NATIONWIDE TRACKING OF COVID-19 SPREAD BY WASTEWATER BASED EPIDEMIOLOGY (WBE)

TURKISH WATER INSTITUTE
SUEN
TÜRKİYE SU ENSTİTÜSÜ

NATIONWIDE TRACKING OF COVID-19 SPREAD BY WASTEWATER BASED EPIDEMIOLOGY (WBE)

FEBRUARY 2022

NATIONWIDE TRACKING OF COVID-19 SPREAD BY WASTEWATER BASED EPIDEMIOLOGY (WBE)

Publisher

Ministry of Agriculture and Forestry of Republic of Türkiye
Prof. Ahmet Mete SAATÇI on behalf of the Turkish Water Institute (SUEN)

Report Coordinator

Mustafa Salih SARIKAYA
SUEN, Coordinator for Trainings and Publications

Authors

Assoc. Prof. Bilge ALPASLAN KOCAMEMI
(Marmara University, Environmental Engineering Department)
Asst. Prof. Esra ERKEN
(Marmara University, Environmental Engineering Department)

Graphic Design by

Emine KABAKTEPE

ISBN: 978-625-8451-39-9

Turkish Water Institute (SUEN)
Libadiye Cad. No: 54 Kucukcamlıca Uskudar 34696 Istanbul / Türkiye
www.suen.gov.tr

"Science is the only true guide in life..."

K. Atatürk

FOREWORD

The novel corona virus (SARS-CoV-2), which emerged in Wuhan, China in December 2019, spread all over the world in a short time and, consequently, the World Health Organization (WHO) declared a global pandemic on March 11, 2020. The outbreak, which has affected all layers of our daily lives, once again demonstrated the critical importance of uninterrupted access to water and sanitation services.

In the first period of the pandemic, a number of countries conducted scientific studies on the presence of the virus in wastewater and its possible negative effects thereof in order to generate information concerning the distribution and trend of the virus throughout the country and as such take necessary preventive measures. Our country has become one of the few countries to carry out these studies nationwide with the project, “Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)”.

The studies are carried out through the coordination of the Turkish Water Institute (SUEN) under our Ministry and with the support and contributions of the General Directorate of Food and Control and the General Directorate of State Hydraulic Works (DSI). Marmara University as project coordinator, University of Health Sciences as scientific consultant, and the municipalities in the provision of wastewater samples have also contributed significantly from the very beginning. For this reason, the project also set a good example with regard to collaboration and coordination of different organizations.

I hope that this publication prepared to introduce the concept of wastewater-based epidemiology and to share the knowledge and experience gained will be to the benefit of all relevant local and foreign experts, and once again would like to express my gratitude to all researchers and staff who made this valuable study happen.

Bekir PAKDEMİRLİ, PhD
Minister of Agriculture and Forestry

PREFACE

As the world entered 2020, no one predicted that the infections reported initially in China would lead to a pandemic. With the announcement of the new type of corona virus (SARS-CoV-2) infection as a pandemic by the World Health Organization in March 2020, all aspects of our lives have been affected in a way that we had never experienced before.

Although the wastewater-based epidemiology concept is not new, the importance of this practice has become better understood with the Covid-19 outbreak. Wastewater surveillance studies in this context reveal the prevalence and trends of infection in the community and serve as a cost-effective early warning tool for public health decision makers. With this approach, which can scan asymptomatic cases as well as symptomatic cases, increase and decrease of cases can be detected at least 4-5 days in advance. In particular, with the rise in the number of asymptomatic cases following vaccination practices, the number of clinical diagnostic tests descended. At this point, the spread of infection in the community is possible only by monitoring wastewater samples.

Immediately after the Covid-19 pandemic was declared, various countries including the Netherlands and Australia, began conducting scientific research on the presence and possible negative effects of the novel coronavirus in wastewater.

By closely following these developments, our country has become one of the few first countries that carry out a nationwide SARS-CoV-2 monitoring study in wastewaters. The results and experiences obtained from these studies have been shared with many countries through different platforms. Also, a systematic methodology has been developed that could be used in possible future outbreaks. Furthermore, these experiences have the potential to be benefitted from in studies (e.g. monitoring of antibiotic residues in wastewater, virus and pathogen monitoring in reused wastewater as agricultural irrigation water, etc.) within the ‘One Health' approach that aims the development of global health security.

I wish that this guide document, which is prepared to share the knowledge and experience gained from the studies will be useful to all relevant experts, and I would like to express my sincere gratitude to all who have devotedly supported and contributed to the studies, in particular to our Minister Dr. Bekir Pakdemirli.

Prof. Dr. Ahmet Mete SAATÇI
President of Turkish Water Institute

CONTENT

01 | Wastewater Based Epidemiology (WBE)

Human urine and feces are conveyed to wastewater treatment plants (WWTP) through sewer systems (Figure 1). Therefore, WWTPs are important footprints of the community's lifestyle habits and health owing to the human excretion products.

WBE enables almost real-time identification and quantification of chemical and/or biological markers using samples from the WWTPs, thus provides information on community circulation of these markers.

***Figure 1.** Extent of WBE Studies*

WBE is a very efficient tool for detection and population surveillance of illicit drugs, personal care products, industrial chemicals and human enteric viruses (Poliovirus, Hepatitis A, Adenovirus, Corona viruses etc.). It is also a non-invasive tool, which does not require repeated sampling of individuals.

1.1 History

Monitoring of pathogen organisms in wastewater dates back to 1920's (Figure 2). Since the launch of the Global Polio Eradication Initiative (GPEI) in 1988, WBE studies have gained importance and been used for the monitoring of illicit and pharmaceutical drugs, and personal care product consumption since 2005.

In Türkiye, WBE was first implemented to monitor illicit drugs and physcoactive substances in 2017 by cooperation of Yeşilay (Green Crescent) and Istanbul University Institute of Forensic Sciences. A similar study led by the Turkish Ministry of Interior is in progress since 2019.

A nationwide SARS-CoV-2 WBE study has been initiated by the Ministry of Agriculture and Forestry of Türkiye, under the coordination of Turkish Water Institute (SUEN) to monitor the spread of COVID-19 outbreak throughout the country since the beginning of the pandemic (April 2020) and is still on-going.

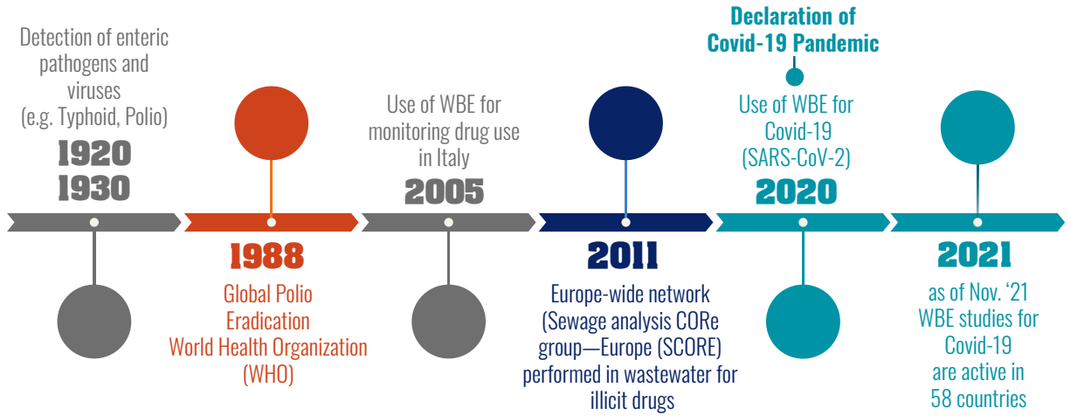


***Figure 2.** History of WBE Studies*

Global Polio Eradication

The virus that causes polio was isolated from wastewater for the first time in 1932. Following the Global Polio Eradication Program of the World Health Assembly in 1988, systematic sewage surveillance has gained importance to track and prevent circulation of infectious diseases.

SCORE
Sewage Analysis CORe Group Europe

WBE studies was first implemented in Italy to monitor drug use. Since 2011, the SCORE (Sewage Analysis Core Group Europe) network has been conducting an extensive study and has analysed drugs in wastewater samples from the wastewater treatment plants in participating European cities.

COVID-19 Pandemic

The beginning of COVID-19 pandemic (2020) was a global milestone in WBE studies. Since 2021, WBE has been actively used to monitor SARS-CoV-2 in 58 countries.

1.2 Global WBE Studies

Following the declaration of Covid-19 pandemic on 11 March 2020, monitoring of Covid-19 with WBE was first carried out in the Netherlands in certain regions. Türkiye, concurrently, initiated the "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" project commissioned by the Ministry of Agriculture and Forestry of Türkiye in all of the cities (81 city) of the country. This study has been the first nationwide study and is still on-going since November 2021.

As of November 2021 monitoring efforts have been made all over the world in 58 countries with samples from 3145 different locations. Majority of these studies are small and medium-scale research efforts (Figure 3).

Figure 3. Covid-19 Dashboard of Global Scale WBE Studies

(www.covid19wbec.org/covidpoops19, 16.11.2021)

First Studies: The Netherlands Case

During Covid-19 pandemic, WBE studies have first been implemented in the Netherlands by conducting SARS-CoV-2 q-PCR analyses on samples taken from the inlets of 7 wastewater treatment plants and the Schiphol international airport. The virus was quantified in sewage 6 days before the first cases were reported.

First Studies: Türkiye Case

Immediately after the declaration of the pandemic, in April 2020, Türkiye has initiated a a nationwide SARS-CoV-2 surveillance study in the wastewaters of Türkiye's 81 cities commissioned by the Turkish Ministry of Agriculture and Forestry and conducted through the coordination of Turkish Water Institute (SUEN). As from September 2020, the study covers 22 pilot cities selected as to represent the whole country. Also, 27 touristic destinations located in the Aegean and Mediterranean coast line have routinely been monitored during summer season.

Studies Worldwide

Information on global WBE studies have been shared via a dashboard: **www.covid19wbec.org/covid19poops19.** Scientific papers as outcomes of these studies can be accessed on **www.covid19wbec.org/**

02 | Public Health Monitoring with Covid-19 WBE Studies

2.1 Covid-19 Diagnosis and Monitoring Methods

Methods that can be used for Covid-19 monitoring are;

a) Detection of SARS-CoV-2 virus in nasopharyngeal swabs with qPCR,
b) Serological tests based on the detection of SARS-CoV-2 antibodies in the blood,
c) WBE based on detection of SARS-CoV-2 virus with qPCR in wastewater.

For the diagnosis of nasopharyngeal disease, serological tests are used to detect people who are immune to the disease in the community, whereas WBE is used to track the spread of all Covid-19 patients in the community whether or not they show any symptoms.

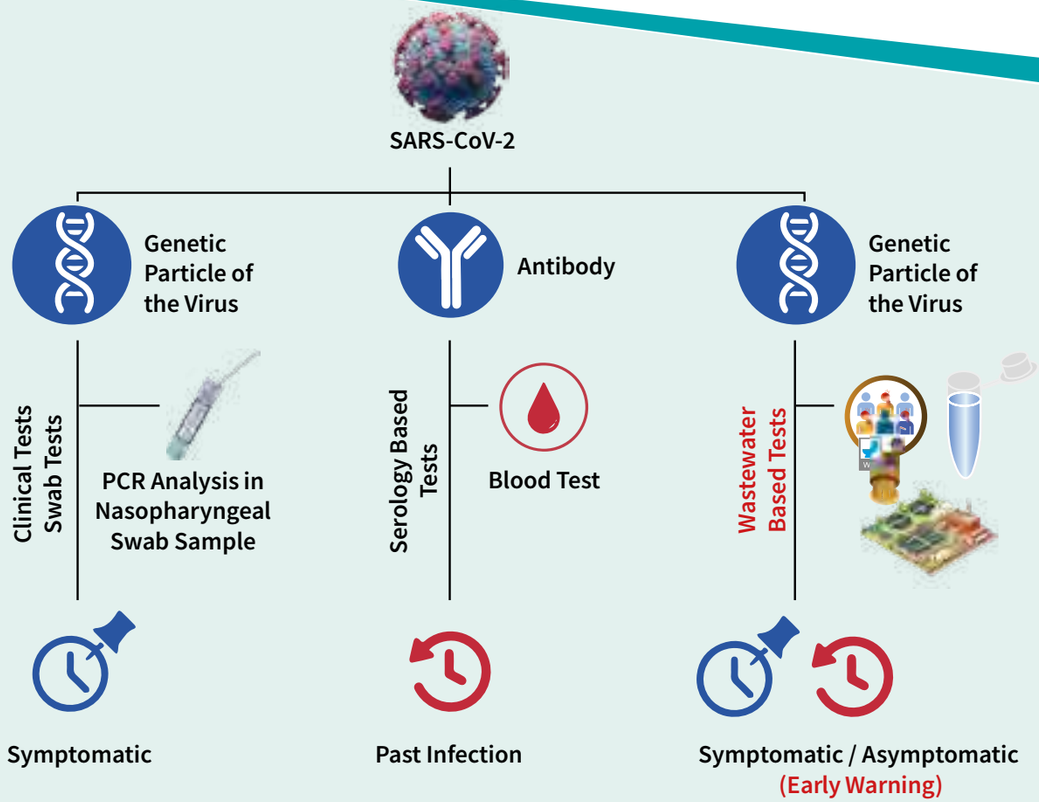


Figure 4. Covid-19 Diagnosis and Monitoring Methods

2.2 Importance of Covid-19 WBE Studies

Minimizing or reducing the impact of an epidemic on public is only possible by conducting a sufficient number of clinical diagnostic tests. However, both the cost of these tests and the limitations on the production and logistics caused by closure during the pandemic makes it impossible to achieve the targeted number of tests. In addition, clinical diagnostic tests are only performed on infected individuals resorting to health centers and showing symptoms, so they do not reflect the results of infected individuals who have not yet shown symptoms (presemptomatic), mild symptoms, or symptoms (asymptomatic) (Figure 5).

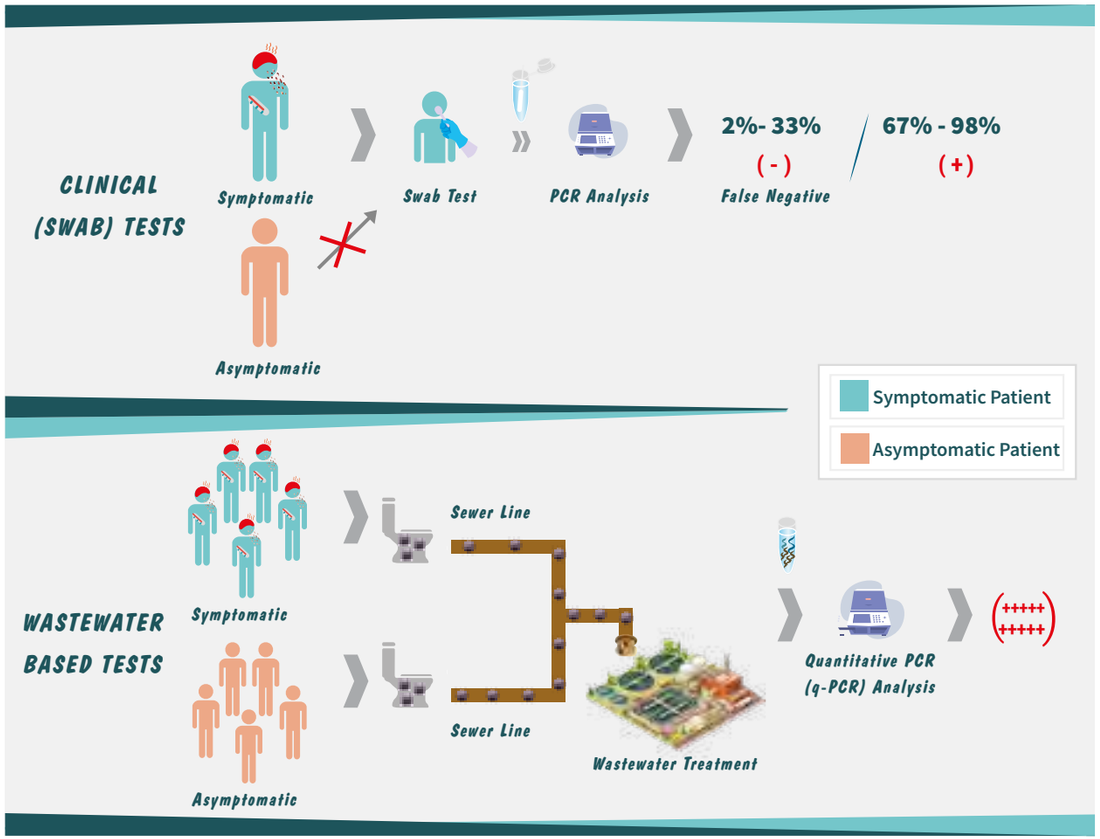


Figure 5. *Covid-19 Diagnosis and Tracking in a Community*

Therefore, unlike clinical diagnostic tests, WBE is an important tool that can be used to monitor the footprint of infectious diseases such as Covid-19 in society and their presence and spread in real time and without presemptomatic/symptomatic/asymptomatic distinction with a small number of samples in a cost-effective manner. WBE studies and routine monitoring of the spread of Covid-19 in regions enable decision-makers to quickly implement quarantine measures in the right regions at the right time, depending on the trend of increasing disease intensity.

2.3 WBE Studies in Non-Sewered Countries

In underdeveloped/developing countries, clinical diagnostic tests are not widely available due to their high costs and limited availability. For this reason, WBE studies that make it possible to collect mass case numbers at low costs are highly important for these countries. However, since most of these countries are non-sewered (Figure 6), sampling and measurements for WBE should be different from sewered countries.

During the Covid-19 pandemic, the South African National Institute of Infectious Diseases (NICD), the South African Medical Research Council (SAMRC) and the Water Research Commission (WRC) consortium have been conducting pioneering studies in this direction.

Figure *6. Wastewater Discharge in Non-sewered Countries*

03 | WBE Studies as an Early Warning Approach

Correlation studies that will enable the transition from the results of wastewater based epidemiology studies to the number of cases in a given community are still under way. However, as it is proven by many studies that, thanks to wastewater monitoring, the increase in the number of cases in a community can be observed beforehand on a daily/weekly basis, WBE is accepted as an effective early warning approach.

It is possible to detect the new surge waves and possible new epidemics with WBE studies.

Early Warning Examples in Türkiye during the Pandemic

Within the scope of WBE studies that have been carried out throughout the country since April 2020, the rise in the number of cases in many of the cities could be detected ~1-2 week beforehand with wastewater samples (Figure 7).

Figure 7. *Early Warning Examples in Türkiye during the Pandemic*

3.1 Hotspots/Location of Interests

WBE studies are particularly important for hotspots/location of interests.

These hotspots can be examined in two different groups namely; Stationary and Mobile. Stationary groups include university campuses, student dormitories, military bases, homeless shelters, industrial sites, refugee camps, prisons, orphanages, and resorts (Figure 8), whereas mobile groups include metro stations, airports, bus and train stations and ferry docks (Figure 9).

Figure 8. Stationary Hotspots

Routine analyses of wastewater samples allow quick and economic pre-screening of asymptomatic patients in stationary hotspots where a large number of individuals live together and there is a high risk of infecting each other rapidly.

These screening results provide early warning before the number of cases goes up, allowing necessary measures to be taken (e.g. increasing clinical diagnostic tests).

Routine wastewater sampling in mobile hotspots allow monitoring of intercity, intercountry and intercontinental spread of the disease and thus, provides necessary measures (closure of flights, prohibition of entrances and exits to cities, etc.) are taken regarding air, sea and highway traffic as well as travel destinations.

Figure 9. Mobile Hotspots

3.2 Detection of Current and Possible Future Variants

The SARS-CoV-2 RNA virus, which causes the Covid-19 disease, transfers its RNA into the viable cell, copies them and multiplies. Any problem that will cause the virus to change its genetic sequence while copying its RNA is called a mutation, while mutating viruses are called variants. Variants are detected by a molecular method known as Next Generation Sequence (NGS).

As of November 2021, 5 variants have been identified on a global scale. These are named as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Omicron (B.1.1.529) (Figure 10).

With each newly formed variant, the symptoms of the disease, its contagiousness and the treatments applied can change.

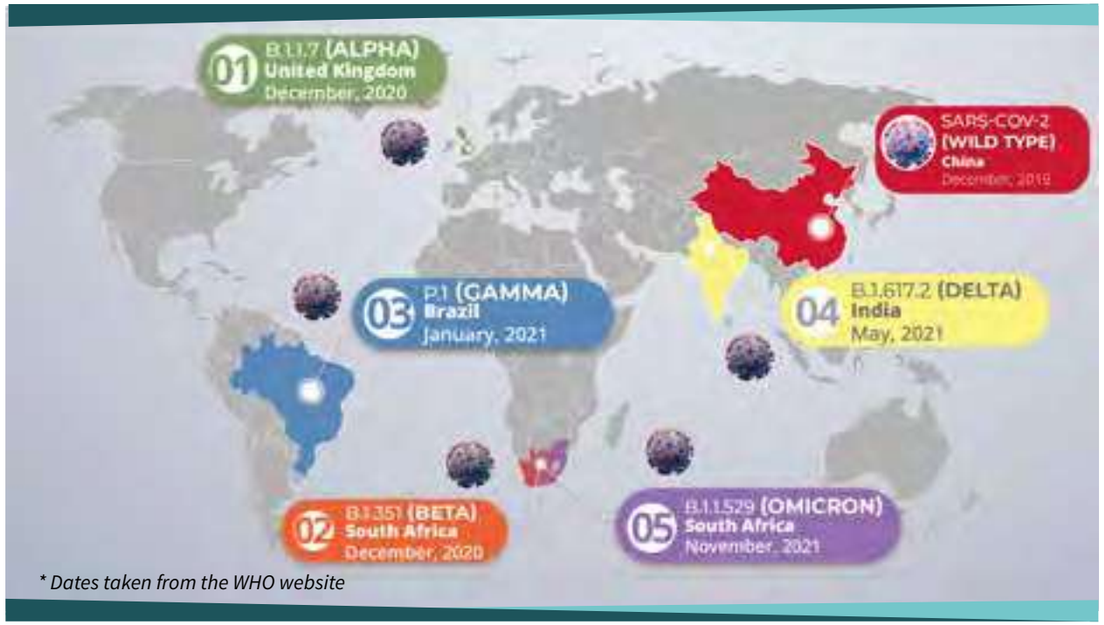


***Figure 10.** SARS-CoV-2 Variants (as of December 2021)*

Once a new variant appears in a population, it is very challenging to detect that variant with clinical testing before it reaches a certain transmission level. Therefore, routine next-generation sequencing (NGS) analyses to be performed on samples taken from wastewater treatment plants serving high-density population areas in the community allow for rapid and inexpensive detection of variants.

3.3 Covid-19 Monitoring After Vaccinations

Worldwide vaccination activities started simultaneously in many countries in December 2020, 8 months after the pandemic was declared. The first coronavirus vaccine approved for emergency use in Türkiye was administered to the Minister of Health on January 13, 2021. As of December 15, 2021, the 1st and 2nd dose vaccination rates in Türkiye were 67.68% and 61.04%, respectively. Particularly after the 2nd vaccination dose, there has been an increase in the number of infected people who do not show any symptoms (i.e. asymptomatic) which in turn has led to a reduction in the number of clinical diagnostic tests. Thus, the actual Covid-19 transmission in the community remains unknown.

However, since the vaccine does not prevent the presence of the Covid-19 virus in urine and stool, even when the patient is asymptomatic, WBE studies have become even more instrumental to monitor the real status of the spread of Covid-19 in the community after vaccination.

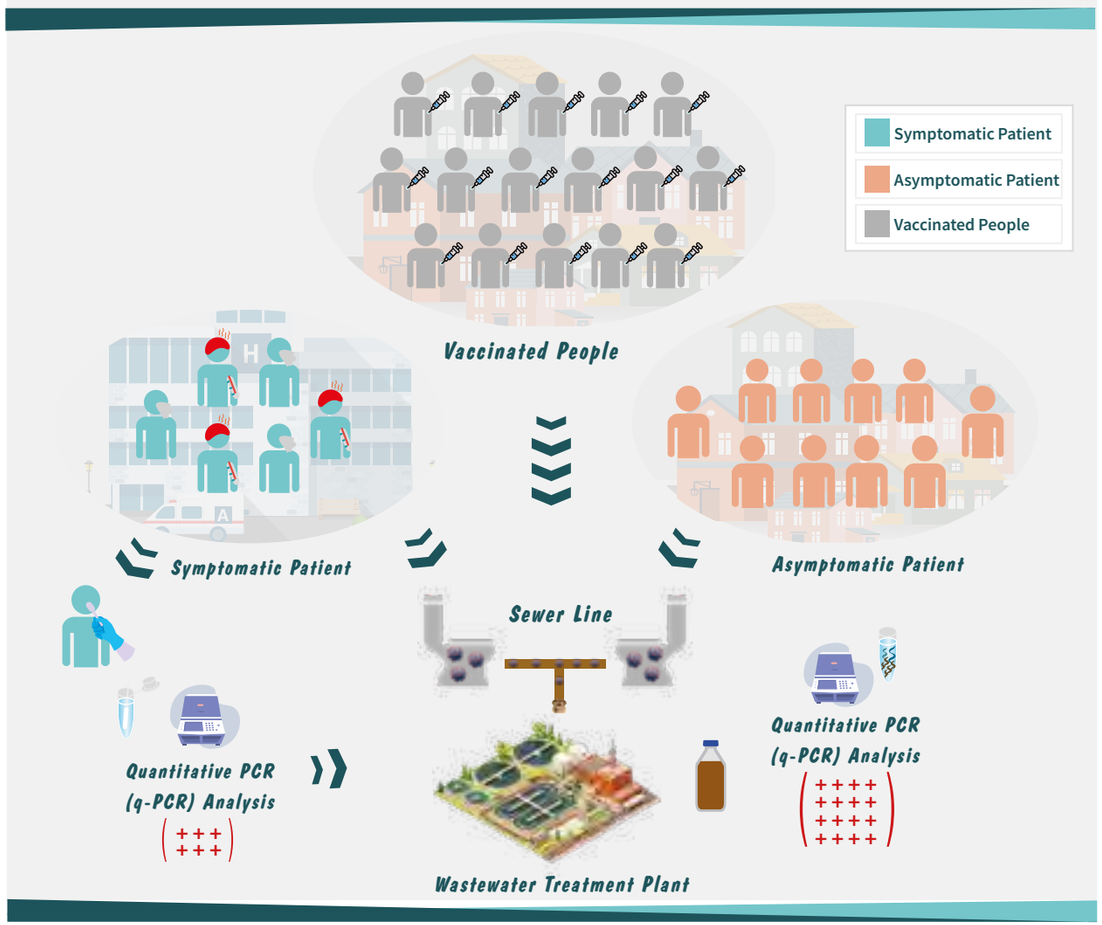


***Figure 11.** Importance of WBE after Covid-19 Vaccination*

04 | Stages of WBE Studies

Establishing the logistics of the work steps in the systematic and sustainable functioning of WBE studies is a critical and challenging process.

The 3 basic steps to be planned within this process are (Figure 12);

I. Sampling and transfer

II. Preliminary steps, analysis and

III. Reporting

sequentially.

When applying these steps, a careful assessment of the following points is essential for the smooth functioning of the studies:

- Sample transfer capabilities,
- Biosafety level 2-3 laboratories,
- Equipment (refrigerated centrifuges, RT-qPCR, etc.),
- Competent staff: Team specialized in molecular analysis and interpretation (laboratory technicians, virologists/microbiologists/molecular biologists/ environmental engineers),
- Supply and delivery times of consumables

If WBE studies are planned to be carried out nationwide, as in the case of Türkiye, the need for central government support is a crucial factor.

4.1 Sampling and Transfer

Wastewater samples used in WBE studies are grab or 24-hour composite samples collected from the inlets of wastewater treatment plants where wastewater infrastructure (Figure 12-A) is available. There are also studies in which sludge (primary and activated) and effluent samples are analyzed in addition to the influent samples.

In some areas, samples can also be taken from manholes prior to the entry into the wastewater treatment plant.

In non-sewered areas, samples are taken from septic tanks or surface water bodies into which wastewater is discharged.

The weather conditions are critical in sampling. Rainy and snowy weather conditions can cause delays in the sampling schedule as the virus concentration in the wastewater is diluted.

Türkiye WBE Case: Sampling

A total of 501 samples (177 influents, 167 effluents, 35 primary and 122 active sludge) were collected from 189 plants in 81 cities of Türkiye, in the period of May-June 2020 within the scope of the "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" project, which has been carried out since April 2020. https://covid19.tarimorman.gov.tr/Galeri/ResimGaleriDetay/1546.

As part of the routine monitoring that has been active since June 2020, only inlet samples are collected from several wastewater treatment plants in 22 pilot cities. Of these treatment plants, 24 h composite samples are collected from 21 of them with a refrigerated composite sampler, while grab samples are collected from 7 of them (https://covid19.tarimorman.gov.tr/Galeri/ResimGaleriDetay/1548). Samples are collected in 250 mL sterile bottles, in duplicate.

Türkiye WBE Case: Sample Transfer

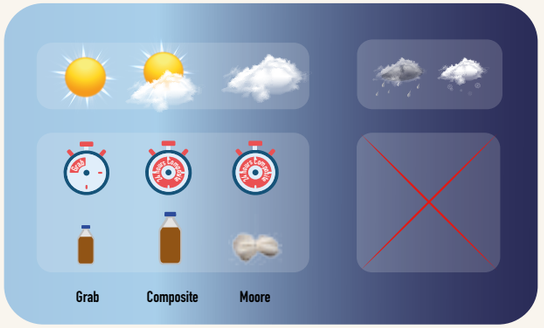
From the beginning of the project, samples are transferred by State Hydraulic Works (DSI) of the Ministry of Agriculture and Forestry to BSL-2 laboratories located in Veterinary Research Institutes (İstanbul Pendik and Samsun) of Food and Control General Directorate under cold chain by highway transport (see Section 5). Only in the May 2020 period of the project, samples were transferred to Ankara Veterinary Research Institute as well. Collected samples are transferred under cold chain protocols (< 4°C) to keep the virus intact.

In some studies, samples can be filtered on-site (at the wastewater treatment plants) and these filters which are contained in specific solutions to prevent RNA degradation can be transferred to laboratories at ambient temperature. The sample volume may change depending on the concentration method to be used.

SEWER LINE

WASTEWATER TREATMENT PLANT

A) SAMPLING AND TRANSPORTATION



COLD CHAIN

B) PRELIMINARY STUDIES

BSL-2

REFRIGERATED CENTRIFUGE

ULTRACENTRIFUGE

PEG METHOD

ULTRAFILTRATION

ELECTRONEGATIVE MEMBRANE

$Al(OH)_3$

SKIMMED MILK

RNA INSULATION

BSL-2

C) MOLECULAR ANALYSES

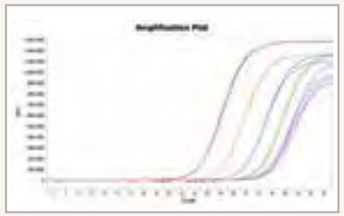
qPCR / ddPCR

NEXT GENERATION SEQUENCE (NGS)

ACTIVITY

BSL-3

D) REPORTING



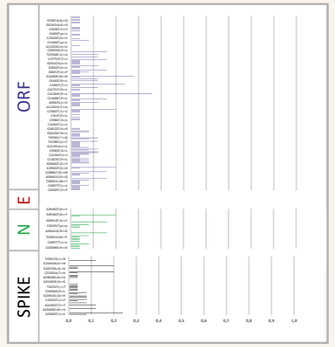
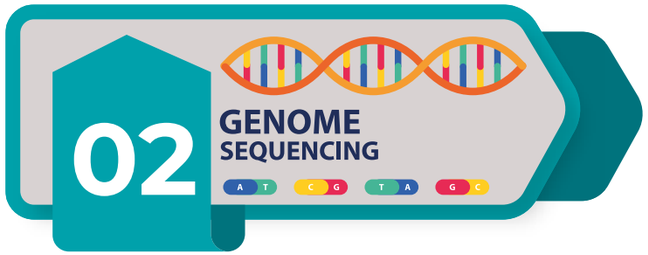


Figure 12. Stages of WBE Studies

4.2 Preliminary Steps and Analysis

3 different analyses can be performed for the SARS-CoV-2 virus in wastewater samples:

- **RT-qPCR analyses** to determine the presence and concentration of the virus
- **Next-generation sequence (NGS)** analyses to determine the virus strains and variants
- **RT-qPCR analysis** for variants of concern **(VOC)**
- **Activity tests** to determine the potential of the virus in wastewater to cause infection



Preliminary steps prior to RT-qPCR

As wastewater is a matrix rich in solids, solids are removed from the water by refrigirated centrifuges prior to these analyses. The virus particles diluted in the wastewater are then concentrated using different methods, depending on the options available (Figure 12-B):

- Ultracentrifuge Polyethyleneglicol (PEG) adsorption
- Electronegative membrane
- Ultrafiltration
- Skim milk
- Aluminum hydroxide

Concentrated virus particles are separated from the water by centrifuges and RNA isolation is performed.

SARS-CoV-2 Virus Detection with RT-qPCR Measurements

The presence and concentration analysis of viruses in wastewater is carried out in BSL-2 laboratories by a molecular method known as quantitative polymerase chain reaction (RT-qPCR) performed in thermal cycle devices (Figure 12-C).

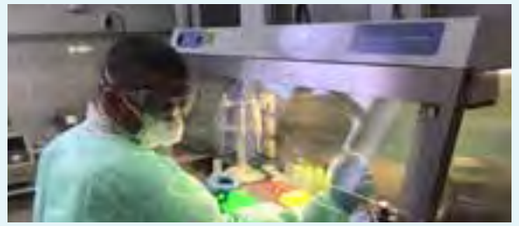
The most important components of the test are so-called primary chemicals that bind to different gene regions of the RNA in the virus genome. These chemicals are produced in light of data generated through a full genome analysis of the virus.

Another new method that can be used as an alternative to the RT-qPCR method is droplet digital PCR (ddPCR). However, the cost of the method is currently higher than that of qPCR.

Türkiye WBE Case: Preliminary Steps and RT-qPCR Analysis

As part of the "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" project, samples collected from wastewater treatment plants are analyzed by the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) method in Biosafety Level 2 (BSL-2) laboratories of Istanbul Pendik and Samsun Veterinary Research Institutes.

Prior to the analyses, the samples are centrifuged, and SARS-CoV-2 virus in the filtered supernatant is concentrated by the PEG 8000 method. Total RNA extraction is carried out on the concentrated phase. The analyses are performed in triplicates.



Detection of SARS-CoV-2 Variants with Next Generation Sequence (NGS) Analysis

RNA viruses such as SARS-CoV-2 develop mutations in their genome as they multiply and spread. Although most of these mutations do not have a meaningful effect, some mutations may provide advantages to the virus, such as increased transmission. The World Health Organization (WHO) classifies variants of mutations in two groups: “Variants of Interest - VOI” and “Variants of Concern – VOC”. As of November 2021, 5 VOCs (Alpha, Beta, Gamma, Delta, Omicron), 2 VOIs (Lambda, Mu) have been identified by WHO.

New generation sequencing (NGS) genetic technology is used to detect VOCs and VOIs.

NGS is an expensive technique that takes time and requires a complex, experienced team. This makes it difficult to monitor the spread of existing variants and to identify possible new variants with NGS analyses performed in swab samples of patients admitted to the hospital with symptoms.

On the other hand, NGS analysis on a single sample collected from a wastewater treatment plant as part of WBE studies enables an cost-effective monitoring of all variants in the community living in that region represented by this sample. However, the complex structure of the wastewater matrix, the possibility of RNA degradation during the time the wastewater travels along the sewer lines, and the need for laboratory infrastructure and trained bioinformatics specialists for tests make it difficult to use these tests widely.

Detection of Variants of Concern (VOC) with RT-qPCR Analysis

The primer sets, created after determining the whole genome sequences of variants of concern with next-generation sequencing analyses, allow tracking spread of these variants in the community with RT-qPCR measurements, as well.

Türkiye WBE Case: Tracking SARS-CoV-2 Variants

In Türkiye, the Next Generation Sequencing studies of wastewater samples started in April 2021. These analyses are mainly implemented on wastewater samples taken from the megacity Istanbul where there is a heavy international flight traffic and from the border cities.

Pangolin lineage and nextstrain clade information obtained as a result of VOC and VOI monitoring studies in Türkiye are published real time on the Covid-19 WBE website (covid19.tarimorman.gov.tr) of the Ministry of Agriculture and Forestry under the SARS-CoV-2 Genome Sequencing Dashboard along with mutations observed in the SARS-CoV-2 virus spike gene and protein.

As of April 2021, all samples that tested positive for RT-qPCR are also scanned with the current mutant mix RT-qPCR primers and the results are shared on the same webpage.

Activity Tests

The SARS-CoV-2 virus, the cause of the Covid-19 disease, is more sensitive to environmental factors since it is an enveloped RNA virus, and the RNA structure deteriorates quickly and can be easily inactivated. Therefore, SARS-CoV-2 virus is not expected to remain active in wastewater.

In regions where there is wastewater infrastructure (sewered regions), wastewater is discharged to the receiving water bodies such as stream, river, lake, sea that have human contact either without treatment or after preliminary/biological treatment/advanced biological treatment. Also, in some areas advanced biological treatment plant effluent can be used as agricultural irrigation water.

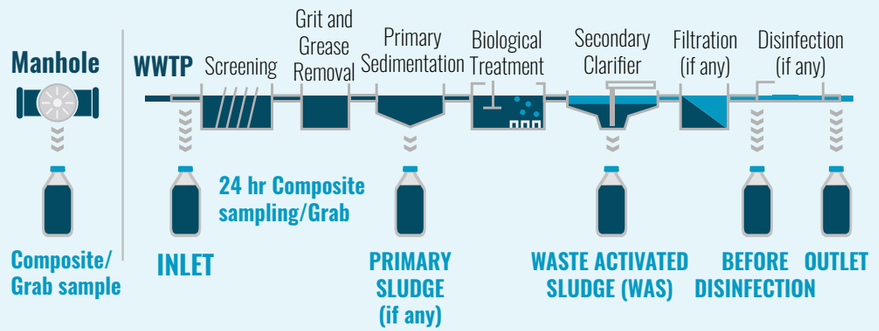
This makes it important to monitor the activity of SARS-CoV-2 virus in wastewater. In non-sewered regions where septic tanks are in use, there is a risk that wastewater can leach to groundwater. In addition, receiving water bodies such as streams, rivers, lakes, and seas into which wastewater is directly discharged have human contact and can be used as potable water supply. Therefore, SARS-CoV-2 virus activity tests in these regions are much more important than in regions with wastewater infrastructure.

Activity tests require virus isolation studies in cell culture and can be performed in laboratories with at least Biosafety Level 3 due to the risk of transmission of the virus.

Türkiye WBE Case: Activity Tests

Activity tests have been performed on 70 SARS-CoV-2 positive samples collected from various points of wastewater treatment plants (shown below) that have different configurations by inoculation of cell culture as part of the ‘Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)’ project, ongoing since April 2020.

Test were conducted in BSL-3 laboratories of the Pendik Veterinary Control Institute. In only one (primary sludge) of the 70 inoculums, the cytopathological effect (CPE) of the reproductive foci were detected in the virus control eyes.



4.3 Reporting

The use of the results of WBE studies for the protection of public health depends on an understandable and functional reporting format of the results obtained. Findings should be reported in a way that, not only the scientists, but the whole community, especially policy makers, can easily understand.

Many of the WBE studies carried out worldwide during the Covid-19 pandemic remain in the scientific data format (the virus observed in wastewater titers/liter). However, in nationwide WBE studies, particularly in the case of Türkiye, various reporting techniques have been developed based on feedback from decision-making authorities and community during the pandemic.

Some countries report their wastewater virus concentration levels along with the number of cases, while others track the spread of Covid-19 across the country based solely on wastewater data.

Visual reports (maps, color scale maps) and regular sharing of these reports with community from platforms such dashboards make WBE studies easier to be understood by decision makers and the community and enable its functional use.

Türkiye CASE

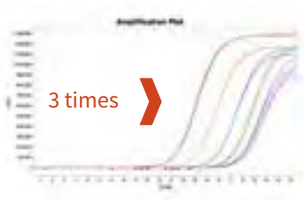
RT-qPCR test results (virus titer/Liter) of samples -routinely collected every 2 weeks (once a week in Istanbul case) from wastewater treatment plants of 21 cities - are normalized with the flow rates of the plants and converted to viral load (virus titer/day) within the framework of the national WBE project carried out in Türkiye.

Calculated viral loads are transferred to color-scaled spread maps for easy and understandable monitoring of Covid-19 spread throughout Türkiye. In these maps, viral loads are shown on a scale of 4 colors from low to high: **"very low case-no case (yellow)"**, **"low case (light green)"**, **"average case (green)"** and **"high case (dark green)"**. These maps are published on the 'covid19.tarimorman.gov.tr' website 3-4 days after the samples are collected for all of Türkiye and 1-2 days after sampling for Istanbul. Since there is also an English version of the site, the data to be tracked from abroad.

The **covid19.tarimorman.gov.tr** website includes up-to-date maps as well as all past data, enabling rapid monitoring of the rise/fall trends of regional Covid-19 cases across the country.

Türkiye: WBE Case
Reporting: Color Scaled Covid-19 Maps

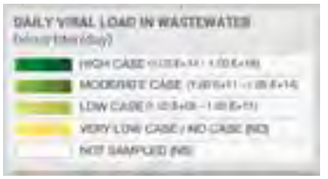
RT-qPCR (Virus Titer/Liter)



Normalization with Flow (Viral load)

RT-qPCR
(Virus Titer/Liter)
X
Q plant
(m^3/d)

Viral Load Colour Based Scale



Covid-19 (SARS-CoV-2) Viral Load Distribution Map

https://covid19.tarimorman.gov.tr/

05 | Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE) Project

5.1 About Project

The "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" project was launched in April 2020 simultaneously with a few countries in the world under the coordination of Turkish Water Institute (SUEN).

State Hydraulic Works and General Directorate of Food and Control which are the units of the Ministry of Agriculture and Forestry of Türkiye provide support and contribute to this project. Marmara University Environmental Engineering Department is the principal investigator and scientific advisor of the project.

Health Sciences University is the scientific advisor of molecular analyses.

At the outset, until May 2020, “Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)” project scanned the Covid-19 distribution in 81 cities of Türkiye. In June 2020, 22 cities have been designated as representatives of 7 geographical regions of Türkiye and since then bi-weekly samples have been collected from these cities, except Istanbul. Istanbul which is the most populous city of Türkiye has been scanned with weekly samples. During summer months, bi-weekly samples have been collected from 27 touristic districts for routine analyses.

The project also aims to scan the spread of mutant viruses throughout Türkiye and to develop an early warning system which will allow announcement of new mutants originating from abroad and/or forming locally before causing an increase in case numbers in a fast, accurate and economic manner.

The chronological summary of the studies conducted within the project, which has been running for 21 months as of December 2021, is given in Figure 13.

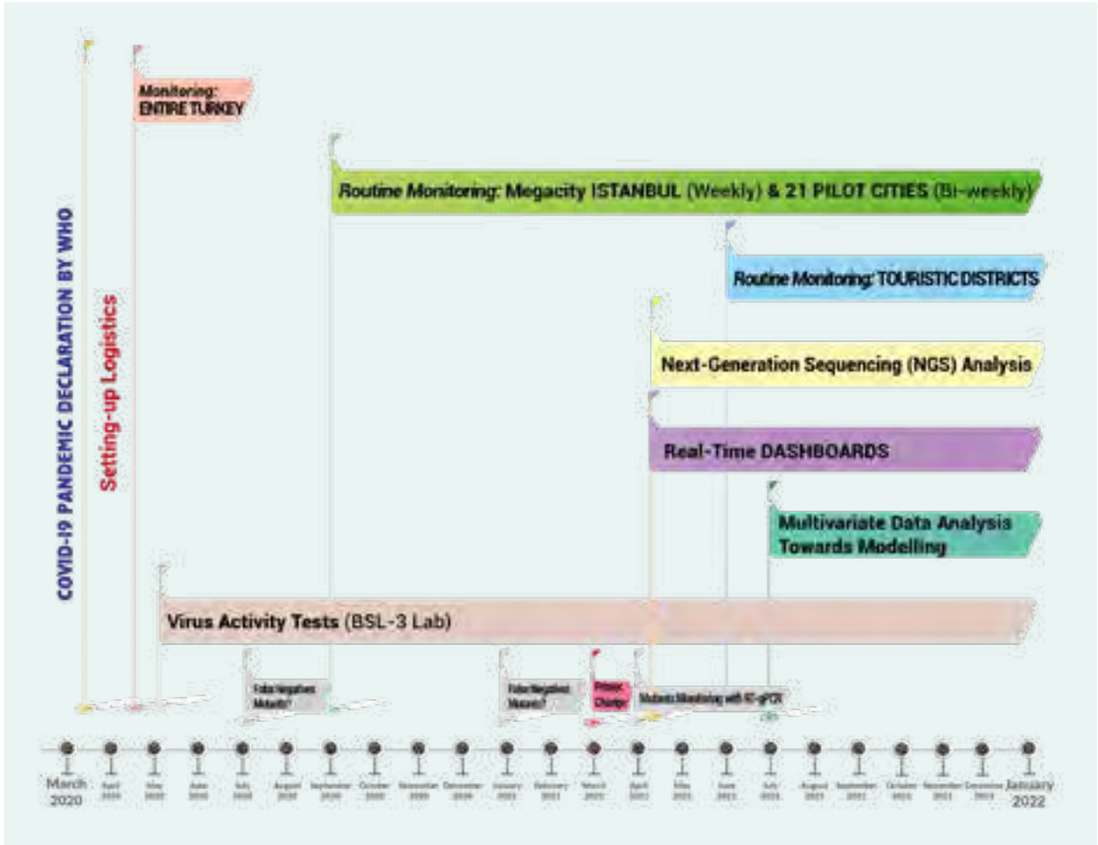


Figure 13. *Timeline of "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE) Project"*

5.2 Project Team

The "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE) Project" was initiated by the Ministry of Agriculture and Forestry of Türkiye under the coordination Turkish Water Institute (SUEN). Under this unique project, which is in progress for 21 months, high-level governmental representatives, governmental agencies (many publicly qualified institutions), municipalities, wastewater treatment plant employees and universities are working together.

Marmara University Environmental Engineering Department is the principal investigator and scientific advisor of the project. The samples from wastewater treatment plants have been collected by the authorized employees of the treatment plants. The cold chain transfer of the picked samples has been done by State Hydraulic Works.

The RT-qPCR measurements of the samples have been made in Veterinary Control Institutes under the General Directorate of Food and Control. At the beginning of pandemic (April-May 2020), the RT-qPCR measurements were done in Ankara Etlik Veterinary Control Central Research Institute. Since May 2020, the RT-qPCR measurements are done in Istanbul Pendik and Samsun Veterinary Control Institutes.

Health Sciences University is the scientific advisor of molecular analyses performed in Veterinary Control Institutes.

Figure 14. Project Team

TURKISH WATER INSTITUTE (SUEN)

PROJECT COORDINATOR

Prof. Ahmet Mete SAATCI
President of SUEN

SUEN is a national think tank established to develop national water policies, provide consultation to decision makers, coordinate between organizations & institutions and enhance scientific research and strategic ideas with a focus on creating a common platform for water management.

SUEN works in cooperation with national and international water-related institutions on issues such as sustainable water management, developing water policies, sustainable energy and capacity building for solving local and global water problems.

TURKISH WATER INSTITUTE (SUEN)

· Prof. Dr. Ahmet Mete SAATCI (President) · Mustafa Salih SARIKAYA, M.Sc. · Tugba Evrim MADEN, Ph.D
· Burcu CALLI, M.Sc. · Elec. Eng. Akif Murat CEYLAN, M.Sc. · Env. Eng. Çigdem KUS, M.Sc
· Env. Eng. Hatice TANER ÖZER, M.Sc · Env. Eng. Nuray AKALIN, M.Sc · Env. Eng. Burcu YAZICI, M.Sc
· Graphic Design Emine KABAKTEPE · Env. Eng. Salim YAYKIRAN, M.Sc

Marmara University Environmental Engineering Department

· Assoc. Prof. Bilge ALPASLAN KOCAMEMİ (Principal Investigator and Scientific Advisor)
· Asst. Prof. Esra ERKEN · Asst. Prof. Nur ORAK · Env. Eng. Sümeyye CELIK, M.Sc

University of Health Sciences

· Asst. Prof. Halil KURT (Scientific Advisor of Molecular Analyses)

GENERAL DIRECTORATE OF FOOD AND CONTROL

Harun SEÇKİN
Director General

· DG Harun SEÇKİN
· Deputy DG Sedat ILDIZ
· Head of Department Bayram SERTKAYA
· Dvm. Alper KARAPINAR

Istanbul Pendik Veterinary Control Research Institute

· Dvm. Ph.D. Director Fahriye SARAC
· Dvm. Ph.D. Ahmet SAIT

Veterinarians:

· Dvm. Ayse PARMAKSIZ · Dvm. Mustafa TÜRKDOĞAN
· Dvm. Merve DEMİR

Veterinary Technicians:

· Vet. Tech. Nuray MALAL

Samsun Veterinary Control Research Institute

· Dvm. Director Ismail AYDIN
· Dvm. Ph.D. Hekim Hamza KADI

Veterinarians:

· Dvm. Fatih DOĞAN · Dvm. Serdar YILDIRIM
· Dvm.Hakan TÜTÜNCÜ

Veterinary Health Technicians:

· Vet. Tech. Mustafa BULUT ·
Vet. Tech. Muhammed ÖVECEK · Vet. Tech. Barış VAR

Ankara Etlik Veterinary Control Central Research Institute

· Dvm. Director Cevdet YARALI
· Dvm. Ph.D. Sabri HACIOGLU · Dvm. Samiye Oznur YESIL

GENERAL DIRECTORATE OF STATE HYDRAULIC WORKS

Kaya YILDIZ
Director General

Technical Research and Quality Control Department

· DG Kaya YILDIZ

· Deputy DG Dincer AYDOĞAN

· Head of Technical Research and Quality Control Department Dr. Nurettin PELEN

STAFF OF STATE HYDRAULIC WORKS REGIONAL DIRECTORATES TRANSFERRING NATIONWIDE COLLECTED SAMPLES AT THE BEGINNING OF PANDEMIC

ADANA (6th REGION)

ANKARA (5th REGION)

AYDIN (21st REGION)

DIYARBAKIR (10th REGION)

EDIRNE (11th REGION)

ELAZIG (9th REGION)

ERZURUM (8th REGION)

ESKISEHIR (3rd REGION)

IZMIR (2nd REGION)

KASTAMONU (23rd REGION)

KAYSERI (12th REGION)

KONYA (4th REGION)

SAMSUN (7th REGION)

SANLIURFA (15th REGION)

TRABZON (22nd REGION)

VAN (17th REGION)

STAFF OF STATE HYDRAULIC WORKS REGIONAL DIRECTORATES TRANSFERRING NATIONWIDE ROUTINE COLLECTED SAMPLES


ANTALYA (13th REGION)


ARTVIN (26th REGION)


BALIKESIR (25th REGION


BURSA (1st REGION)


ISPARTA (18th REGION)


ISTANBUL (14th REGION)

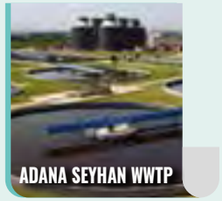

SIVAS (19th REGION)


K.MARAS (20th REGION)


KARS (24th REGION)

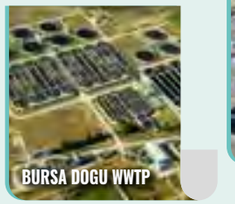

MARDIN (16th REGION)

ROUTINELY SAMPLE TRANSFERRING TREATMENT PLANTS ACROSS TÜRKİYE

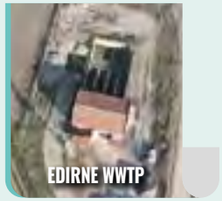

ADANA SEYHAN WWTP


ANKARA TATLAR WWTP


ANTALYA HURMA WWTP

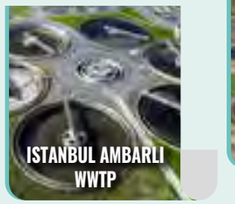

BURSA DOGU WWTP


DIYARBAKIR SUR WWTP


EDIRNE WWTP


ELAZIG MERKEZ WWTP


ERZURUM AZIZIYE WWTP


ISTANBUL AMBARLI WWTP


IZMIR CIGLI WWTP

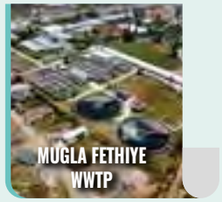

KAYSERI-KOCASINAN WWTP

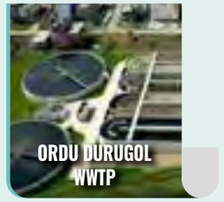

KONYA-KARATAY WWTP


KUTAHYA MERKEZ WWTP

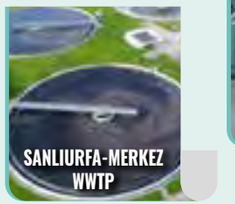

MALATYA MERKEZ WWTP


MERSIN KARADUVAR WWTP


MUGLA FETHIYE WWTP


ORDU DURUGOL WWTP


SAMSUN-TEKKEKOY WWTP


SANLIURFA-MERKEZ WWTP


TRABZON-ORTAHISAR WWTP


VAN-TUSBA WWTP


ZONGULDAK WWTP

ROUTINELY SAMPLED TREATMENT PLANTS ACROSS ISTANBUL AT THE BEGINNING OF PANDEMIC

Istanbul Water and Sewerage Administration (ISKI) – Department of Wastewater

· Asian Side Wastewater Treatment Branch Manager Abdülkerim UZUNTEPE
· European Side Wastewater Control Branch Manager İmdat DEMİR
· Environmental Engineer, M.Sc., Mihriban HACI GÖKTAŞ · Head of Wastewater Treatment Department Celil ASLAN
· European 1st District Wastewater Treatment Branch Manager Serhat KUŞ
· Asian Side Wastewater Control Branch Manager Hasan ONUR

Advanced Biological Treatment Plants

AMBARLI WWTP

PASAKOY WWTP

TUZLA WWTP

ATAKOY WWTP I-II

BUYUKCEKMECE WWTP

SILIVRI-CANTA-SELIMPASA WWTP

TERKOS WWTP

Preliminary Treatment Plants

BALTALIMANI WWTP

KADIKOY WWTP

KUCUKCEKMECE WWTP

KUCUKSU - P.BAHCE WWTP

SILE KUMBABA WWTP

YENIKAPI WWTP

Biological Treatment Plants

AGVA, KURNAKOY, DOGANCALI, KOMURLUK WWTP

IZZETTIN, BINKILIC, CIFTLIKKOY, KARABURUN, ORENCIK WWTP

ROUTINELY SAMPLED TREATMENT PLANTS ACROSS TÜRKİYE AT THE BEGINNING OF PANDEMIC

Routine Sampling

BURSA DOGU WWTP

Routine Sampling

BURSA BATI WWTP

BILECIK WWTP

BOLU WWTP

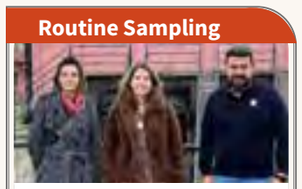
DUZCE WWTP

ESKISEHIR WWTP

KOCAELI WWTP

SAKARYA - KARAMAN WWTP

YALOVA WWTP


Routine Sampling

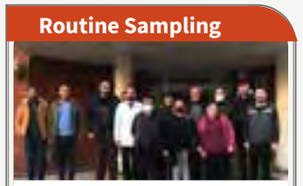
EDIRNE WWTP


BALIKESIR WWTP


CANAKKALE WWTP


KIRKLARELI WWTP


TEKIRDAG - CORLU, SARKOY, BATI WWTP


Routine Sampling

DIYARBAKIR WWTP


Routine Sampling

SANLIURFA WWTP


ADIYAMAN WWTP


BATMAN WWTP


GAZIANTEP WWTP

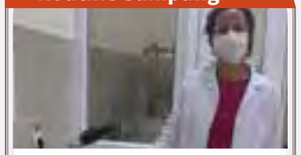
KILIS WWTP


MARDIN WWTP


SIIRT WWTP


SIRNAK WWTP

Routine Sampling



IZMIR DOGANBEY WWTP

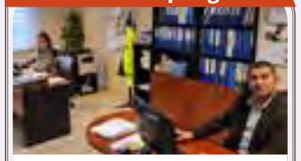
Routine Sampling

IZMIR CIGLI WWTP

Routine Sampling

MUGLA GUMBET WWTP

Routine Sampling



MUGLA FETHIYE WWTP

AFYONKARAHISAR WWTP

AYDIN KUSADASI WWTP

DENIZLI WWTP

KUTAHYA WWTP

MANISA MERKEZ WWTP

MANISA TURGUTLU WWTP

USAK WWTP

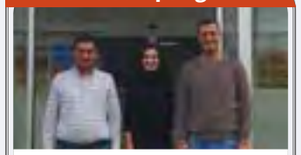
Routine Sampling

ADANA WWTP

Routine Sampling

ANTALYA HURMA - LARA WWTP

Routine Sampling



MERSIN WWTP

ADANA - YUREGIR WWTP

BURDUR WWTP

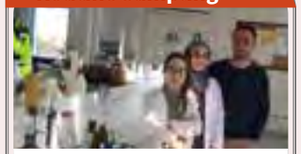
HATAY WWTP

ISPARTA WWTP

KAHRAMANMARAS WWTP

OSMANIYE WWTP

Routine Sampling



ANKARA TATLAR WWTP

Routine Sampling

KAYSERI WWTP

Routine Sampling

KONYA WWTP

AKSARAY WWTP

KARAMAN WWTP

KIRIKKALE WWTP

KIRSEHIR WWTP

NEVSEHIR WWTP

NIGDE WWTP

SIVAS WWTP

YOZGAT WWTP


Routine Sampling
ORDU DURUGOL WWTP


Routine Sampling
ORDU FATSA WWTP


Routine Sampling
SAMSUN WWTP


Routine Sampling
TRABZON WWTP


Routine Sampling
ZONGULDAK WWTP


AMASYA WWTP


ARTVIN WWTP


BARTIN WWTP


CANKIRI WWTP


CORUM WWTP


GIRESUN WWTP


GUMUSHANE WWTP

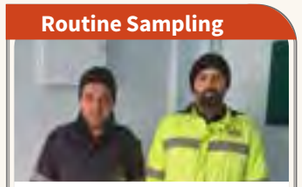

KARABUK WWTP


KASTAMONU WWTP


RIZE WWTP


SINOP WWTP


TOKAT WWTP


Routine Sampling

ELAZIG WWTP


Routine Sampling

ERZURUM WWTP


Routine Sampling

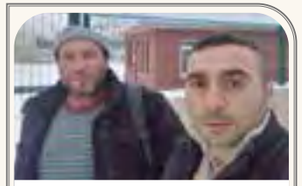
MALATYA WWTP


Routine Sampling

VAN WWTP

AGRI WWTP

ARDAHAN WWTP



BAYBURT WWTP

BINGOL WWTP

BITLIS WWTP

ERZINCAN WWTP

HAKKARI WWTP

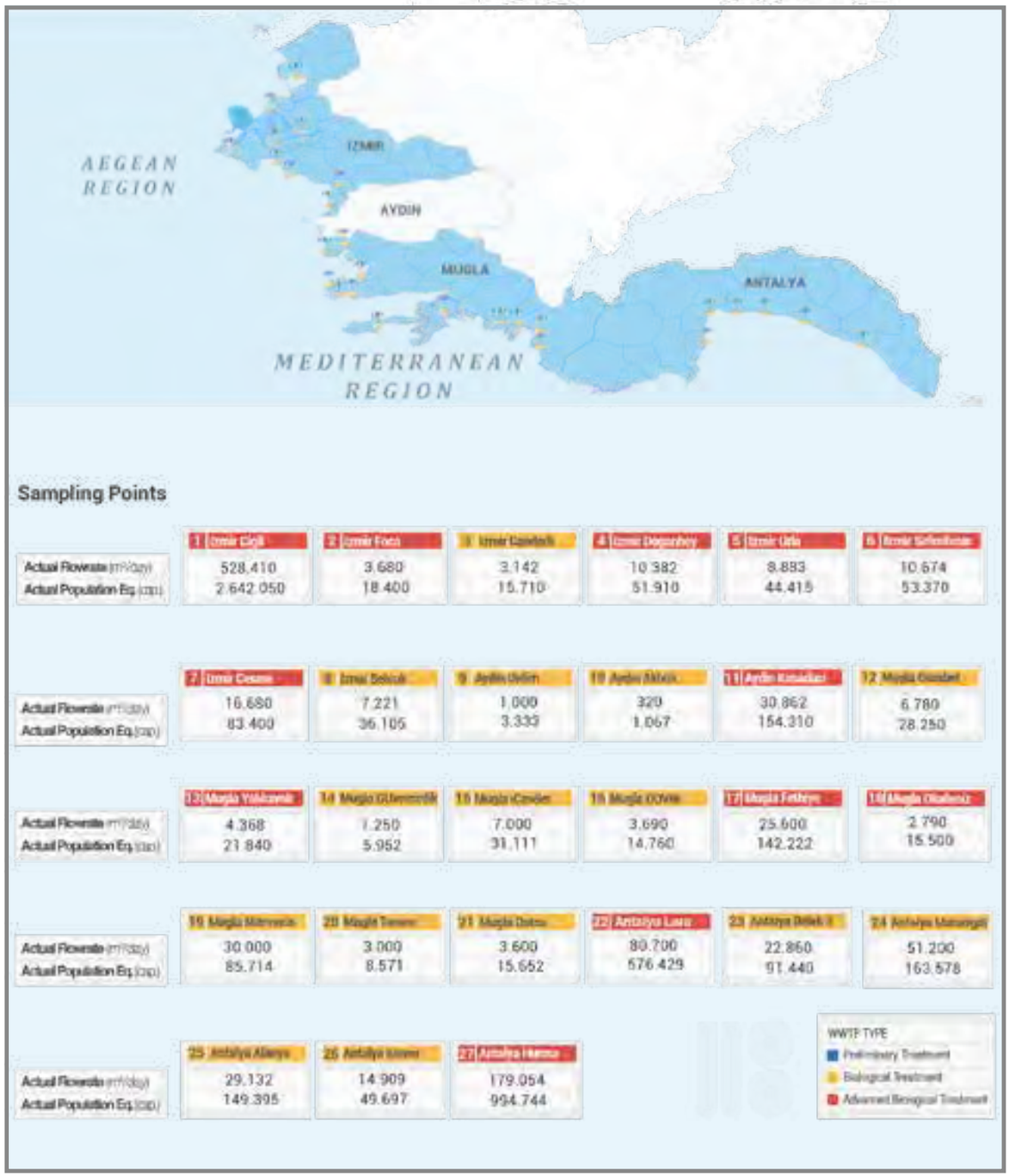
IGDIR WWTP

KARS WWTP

MUS WWTP

TUNCELI WWTP

ROUTINELY SAMPLED TREATMENT PLANTS ACROSS TOURISTIC DISTRICTS



	1 İzmir Çiğli	2 İzmir Foça	3 İzmir Gaziemir	4 İzmir Doğanbey	5 İzmir Urla	6 İzmir Seferihisar
Actual Flowrate (m³/day)	528.410	3.680	3.142	10.382	8.883	10.674
Actual Population Eq. (cap.)	2.642.050	18.400	15.710	51.910	44.415	53.370

	7 İzmir Çeşme	8 İzmir Selçuk	9 Aydın Didim	10 Aydın Akbük	11 Aydın Kuşadası	12 Muğla Güvercinlik
Actual Flowrate (m³/day)	16.680	7.221	1.000	320	30.862	6.780
Actual Population Eq. (cap.)	83.400	36.105	3.333	1.067	154.310	28.250

	13 Muğla Yalıkavak	14 Muğla Gümüşlük	15 Muğla Içmeler	16 Muğla Ölüdeniz	17 Muğla Fethiye	18 Muğla Göcek
Actual Flowrate (m³/day)	4.368	1.250	7.000	3.690	25.600	2.790
Actual Population Eq. (cap.)	21.840	5.952	31.111	14.760	142.222	15.500

	19 Muğla Marmaris	20 Muğla Turunç	21 Muğla Datça	22 Antalya Lara	23 Antalya Belek 2	24 Antalya Manavgat
Actual Flowrate (m³/day)	30.000	3.000	3.600	80.700	22.860	51.200
Actual Population Eq. (cap.)	85.714	8.571	15.652	576.429	91.440	163.578

	25 Antalya Alanya	26 Antalya Kemer	27 Antalya Hurma
Actual Flowrate (m³/day)	29.132	14.909	179.054
Actual Population Eq. (cap.)	149.395	49.697	994.744


IZMIR CIGLI WWTP


IZMIR FOCA WWTP


IZMIR CANDARLI WWTP


IZMIR DOGANBEY WWTP


IZMIR URLA WWTP


IZMIR SEFERHISAR WWTP


IZMIR CESME WWTP


IZMIR SELCUK WWTP


AYDIN DIDIM-AKBUK WWTP


AYDIN KUSADASI WWTP


MUGLA ATIKSU SUBE


MUGLA YALIKAVAK WWTP


MUGLA GUVERCINLIK WWTP


MUGLA ICMELER WWTP


MUGLA GOCEK WWTP


MUGLA GUMBET WWTP


MUGLA FETHIYE WWTP


MUGLA OLUDENIZ WWTP


MUGLA MARMARIS WWTP


MUGLA TURUNC WWTP


MUGLA DATCA WWTP


ANTALYA HURMA - LARA WWTP


ANTALYA ALANYA-MANAVGAT-KEMER-BELEK WWTP

5.3 Project Logistics

As part of the “Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)” project there are 4 basic steps applied in each sampling period:

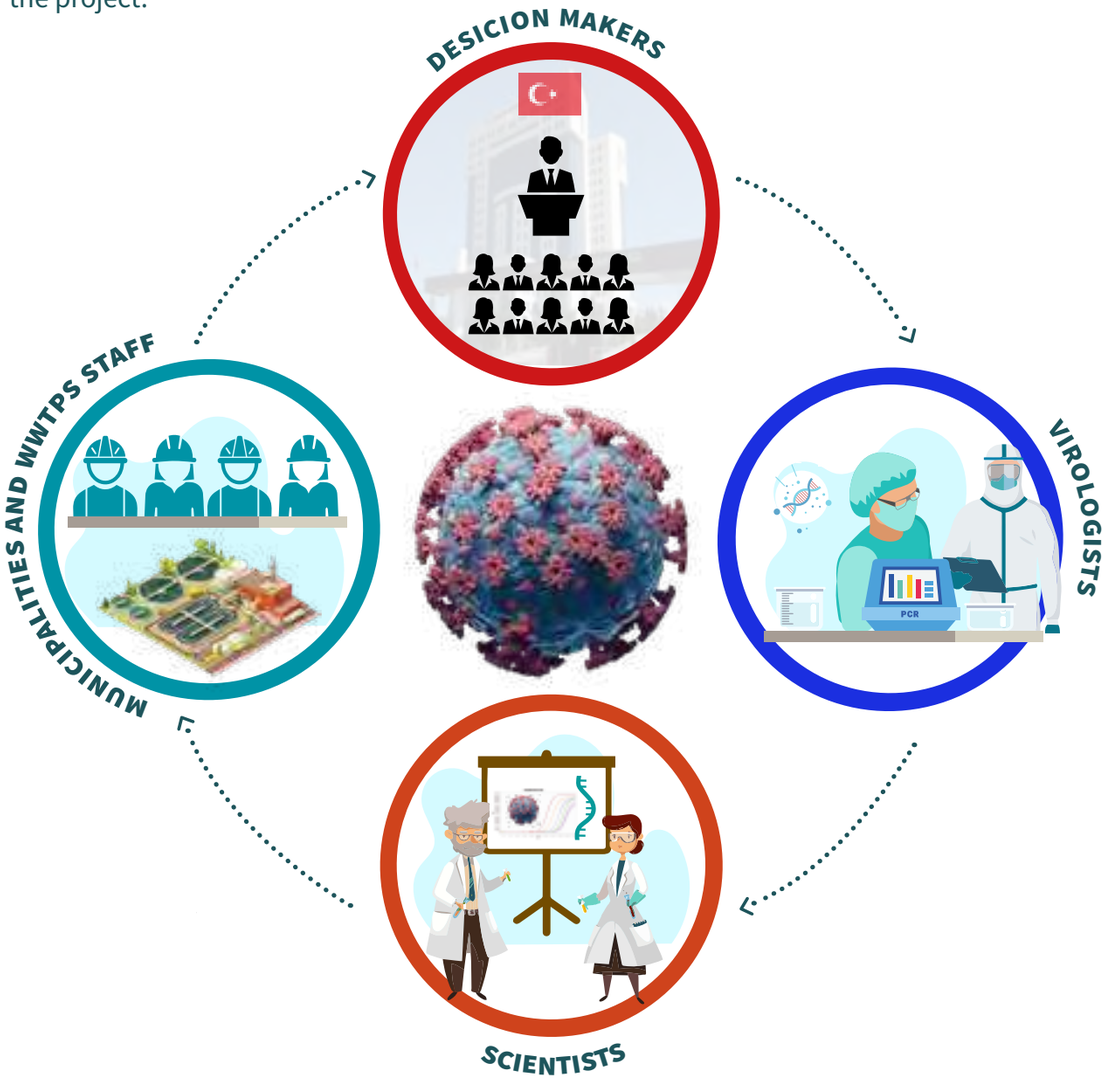
I. Planning of Sampling

II. Sampling and Transfer

III. Analysis and Interpretation of Results

IV. Reporting

In each of these steps, many units work together in a coordinated manner, and according to the experience gained during the course of the project, flow charts have been determined in these steps. The plan is to digitize the steps into workflow diagrams in the later stages of the project.



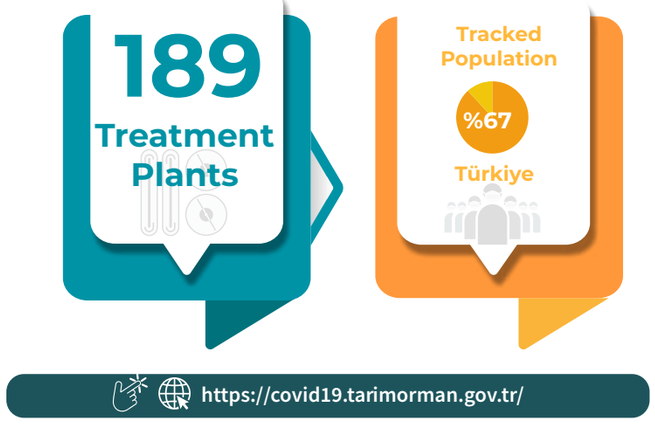
According to the flowcharts set out in logistics, the results of the analysis, generated from weekly samples collected every two weeks from all over Türkiye, are available at **https://covid19.tarimorman.gov.tr/** as Covid-19 Distribution Maps. In these maps, regional case spreads are illustrated with colors that vary from light green to dark green on 4 separate scales, namely low case, average case and high case while results below the detection limit of RT-qPCR equipment are shown in yellow to show low/no cases.

5.4 WBE Scanning Throughout Türkiye at the Beginning of the Pandemic

After the declaration of Covid-19 pandemic, the spread of the disease throughout the country in the period between April 2020 and May 2020 was followed by collecting samples from 81 cities. During this period 177 inlet, 167 effluent, 35 primary sludge and 122 active sludge samples were collected from 189 wastewater treatment plants (65 advanced biological treatment plants, 92 conventional activated sludge treatment plants, 22 pre-treatment plants) and 10 manholes in 81 cities and RT-qPCR measurements were performed in triplicate. These analyses enabled rapid screening of 67% (58 million) of Türkiye's total population at the beginning of the pandemic and identified 7 cities with high Covid-19 cases.

The results that are normalized by flow rates of the plants at the time of sampling and displayed on color scaled (from light green to dark green) distribution maps (Figure 15), are available at https://covid19.tarimorman.gov.tr/ under tabs Past Data, May-June 2020, Türkiye (81 cities).

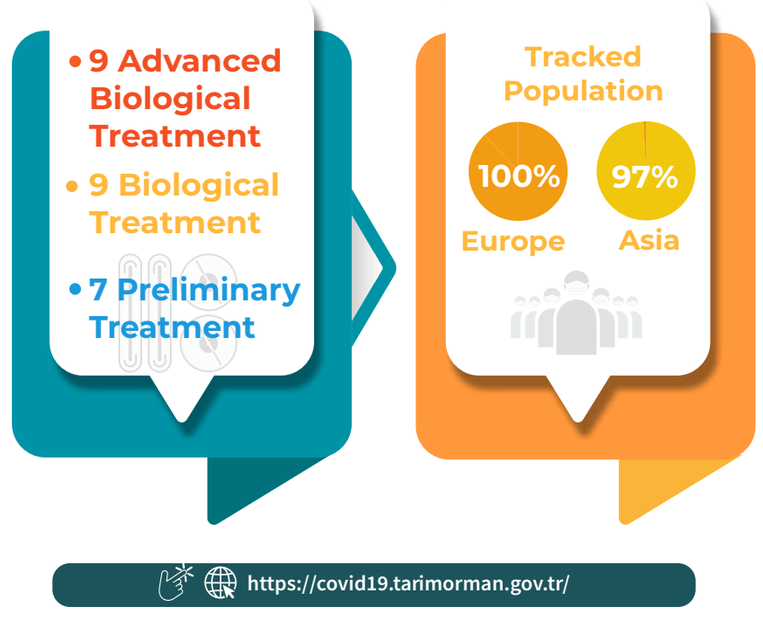
Figure 15. Covid-19 Distribution Maps Throughout Türkiye At the Beginning of Pandemic



5.5 WBE Scanning in Istanbul at the Beginning of the Pandemic

During the same period, 67 samples were collected from 25 treatment plants (9 advanced biological treatment plants, 9 conventional activated sludge treatment plants and 7 preliminary plants) in Istanbul, which is among the few megacities in the world with a population of more than 15 million, and 99% of the total population of Istanbul (15.3 million people) were scanned and the Covid-19 spread maps (Figure 16) were created.

Figure 16. Covid-19 Distribution Maps Across Istanbul At the Beginning of Pandemic

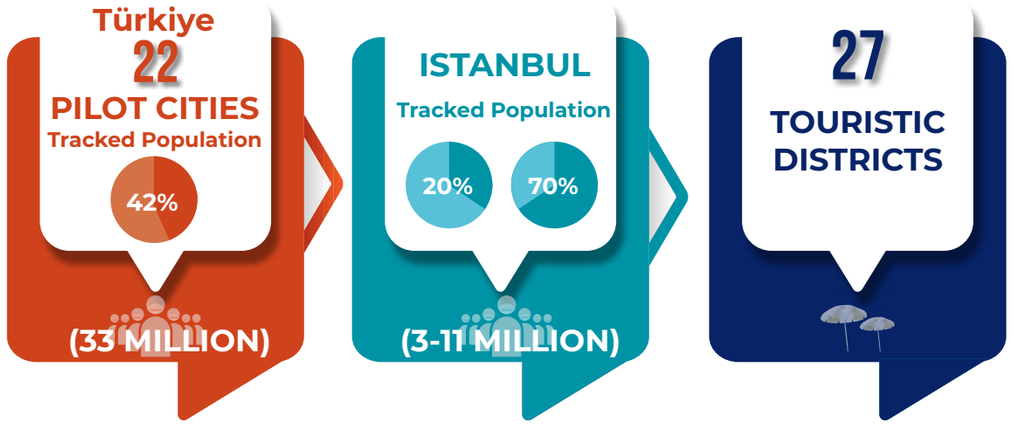


5.6 Routine WBE Monitoring of Türkiye and Istanbul

As part of the “Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)” project routine analyses are carried out at 15-day intervals from 21 pilot cities which are determined to represent all geographic regions across Türkiye and weekly in Istanbul since June 2020 (Figure 17).

With these analyses, **42%** (approximately 33 million people) of **Türkiye and 20-70%** of **Istanbul's population** (approximately 3-11 million people) are screened weekly for Covid-19 spread. Covid-19 distribution maps, prepared with high case, average case, low case and very low case color scales, are published simultaneously with measurements in Turkish and English at **https://covid19.tarimorman.gov.tr.** Distribution maps created starting from June 2020 for Türkiye and Istanbul are given in Appendix-1, respectively.

Türkiye is a country with a coastline on the Aegean and the Mediterranean Seas and has heavy tourist traffic during the summer months. During the Covid-19 pandemic, 27 touristic districts are routinely screened for Covid-19 spread at 15-day intervals between June and October. Color scaled distribution maps prepared for Türkiye and Istanbul are also prepared for these touristic districts and published instantaneously with measurements on **https://covid19.tarimorman.gov.tr** in Turkish and English.



The spread of mutant viruses throughout Türkiye is also being monitored through sequencing studies in wastewater. These analyses aim to track Variants of Concern (VOCs) and Variants of Interest (VOIs). However, while the difficulty of the wastewater matrix does not allow direct VOC and VOI detection in some cases, mutations observed in different gene regions of the SARS-CoV-2 genome can indirectly shed light on mutants present. As part of this project, the results of the sequence analysis carried out since April 2021 are published on **https://covid19.tarimorman.gov.tr.**

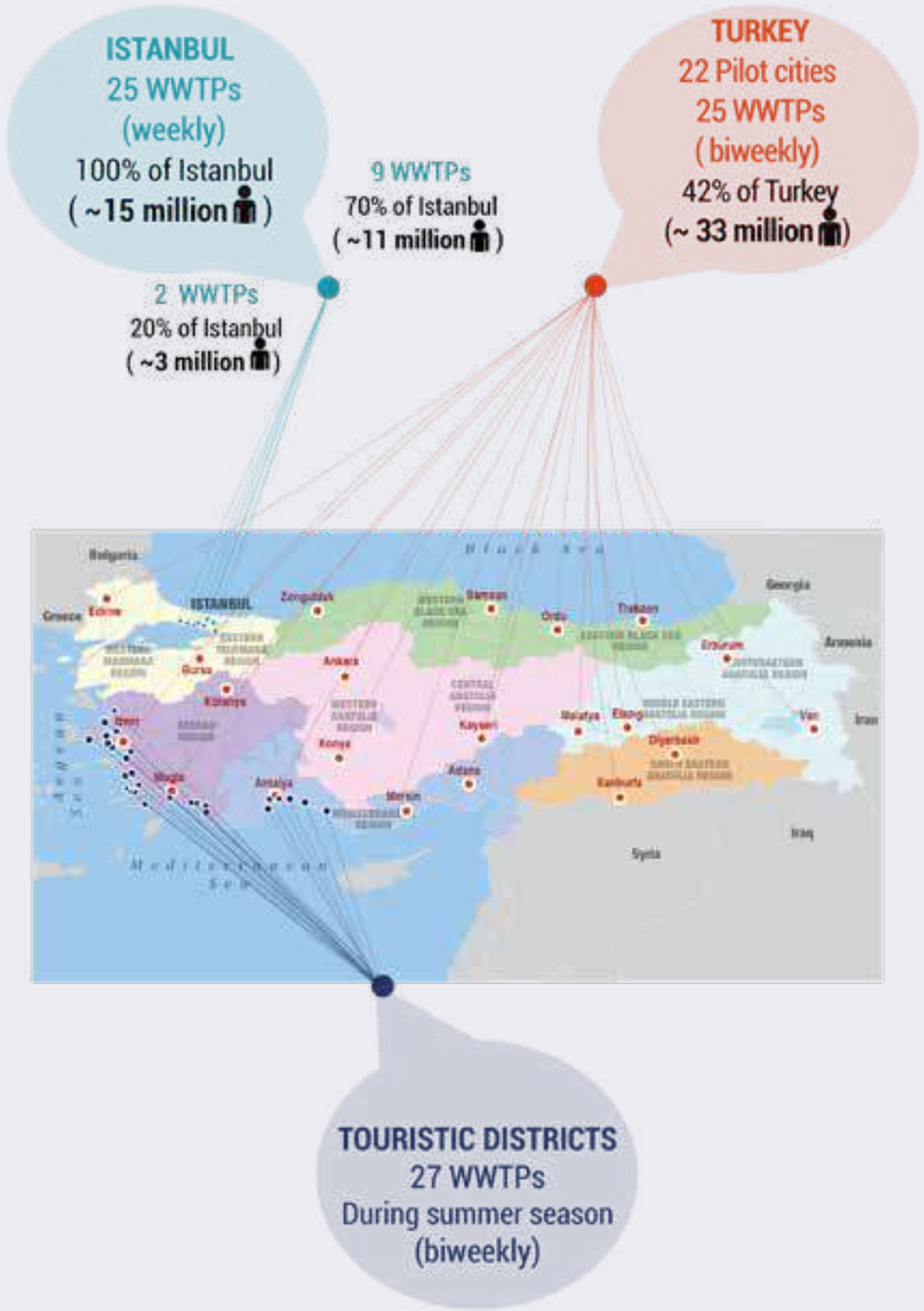


Figure 17. *Routine Sampling Points Throughout Türkiye and Istanbul*

06 | Dissemination of the "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" Project

6.1 Media: TV and Press

The fact that the history of wastewater epidemiology studies (Section 1.1) does not date back much leads to a lesser awareness of the usefulness of these studies among decision makers and society. These studies have gained momentum to be used by scientists around the world as an early warning system for outbreaks during the Covid-19 pandemic.

Wastewater based epidemiology studies have been proven to be an effective, fast and economical approach to pandemic management. However, the active use of this approach requires that decision-makers and society be well-informed on this topic.

Unlike many similar studies, the "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" project is commissioned by the Ministry of Agriculture and Forestry, hence, is well known and followed by decision makers.

Dissemination of the project findings to local governments, professional chambers, medical doctors (especially public health professionals) and society was implemented through television statements, press, participation in national and international webinars, publication of technical notes, chapters and pre-prints.

All project-related information and maps (dashboard) are published in Turkish and English on the Ministry of Agriculture and Forestry website.

https://covid19.tarimorman.gov.tr/

***Figure 18.** Examples from TV Statements related to ""Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)"*

***Figure 19.** Examples from Press Releases related to ‘‘Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)"*

6.2 Webinars

As part of the "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" project, Turkish Water Institute (SUEN) participated in 6 national webinars (Figure 20), 28 international webinars/meetings (Figure 21), 4 international conferences (Figure 22) as invited speaker to share and disseminate project findings and to create collaborations between different disciplines.

Turkish Water Institute also has organized 1 international and 2 national webinars as part of this project (Figure 23).

Several participants from 7 different continents and 24 different countries attended the webinar titled 'Lessons Learned from SARS-CoV-2 in Wastewater: Surveillance, Genomics, Early Warning' held by SUEN on August 24, 2020.

On January 19, 2021, the entire project team participated in the national webinar (Figure 25) titled 'Monitoring Spread of Covid-19 through Routine SARS-CoV-2 Analysis in Wastewaters across Türkiye', with high participation from the sanitation sector and academia, particularly municipal sanitation workers.

The 'Monitoring the Spread of Covid-19 Across Türkiye, Routine Follow-up, Early Warning, Mutant Detection, Dashboard Applications' national webinar held on June 11, 2021 targeted dissemination of the project findings to environmental engineering students and academics.

Date	Organization	Topic	Speaker
06.08.2020	Chamber of Environmental Engineers, UCTEA, Türkiye	Covid-19 in Turkish Wastewaters	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
10.27.2020	Bursa Governorship Bursa Province Water Management Coordination Board	Bursa Provincial Water Management Coordination Board of Directors 2020 Meeting - Scanning of SARS-CoV-2 in wastewater and sludges of Türkiye	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
12.12.2020	Chamber of Environmental Engineers, UCTEA, Türkiye	Routine Monitoring of Covid-19 Spread across Türkiye through SARS-CoV-2 Analysis in Wastewater	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
01.27.2021	Public Health Professionals Association, (HASUDER)	Covid-19 Scanning in Türkiye through SARS-CoV-2 Monitoring in Wastewater	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
04.07.2021	Public Health General Directorate	SUEN Activities and Covid-19 Monitoring in Wastewater	Prof. Ahmet Mete SAATCI
06.28.2021	Bursa Governorship Bursa Province Water Management Coordination Board	Bursa Provincial Water Management Coordination Board of Directors 2020 Meeting_ Monitoring the Spread of Covid-19 with SARS-CoV-2 Screening in Wastewaters of Türkiye Project Developments: Routine Follow-ups, Early Warning, Mutant Detection, Dashboard	Assoc. Prof. Bilge ALPASLAN KOCAMEMI

***Figure 20.** National webinars attended as part of the "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" project*

Date	Organization	Topic	Speaker
04.27.2020	Union for the Mediterranean IME/UfMs 2nd Brainstorming session on Water, Sanitation and COVID-19", Spain	Brainstorming session on Water, Sanitation and COVID-19	Prof. Ahmet Mete SAATCI
06.04.2020	76th Scientific Community Meeting European Environment Agency of European Union	SARS- CoV-2 Detection in wastewater	Prof. Ahmet Mete SAATCI
06.08.2020	"1st Town Hall Meeting" ENV, Water Europe and EurEau	SARS- CoV-2 monitoring employing sewers a first gathering to share knowledge	Dr. Halil KURT
06.17.2020	Word Water Council, France	SARS- CoV-2 Detection of Wastewater and Sludges in Türkiye	Prof. Ahmet Mete SAATCI
06.17.2020	"SARS- CoV-2 in Sanitation and wastewater Sludge" Portuguese Association of Water and Wastewater services (APDA), Portugal	SARS- CoV-2 Surveillance Study for wastewater and Sludges in all of the 81 cities in Türkiye	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
07.22.2020	"2nd Town Hall Meeting /UN – WWQA Part 2 Meeting" EU Umbrella Study @ UN World Water Quality Alliance	SARS- CoV-2 Surveillance Study for wastewater and Sludges in all of the 81 cities in Türkiye	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
07.22.2020	"TAIEX online Workshop on Wastewater use in sanitation Systems" Technical Assistance and Information Exchange (TAIEX), Türkiye	SARS- CoV-2 Surveillance Study for wastewater and Sludges in all of the 81 cities in Türkiye	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
07.23.2020	"COVID-19 Implication on Water Management in Megacities" UNESCO	COVID-19 Surveillance Study for Wastewater and sludges in Istanbul	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
07.23.2020	"COVID-19 Implication on Water Management in Megacities" UNESCO	COVID-19 Surveillance Study for Wastewater and sludges in Istanbul	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
08.19.2020	"WRC/SALGA Programme on water Quality, Sanitation and Health in Light of COVID-19" South African Local Government Association (Salga), Water Research Commission (WRC)	SARS- CoV-2 Surveillance Study for wastewater and Sludges in all of the 81 cities in Türkiye	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
08.23.2020	G20	SARS- CoV-2 Surveillance Study for wastewater and Sludges in all of the 81 cities in Türkiye	Burcu CALLI

***Figure 21. (cont.)** International webinars attended as part of the "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" project*

Date	Organization	Topic	Speaker
09.11.2020	"8th series mini-conference WWN SARS/CoV- 2 in wastewater: State of the knowledge and research communication" American Society of Civil Engineers (ASCE) Women-Water Nexus	SARS- CoV-2 Surveillance Study for Wastewater and sludges in Türkiye	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
09.12.2020	Innovation Pathway 2020 Águas do Tejo Atlântico, Portugal	SARS- CoV-2 Surveillance Study for wastewater and Sludges in Türkiye	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
09.30.2020	"Online workshop COVID-19 research and Innovation programmes and Projects" Water Research Commission of South Africa and Indian Ocean Rim Association (IORA)	SARS- CoV-2 Surveillance Study for wastewater and Sludges inTürkiye	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
09.30.2020	Wastewater and COVID-19: Getting the Facts Right" The United Nations Environment Programme (UNEP)	SARS- CoV-2 Surveillance Study for Wastewater and sludges in Istanbul	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
10.29.2020	Impact and implications of COVID-19 in the WASH sector Islamic Development Bank (IsDB)	Logistics, Methods, Findings and Challenges of SARS-CoV-2 Surveilance Study for wastewater and Sludges in Türkiye	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
12.02.2020	SARS-CoV-2 Monitoring employing Sewers From an EU Umbrella to Sentinel System	Routine wastewater surveillance in Türkiye to Control Covid-19 outbreak	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
12.08.2020	The Sixteenth Meeting of The High-level Experts and Leaders Panel on Water and Disasters (HELP)	Wastewater surveillance as pandemic disaster management tool	Prof. Ahmet Mete SAATCI
12.10.2020	Science Forum South Africa 2020 Igniting conversations about science Water Research Commission of South Africa and Indian Ocean Rim Association (IORA)	Routine wastewater surveillance in Türkiye to Control Covid-19 outbreak	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
03.22.2021	"4th Town Hall Meeting SARS CoV-2 Monitoring System Designing the EU Sewer Sentinel System for SARS-CoV-2 (EUS4)	The Turkish Dashboard for SARS-CoV-2 Sewer Surveillance: Mega City Istanbul and 21 pilot cities	Assoc. Prof. Bilge ALPASLAN KOCAMEMI

***Figure 21 (cont.)** International webinars attended as part of the "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" project*

Date	Organization	Topic	Speaker
04.01.2021	World Water Council Governors Meeting	Wastewater and Covid-19 Work Group	Prof. Ahmet Mete SAATCI
05.25.2021	Watershare 3rd COVID Sewage Surveillance Strategic Meeting Perspectives and Updates from across the Globe	Tools to Translate Systematic Nationwide Wastewater-Based Surveillance Data to Authorities and Public	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
07.07.2021	"5th Town Hall Meeting Sewage Sentinel System for SARS CoV-2 (EU4S)	Current state-of-play in Nationwide Wastewater-Based COVID-19. Surveillance of Türkiye: City-based live distribution maps, Variants and Real-time government dashboard for public	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
11.03.2021	Wastewater Surveillance for SARS-CoV-2:Fall RCN Meeting National Science Foundation (NSF) NSF Research Coordination Network Fall Meeting	Nationwide Wastewater-Based Epidemiology in Türkiye: Routine Monitoring of Covid-19	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
11.23.2021	"6th Town Hall Meeting The EU Sewage Sentinel System for SARS-CoV-2 (EU4S) From an idea to an institutional instrument	Update from the Türkiye nationwide wastewater-based Covid-19 Surveillance	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
11.24.2021	XXIV Simpósio Brasileiro De Recursos Hídricos (SBRH) "Sewage monitoring as a tool for population health surveillance: experiences and lessons learned in the world with COVID-19" ABRHidro and the World Water Council	Nationwide wastewater-based surveillance of Covid-19 outbreak in Türkiye	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
11.26.2021	FCIC INTERNATIONAL ENGINEERING FORUM "Effects of Climate Change and Covid 19 on Consulting Services And FCIC Updated Strategy" Dış Ekonomik İlişkiler Kurulu (DEİK)	Nationwide SARS-CoV-2 Surveillance Study of Türkiye for Monitoring of Covid-19 Spread	Assoc. Prof. Bilge ALPASLAN KOCAMEMI

***Figure 21 (cont.)** International webinars attended as part of the "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" project*

Date	Organization	Topic	Speaker
04.17.2021	III. International City, Environment and Health Congress Aydin Adnan Menderes University, Medical School, Department of Public Health	Routine Monitoring of Covid-19 in Türkiye through SARS-CoV-2 Analysis in Wastewaters	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
09.05.2021	4th IWA Resource Recovery Conference – IWARR2021 Türkiye (On-line)	Nationwide SARS-CoV-2 Surveillance Study of Türkiye for Monitoring of Covid-19 Spread	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
11.05.2021	V International Symposium Current Challenges in Sustainability University of Stuttgart	Wastewater-Based Surveillance of Covid-19 Outbreak in Türkiye: Regional SARS-CoV-2 Distribution maps, Variants Tracking, Dashboards	Assoc. Prof. Bilge ALPASLAN KOCAMEMI
12.04.2021	ENVIRONMENTAL CHANGE AND WASTEWATER EPIDEMIOLOGY SURVEILLANCE DURING COVID-19 Capital Medical University	Nationwide Wastewater-based Surveillance of COVID-19 in Türkiye: Regional Distribution Maps, Early Warning, Variants, Dashboards	Assoc. Prof. Bilge ALPASLAN KOCAMEMI

***Figure 22.** International conferences attended as part of the "Nationwide SARS-CoV-2 Surveillance Study in the Wastewaters of Türkiye" project*

Date	Organization	Topic	Speaker
08.27.2020	Turkish Water Institute (SUEN)	Lessons Learned from SARS-CoV-2 in Wastewater: Surveillance, Genomics, Early Warming	Prof. Ahmet Mete SAATCI Assoc. Prof. Bilge ALPASLAN KOCAMEMI Prof. Dr. Kartik Chandran Jay Bhagwan
01.19.2021	Turkish Water Institute (SUEN) Marmara University, Türkiye	Routine Monitoring of Covid-19 in Türkiye through SARS-CoV-2 Analysis in Wastewaters	Akif ÖZKALDI Prof. Ahmet Mete SAATCI Assoc. Prof. Bilge ALPASLAN KOCAMEMI DG Harun SEÇKİN Deputy DG Murat DAĞDEVİREN
06.11.2021	Marmara University, Environmental Engineering Department Seminar	Monitoring of Covid-19 Spread across Türkiye via Wastewater Based Epidemiology Studies	Prof. Ahmet Mete SAATCI Assoc. Prof. Bilge ALPASLAN KOCAMEMI

***Figure 23.** National and International webinars attended as part of the "Nationwide SARS-CoV-2 Surveillance Study in the Wastewaters of Türkiye" project*

***Figure 24.** Pictures from the "Lessons Learned from SARS-CoV-2 in Wastewater: Surveillance, Genomics, Early Warning" international webinar*

***Figure 25.** Pictures from "Routine Monitoring of Covid-19 Spread in Wastewater throughout Türkiye with SARS-CoV-2 Analyses" organized by SUEN*

***Figure 26.** Pictures from the national webinar "Wastewater Epidemiology Studies and Covid-19 Spread Monitoring Studies throughout Türkiye" organized by SUEN*

6.3 Publications

Findings and achievements of the "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" project were published by the Turkish Water Institute (SUEN) as 1 SCI indexed journal, 4 pre-prints, 1 international book chapter, 2 international conference abstracts, 2 national and 1 international technical journal articles and 2 corporate handbooks including this booklet both in English and Turkish.

Books can be accessed through link

07 | World Water Council (WWC) Covid-19 Task Force

The World Water Council (WWC) is an international, multi-stakeholder organization that works to raise awareness and make policy commitments at all levels of society, including senior decision-makers on water issues critical to effective protection, development, planning, management and use of water resources on an environmentally sustainable basis for the benefit of all life on earth. The World Water Council organizes a "World Water Forum" in a different country every three years. The forum is the largest water organization in the world and the fifth of the forums was held in Istanbul in 2009 with the participation of over 30,000 local and foreign experts.

The Turkish Water Institute (SUEN) has been designated by the World Water Council (WWC) to establish a new task force named as "Covid-19 and Resilence" in addition to the 4 main Task Forces (Water Safety, Water Finance, Global Changes, Integrated Water Resources Management) with its ongoing work starting in February 2021. SUEN has included the European Commission Joint Research Centre (EC JRC), the Water Research Center Netherlands (KWR), and the South African Water Research Commission (WRC), as its main partners, who are expert groups on Covid-19 monitoring in wastewater (Figure 27). World Bank, SuSanA and Watershare will also support the events organized by SUEN as co-convening partners in many aspects including dissemination of session outcomes.

The 9th World Water Forum will be held on 21 - 26 March 2022 in Dakar, Senegal, and the main theme of the forum is "Water Security for Peace and Development". The Covid-19 task force, of which SUEN is the chair, will hold 2 sessions as part of the forum.

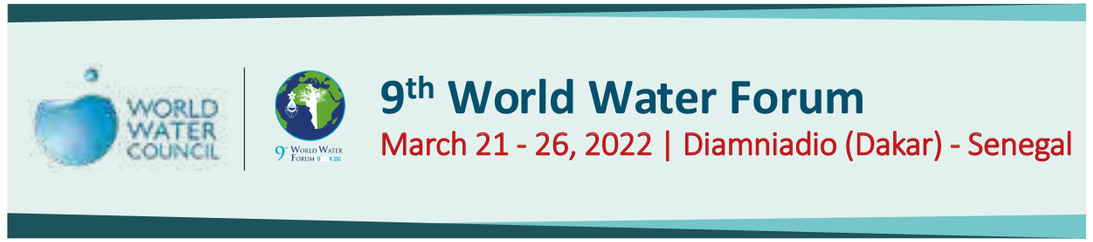


Figure 27. *9th World Water Forum Covid-19 Task Force Activities*

These sessions aim to promote the use of wastewater-based epidemiology studies in monitoring Covid-19 spread and to enhance global cooperation in the field of Water, Sanitation, Hygiene (WASH) during the Covid-19 pandemic.

In each session, speakers from different continents of the world will share their knowledge on WBE and WASH issues, and aim to shed light on decision-making authorities so that these studies can be used as a means of protecting public health in line with the 'One Health' approach now and in the future.

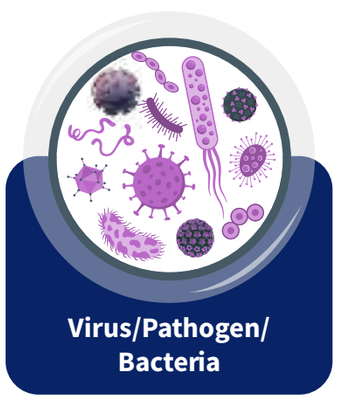
08 | Recommendations for Sustainable Use of WBE Studies in the Post-Covid-19 Period

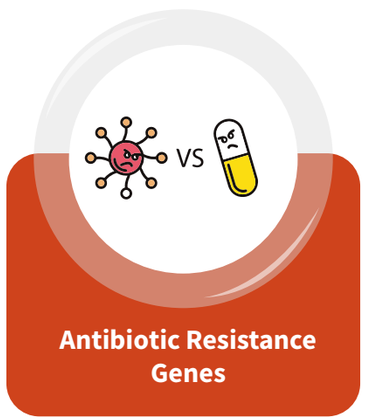
The experiences gained from the WBE studies accomplished during the course of Covid-19 will ensure preparedness against possible future pandemics. In addition, these studies have the potential to be used in tracking viruses and pathogens, as well as and antibiotic resistant genes present in wastewater. This potential will enable wider use of treated wastewater in agricultural irrigation without endangering public health.

WBE studies are expected to reveal advances in biosensing technologies that will enable real-time, on-site measurements of virus, pathogen and antibiotic resistance genes in the near future.

With the Covid-19 pandemic, the “One Health” approach, which aims to develop global health security, has become even more relevant worldwide by mapping human, animal and environmental health at national and global levels.

Within this approach, food safety, sustainable agriculture, zoonoses and antimicrobial resistance are the main goals and WBE studies in wastewater will be an valuable tool to achieve all these goals.





App 1 | Outcomes of "Nationwide Tracking of Covid-19 Spread by Wastewater Based Epidemiology (WBE)" Project

Appendix 1.1 Outcomes of Türkiye's 21 Pilot Cities

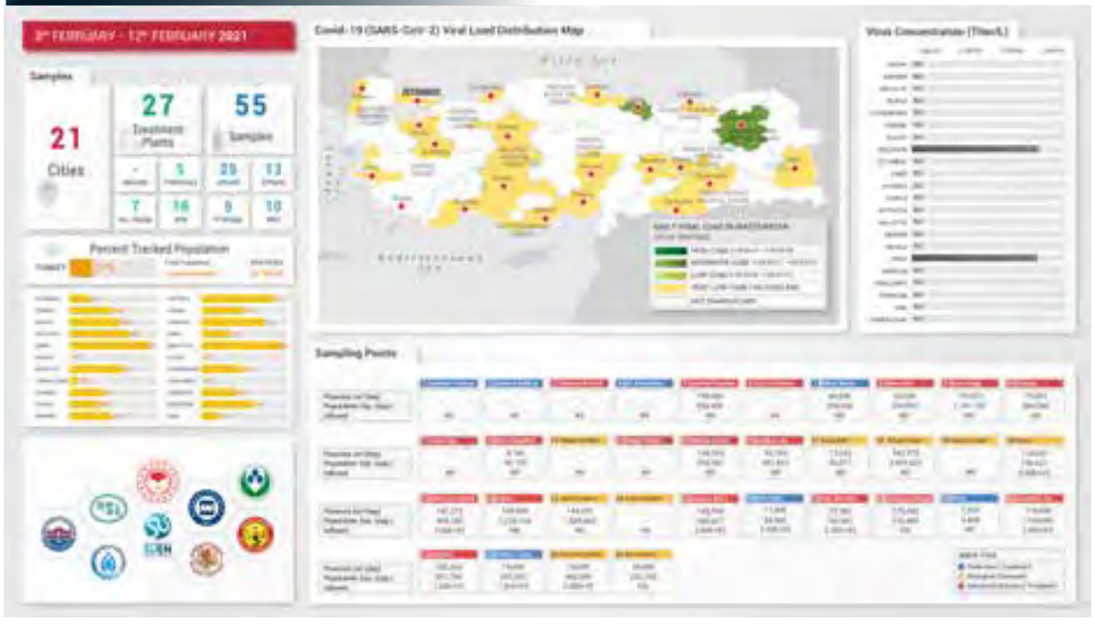
15th SEPTEMBER - 15th NOVEMBER 2020

15th NOVEMBER - 15th DECEMBER 2020

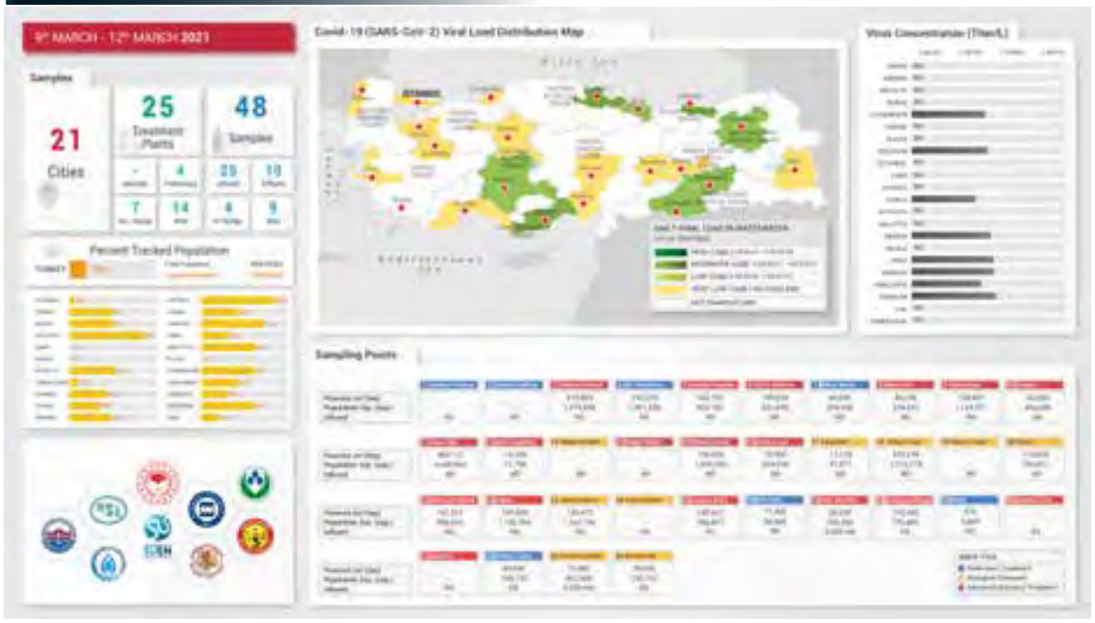
15th DECEMBER - 1st JANUARY- 2021

1st JANUARY - 8th JANUARY- 2021

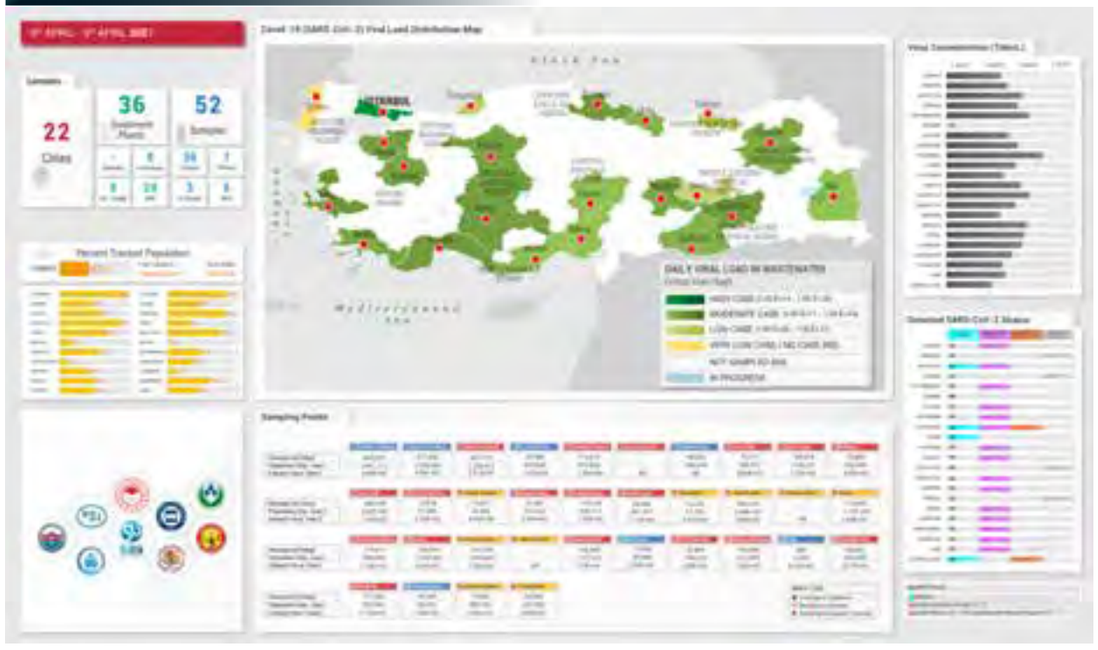
8th FEBRUARY - 12th FEBRUARY 2021



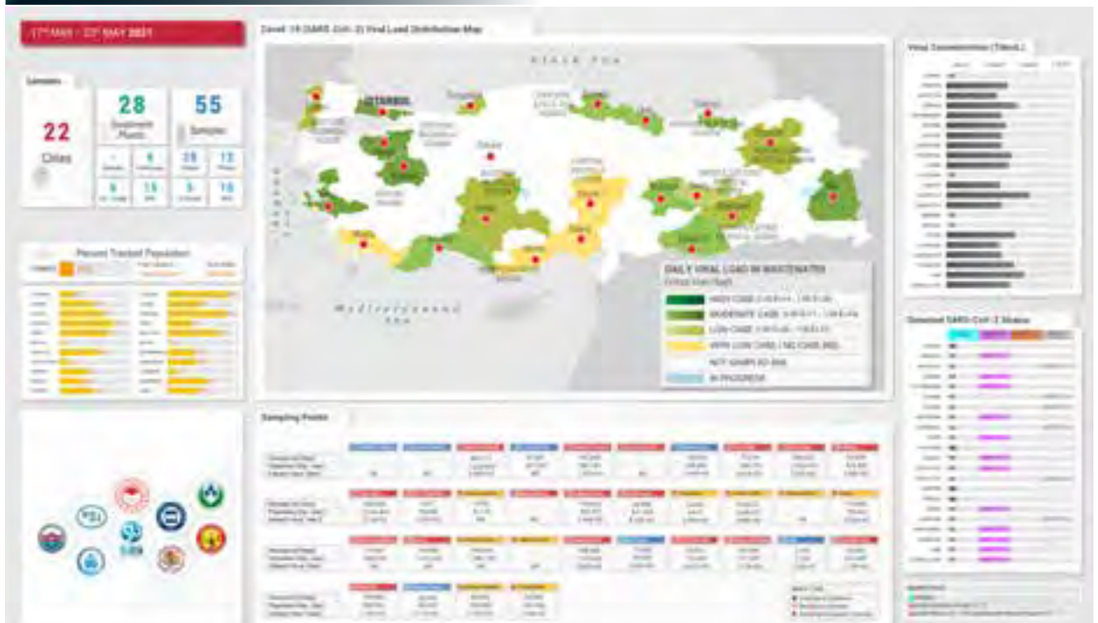
9th MARCH - 12th MARCH 2021



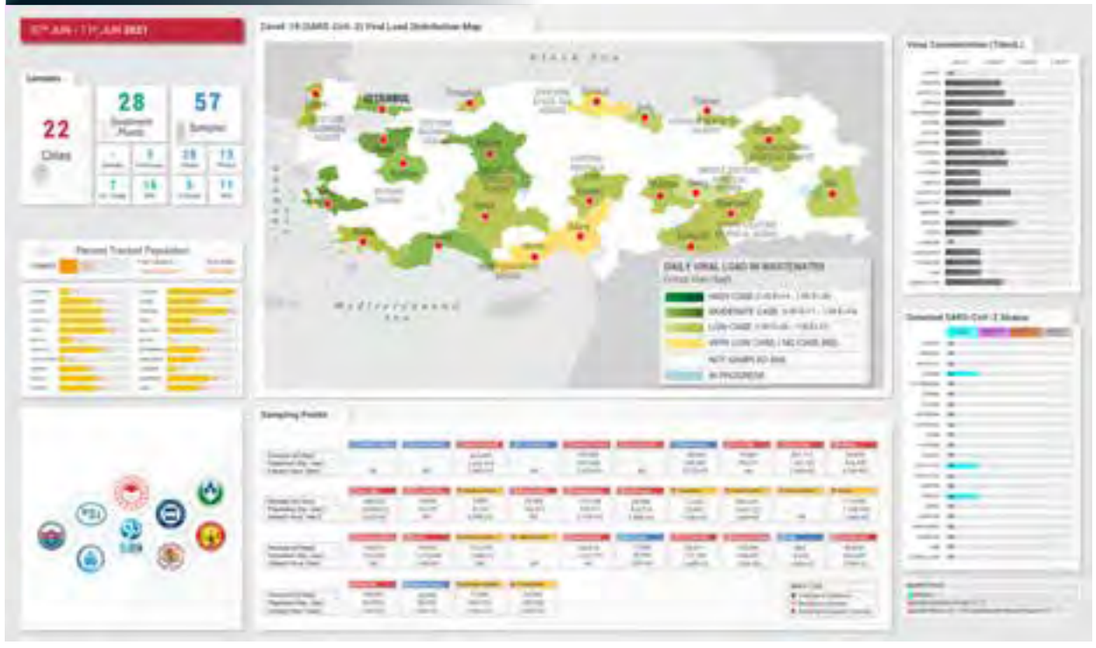
5th APRIL - 9th APRIL 2021



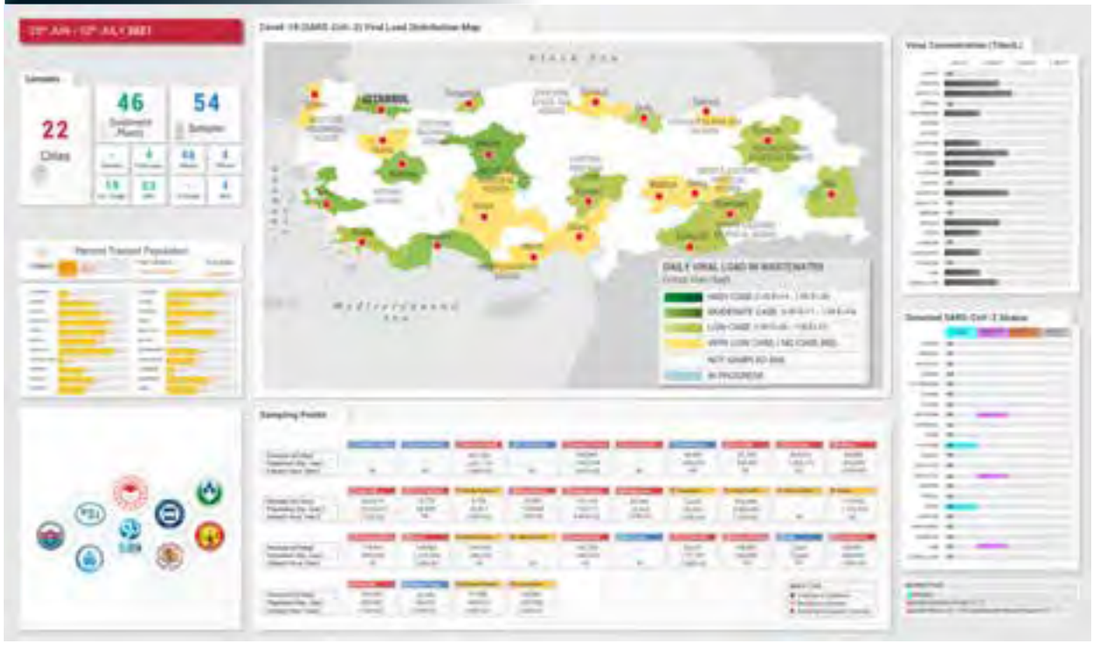
1th MAY - 23th MAY 2021



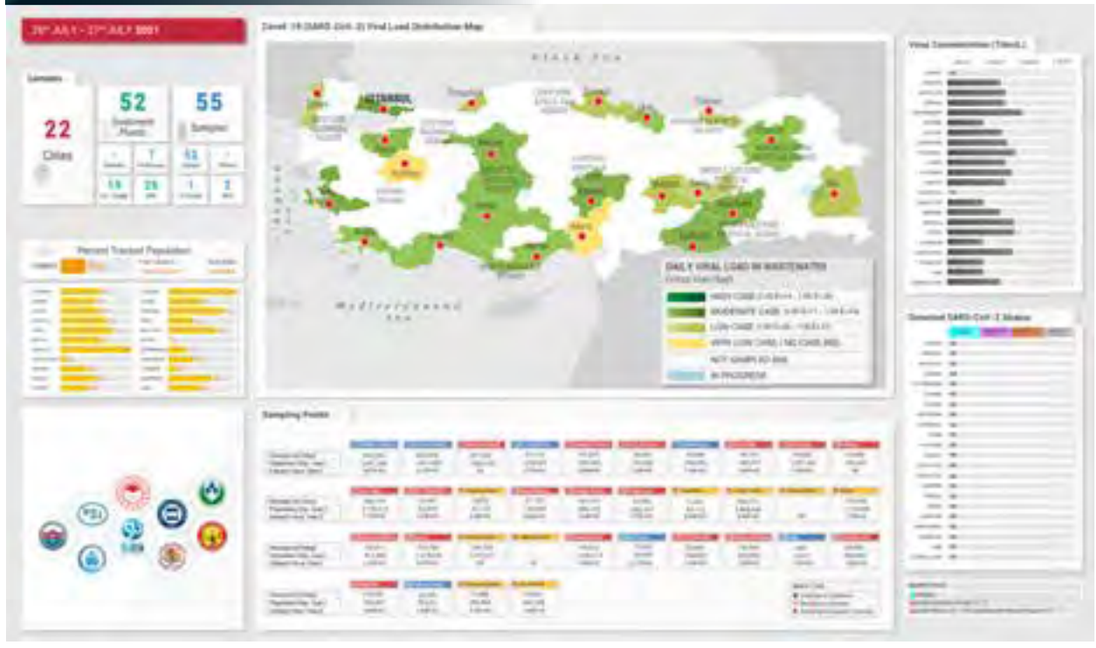
7th JUN - 11th JUN 2021



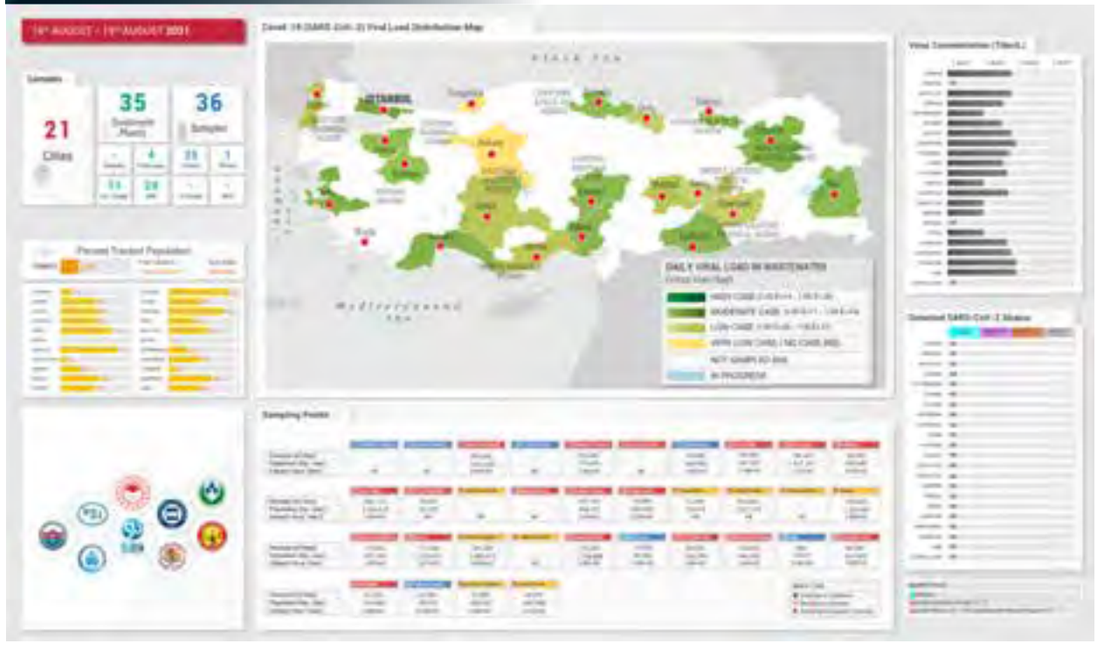
28th JUN - 02th JULY 2021



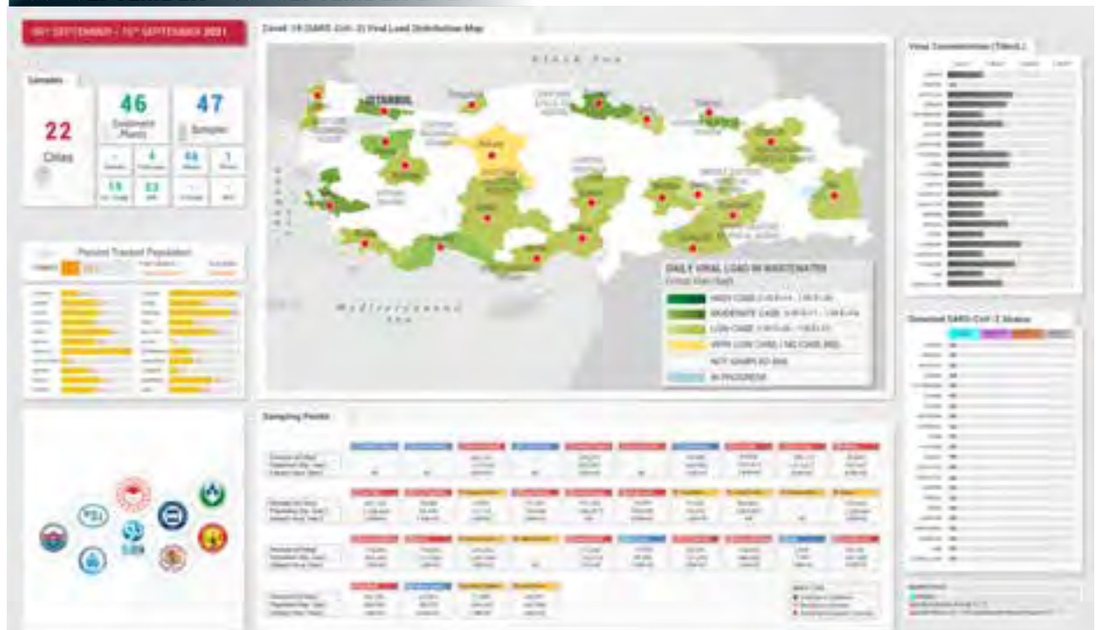
26th JULY - 27th JULY 2021



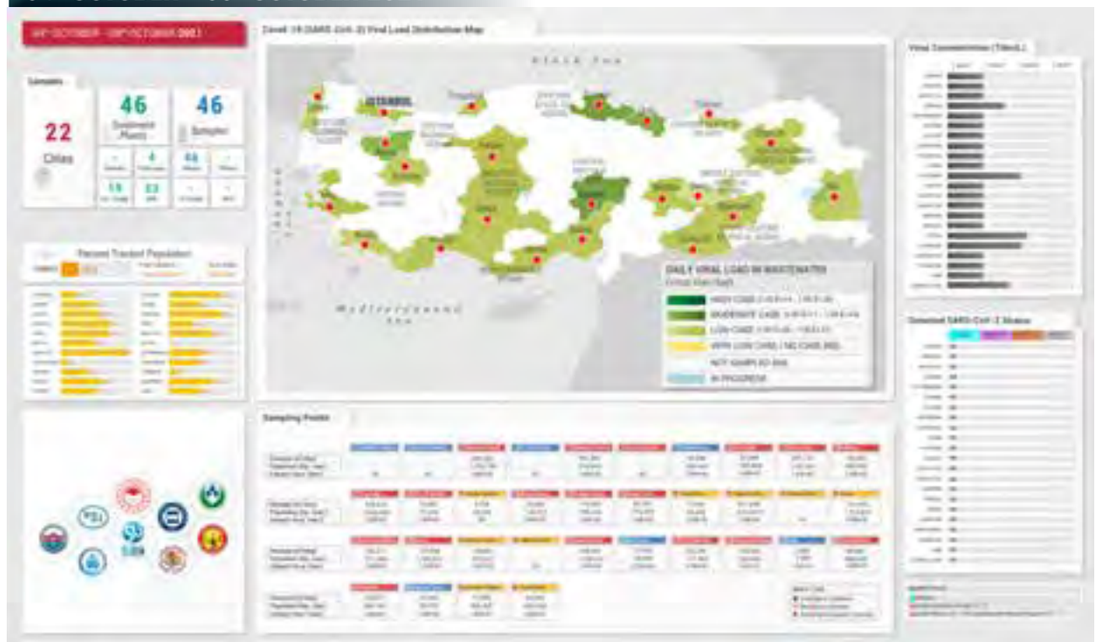
16th AUGUST - 19th AUGUST 2021



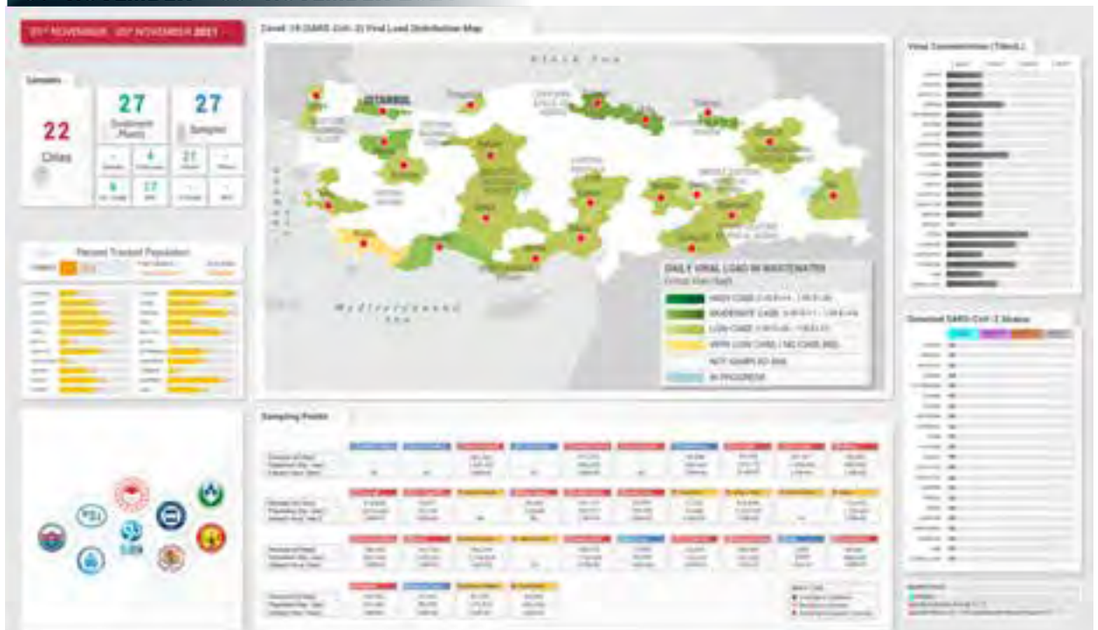
06th SEPTEMBER - 10th SEPTEMBER 2021



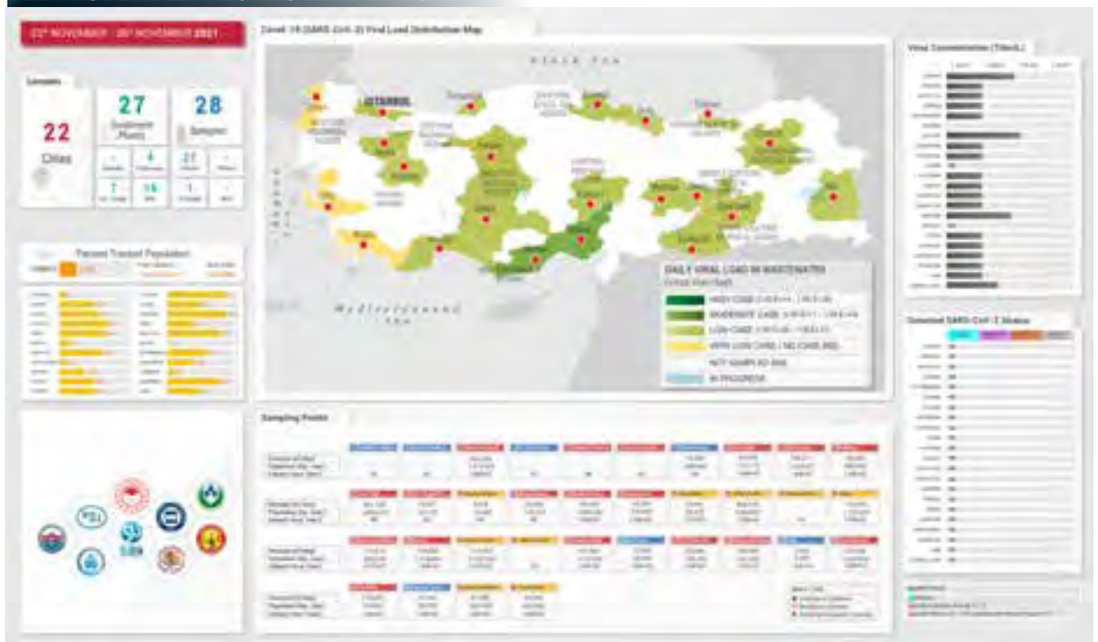
04th OCTOBER - 08th OCTOBER 2021



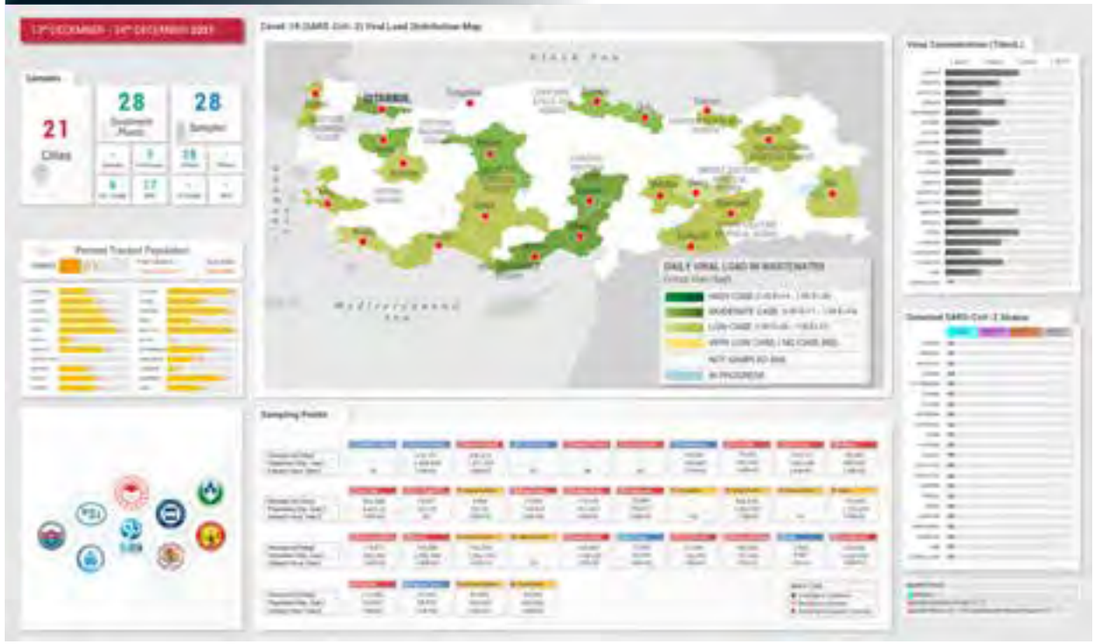
01th NOVEMBER - 05th NOVEMBER 2021



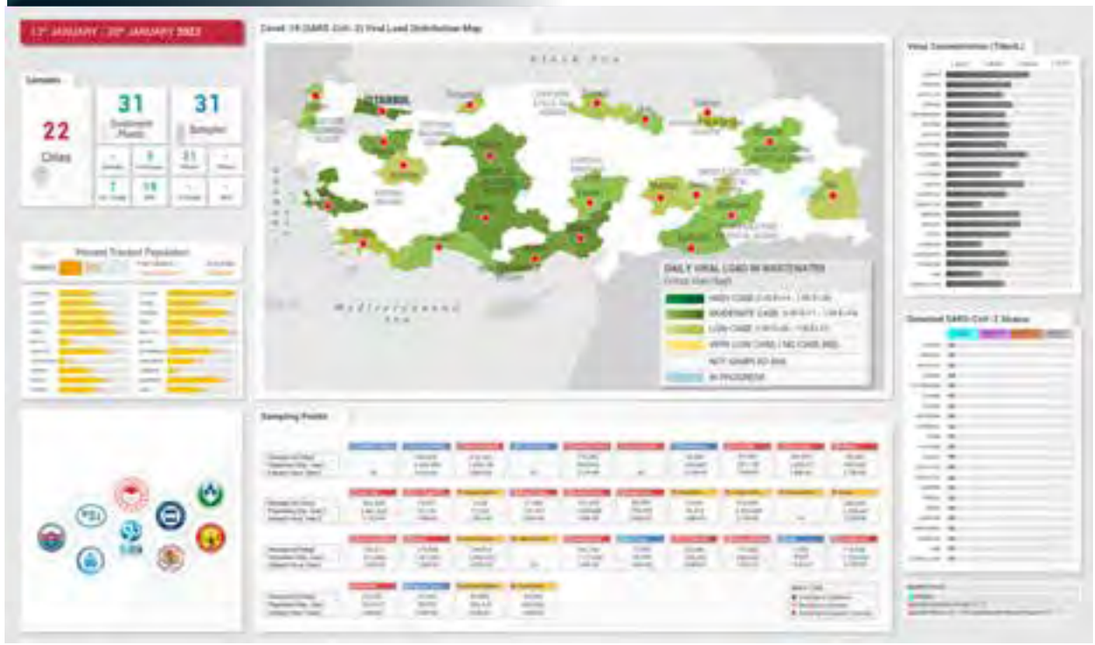
22th NOVEMBER - 26th NOVEMBER 2021



13th DECEMBER - 24th DECEMBER 2021



12th JANUARY - 20th JANUARY 2022



Appendix 1.2 Outcomes of City of Istanbul

JUNE 2020 - WEEK 1

JUNE 2020 - WEEK 2

JUNE 2020 - WEEK 3

JUNE 2020 - WEEK 4

JULY 2020 - WEEK 1

JULY 2020 - WEEK 2

JULY 2020 - WEEK 3

JULY 2020 - WEEK 4

AUGUST 2020 - WEEK 4

SEPTEMBER 2020 - WEEK 1

SEPTEMBER 2020 - WEEK 3

SEPTEMBER 2020 - WEEK 4

SEPTEMBER 2020 - WEEK 5

OCTOBER 2020 - WEEK 1

OCTOBER 2020 - WEEK 3

OCTOBER 2020 - WEEK 4

OCTOBER 2020 - WEEK 5

NOVEMBER 2020 - WEEK 1

NOVEMBER 2020 - WEEK 2

NOVEMBER 2020 - WEEK 3

NOVEMBER 2020 - WEEK 4

DECEMBER 2020 - WEEK 1

DECEMBER 2020 - WEEK 2

DECEMBER 2020 - WEEK 3

DECEMBER 2020 - WEEK 4

DECEMBER 2020 - WEEK 5

JANUARY 2021 - WEEK 1

JANUARY 2021 - WEEK 4

JANUARY 2021 - WEEK 5

FEBRUARY 2021 - WEEK 1

FEBRUARY 2021 - WEEK 2

FEBRUARY 2021 - WEEK 4

MARCH 2021 - WEEK 1

MARCH 2021 - WEEK 2

MARCH 2021 - WEEK 3

MARCH 2021 - WEEK 4

APRIL 2021 WEEK 1

APRIL 2021 WEEK 2

APRIL 2021 WEEK 3

APRIL 2021 WEEK 4

APRIL 2021 WEEK 5

MAY 2021 WEEK 1

MAY 2021 WEEK 2

MAY 2021 WEEK 3

MAY 2021 WEEK 4

JUN 2021 WEEK 1

JUN 2021 WEEK 2

JUN 2021 WEEK 4

JULY 2021 WEEK 1

JULY 2021 WEEK 2

JULY 2021 WEEK 3

JULY 2021 WEEK 5

AUGUST 2021 WEEK 1

AUGUST 2021 WEEK 2

AUGUST 2021 WEEK 3

AUGUST 2021 WEEK 4

SEPTEMBER 2021 WEEK 1

SEPTEMBER 2021 WEEK 2

SEPTEMBER 2021 WEEK 3

SEPTEMBER 2021 WEEK 5

OCTOBER 2021 WEEK 1

OCTOBER 2021 WEEK 2

OCTOBER 2021 WEEK 3

OCTOBER 2021 WEEK 4

NOVEMBER 2021 WEEK 1

NOVEMBER 2021 WEEK 2

NOVEMBER 2021 WEEK 3

NOVEMBER 2021 WEEK 4

NOVEMBER 2021 WEEK 5

DECEMBER 2021 WEEK 1

DECEMBER 2021 WEEK 3

DECEMBER 2021 WEEK 4

JANUARY 2022 WEEK 1

JANUARY 2022 WEEK 3

Appendix 1.3 Outcomes of Touristic Destinations

JUNE 2021

JULY 2021

AUGUST 2021

SEPTEMBER 2021

OCTOBER 2021

Appendix 1.4 Genome Sequence Monitoring

NİSAN - KASIM 2021

TURKISH WATER INSTITUTE
SUEN
TÜRKİYE SU ENSTİTÜSÜ

9 786258 451399