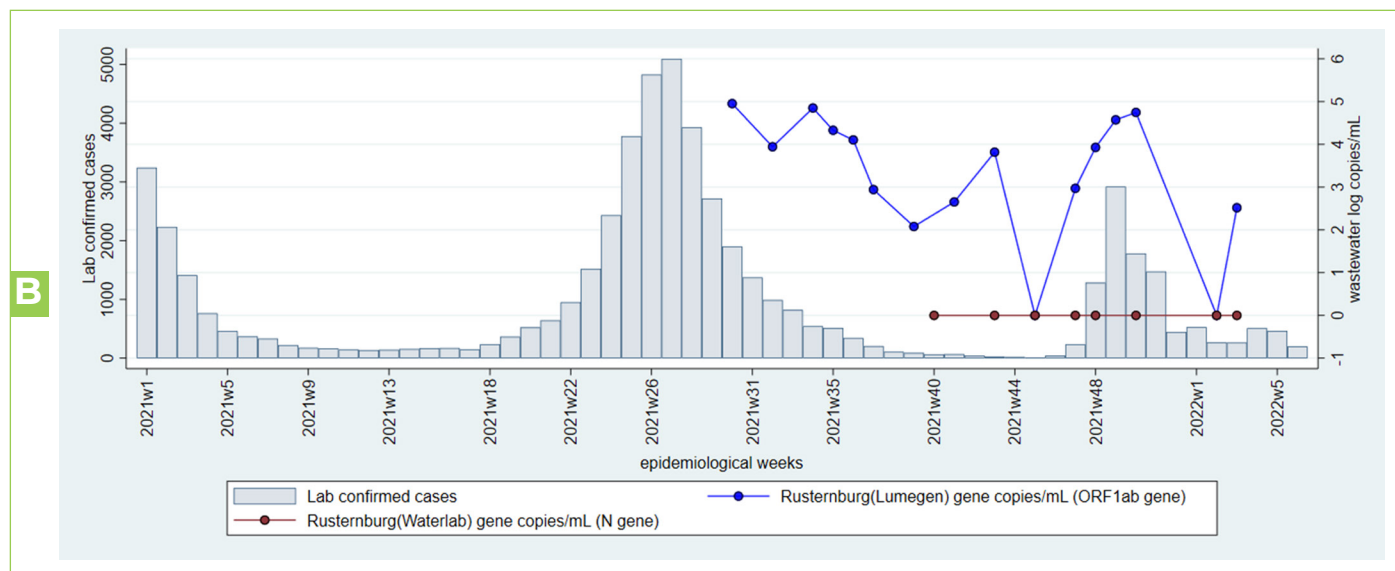
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North West Province

A: JB Marks Local Municipality



B: Bojanala District Municipality



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C: City of Matlosana Municipality

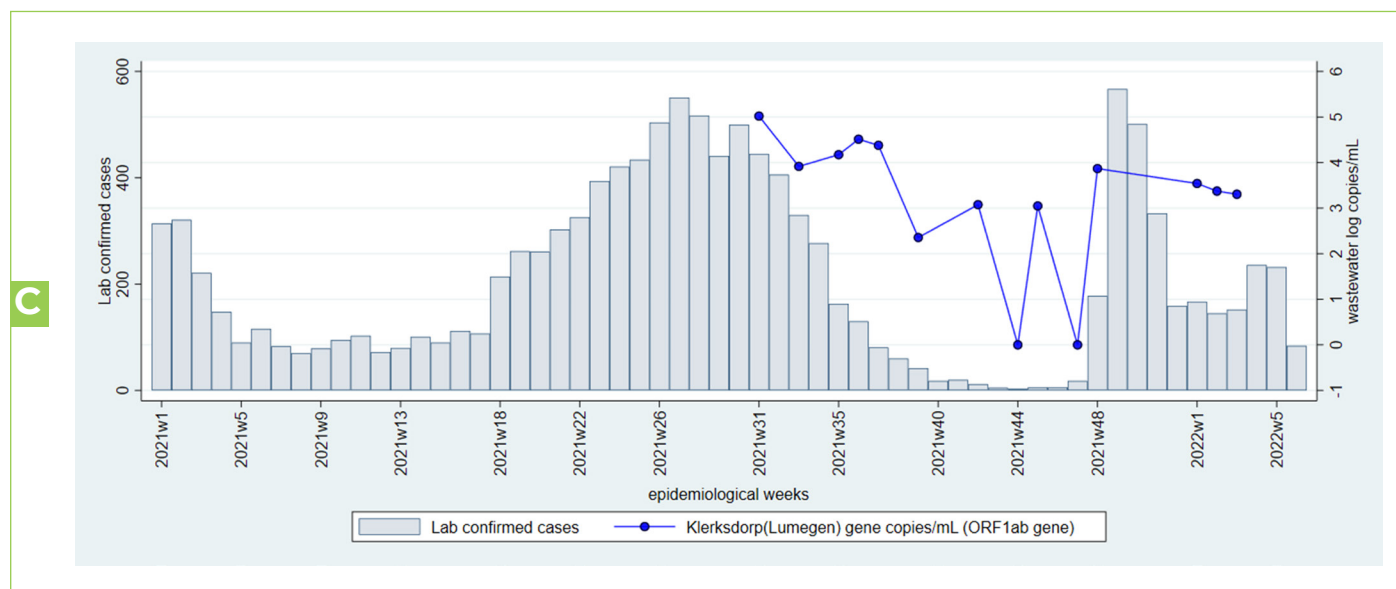


Figure 7A-C. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Potchefstroom, JB Marks District (A) Rustenberg, Bojanala District (B), and City of Matlosana, Northwest Province during epidemiological weeks 1, 2021 to 3, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

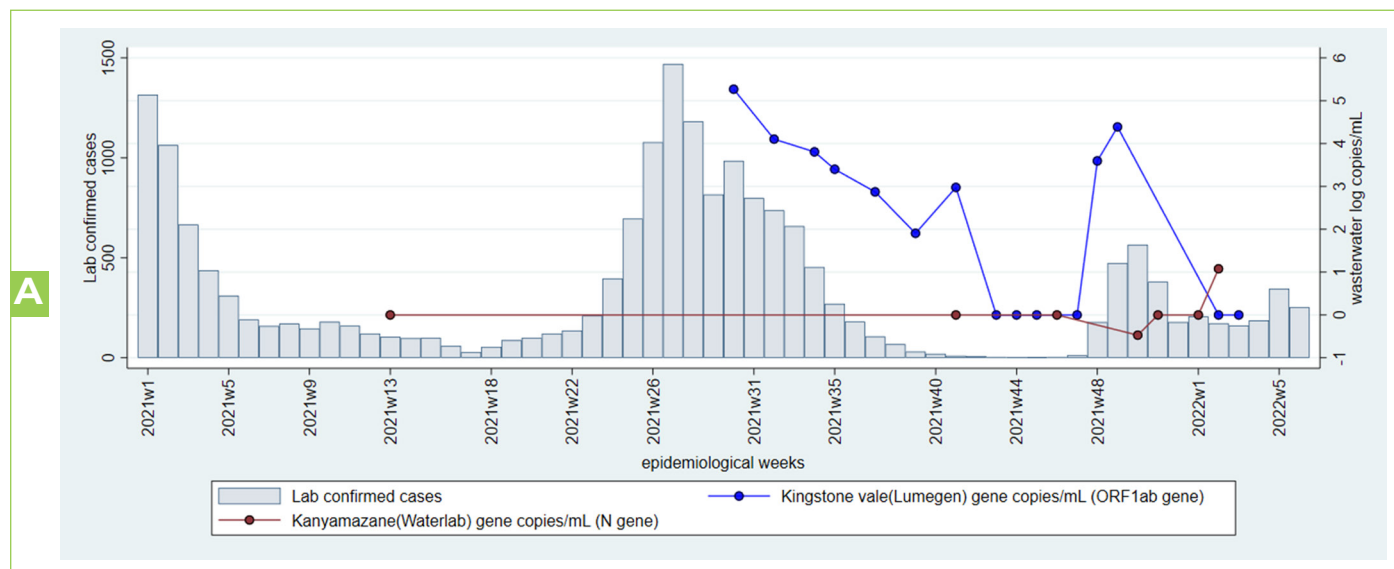
The SARS-CoV-2 levels in Potchefstroom (JB Marks), Rustenburg (Bojanala) and Klerksdorp (Matlosana) as of week 3 were around 3log copies/ml of wastewater suggesting ongoing population transmission by the Omicron subvariant. However, more recent and consistent results are required in this area. The public health authorities should continue surveillance for cases, promote vaccination and non-pharmaceutical interventions.

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Mpumalanga Province

A: Mbombela Local Municipality



B: Emalahleni Local Municipality

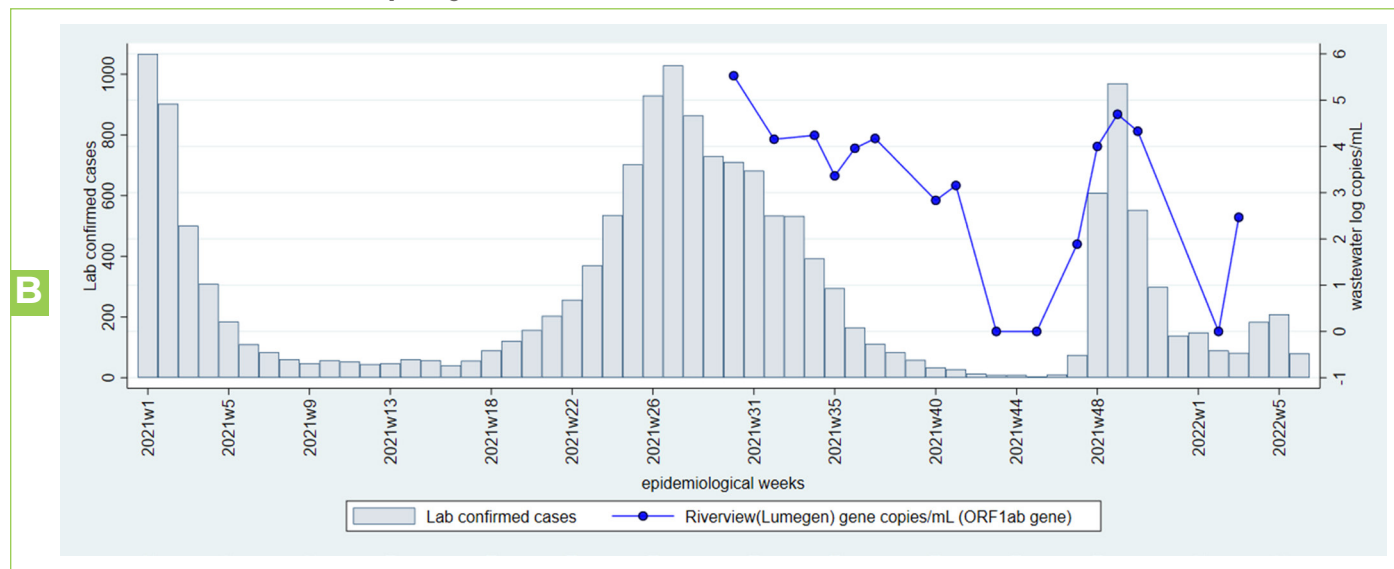


Figure 8A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Mbombela and Emalahleni Local Municipality, Mpumalanga Province during epidemiological weeks 30-45, 2021. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP. Note that comparisons of levels over time should only be made for specimens tested in the same laboratory.

The SARS-CoV-2 levels in Mbombela (Kingstonvale) have consistently decreased corresponding to the decrease in clinical cases. Although the levels in Riverview increased in week 3 suggesting ongoing population transmission by the Omicron subvariant, more recent and consistent results are required in this area. The public health authorities should continue surveillance for cases, promote vaccination and non-pharmaceutical interventions.

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Limpopo Province

Polokwane Local Municipality

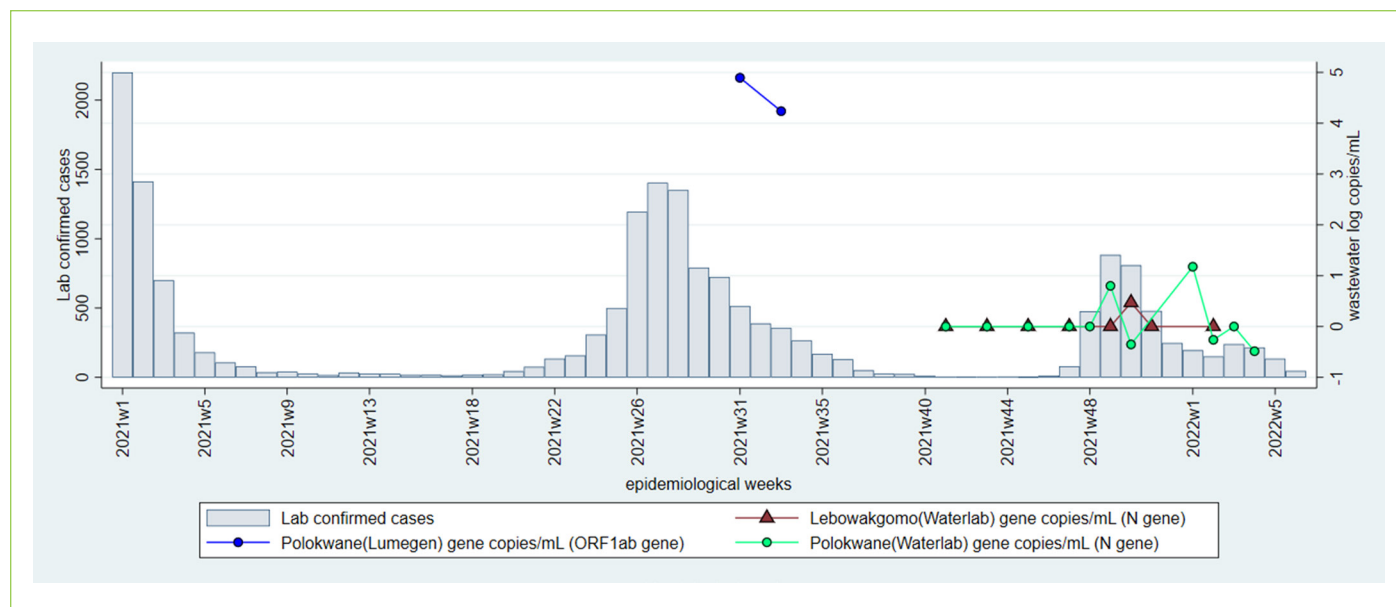


Figure 9. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Polokwane Local Municipality, Limpopo Province during epidemiological weeks 31-33, 2021.

Results are difficult to interpret, but levels are declining in Polokwane corresponding to decline in clinical case load. Current samples are needed for this area.

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LIMITATIONS

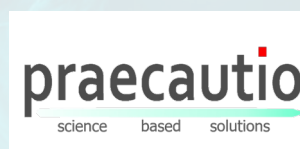
It is not possible to estimate population burden of disease using wastewater testing of SARS-CoV-2 as sources of variability are multiple, including variation in length and concentration of SARS-CoV-2 excretion by infected persons, variation in degradation rate of viral RNA in wastewater and sampling error. Interpretation of results from quantitative testing of SARS-CoV-2 in wastewater is enhanced when the population served by the wastewater treatment plants is well characterised in terms of SARS-CoV-2 testing rates, health seeking behaviour, hospital admissions and deaths due to SARS-CoV-2, as well as other general indicators of health. Further exploration of the relationship between quantitative SARS-CoV-2 results, local trends in clinical case burden, environmental factors, and test methodology will support the interpretation of observed fluctuations in RNA levels. Quality assessment and inter-laboratory comparisons are underway to ensure participating laboratories are providing consistent and comparable results.

CONCLUSION

SARS-CoV-2 data from wastewater at South African sentinel sites show concordance with clinical, epidemiologic curves in the respective locations, illustrating the potential of the SACCESS network to provide descriptive epidemiological data pertaining to geographic variation and burden of SARS-CoV-2.

ACKNOWLEDGEMENTS

- The contributions of local government and wastewater treatment staff to sample collection and transport is acknowledged and appreciated.
- The Water Research Commission is thanked for their vision and support.
- The NICD SARS-CoV-2 epidemiology and IT team members are thanked for sharing district and sub-district case burdens in order to generate graphs. These team members include Andronica Moipone Shonhiwa, Genevieve Ntshoe, Joy Ebonwu, Lactatia Motsuku, Liliwe Shuping, Mazvita Muchengeti, Jackie Kleynhans, Gillian Hunt, Victor Odhiambo Olago, Husna Ismail, Nevashan Govender, Ann Mathews, Vivien Essel, Veerle Msimang, Tendesayi Kufa-Chakezha, Nkengafac Villyen Motaze, Natalie Mayet, Tebogo Mmaborwa Matjokotja, Mzimasi Neti, Tracy Arendse, Teresa Lamola, Itumeleng Matiea, Darren Muganiri, Babongile Ndlovu, Khuliso Ravhuhali, Emelda Ramutshila, Salaminah Mhlana, Akhona Mzoneli, Nimesh Naran, Trisha Whitbread, Mpho Moeti, Chidozie Iwu, Eva Mathatha, Fhatuwani Gavhi, Masingita Makamu, Matimba Makhubele, Simbulele Mdeleleni, Tsumbedzo Mukange, Trevor Bell, Lincoln Darwin, Fazil McKenna, Ndivhuwo Munava, Muzammil Raza Bano, Themba Ngobeni.
- The NICD Centre for Respiratory Disease and Meningitis are thanked for their assistance in setting up and troubleshooting PCR testing, and ongoing supportive collaboration.
- Staff of SACCESS network laboratories are thanked for their assistance in generating these results.



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Supplementary Table: Data for all wastewater treatment plants tested by SACCESS network

| S/ No | Wastewater plant name | Province | Metro or District | Official subdistrict SD or Local municipality | Subdistrict as represented on the graphs | Water service authority | Suburbs in drainage reticulation | Testing laboratory | Date quantitative testing started |
|-------|-----------------------|--------------|--|---|--|--|---|--------------------------|-----------------------------------|
| 1 | Central | Eastern Cape | Buffalo City Local Municipality | Amathole district municipality | No subdistrict | Buffalo City Local Municipality | Wisonia, Dawn, Summer Pride, Amalinda Forest, Haven Hills, Buffalo flats ext, Scenery Park | Praecautio | 20-10-2021 |
| 2 | East Bank | Eastern Cape | Buffalo City Local Municipality | Amathole district municipality | No subdistrict | Buffalo City Local Municipality | Blue Bend, Bonza Bay, Nahoon, Beacon Bay | NICD | 13-04-2021 |
| 3 | Gonubie | Eastern Cape | Buffalo City Local Municipality | Amathole district municipality | No subdistrict | Buffalo City Local Municipality | Sunrise-on-Sea, Gonubie Manor, Thorn Ridge, Cyprus Dale, Bay View, Donny-brook, Gonubie, Gonubie Park | Praecautio | 15-09-2021 |
| 4 | Mdantsane | Eastern Cape | Buffalo City Local Municipality | Amathole district municipality | No subdistrict | Buffalo City Local Municipality | Mdantsane Newlands | NICD | 01-06-2021 |
| 5 | Potsdam | Eastern Cape | Buffalo City Local Municipality | Amathole district municipality | No subdistrict | Buffalo City Local Municipality | Zone 12 to Zone 18, Unit P, Potsdam, Khayelitsha, WSU Potsdam, Campus, Mbekweni | Praecautio | 20-10-2021 |
| 6 | Reeston | Eastern Cape | Buffalo City Local Municipality | Amathole district municipality | No subdistrict | Buffalo City Local Municipality | Reeston, Chicken Farm, Newlife | Praecautio | 15-09-2021 |
| 7 | West Bank | Eastern Cape | Buffalo City Local Municipality | Amathole district municipality | No subdistrict | Buffalo City Local Municipality | Duncan Village, Leach Bay, Nahoon | Praecautio | 15-09-2021 |
| 8 | Zwelitsha | Eastern Cape | Buffalo City Local Municipality | Amathole district municipality | No subdistrict | Buffalo City Local Municipality | Sweet Waters, Zwelitsha, Phakamisa, Ilitha Park | Greenhill and Praecautio | 20-10-2021 |
| 9 | Brickfield | Eastern Cape | Nelson Mandela Metropolitan Municipality | N Mandela A SD | No subdistrict | Nelson Mandela Metropolitan Municipality | KwaNobuhle, Uitenhage, Van Riebeeckhoogte | NICD | 13-04-2021 |
| 10 | KwaNobuhle | Eastern Cape | Nelson Mandela Metropolitan Municipality | N Mandela A SD | No subdistrict | Nelson Mandela Metropolitan Municipality | KwaNobuhle, Uitenhage | NICD | 13-04-2021 |

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| S/ No | Wastewater plant name | Province | Metro or District | Official subdistrict SD or Local municipality | Subdistrict as represented on the graphs | Water service authority | Suburbs in drainage reticulation | Testing laboratory | Date quantitative testing started |
|-------|-----------------------|------------|--|---|--|--|--|--------------------|-----------------------------------|
| 11 | Bainsvlei | Free State | Mangaung | Bloemfontein SD | Bloemfontein | Mangaung | Bloemfontein, Bain's Vlei | Lumegen | 01-09-2021 |
| 12 | Bloemspruit | Free State | Mangaung | Bloemfontein SD | Bloemfontein | Mangaung | Langenhoven Park, Bloemfontein | NICD | 16-03-2021 |
| 13 | Northeastern works | Free State | Mangaung | Bloemfontein SD | Bloemfontein | Mangaung | Bloemfontein Maselapoort, Rustig | Lumegen | 01-09-2021 |
| 14 | Sterkwater | Free State | Mangaung | Bloemfontein SD | Bloemfontein | Mangaung | Fontejntjie, Rooidam | NICD | 16-03-2021 |
| 15 | Botshabelo | Free State | Mangaung | Botshabelo SD | Botshabelo | Mangaung | Bonolo, Botshabelo, Poklenberg, Dankbaar, Roodekop | Lumegen | 04-10-2021 |
| 16 | Welvaart | Free State | Mangaung | Botshabelo SD | Botshabelo | Mangaung | Kagisanong, Fichardtpark, Bochebela, Phahameng, Generaal deWet, Willows, Batho, Rocklands, Universitas | Lumegen | 09-09-2021 |
| 17 | Northern Works | Free State | Mangaung | Bloemfontein SD | Bloemfontein | Mangaung | Midway, Bloemspruit, Grasslands | Lumegen | 01-09-2021 |
| 18 | Dewetsdorp | Free State | Mangaung | Naledi SD | Naledi and Thabanchu | Mangaung | Dewetsdorp, Frankfort, Glengary | Lumegen | 01-09-2021 |
| 19 | Thaba Nchu | Free State | Mangaung | Thaba N'chu SD | Naledi and Thabanchu | Mangaung | Thaba Nchu, Mokwena, Selosha, Abramskraal, Roodekop, Strydom College, Bultfontein Number Three, Ratlau, Serwalo, Bultfontein Number One, Bultfontein Number Two, Motlala, Lusaka | Lumegen | 01-09-2021 |
| 20 | Ennerdale | Gauteng | City of Johannesburg Metropolitan Municipality | Johannesburg G SD | No subdistrict | City of Johannesburg Metropolitan Municipality | Walkerville, Hartsenberfontein, Althea, Golfview, Blignautrus | NIOH | 04-10-2021 |

WASTEWATER-BASED EPIDEMIOLOGY FOR SARS-COV-2 SURVEILLANCE IN SOUTH AFRICA

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| S/ No | Wastewater plant name | Province | Metro or District | Official subdistrict SD or Local municipality | Subdistrict as represented on the graphs | Water service authority | Suburbs in drainage reticulation | Testing laboratory | Date quantitative testing started |
|-------|-------------------------------------|----------|--|---|--|--|--|----------------------|-----------------------------------|
| 21 | Northern Wastewater Treatment Works | Gauteng | City of Johannesburg Metropolitan Municipality | Johannesburg A SD | No subdistrict | City of Johannesburg Metropolitan Municipality | Strydompark, Olivedale, Rivonia, Jukskei Park, Douglasdale, Ferndale, Lone Hill, Sandton, North Riding, Fourways, Paulshof | NICD | 06-04-2021 |
| 22 | Goudkoppies | Gauteng | City of Johannesburg Metropolitan Municipality | Johannesburg D SD | No subdistrict | City of Johannesburg Metropolitan Municipality | Soweto, Rivasdale | NICD | 24-05-2021 |
| 23 | Bushkoppies | Gauteng | City of Johannesburg Metropolitan Municipality | Johannesburg G SD | No subdistrict | City of Johannesburg Metropolitan Municipality | Baragwanath, Pimville, Johannesburg South, Dube, Willowdene, Nancefield | Waterlab/UP | 11-10-2021 |
| 24 | Olifantsvlei | Gauteng | City of Johannesburg Metropolitan Municipality | Johannesburg G SD | No subdistrict | City of Johannesburg Metropolitan Municipality | Soweto, Eldorado, Lenasia | Waterlab/UP | 11-10-2021 |
| 25 | Driefontein | Gauteng | City of Johannesburg Metropolitan Municipality | Mogale City LM | No subdistrict | City of Johannesburg Metropolitan Municipality | Kelvin, Morningside Manor, Edenburg, Lone Hill, Rivonia, Sandton, Northdene, Fourways, Paulshof | NIOH | 04-10-2021 |
| 26 | Bronk-hortspruit | Gauteng | City of Tshwane Metropolitan Municipality | Thembisile Hani LM | Tshwane North (sub-districts 3,4,6,7) | City of Johannesburg Metropolitan Municipality | Wilgerivier, Wonderfontein, Graley Crown Douglas, Bronkhorst | NICD and Waterlab/UP | 04-10-2021 |
| 27 | Klipgat | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 1 SD | Tshwane North (sub-districts 1,2) | City of Tshwane Metropolitan Municipality | Klipgat, Boekenhoutfontein, Soshanguve, Mabopane, Honeyvale, Boekenhoutfontein, Lebaleng | SAMRC-TB | 02-11-2021 |
| 28 | Sandspruit | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 1 SD | Tshwane North (sub-districts 1,2) | City of Tshwane Metropolitan Municipality | Medunsa, Hebron, Rosslyn, Strydfontein, Hornsnek, Kruisfontein | Waterlab/UP | 11-09-2021 |
| 29 | Rooiwal Eastern Works | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 2 SD | Tshwane North (sub-districts 1,2) | City of Tshwane Metropolitan Municipality | Atteridgeville, Pretoria CBD, Pretoria North, Rosslyn | NICD | 23-03-2021 |
| 30 | Temba | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 2 SD | Tshwane North (sub-districts 1,2) | City of Tshwane Metropolitan Municipality | Majanan, Hammanskraal, Mabopane, Soshanguve, Pyramid, Doornpoort | Waterlab/UP | 26-09-2021 |

WASTEWATER-BASED EPIDEMIOLOGY FOR SARS-COV-2 SURVEILLANCE IN SOUTH AFRICA

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| S/ No | Wastewater plant name | Province | Metro or District | Official subdistrict SD or Local municipality | Subdistrict as represented on the graphs | Water service authority | Suburbs in drainage reticulation | Testing laboratory | Date quantitative testing started |
|-------|-------------------------------------|----------|---|---|--|---|---|--------------------|-----------------------------------|
| 31 | Daspoort Wastewater Treatment Works | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 3 SD | Tshwane North (sub-districts 3,4,6,7) | City of Tshwane Metropolitan Municipality | Groenkloof, Arcadia, Pretoria South, Gezina, Hercules, Rietfontein, Pretoria Central, Sunnyside, Pretoria East, Prinshof, Daspoort, Villieria, Capital Park, Pretoria West, Wonderboom South, Pretoria-Wes, Innesdale | NICD | 02-03-2021 |
| 32 | Sunderland Ridge | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 4 SD | Tshwane North (sub-districts 3,4,6,7) | City of Tshwane Metropolitan Municipality | Centurion, Olivenhoutbosch and some parts of Midrand. | NIOH | 18-08-2021 |
| 33 | Babelegi | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 5 SD | Tshwane North (sub-districts 5) | City of Tshwane Metropolitan Municipality | Industrial sites | NIOH | 18-08-2021 |
| 34 | Baviaanspoort | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 5 SD | Tshwane North (sub-districts 5) | City of Tshwane Metropolitan Municipality | Elandsfontein, Cullinan, Sonderwater | NIOH | 18-08-2021 |
| 35 | Refilwe | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 5 SD | Tshwane North (sub-districts 5) | City of Tshwane Metropolitan Municipality | Cullinan | NIOH | 05-10-2021 |
| 36 | Zeekoegat | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 5 SD | Tshwane North (sub-districts 5) | City of Tshwane Metropolitan Municipality | Zeekoegat, Magalies Water, Buffelsdrif | Waterlab/UP | 04-10-2021 |
| 37 | Godrich | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 7 SD | Tshwane North (sub-districts 3,4,6,7) | City of Tshwane Metropolitan Municipality | Bronkspruit town Rhema Park Caltura park Venster Park Zithobeni | SAMRC-TB | 13-09-2021 |
| 38 | Summer Place Pack-age Plant | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 7 SD | Tshwane North (sub-districts 3,4,6,7) | City of Tshwane Metropolitan Municipality | Summerplace | SAMRC-TB | 01-09-2021 |
| 39 | Rietgat | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 2 SD | Tshwane North (sub-districts 1,2) | City of Tshwane Metropolitan Municipality | Soshanguve | NIOH | 05-10-2021 |
| 40 | Thaba Tshwane | Gauteng | City of Tshwane Metropolitan Municipality | Tshwane 2 SD | Tshwane North (sub-districts 1,2) | City of Tshwane Metropolitan Municipality | Thaba Tshwane, Generaal Kemp Heuwel Radio Uitkyk | Waterlab/UP | 05-10-2022 |

WASTEWATER-BASED EPIDEMIOLOGY FOR SARS-COV-2 SURVEILLANCE IN SOUTH AFRICA

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| S/ No | Wastewater plant name | Province | Metro or District | Official subdistrict SD or Local municipality | Subdistrict as represented on the graphs | Water service authority | Suburbs in drainage reticulation | Testing laboratory | Date quantitative testing started |
|-------|--|----------|--------------------------------------|---|--|--------------------------------------|---|---------------------|---|
| 41 | Daveyton WasteWater Treatment Works | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni E1 SD | Ekurhuleni East (E1, E2) | Ekurhuleni Metropolitan Municipality | Welgedag, Persida | NICD and CSIR | NICD:02-03-2021 CSIR: 21-09-2021 |
| 42 | Rynfield | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni E1 SD | Ekurhuleni East (E1, E2) | Ekurhuleni Metropolitan Municipality | New Modder, Lakefield, Benoni, Boksburg, Northmead, Atlasville | CSIR | 21-09-2021 |
| 43 | Ancor | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni E2 SD | Ekurhuleni East (E1, E2) | Ekurhuleni Metropolitan Municipality | Welgedag, Payneville, Selcourt, Casseldale, Springs | Waterlab/UP | 21-09-2021 |
| 44 | Carl Grun-dlingh | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni E2 SD | Ekurhuleni East (E1, E2) | Ekurhuleni Metropolitan Municipality | Nigel, Bultfontein, Laversburg | CSIR | 21-09-2021 |
| 45 | Jan Smuts | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni E2 SD | Ekurhuleni East (E1, E2) | Ekurhuleni Metropolitan Municipality | Dalpark, Brakpan, Dersley, Dalview, Benoni, New Modder, Schapenrust | CSIR | 21-09-2021 |
| 46 | Tsakane | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni E2 SD | Ekurhuleni East (E1, E2) | Ekurhuleni Metropolitan Municipality | Benoni, Dersley, Dalpark, Brakpan, Dalview, Schapenrust | Waterlab/UP | 05-10-2021 |
| 47 | Welgedacht | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni E2 SD | Ekurhuleni East (E1, E2) | Ekurhuleni Metropolitan Municipality | KwaThema, Brakpan, Dersley, Schapenrust | Waterlab/UP | 21-09-2021 |
| 48 | Hartebees-fontein WasteWater Treatment Works | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni N1 SD | Ekurhuleni North (N1, N2) | Ekurhuleni Metropolitan Municipality | Mid-Ennerdale, Althea, Grasmere, Elandsfontein | NICD Waterlab/UP | NICD:02-03-2021 Waterlab/UP : 05-10-2021 |
| 49 | Herbert Bickley | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni N1 SD | Ekurhuleni North (N1, N2) | Ekurhuleni Metropolitan Municipality | Jameson Park | CSIR | 21-09-2021 |
| 50 | Olifantsfontein WasteWater Treatment Works | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni N1 SD | Ekurhuleni North (N1, N2) | Ekurhuleni Metropolitan Municipality | Pinedene, Clayville, Tembisa, Midstream Estates, Olifantsfontein | CSIR and NICD | CSIR: 21-09-2021 NICD: 02-03-2021 |
| 51 | Benoni | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni N2 SD | Ekurhuleni North (N1, N2) | Ekurhuleni Metropolitan Municipality | Northmead, Dalpark, Dalview, Lakefield, Benoni, New Modder | Waterlab/UP | 05-10-2021 |

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| S/ No | Wastewater plant name | Province | Metro or District | Official subdistrict SD or Local municipality | Subdistrict as represented on the graphs | Water service authority | Suburbs in drainage reticulation | Testing laboratory | Date quantitative testing started |
|-------|---------------------------------------|---------------|--------------------------------------|---|--|--------------------------------------|---|--------------------|--------------------------------------|
| 52 | J.P. Marais | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni N2 SD | Ekurhuleni North (N1, N2) | Ekurhuleni Metropolitan Municipality | Northmead, Atlasville, New Modder, Lakefield, Benoni | CSIR | 21-09-2021 |
| 53 | Dekema | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni S1 SD | Ekurhuleni South (S1, S2) | Ekurhuleni Metropolitan Municipality | Katlehong, Natalspruit, Randhart, Alrode | Waterlab/UP | 05-10-2021 |
| 54 | Rondebult | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni S1 SD | Ekurhuleni South (S1, S2) | Ekurhuleni Metropolitan Municipality | Bartlett, Atlasville, Boksburg North, Lakefield, Bonaero Park, Ravenswood, Witfield, Boksburg | Waterlab/UP | 21-09-2021 |
| 55 | Vlakplaats WasteWater Treatment Works | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni S2 SD | Ekurhuleni South (S1, S2) | Ekurhuleni Metropolitan Municipality | Vosloorus | NICD and CSIR | NICD: 22-02-2021 CSIR: 21-09-2021 |
| 56 | Waterval WWTW | Gauteng | Ekurhuleni Metropolitan Municipality | Ekurhuleni S2 SD | Ekurhuleni South (S1, S2) | Ekurhuleni Metropolitan Municipality | Kliprivier, Henley on Klip, Ophir, Glen Donald, Chrissiefontein, Rothdene, Riversdale, Meyerton Farms | Waterlab/UP | 21-09-2021 |
| 57 | Flip Human | Gauteng | West Rand | West Rand *(Johannesburg C SD) | No Subdistrict | Mogale City Local Municipality | Rietvallei, Bhongwem, Brink's Vlakfontein | Waterlab/UP | 12-10-2021 |
| 58 | Magaliesburg | Gauteng | West Rand | West Rand (Mogale City LM) | No Subdistrict | Mogale City Local Municipality | Magaliesburg, Mogale City | Waterlab/UP | 12-10-2021 |
| 59 | Percy Steward | Gauteng | West Rand | West Rand (Mogale City LM) | No Subdistrict | Mogale City Local Municipality | Lewisham, Krugersdorp North | Waterlab/UP | 12-10-2021 |
| 60 | Hammarsdale | Kwazulu-Natal | eThekweni Metropolitan Municipality | eThekweni MM Sub | eThekweni West | eThekweni Metropolitan Municipality | Hammarsdale, Elangeni, Mpumalanga | GreenHill | 02-09-2021 |
| 61 | Hillcrest | Kwazulu-Natal | eThekweni Metropolitan Municipality | eThekweni MM Sub | eThekweni West | eThekweni Metropolitan Municipality | New Germany, Pinetown, Clermont, Pinelands, KwaDabeka | GreenHill | 02-09-2021 |

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| S/ No | Wastewater plant name | Province | Metro or District | Official subdistrict SD or Local municipality | Subdistrict as represented on the graphs | Water service authority | Suburbs in drainage reticulation | Testing laboratory | Date quantitative testing started |
|-------|-----------------------|---------------|-------------------------------------|---|--|-------------------------------------|--|--------------------|---|
| 62 | Isipingo | Kwazulu-Natal | eThekweni Metropolitan Municipality | eThekweni MM Sub | eThekweni South | eThekweni Metropolitan Municipality | Umbogintwini, Malukaze, Egolokodo, KwaMakhutha, Umlazi | DUT | 10-08-2021 |
| 63 | Central | Kwazulu-Natal | eThekweni Metropolitan Municipality | eThekweni MM Sub | eThekweni North | eThekweni Metropolitan Municipality | Brighton Beach, Grosvenor, King's Rest, Ocean View, Fynnland and Treasure Beach | NICD and DUT | NICD: 22-02-2021 DUT: 10-08-2021 for DUT |
| 64 | KwaMashu | Kwazulu-Natal | eThekweni Metropolitan Municipality | eThekweni MM Sub | eThekweni South | eThekweni Metropolitan Municipality | La Lucia, Umhlanga, Prestondale, Phoenix, Duff's Road, Glen Ashley, Mount Edgecombe | DUT | 10-08-2021 |
| 65 | Northern | Kwazulu-Natal | eThekweni Metropolitan Municipality | eThekweni MM Sub | eThekweni North | eThekweni Metropolitan Municipality | Newlands, KwaMashu, Greenwood Park, Park Hill | NICD | 22-02-2021 |
| 66 | Phoenix | Kwazulu-Natal | eThekweni Metropolitan Municipality | eThekweni MM Sub | eThekweni South | eThekweni Metropolitan Municipality | Rietrivier, KwaMashu, Duff's Road, Mount Edgecombe, Phoenix, Richmond, Inanda | DUT | 10-08-2021 |
| 67 | Fraser's | Kwazulu-Natal | eThekweni Metropolitan Municipality | eThekweni MM Sub | eThekweni North | iLembe District municipality | Salt Rock, Ballitoville, Umhlali, Fraser, Zimbali, Shaka's Rock, Ballito | Waterlab/UP | 11-10-2021 |
| 68 | Darvill | Kwazulu-Natal | uMgungundlovu District municipality | Msunduzi LM | No subdistrict | The Msunduzi Local Municipality | Pelham, Hayfields, New England, Northdale, Hay Paddock, Scottsville, Cleland, Bishopstowe, Sobantu | GreenHill | 02-09-2021 |
| 69 | Lynfield Park | Kwazulu-Natal | uMgungundlovu District municipality | Msunduzi LM | No subdistrict | The Msunduzi Local Municipality | Thornville, Hayfields, Hay Paddock, Lynnfield Park, Cleland | GreenHill | 02-09-2021 |
| 70 | Mpofana | Kwazulu-Natal | Umgungundlovu District municipality | Mpofana LM | No subdistrict | Mpofana Local Municipality | Bruntville, Brown Stones, Windy, Weston, Moorivier | Waterlab/UP | 28-09-2021 |
| 71 | Howick | Kwazulu-Natal | Umgungundlovu District municipality | uMngeni LM | No subdistrict | UMgungundlovu District municipality | Riversdale, Merrivale, Cedara | GreenHill | 02-09-2021 |

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| S/ No | Wastewater plant name | Province | Metro or District | Official subdistrict SD or Local municipality | Subdistrict as represented on the graphs | Water service authority | Suburbs in drainage reticulation | Testing laboratory | Date quantitative testing started |
|----------|----------------------------|------------------|------------------------------------|--|--|------------------------------------|---|------------------------------|--|
| 72 | Polokwane | Limpopo | Capricorn District municipality | Polokwane LM | No subdistrict | Polokwane Local Municipality | Westenburg, Nirvana, Bendor, Welgelegen, Moregloed, Annadale, Ivydale, Flora Park, Fauna Park, Penina Park, Ivy Park, Ster Park, Dalmada, Broadlands, Woodlands, and Thornhill | Lumegen: /Waterlab/ UP | Lumegen: 02-08-2021 Waterlab/UP: 11-10-2021 |
| 73 | Lebowakgo- mo | Limpopo | Capricorn District municipality | Lepelle-Nkumpi LM | No subdistrict | Lepelle-Nkumpi LM | Thabamoopo, Vaalboschlaagte, Lekhuswaneng, Moepeng, Makurung, Sekurwaneng, Ga- Matshele, Makurun | Waterlab/ UP | 12-10-2021 |
| 74 | Emalahleni (Riverview) | Mpumalanga | Nkangala District Municipality | Emalahleni LM | No subdistrict | Emalahleni LM | Lynnville, Duvhapark, Paxton, Klipfontein | Lumegen | 26-07-2021 |
| 75 | Kanyamazane | Mpumalanga | Ehlanzeni District | City of Mbombela LM | No subdistrict | Mbombela/ Umjindi | Daantjiekasie, eNyamazani | Waterlab/ UP | 30-03-2021 |
| 76 | Mbombela (Kingstonvale) | Mpumalanga | Ehlanzeni District | Mbombela/ Umjindi | No subdistrict | Mbombela/ Umjindi | Gutshwa, eMpumalanga, eNyalungu, Dwaleni, Hlahlau, Phasha, Ngodini | Lumegen | 26-07-2021 |
| 77 | Calvina | Northern Cape | Namakwa | Hantam Local Municipality | No subdistrict | Hantam Local Municipality | Calvinia | NICD | 06-07-2021 |
| 78 | Homevale Kimberley | Northern Cape | Frances Baard | Sol Plaatjie Local Municipality | No subdistrict | Sol Plaatjie Local Municipality | Remount Camp, Kenilworth, Homevale | Lumegen | 28-08-2021 |
| 79 | Potchefstroom | North- West | JB Marks Local Municipality | JB Marks LM | No subdistrict | JB Marks Local Municipality | Harpington, Vyfhoek, Mooibank, Wilgeboom | Lumegen | 17-08-2021 |
| 80 | Klerksdorp main | North- West | Matlosana Local Municipality | City of Matlosana LM | No subdistrict | Matlosana Local Municipality | Boetrand, Wilkoppies | Lumegen | 02-08-2021 |

WASTEWATER-BASED EPIDEMIOLOGY FOR SARS-COV-2 SURVEILLANCE IN SOUTH AFRICA

WEEK 6 2022

| S/ No | Wastewater plant name | Province | Metro or District | Official subdistrict SD or Local municipality | Subdistrict as represented on the graphs | Water service authority | Suburbs in drainage reticulation | Testing laboratory | Date quantitative testing started |
|-------|-----------------------|--------------|---|---|--|---|--------------------------------------|-------------------------|---|
| 81 | Rustenburg | North-West | Rustenburg Local Municipality | Rustenburg Local Municipality | No subdistrict | Rustenburg Local Municipality | Rustenburg | Lumegen and Waterlab/UP | Lumegen: 26-07-2021 Waterlab: 11-10-2021 |
| 82 | Bellville | Western Cape | City of Cape Town Metropolitan Municipality | CT Northern SD | No subdistrict | City of Cape Town Metropolitan Municipality | Parow, Belhar, Brackenfell | MRC-BRIP | Not testing quantitatively |
| 83 | Cape Flats | Western Cape | City of Cape Town Metropolitan Municipality | CT Southern SD | No subdistrict | City of Cape Town Metropolitan Municipality | Khayelitsha, Mitchells Plain | Waterlab/UP | 06-10-2022 |
| 84 | Athlone | Western Cape | City of Cape Town Metropolitan Municipality | CT Tygerberg | No subdistrict | City of Cape Town Metropolitan Municipality | Crawford, Gleemore, Rondenbosch East | Waterlab/UP | 06-10-2021 |
| 85 | Borchard's Quarry | Western Cape | City of Cape Town Metropolitan Municipality | CT Tygerberg SD | No subdistrict | City of Cape Town Metropolitan Municipality | Belhar, Elsiesrivier | NICD | 09-03-2021 |
| 86 | Zandvliet | Western Cape | City of Cape Town Metropolitan Municipality | Stellenbosch LM | No subdistrict | City of Cape Town Metropolitan Municipality | Faure, Macassar, Somerset West | NICD | 09-03-2021 |