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

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

WEEK 12 2022

OVERVIEW

This report summarises and interprets findings from detection, quantification and sequencing of SARS-CoV-2 by the National Institute for Communicable Diseases (NICD), Centre for Vaccines and Immunology from influent (untreated) wastewater in 17 wastewater treatment plants (WWTP) across five South African provinces. The report also presents results and interpretations of levels of SARS-CoV-2 in wastewater from 70 additional plants across South Africa tested by SACCESS partners including the National Institute for Occupational Health, Lumegen Laboratories, GreenHill Laboratories, SAMRC-TB Platform (until March 2022) and Praecautio to cover all provinces in South Africa. Levels of SARS-CoV-2 in wastewater correlate with population levels of SARS-CoV-2 over time and indicate the geographic distribution of disease. Variants of SARS-CoV-2 can be identified in wastewater through detection of single-nucleotide polymorphisms (SNPs) that are specific to each variant. These variants are shown to correspond to variants prevalent in clinical cases, across time and place. 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 31st March 2022 (epidemiological week 12). Results from wastewater testing should be read and interpreted together with testing and genomic reports generated by the Centre for Respiratory Diseases and Meningitis found at https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/

- Part 1 of this report presents methods and results of quantitative testing of wastewater.
- Part 2 of this report presents methods and results from sequencing of SARS-CoV-2 RNA fragments in wastewater.

Generally, SARS-CoV-2 in wastewater remains at low levels across the country corresponding to the low numbers of clinical cases after the 4th wave. Omicron variant is present in wastewater across the country. Detailed analyses are described below.

HIGHLIGHTS

- **SARS-CoV-2 levels in wastewater:** Most wastewater plants across South Africa are showing low levels of SARS-CoV-2. However, sustained increases of three weeks' duration were observed in Mabopane, City of Tshwane (Klipgat WWTP), but sustained increases are not seen in other plants in Tshwane. Levels at three plants in Ekurhuleni increased (Herbert Bickley, Hartebeesfontein and Vlakplaats) but these are presently of uncertain significance. Levels in Soweto (Goudkoppies WWTP) dropped over last week after two successive increases the weeks before.
- **SARS-CoV-2 genomics in wastewater:** Sequencing data show the presence of omicron variant in all recent samples across South Africa (up to week 7, 2022). Mutations, associated with the VOC delta, were found in Gauteng (week 4, Tshwane and week 7, Johannesburg).

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PART 1: 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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7. Greenhill Laboratories
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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 sent by testing laboratories to the National Institute for Communicable Diseases (NICD) via the DATCOV system. From these data sources, 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 eThekwini (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.

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	Skimmed milk flocculation	MagMAX Viral/ 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	Skimmed 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 Platform)	Grab	None – sample is centrifuged then supernatant analysed	ZymoBiomics 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)

wastewater

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.

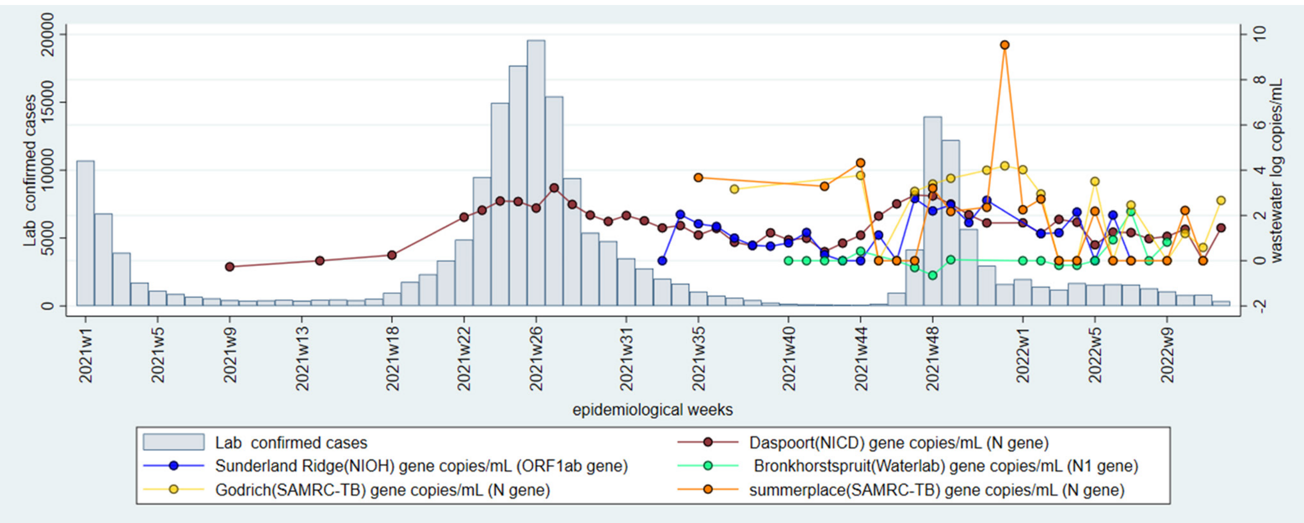
Testing modality	Interpretive principles to support public health responses
Detection of SARS-CoV-2	When a test result changes from · positive to negative, this signifies fewer/no cases in population · negative to positive, this indicates the need for increased population awareness and action · Qualitative results (presence or absence) are comparable between laboratories
Quantification of SARS-CoV-2	*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

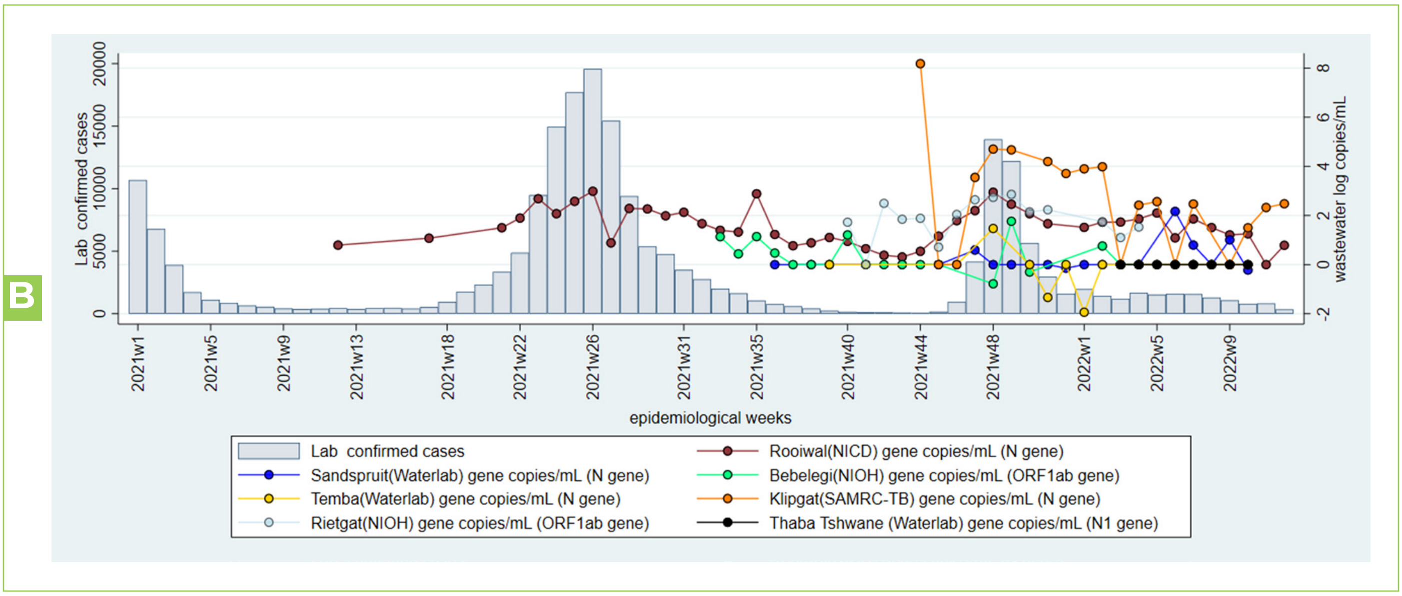
Gauteng Province

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

A



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



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

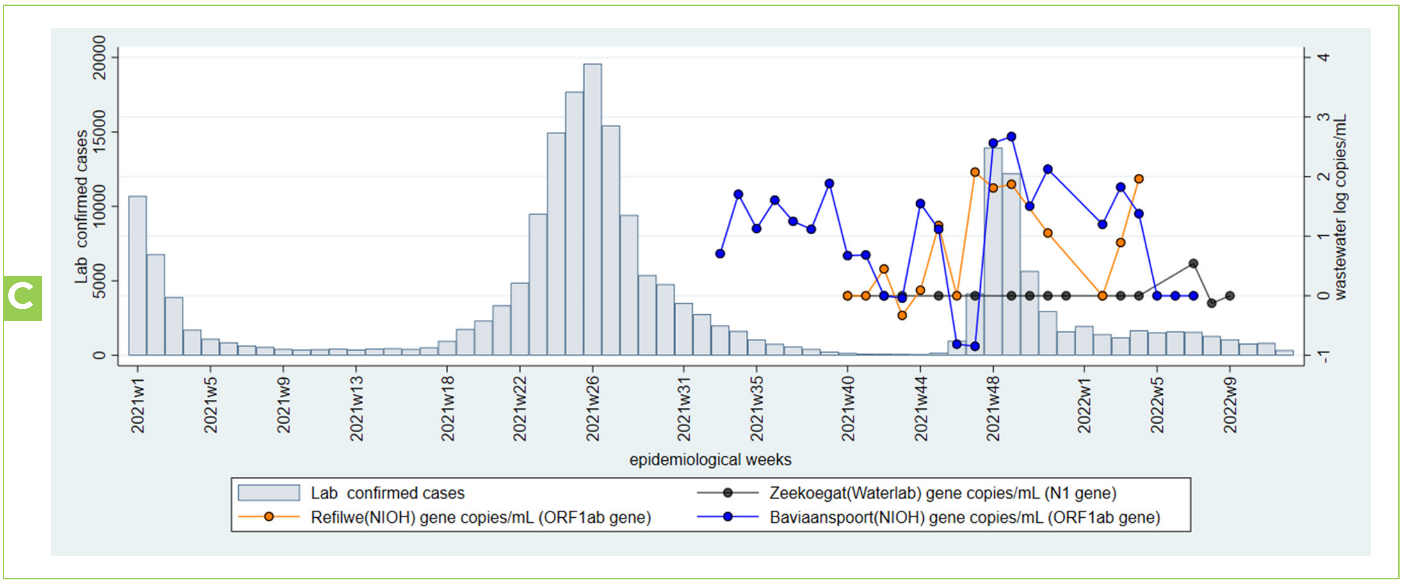


Figure 1 A-C. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) and metropolitan areas in Tshwane District Municipality (Tshwane South and North), Gauteng Province during epidemiological weeks 1 of 2021 to week 12 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.

D: City of Johannesburg Metropolitan Municipality

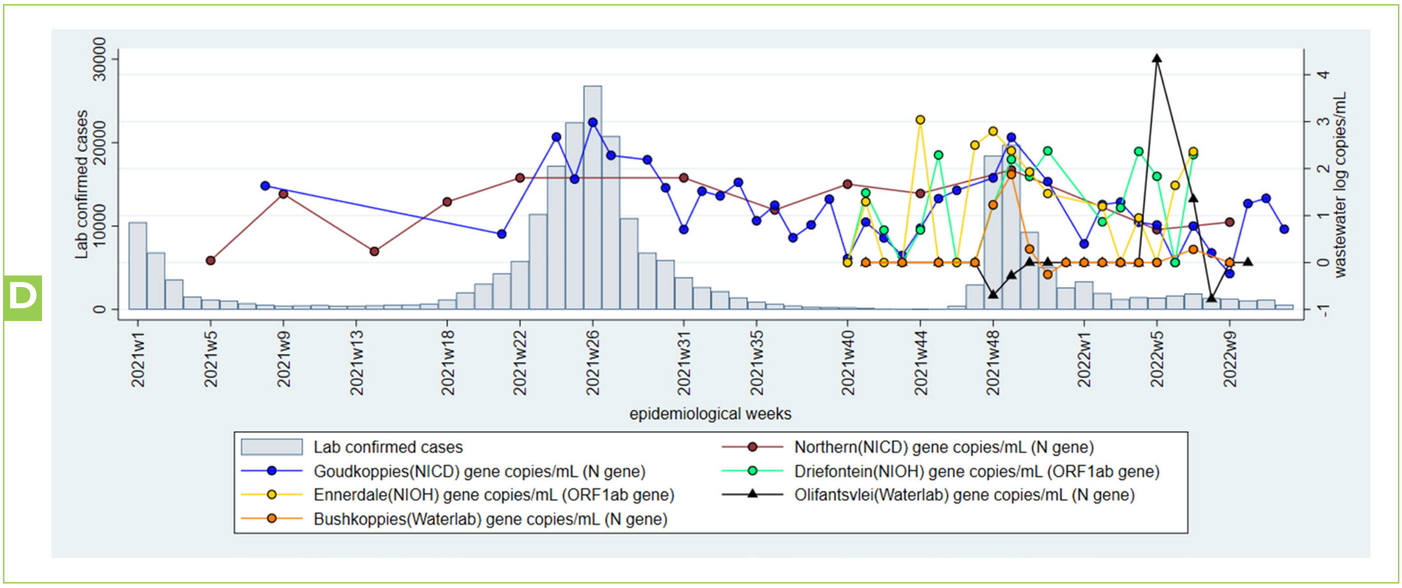
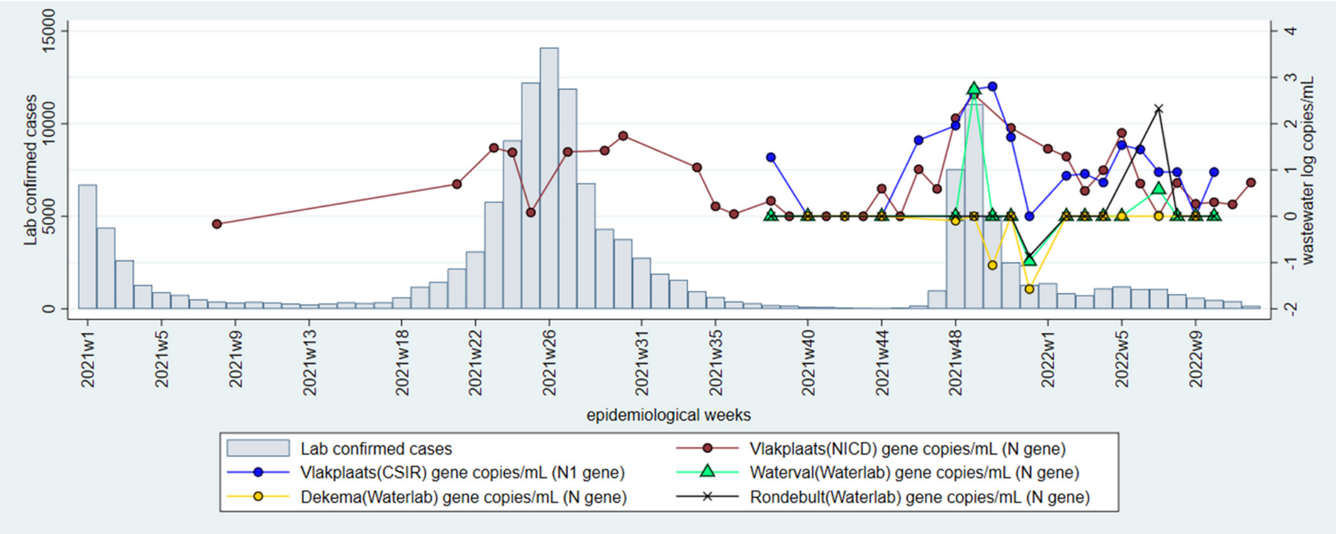


Figure 1 D. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTPs) in the City of Johannesburg Metropolitan Municipality, Gauteng Province during epidemiological weeks 1 of 2021 to week 12 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.

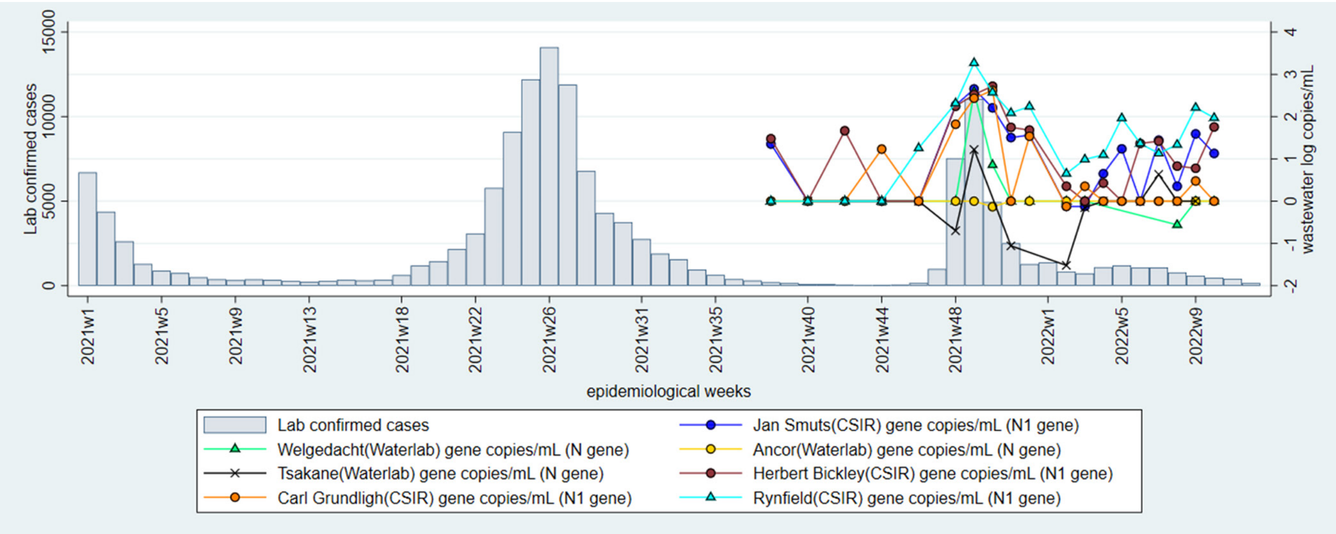
E: Ekurhuleni East (sub-districts D, E or E1, E2)

E



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

F



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

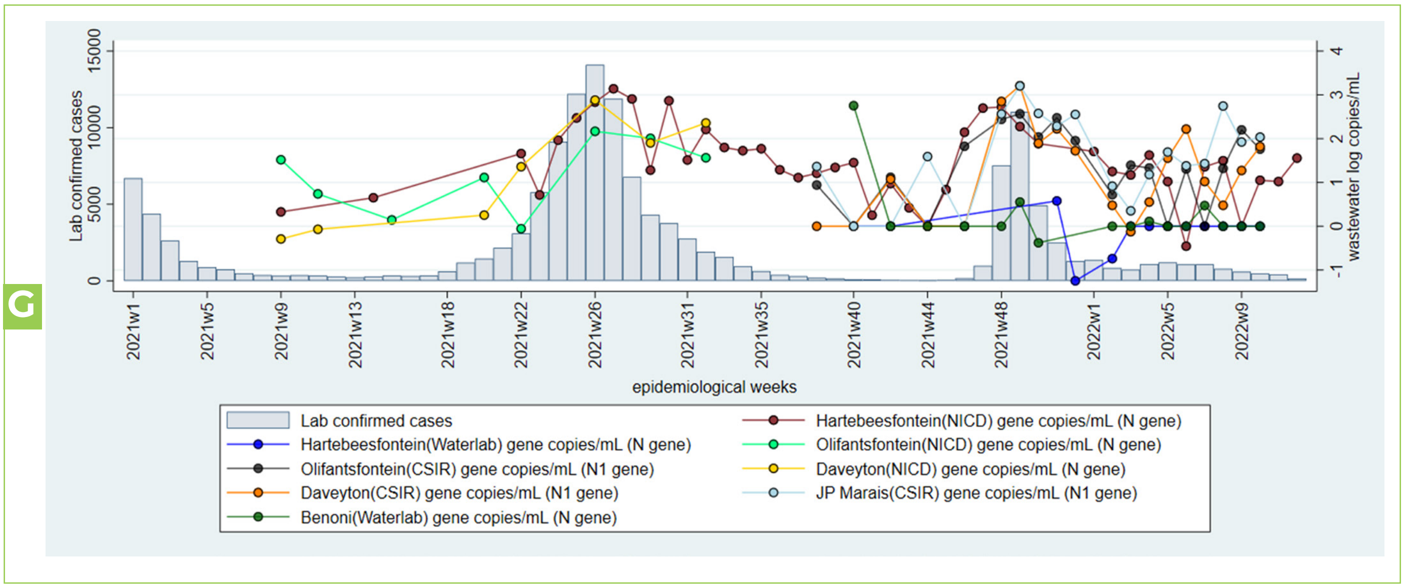


Figure 1 E-G. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) in Ekurhuleni Metropolitan Municipality, Gauteng Province during epidemiological weeks 1 of 2021 to week 12 of 2022. The 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

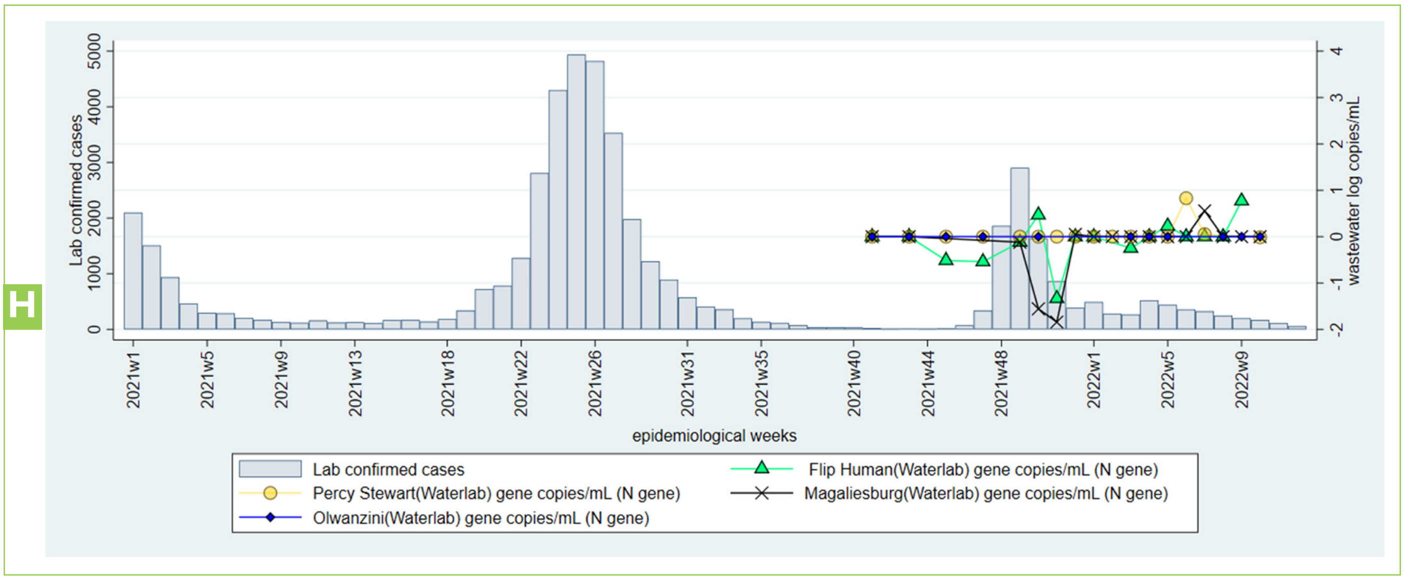


Figure 1 H. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) for selected wastewater treatment plants (WWTP) in West Rand District Municipality, Gauteng Province during epidemiological weeks 1 of 2021 to week 7 of 2022. The testing 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.

In Tshwane South and (sub-district 3, 4, 6 & 7), increases were observed at Godrich and Daspoort WWTPs- almost 2 and greater than 2 logcopies/ml respectively. Also, three successive increases were observed at Klipgat WWTP and a marginal increase in Rooiwal (Tshwane North). In the city of Johannesburg, the SARS-CoV-2 levels in Goudkoppies have just declined to less than 1 log copy/ml.

In Ekurhuleni almost all the WWTPs continue to decrease with corresponding decrease in clinical cases. However, marginal increases in SARS-CoV-2 levels were observed in Vlakplaats in Ekurhuleni South.

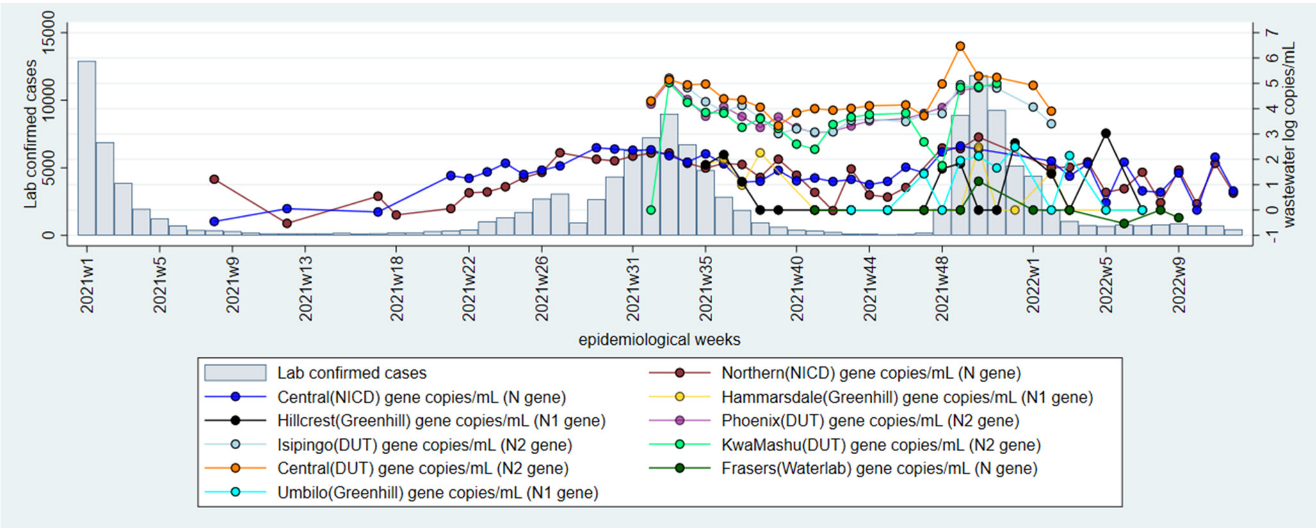
In Hartebeesfontein in Ekurhuleni North (sub-districts B, C or N1, N2), afurther increase was noted from the previous week

The public health authorities should continue to strengthen surveillance for clinical cases in these areas, promote vaccination and non-pharmaceutical interventions in all areas.

KwaZulu-Natal Province

2A: eThekwini Metropolitan Municipality

A



B: uMgungundlovu District Municipality

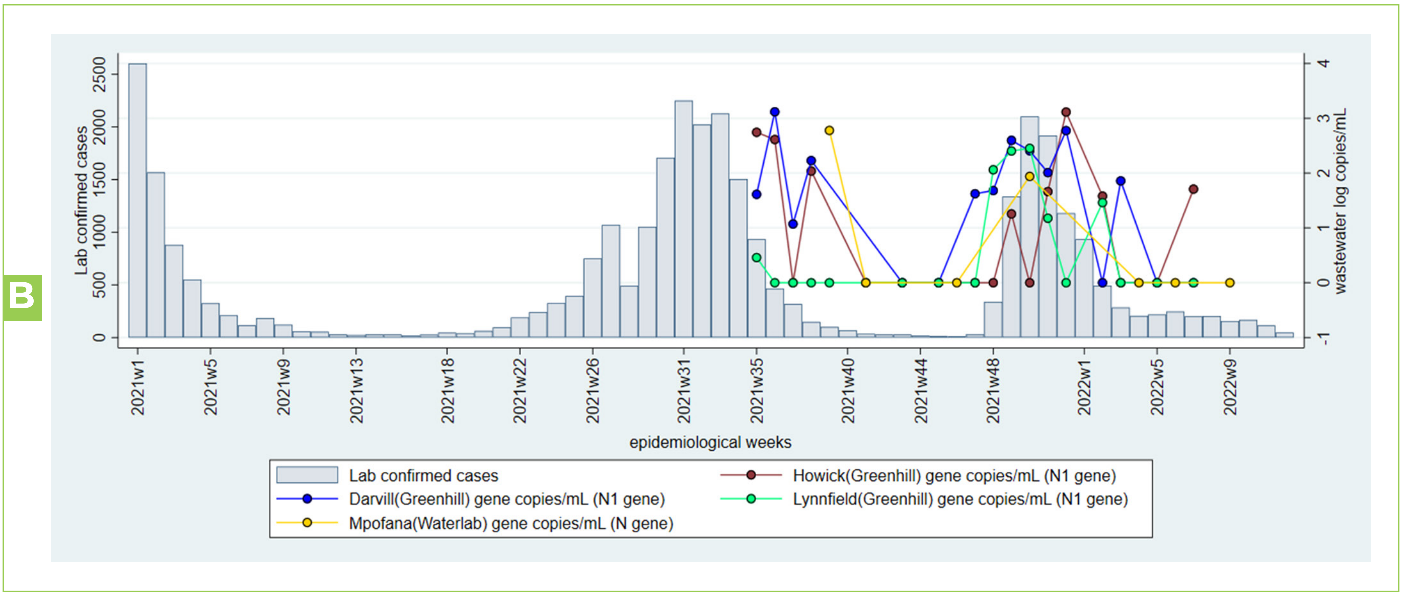


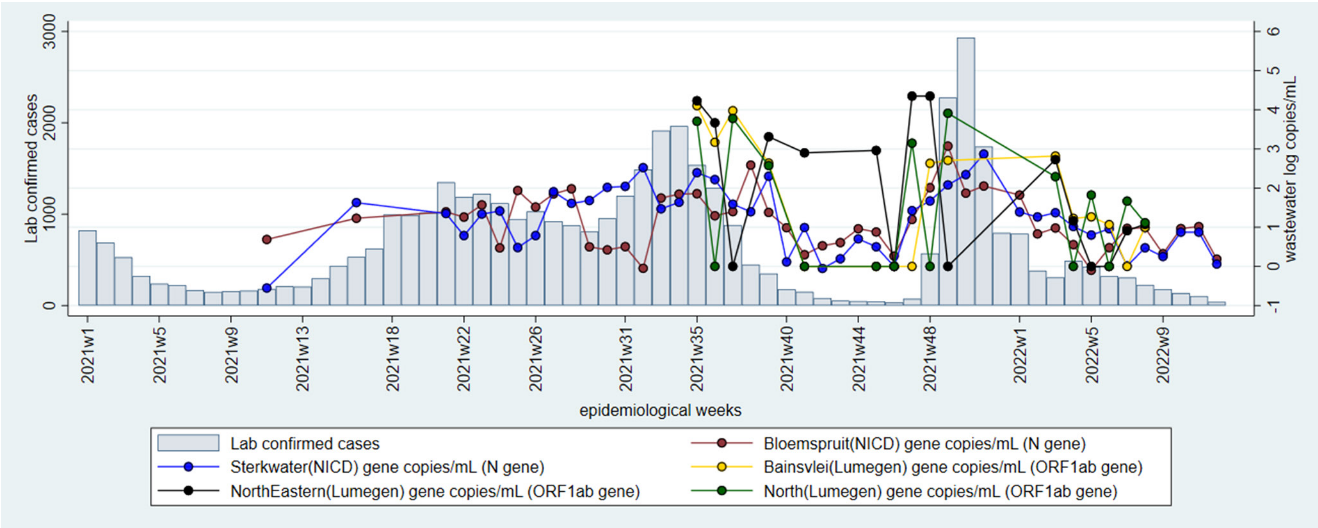
Figure 2A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTP) in eThekwini, (A-B) and uMgungundlovu Metro (C), KwaZulu Natal Province during epidemiological weeks 1-51, 2021 and week 12, 2022. 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 have declined in all the plants with recent results in eThekwini and uMgungundlovu, corresponding to the decrease in clinical cases. We continue to Therefore, the authorities should continue to promote vaccination and non-pharmaceutical interventions in all areas.

Free State Province- Mangaung

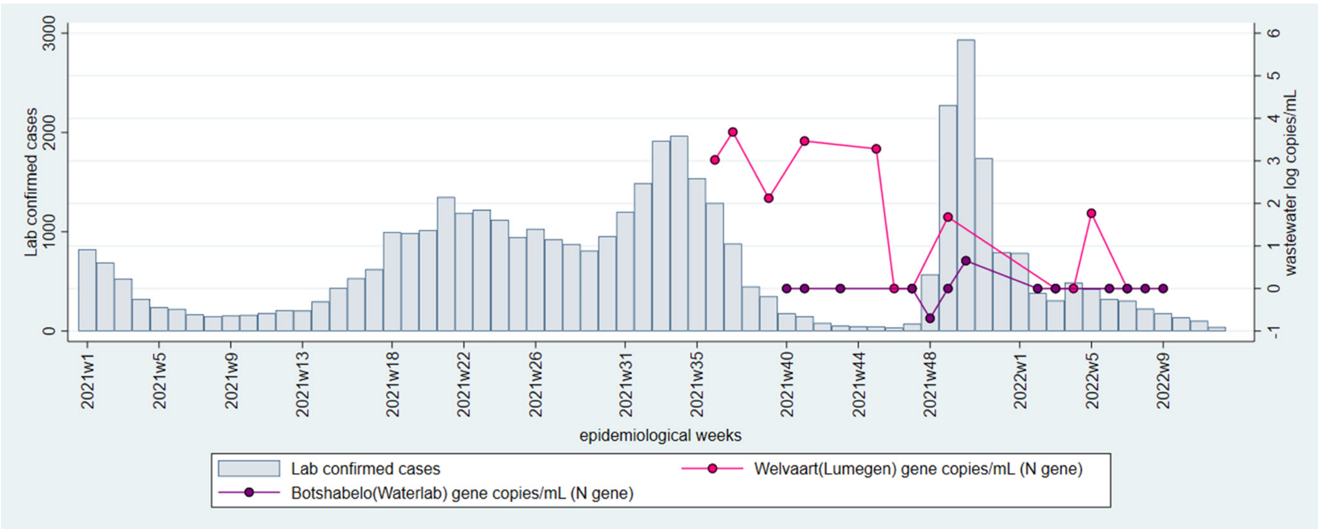
A: Bloemfontein sub-district

A



B. Botshabelo sub-district

B



C. Neledi & ThabaNchu sub-districts

C

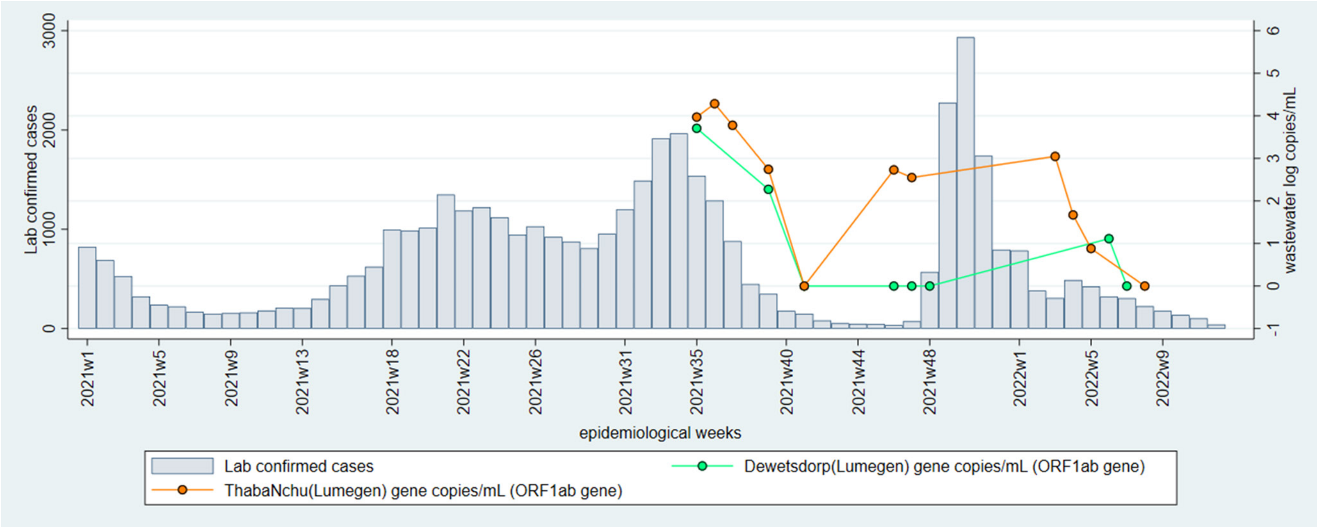


Figure 3 A-C. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Mangaung, Free State Province (Bloemfontein, Botshabelo, Naledi and ThabaNchu) during epidemiological weeks 1, 2021 to 12, 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 majority of 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. The public health authorities should continue surveillance for cases, promote vaccination and non-pharmaceutical interventions.

Eastern Cape Province

A: Nelson Mandela Metropolitan Municipality

A

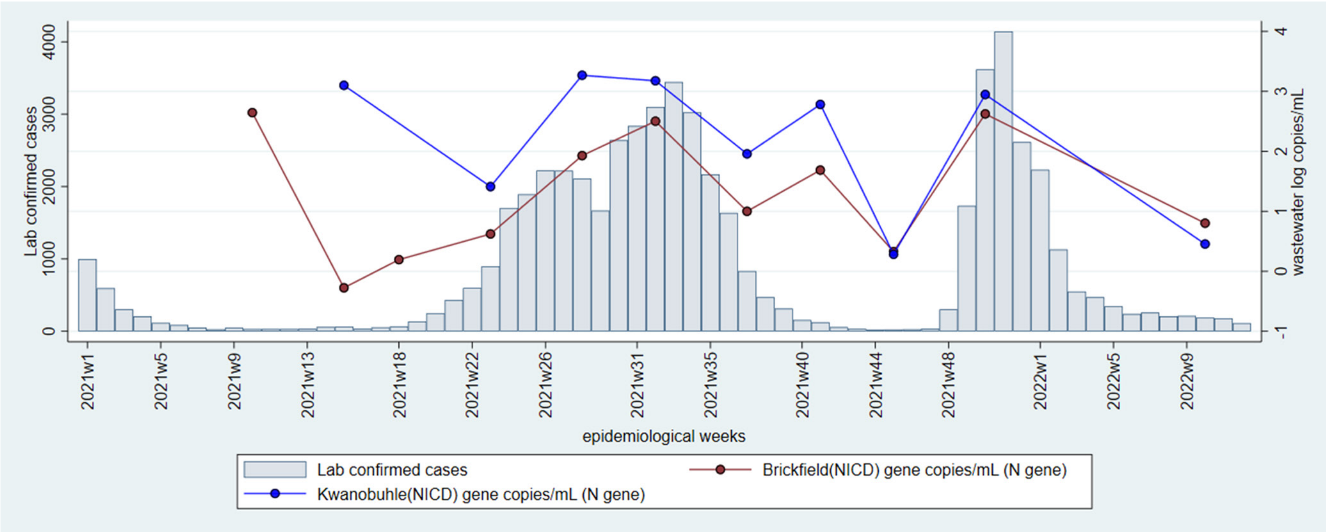


Figure 4A. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Nelson Mandela Metro, Eastern Cape Province during epidemiological weeks 1, 2021 to 10, 2022. The testing laboratory and quantified 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.

B: Buffalo City Metropolitan Municipality

B

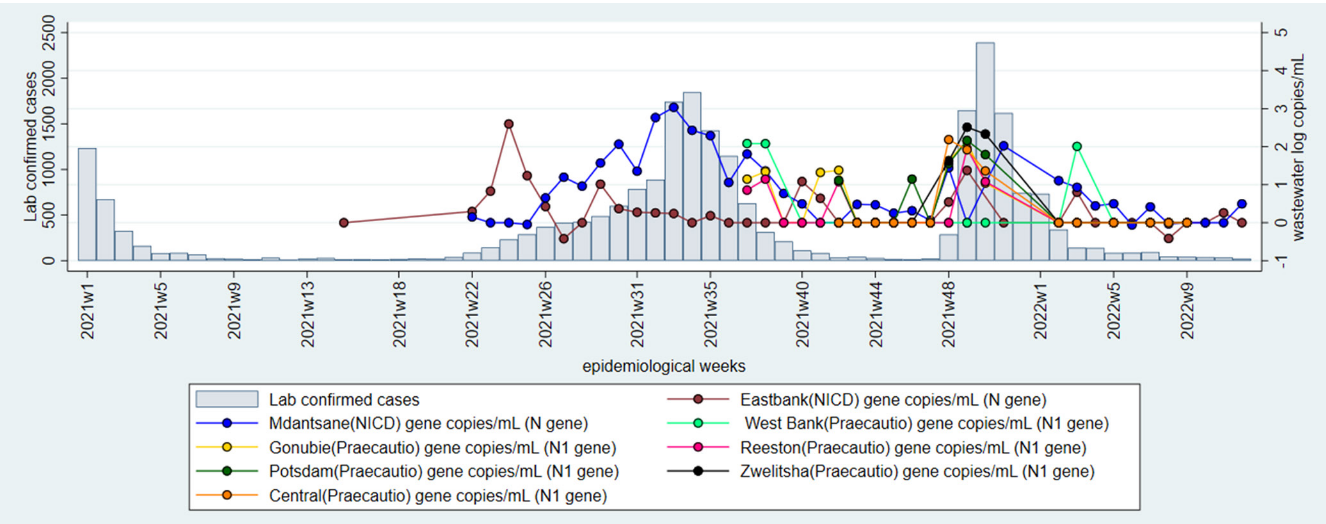


Figure 4B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in Nelson Mandela Metro, Eastern Cape Province during epidemiological weeks 1, 2021 to 12, 2022. The testing laboratory and quantified 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.

In both Nelson Mandela and Buffalo City Metros, the 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. A marginal increase in Mdantsane was observed but we will watch closely for subsequent samples. 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 SAMRC 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:

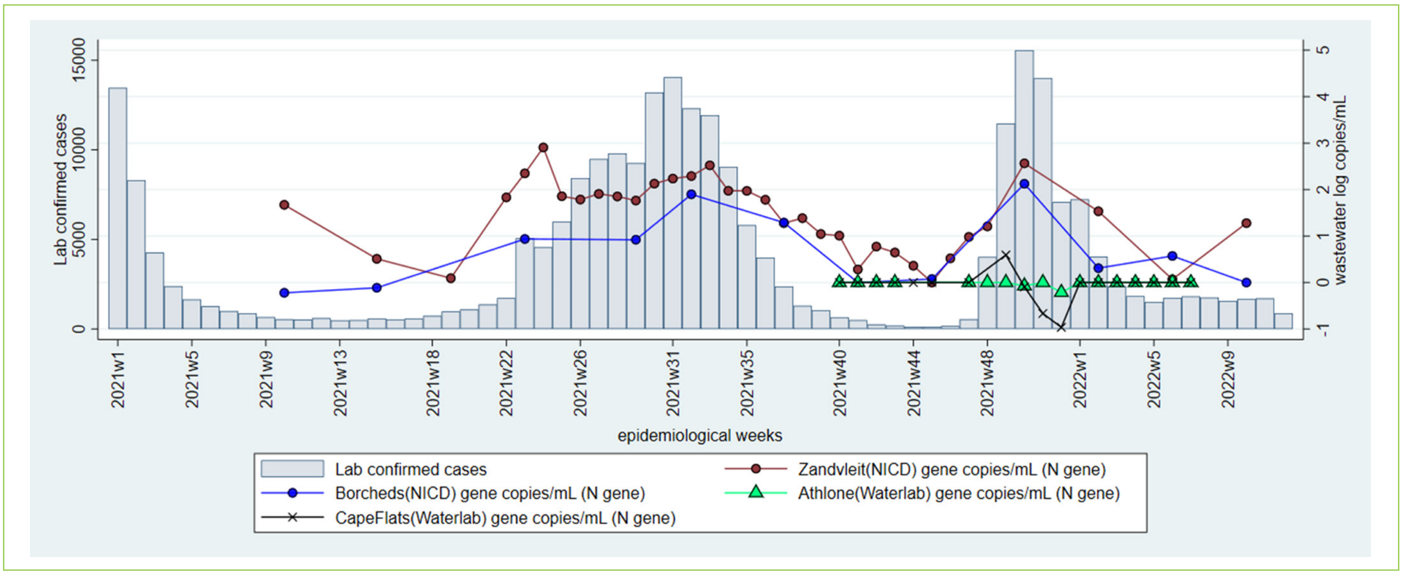


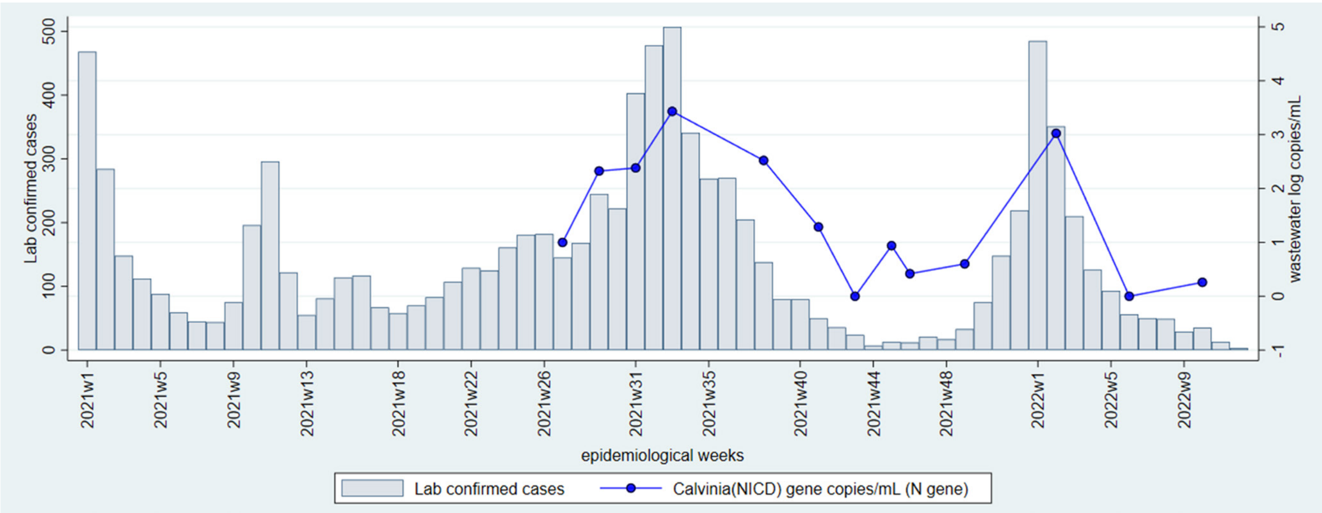
Figure 5. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) from wastewater treatment plants (WWTPs) in the City of Cape Town, Western Cape Province during epidemiological weeks 1, 2021 to 10, 2022. The testing laboratory and quantified SARS-CoV-2 gene are named in brackets after the name of the WWTP.

While the SARS-CoV-2 levels in Borcherds Quarry remain low corresponding to the decline in clinical case load, a marginal increase in the levels in Zandvleit was observed in week 10. 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.

Northern Cape Province

A: Namakwa District Municipality

A



B: Frances Baard District Municipality

B

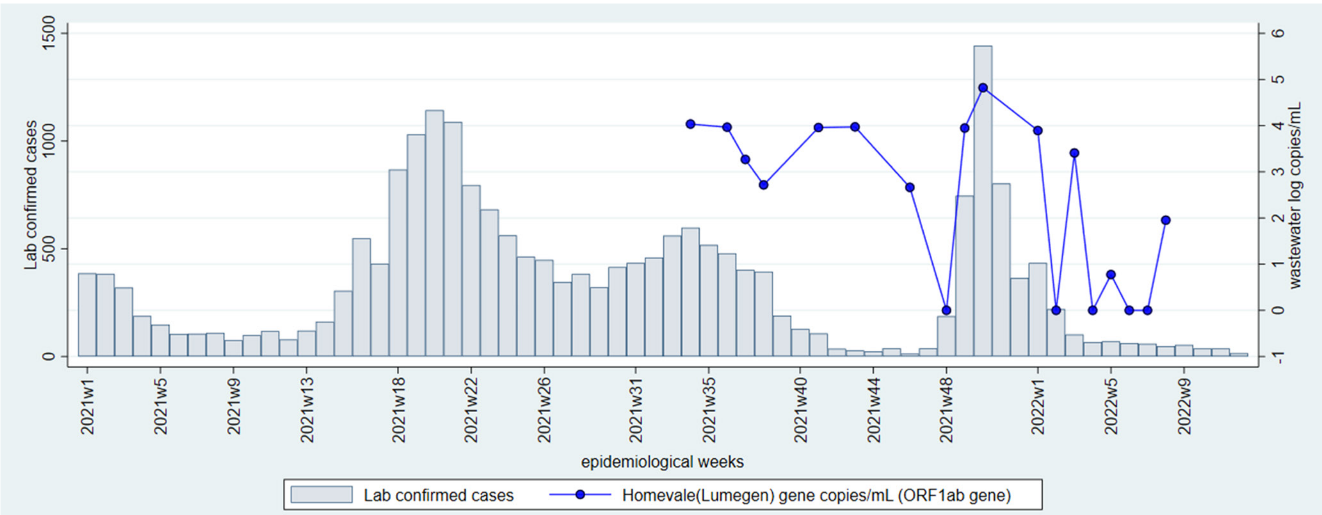
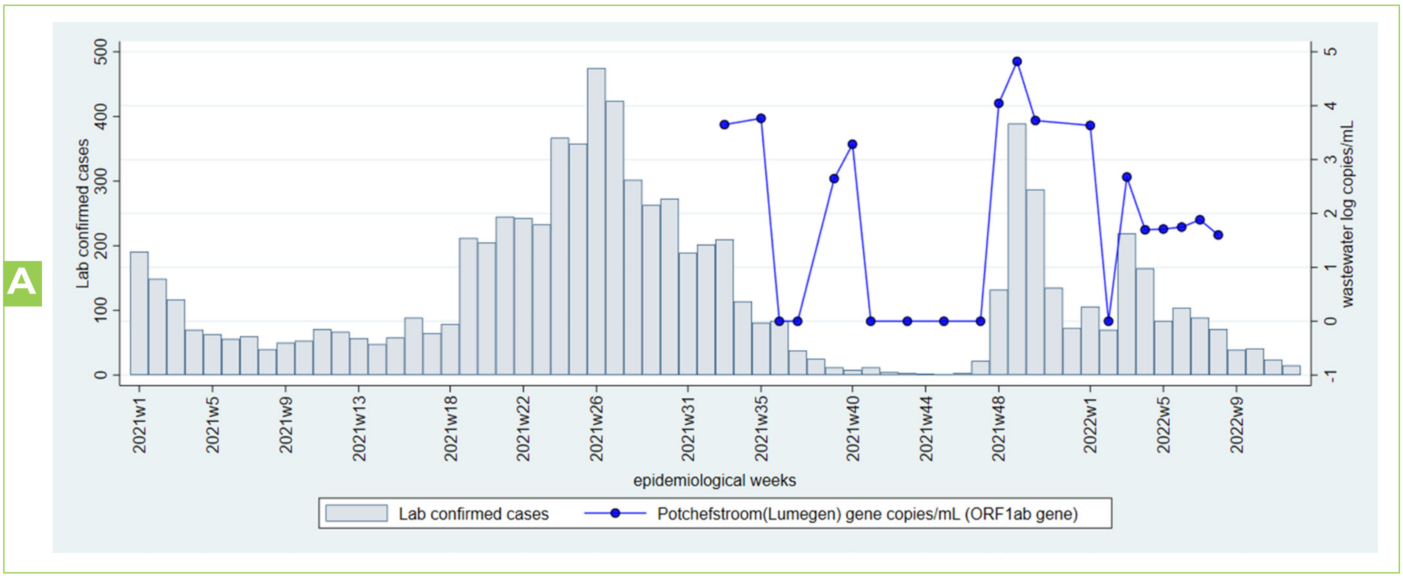


Figure 6A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Calvinia in Namakwa Metro (a) and Kimberly in Frances Baard District (b), Northern Cape Province during epidemiological weeks 1, 2021 to week 10, 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.

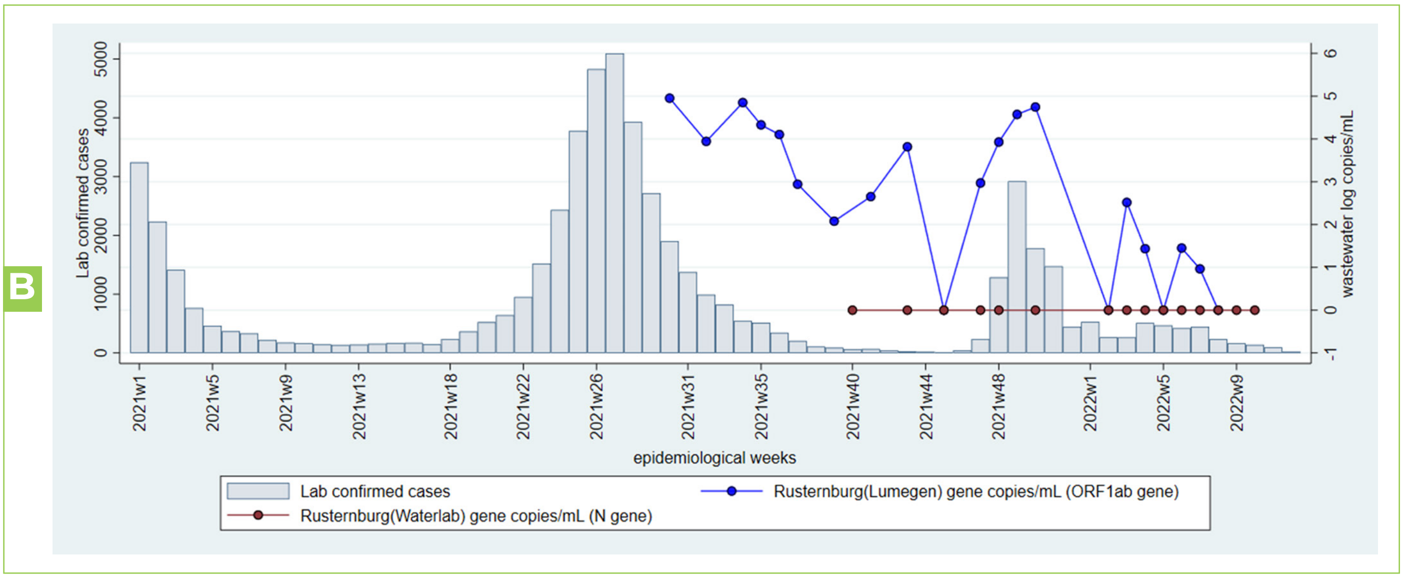
In Calvina and Homevale WWTPs in Namakwa and France Baard subdistricts respectively, the SARS-CoV-2 levels have been declining, corresponding to decline in clinical cases. More recent results are required. On the other hand, a current assessment cannot be made for France Baard due to the absence of recent results. The public health authorities should continue surveillance for cases, promote vaccination and non-pharmaceutical interventions.

North West Province

A: JB Marks Local Municipality



B: Bojanala District Municipality



C: City of Matlosana Municipality

C

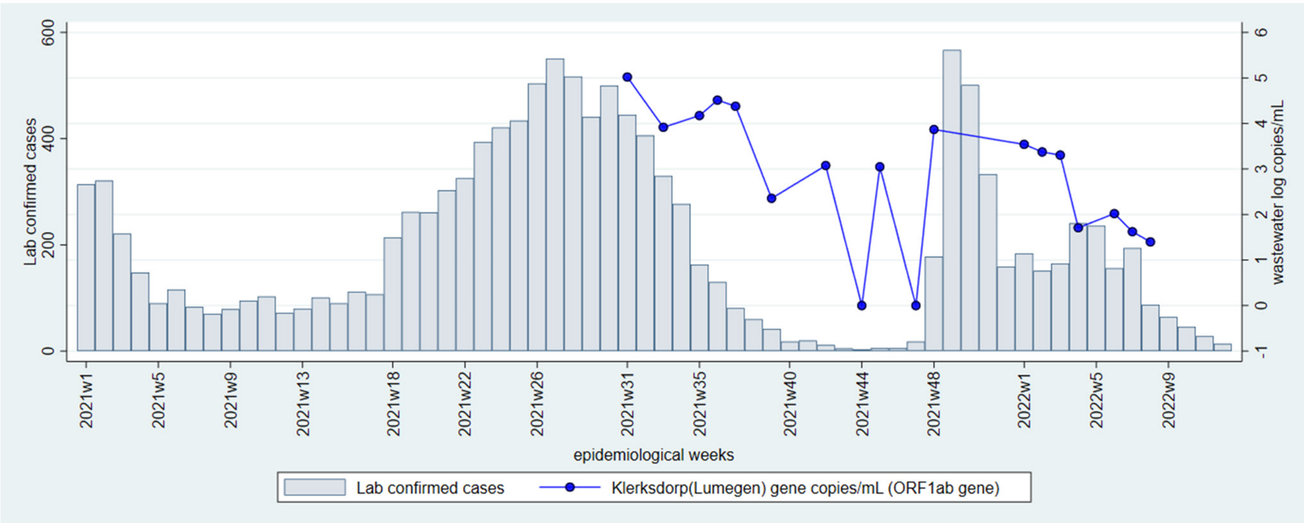


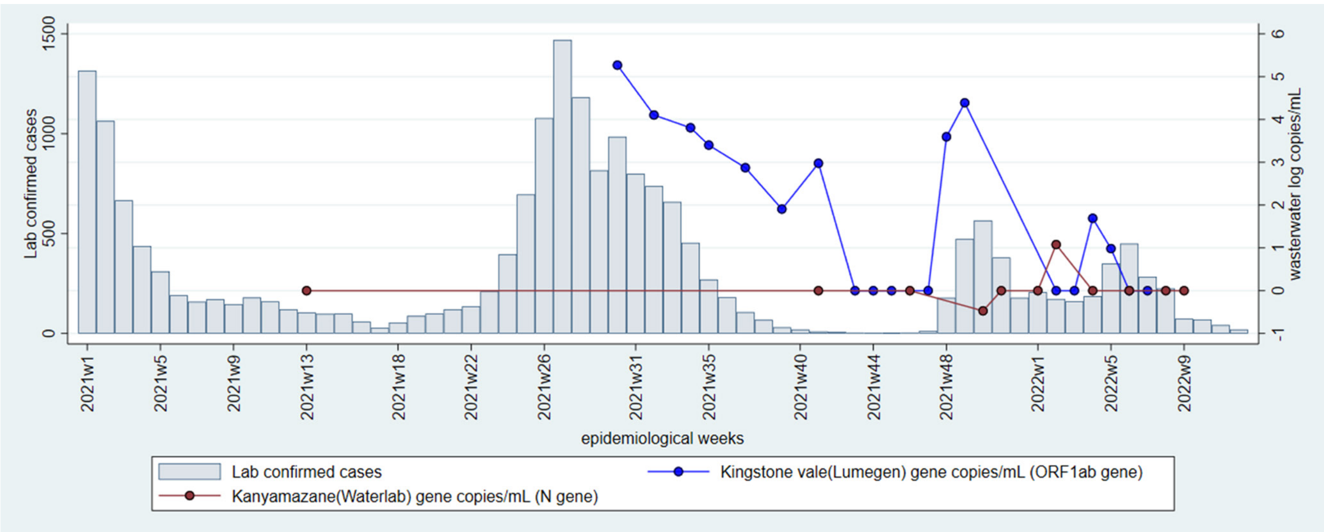
Figure 7A-C. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Potchefstroom, JB Marks District (A) Rustenberg, Bojanala District (B), and City of Matlosana, Northwest Province during epidemiological weeks 1, 2021 to 10, 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 are decreasing in all plants in the NorthWest province corresponding to the decline in clinical case. 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.

Mpumalanga Province

A: Mbombela Local Municipality

A



B: Emalahleni Local Municipality

B

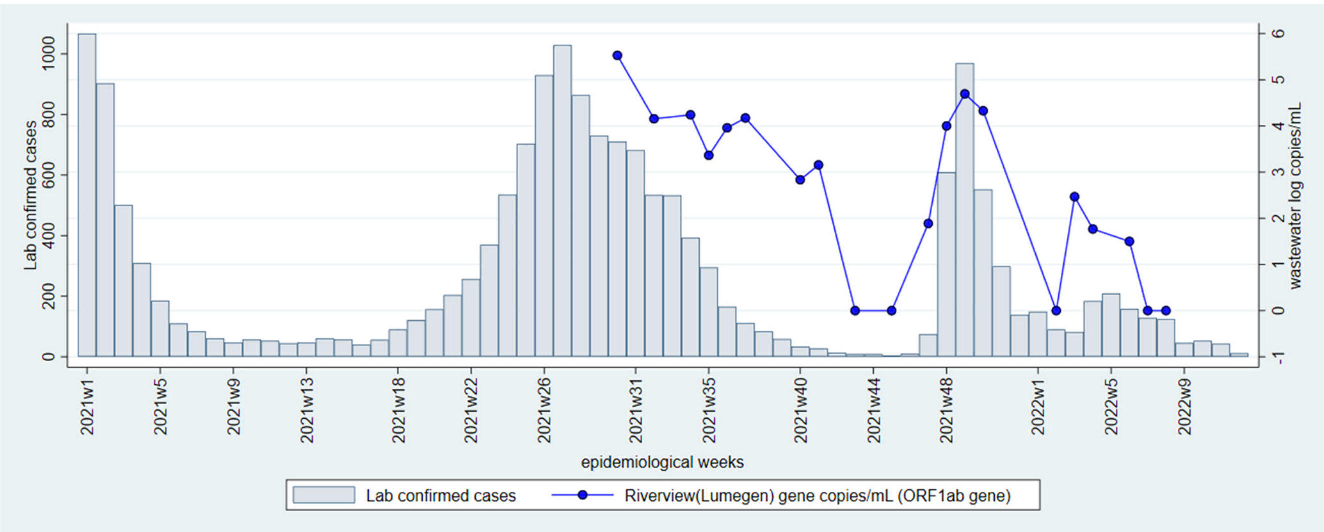


Figure 8A-B. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Mbombela and Emalahleni Local Municipality, Mpumalanga Province during epidemiological weeks 1, 2021 to 9, 2022. The testing 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 Mbombela (Kingstonvale) and Emalahleni (Riverview) have consistently decreased corresponding to the decrease in clinical cases. 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.

Limpopo Province

Polokwane Local Municipality

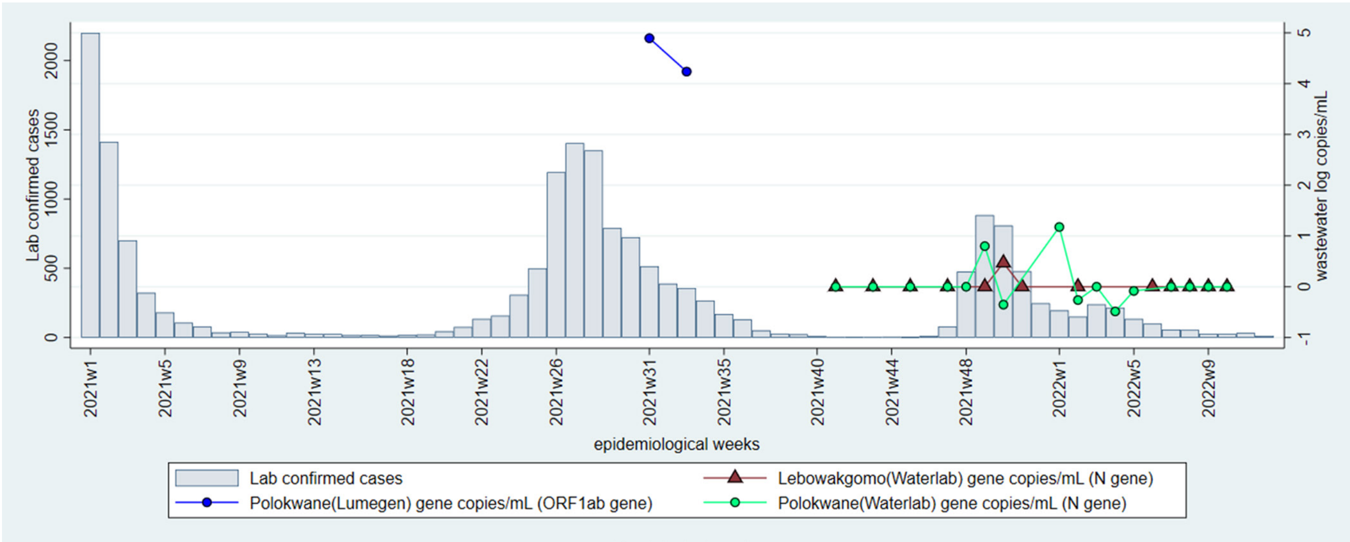


Figure 9. Laboratory confirmed cases of SARS-CoV-2 (bars) and levels of SARS-CoV-2 in log copies/ml of wastewater (coloured lines) in wastewater treatment plants (WWTPs) from Polokwane Local Municipality, Limpopo Province during epidemiological weeks 31-33, 2021.

The levels are low in Polokwane corresponding to low number of clinical cases.

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.

PART 2: RESULTS FROM SEQUENCING OF SARS-COV-2 RNA FRAGMENTS IN WASTEWATER

SARS-CoV-2 is shed into stools and is detectable in wastewater (sewage systems). The South African Collaborative COVID-19 Environmental Surveillance System (SACCESS) detects and quantifies SARS-CoV-2 from wastewater and used these data as a monitoring system for the SARS-CoV-2 epidemic in South Africa. Weekly reports of these data published at the NICD website: (https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/weekly-reports/wastewater-based-epidemiology-for-sars-cov-2-in-south-africa/).

The gold standard method to diagnose COVID-19 infection in clinical samples is reverse-transcriptase polymerase chain reaction (RT-PCR). The test, along with SARS-CoV-2 antigen detection has been used to monitor the epidemiology of the pandemic in South Africa. Epidemiological approaches based on these methods are limited by health seeking behaviour (only symptomatic patients go for testing) and testing practices of health care workers Therefore, this approach does not identify asymptomatically infected persons. Asymptomatic individuals may, however, be driving the force of infection and transmission in the community. Testing of wastewater for SARS-CoV-2 levels overcomes this limitation by allowing population levels of SARS-CoV-2 to be monitored over time, thus adding value to our understanding of SARS-CoV-2 epidemiology of the pandemic.

SARS-CoV-2 has been classified into different variants, that are continually emerging as a result of viral evolution. These variants acquire or lose mutations coding for various epitopes found on key viral proteins which lead to changes in transmissibility dynamics, response to treatment or ability to evade neutralisation by antibodies. WHO classified SARS-CoV-2 variants into variants of concerns (VOCs) and variants of interest (VOIs). VOCs have included Alpha, Beta, Delta, and Gamma, and recently Omicron. Of these, Beta and Omicron were first reported in South Africa. VOIs include Lambda and Mu (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/).

The Network for Genomics Surveillance of South Africa (NGS-SA) monitors the epidemiology of SARS-CoV-2 variants in PCR-confirmed cases in South Africa. In clinical cases, variant detection is performed using whole genome sequencing and other methods such as real-time PCR. During the first wave (June to August 2020), the Wuhan SARS-CoV-2 strain dominated amongst clinical cases while in the second wave (November 2020 to February 2021), the Beta variant was discovered and was predominant. The third wave (May to September 2021) was characterized by the dominance of the Delta variant and the fourth wave (November 2021 to January 2022) by the Omicron variant.

Several groups have sequenced SARS-CoV-2 from wastewater including groups in the Netherlands who generated near whole genome sequence from wastewater (Lara et al., 2020). In the United States, wastewater sequencing provided comparable results to clinical testing and evidenced new mutations before they appeared in clinical samples (Crits-Christoph et al., 2021).

Here, we report on SARS-CoV-2 sequences and variants of concern present in wastewater samples collected at sentinel wastewater treatment plants in South African urban metros from week 14 in 2021 to week 7 of 2022.

METHODS

Wastewater sites

In 2020, the National Institute for Communicable Diseases commenced testing of influent wastewater samples for SARS-CoV-2 from 15 wastewater treatment plants in metropolitan areas, including five in Gauteng Province, four in Eastern Cape province, two in the City of Cape Town (Western Cape Province), two in Mangaung (Free State Province), two in eThekwini (KwaZulu- Natal Province) (Table 1).

Table 1: Characteristics of wastewater treatment facilities and of samples submitted for SARS-CoV-2 sequencing from these sites, 2021-2022

Province	Metro or District	Plant name	Population size served by the facility	Genomic testing		
				Epidemiological week when sequencing started in 2021	# samples submitted for sequencing	# samples yielding good quality sequences
Eastern Cape	Buffalo City Metro	East Bank	141000	15	17	2
		Mdantsane	112900	25	24	7
	Nelson Mandela Metro	Brickfield	40000	15	7	3
		KwaNobuhle	100320	15	7	4
Free State	Mangaung	Sterkwater	200000	16	33	18
		Bloemspruit	350000	16	34	16
Gauteng	Ekurhuleni Metro	Daveyton	100000	20	5	3
	Johannesburg Metro	Northern	1200000	14	7	4
		Goudkoppies	500000	21	23	15
	Tshwane Metro	Rooiwal	unknown	17	33	20
		Daspoort	unknown	14	34	18
Kwazulu-Natal	eThekwini Metro	Northern	316425	17	29	18
		Central	350000	17	30	19
Western Cape	City of Cape Town Metro	Borcherd's Quarry	380000	15	7	1
		Zandvliet	460000	15	25	12

Sample collection, RNA extraction, amplification and sequencing

One litre of grab sewage samples were collected and transported at 4°C. Viruses were concentrated from the sample by ultrafiltration (Ikner, Soto-Beltran and Bright, 2011), and RNA was extracted using the QIAamp Viral RNA kit (Qiagen, GmbH, Germany). SARS-CoV-2 was detected by RT-PCR using AllplexTM 2019- nCoV Assay from Seegene kit (Seoul, Korea). RNA was re-extracted from SARS-CoV-2 positive concentrates and subjected to amplicon-based whole genome sequencing using the Sinai protocol with some modifications (Gonzalez-Reiche et al., 2020). Libraries were prepared using the COVIDSeq Kit (Illumina Inc, USA), and sequencing was performed using Illumina COVIDSeq kits as described in (Bhoyar et al., 2021) at the Sequencing Core Facility at the NICD.

Sequence analysis

The Galaxy pipeline (RC, 2005) was used for sequence analysis. Reads were trimmed and filtered according to published criteria (Khailany, Safdar and Ozaslan, 2020). At least 10 reads required at each nucleotide position for downstream analysis. Mutations present at 10% or less were removed from the analysis. Reads were mapped against the reference genome (Wuhan strain) and amino acid variation was analysed. Figure 2 illustrates an example of amino acids variation file (https://usegalaxy.eu/).

Table 2: Illustration of amino acids variations (Galaxy: : https://usegalaxy.eu/). A shows sample ID. B is QC filter, which is quality indicator. C is the number of reads produced for each sample. D is the effect of the mutation detected in the gene. E is the name of the gene where mutation occurred. F is the mutation detected. G is the frequency of the reads in the mutation.

A	B	C	D	E	F	G
Sample	**QC filtre**	**Number of reads**	**Mutation effect**	**Gene**	**Mutation**	**Frequency of mutations**
ENV-COV-21-285_S337_001.fastq	PASS	12	NON_SYNONYMOUS_CODING	ORF1ab	K790Q	0.833333
ENV-COV-21-285_S337_001.fastq	PASS	644	NON_SYNONYMOUS_CODING	ORF1ab	K798N	0.057453
ENV-COV-21-285_S337_001.fastq	PASS	14	NON_SYNONYMOUS_CODING	ORF1ab	F800L	0.857143
ENV-COV-21-285_S337_001.fastq	PASS	44	SYNONYMOUS_CODING	ORF1ab	G45	0.863636
ENV-COV-21-285_S337_001.fastq	min_af_0.05Xmin_dp_1Xmin_dp_alt_10	44	FRAME_SHIFT	ORF1ab	Y46L?	0.045455
ENV-COV-21-285_S337_001.fastq	PASS	1347	NON_SYNONYMOUS_CODING	ORF1ab	T54P	0.123979
ENV-COV-21-285_S337_001.fastq	PASS	153	SYNONYMOUS_CODING	ORF1ab	T54	0.078431

SARS-CoV-2 in the sewage system is fragmented, and therefore, generation of a consensus sequence for each sample is not meaningful. Therefore, to identify variants at each geographic location, we analysed amino acid variation in each individual sample. For each VOC or VOI, unique single nucleotide polymorphisms were identified by comparing the new lineage with the Wuhan strain in a public database (https://outbreak.info/). Using the amino acid variation data file, we used STATA software (v 17.1) (https://www.stata.com/) to collate s-gene mutations in a matrix such that the columns represented the amino acid positions of the spike protein and each row recorded all mutations detected in each sample at every locus across the spike gene. We included all mutations, including low frequency mutations and recorded the proportion of reads where that mutation was detected (the 'read frequency') as a percentage of total reads. Using the list of unique mutations for each VOC and VOI in the spike protein region (Table 2) we interrogated the matrix for the presence or absence of known signature mutations in each sample using STATA software (Table 2). As new variants were detected and identified in clinical specimens, we added signature mutations to the STATA code, allowing us to identify the presence of new variants both retrospectively and prospectively

Table 3: List of signature mutations which was used to identify VOC and VOI present in wastewater samples from week 14 in 2021 to week 7 of 2022

Omicron	Alpha	Beta	Delta	C.1.2	Gamma	Lambda	Mu
G339D	A570D	D80A	T19R	P9L	T20N	G75V	Y144S
S371L	S982A		R145H	P25L	P26S	T76I	Y145N
S375F	D1118H		E156del	C136F	T1027I	D253N	
Q493R			R158G	Y449H		L452Q	
G496S			A222V			F490S	
Y505H							
T547K							
N856K							
Q954H							
N969K							
L981F							

Results and discussion

Up to the 15th February 2022, a total of 315 wastewater samples from sites listed in Table 1 underwent RNA extraction, amplification and sequencing. Of these 315 samples, 160 (50.8%) yielded SARS-CoV-2 RNA sequences.

Detection of SARS-CoV-2 variants from wastewater samples using signature mutations analysis

Gauteng province

In the Gauteng province, 60 samples yielded sequencing results displayed in figure 1, which illustrates how beta variant was present in north and southern Gauteng province in week 21-22, but was replaced by delta variant across the province from weeks 21 until 32. Two sub-lineages of delta and a variant present only in South Africa. C.1.2 were simultaneously present at all sites with variable but lower read frequencies. During the interwave period (weeks 34-44) most samples submitted for sequencing failed to yield good quality sequence data, most likely on account of low or absent SARS-CoV-2 RNA fragments. Omicron variant was first detected in week 46 and by week 47 was found to be present at all plants across the province. In all plants, the mutation formerly associated with C1.2 was also found to be present at high read frequencies simultaneously with Omicron variant. Omicron variant continues to be present up to week 7 of 2022.

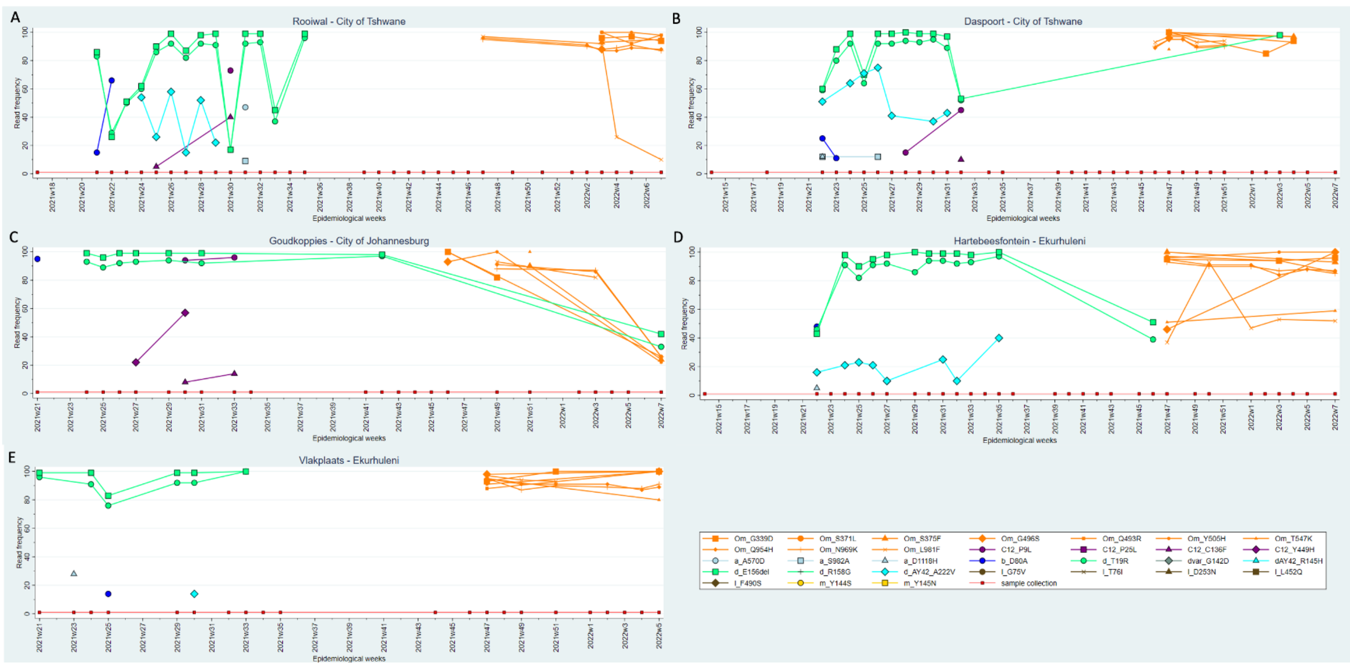


Figure 1: Beta mutation (D80A, dark blue circle). Delta mutations: T19R (green circle), G142D (pale blue diamond), R145H (green triangle), E156G (plus symbol), DEL157/158 (green square), A222V (pale blue diamond). C.1.2 mutations: P9L (purple circle), P25L (purple square), C136F (purple triangle), Y449H purple diamond. Omicron mutations: G339D (black square), S371L (black circle), S375F (black triangle), G496S (black diamond), Q493R (black dot), T547K (black small triangle), Q954H (black small diamond), N969K (black small line), L981F (black small x). Dots on the red line shows the timepoints of sample collection, absence of specific coloured lines means the mutation was not detected at that timepoint. A (Rooiwal plant), B (Daspoort plant), C (Goudkoppies plnat), D (Hartebeesfontein plant), and E (Vlakplaats plant).

KwaZulu- Natal province

In KwaZulu-Natal province, 37 samples yielded good sequences and were included in figure 2. Beta variant was detected in a single sample from Central plant in week 23. Delta variant was present in Northern and Central plants from weeks 21 to 35, and was last detected in week 43 at Central plant. As in Gauteng Province, two sub-lineages of delta and a variant present only in South Africa, C.1.2 were simultaneously present at all sites with variable but lower read frequencies from weeks 21-35. During the interwave period (weeks 34-44) most samples submitted for sequencing failed to yield good quality sequence data, most likely on account of low or absent SARS-CoV-2 RNA fragments. Omicron variant was first detected in week 49 in both plants, along with the mutation formerly associated with C1.2. Omicron variant continues to be present up to week 2 of 2022.

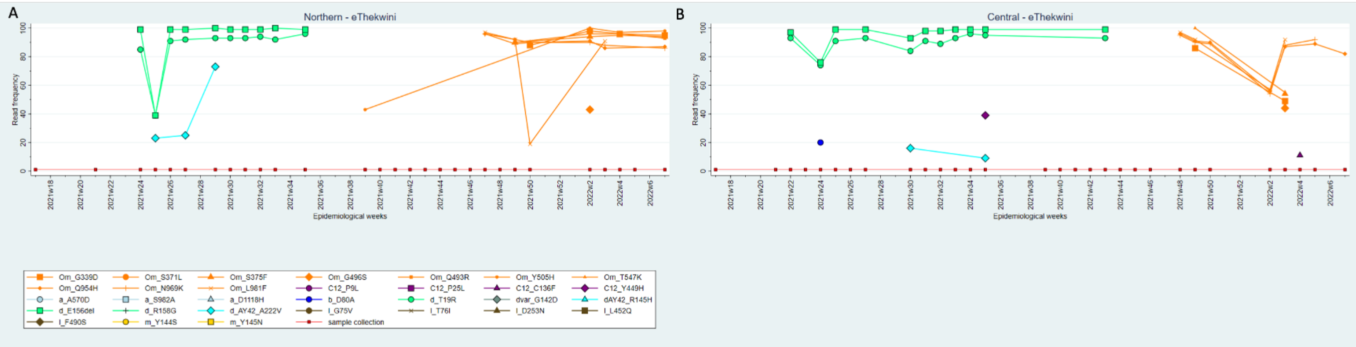


Figure 2: Beta mutation (D80A, dark blue circle). Delta mutations: T19R (green circle), G142D (pale blue diamond), R145H (green triangle), E156G (plus symbol), DEL157/158 (green square), A222V (pale blue diamond). C.1.2 mutations: P9L (purple circle), P25L (purple square), C136F (purple triangle), Y449H purple diamond. Omicron mutations: G339D (black square), S371L (black circle), S375F (black triangle), G496S (black diamond), Q493R (black dot), T547K (black small triangle), Q954H (black small diamond), N969K (black small line), L981F (black small x). Dots on the red line shows the timepoints of sample collection, absence of specific coloured lines means the mutation was not detected at that timepoint. A (Northern eThekwini plant) and B (Central eThekwini plant).

Free State province

In Mangaung, Free State province, 34 samples yielded sequencing results displayed in figure 3. The beta variant was present until week 23 (Sterkwater plant) and 25 (Bloemspruit) when it was co-detected with delta variant. As in Gauteng, delta variant dominated from weeks 23-24, along with two delta sublineanges. Variant C.1.2 was detected in week 31 in Blomespruit plant. No samples yield quality sequence data from weeks 35-46. Omicron was first detected in week 50 at both plants, along with variant C.1. 2.

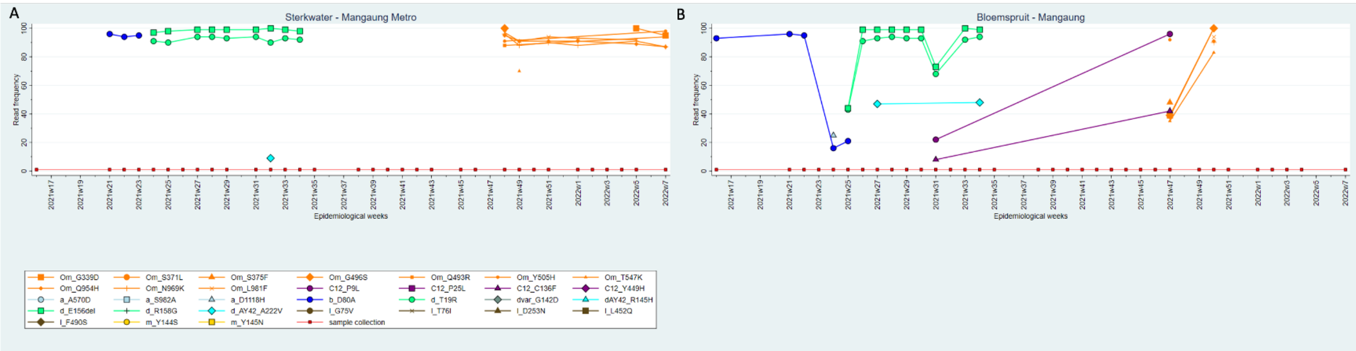


Figure 3: Beta mutation (D80A, dark blue circle). Delta mutations: T19R (green circle), G142D (pale blue diamond), R145H (green triangle), E156G (plus symbol), DEL157/158 (green square), A222V (pale blue diamond). C.1.2 mutations: P9L (purple circle), P25L (purple square), C136F (purple triangle), Y449H purple diamond. Omicron mutations: G339D (black square), S371L (black circle), S375F (black triangle), G496S (black diamond), Q493R (black dot), T547K (black small triangle), Q954H (black small diamond), N969K (black small line), L981F (black small x). Dots on the red line shows the timepoints of sample collection, absence of specific coloured lines means the mutation was not detected at that timepoint. A (Sterkwater plant) and B (Bloemspruit plant).

Western Cape province

In the Western Cape Province, 13 samples yielded sequencing results displayed in figure 4. At Zandvliet plant, delta variant along with subvariants described above were detected from week 25-35. Evidence of C1.2 was found in week 22. No samples yield quality sequence data from week 38 to week 46. In week 47, omicron and variant C.1.2 was detected. A single mutation associated with omicron was observed in week 24, without co-detection of other mutations associated with omicron. At Borcherd's Quarry, the delta variants and subvariant were detected in week 32. No samples yield quality sequence data from week 34, 2021 to week 2, 2022.

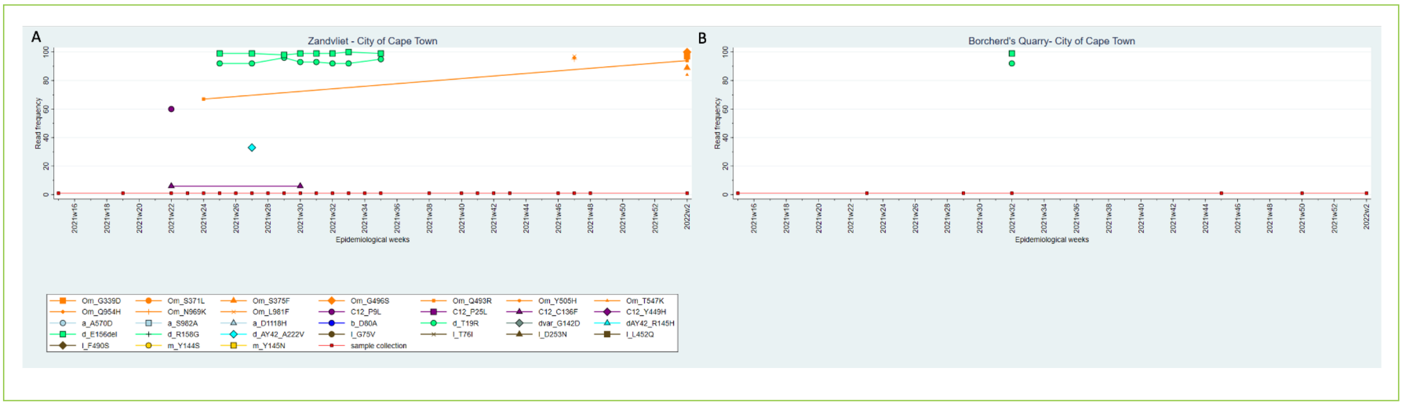


Figure 4: Beta mutation (D80A, dark blue circle). Delta mutations: T19R (green circle), G142D (pale blue diamond), R145H (green triangle), E156G (plus symbol), DEL157/158 (green square), A222V (pale blue diamond). C.1.2 mutations: P9L (purple circle), P25L (purple square), C136F (purple triangle), Y449H purple diamond. Omicron mutations: G339D (black square), S371L (black circle), S375F (black triangle), G496S (black diamond), Q493R (black dot), T547K (black small triangle), Q954H (black small diamond), N969K (black small line), L981F (black small x). Dots on the red line shows the timepoints of sample collection, absence of specific coloured lines means the mutation was not detected at that timepoint. A (Zandvliet plant) and B (Borcherd's Quarry plant).

Eastern Cape province

In the Eastern Cape Province, 16 samples yielded sequencing results displayed in figure 5. The delta variant and subvariant were detected in week 30 until week 35 (Mdantsane plant), week 24 until 29 (Brickfield plant), week 28 until 32 (Brickfield) and week 28 until 41 (Kwanobuhle). Omicron variant was first detected in week 46 and by week 48 at the Mdantsane plant, alongside C1.2 and 3 other omicron subvariants were detected in week 2, 2022. Similarly, omicron and C1.2. variants were detected in week 50 at the Kwanobuhle and Brickfield plant. No omicron and C1.2. variants were detected at the Eastbank plant.

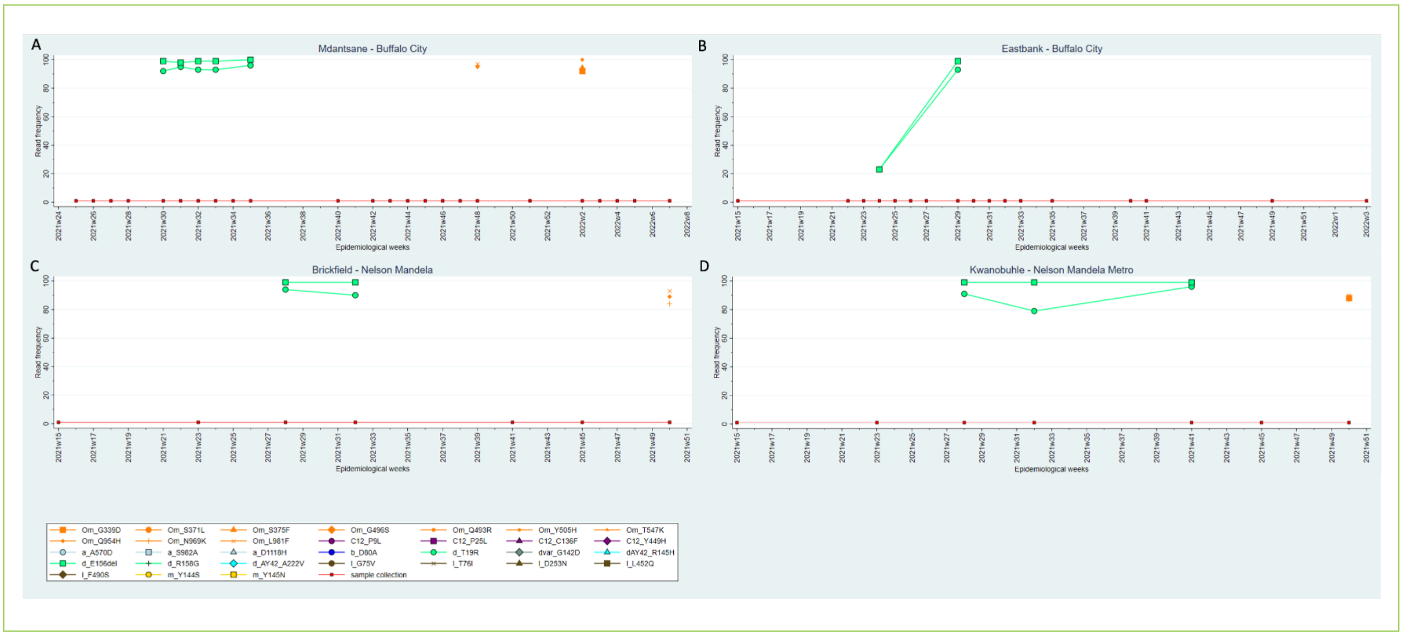


Figure 5: Beta mutation (D80A, dark blue circle). Delta mutations: T19R (green circle), G142D (pale blue diamond), R145H (green triangle), E156G (plus symbol), DEL157/158 (green square), A222V (pale blue diamond). C.1.2 mutations: P9L (purple circle), P25L (purple square), C136F (purple triangle), Y449H purple diamond. Omicron mutations: G339D (black square), S371L (black circle), S375F (black triangle), G496S (black diamond), Q493R (black dot), T547K (black small triangle), Q954H (black small diamond), N969K (black small line), L981F (black small x). Dots on the red line shows the timepoints of sample collection, absence of specific coloured lines means the mutation was not detected at that timepoint. A (Mdantsane plant), B (Eastbank plant) C (Brickfield plant) and D (Kwanobuhle plant).

The distribution of SARS-CoV-2 from wastewater has progressed from the predominance of beta variant in January 2021, to delta variant dominance (June 2021) to Omicron and C1.2 in early 2022 (Figure 6).

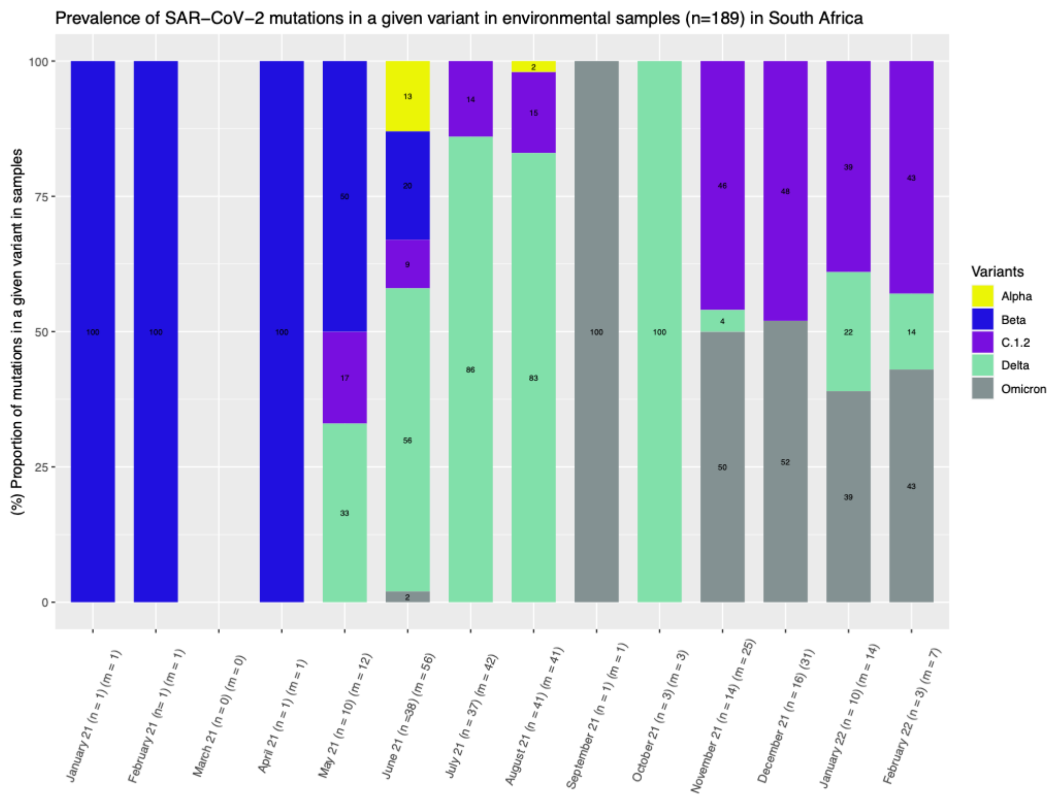


Figure 6: The proportion of mutations in a given variant in the environmental samples by month and year (January 2021-February 2022) from all South African provinces. The number of samples processed each month are indicated as n and the number of mutations present in a sample are represented as m i.e. n = 10 indicates that mutations were successfully detected 10 sample in May, 2021 and m = 12 indicates that 12 mutations were found in which 13% of the 12, are mutations in the alpha variant.

CONCLUSION

Sequencing of SARS-CoV-2 fragments from wastewater is able to identify the presence of variants in known to be present in clinical samples. These data show the presence of omicron variant in all recent samples across South Africa (up to week 7). The significance of the mutation associated with the C1.2 variant (P25L) is presently unclear as other mutations associated with this variant are not present, nor is the mutation found in omicron VOC. These results must be read along with the reports generated by the Centre for Respiratory Diseases and Meningitis found at (https://www.nicd.ac.za/wp-content/uploads/2022/03/Update-of-SA-sequencing-data-from-GISAID-18-Mar-2022_2.pdf).

Sequencing of wastewater has limitations. The ability to identify variants in wastewater relies on the identification of single nucleotide polymorphisms found in clinical strains and which are uniquely associated with these variants. We are not yet able to detect new variants. Sequencing of SARS-CoV-2 from wastewater may not yield good quality sequence data when viral concentration in wastewater is low.

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CONCLUSION

SARS-CoV-2 data from wastewater at South African sentinel sites show concordance with clinical, epidemiologic curves and sequencing data (not shown) in the respective locations, illustrating the potential of the SACCESS network to provide descriptive epidemiological data pertaining to geographic variation, burden and variants of SARS-CoV-2.

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- Staff of SACCESS network laboratories are thanked for their assistance in generating these results.

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 Riebeekhoogte	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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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 Maselspoort, Rustig	Lumegen	01-09-2021
14	Sterkwater	Free State	Mangaung	Bloemfontein SD	Bloemfontein	Mangaung	Fonteintjie, 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, Selosesha, 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, Hartsenbergfontein, Althea, Golfview, Blignautrus	NIOH	04-10-2021

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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 Platform	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 East-ern 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	Majanen, Hammanskraal, Mabopane, Soshanguve, Pyramid, Doornpoort	Waterlab/ UP	26-09-2021

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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 Platform	13-09-2021
38	Summer Place Package 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 Platform	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

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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 Grundlingh	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	Hartebeesfontein 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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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	eThekwini Metropolitan Municipality	eThekwini MM Sub	eThekwini West	eThekwini Metropolitan Municipality	Hammarsdale, Elangeni, Mpumalanga	GreenHill	02-09-2021
61	Hillcrest	Kwazulu-Natal	eThekwini Metropolitan Municipality	eThekwini MM Sub	eThekwini West	eThekwini Metropolitan Municipality	New Germany, Pinetown, Clermont, Pinelands, KwaDabeka	GreenHill	02-09-2021

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62	Isipingo	Kwazulu-Natal	eThekwini Metropolitan Municipality	eThekwini MM Sub	eThekwini South	eThekwini Metropolitan Municipality	Umbogintwini, Malukaze, Egolokodo, KwaMakhutha, Umlazi	DUT	10-08-2021
63	Central	Kwazulu-Natal	eThekwini Metropolitan Municipality	eThekwini MM Sub	eThekwini North	eThekwini 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	eThekwini Metropolitan Municipality	eThekwini MM Sub	eThekwini South	eThekwini Metropolitan Municipality	La Lucia, Umhlanga, Prestondale, Phoenix, Duff's Road, Glen Ashley, Mount Edgecombe	DUT	10-08-2021
65	Northern	Kwazulu-Natal	eThekwini Metropolitan Municipality	eThekwini MM Sub	eThekwini North	eThekwini Metropolitan Municipality	Newlands, KwaMashu, Greenwood Park, Park Hill	NICD	22-02-2021
66	Phoenix	Kwazulu-Natal	eThekwini Metropolitan Municipality	eThekwini MM Sub	eThekwini South	eThekwini Metropolitan Municipality	Rietrivier, KwaMashu, Duff's Road, Mount Edgecombe, Phoenix, Richmond, Inanda	DUT	10-08-2021
67	Frasers	Kwazulu-Natal	eThekwini Metropolitan Municipality	eThekwini MM Sub	eThekwini North	iLembe District municipality	Salt Rock, Ballitoville, Umhlali, Fraser, Zimbali, Shaka's Rock, Ballito	Waterlab/ UP	11-10-2021
68	Umbilo	Kwazulu-Natal	eThekwini Metropolitan Municipality	eThekwini MM Sub	eThekwini North	eThekwini Metropolitan Municipality	Acorn, Albany, Alexander Park	GreenHill	28-10-2021
69	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
70	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
71	Mpofana	Kwazulu-Natal	Umgungundlovu District municipality	Mpofana LM	No subdistrict	Mpofana Local Municipality	Bruntville, Brown Stones, Windy, Weston, Mooirivier	Waterlab/ UP	28-09-2021

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72	Howick	Kwazulu-Natal	Umgungundlovu District municipality	uMngeni LM	No subdistrict	UMgungundlovu District municipality	Riversdale, Merrivale, Cedara	GreenHill	02-09-2021
73	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
74	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
75	Emalahleni (Riverview)	Mpuma-langa	Nkangala District Municipality	Emalahleni LM	No subdistrict	Emalahleni LM	Lynnville, Duvhapark, Paxton, Klipfontein	Lumegen	26-07-2021
76	Kanyamazane	Mpuma-langa	Ehlanzeni District	City of Mbombela LM	No subdistrict	Mbombela/ Umjindi	Daantjielokasie, eNyamazaneni	Waterlab/ UP	30-03-2021
77	Mbombela (Kingstonvale)	Mpuma-langa	Ehlanzeni District	Mbombela/ Umjindi	No subdistrict	Mbombela/ Umjindi	Gutshwa, eMpumalanga, eNyalungu, Dwaleni, Hlauhlau, Phasha, Ngodini	Lumegen	26-07-2021
78	Calvina	Northern Cape	Namakwa	Hantam Local Municipality	No subdistrict	Hantam Local Municipality	Calvinia	NICD	06-07-2021
79	Homevale Kimberley	Northern Cape	Frances Baard	Sol Plaatjie Local Municipality	No subdistrict	Sol Plaatjie Local Municipality	Remount Camp, Kenilworth, Homevale	Lumegen	28-08-2021
80	Potchefst-room	North-West	JB Marks Local Municipality	JB Marks LM	No subdistrict	JB Marks Local Municipality	Harpington, Vyfhoek, Mooibank, Wilgeboom	Lumegen	17-08-2021

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81	Klerksdorp main	North-West	Matlosana Local Municipality	City of Matlosana LM	No subdistrict	Matlosana Local Municipality	Boetrand, Wilkoppies	Lumegen	02-08-2021
82	Rustenburg	North-West	Rustenburg Local Municipality	Rustenburg Local Municipality	No subdistrict	Rustenburg Local Municipality	Rustenburg	Lumegen and Waterlab/ UP	Lumgen: 26-07-2021 Waterlab: 11-10-2021
83	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
84	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
85	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
86	Borcherd'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
87	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