Sustainable Water Resources Management (2023) 9:21
https://doi.org/10.1007/s40899-022-00809-8

ORIGINAL ARTICLE

Investigation of SARS-CoV-2 RNA contamination in water supply resources of Tabriz metropolitan during a peak of COVID-19 pandemic

Hamed Sabzchi-Dehkharghani[1] · Hossein Samadi Kafil[2] · Abolfazl Majnooni-Heris[1] · Abbas Akbarzadeh[3] · Rana Naderi-Ahranjani[4] · Ahmad Fakherifard[1] · Mohammad Mosaferi[5] · Neda Gilani[6] · Mojtaba Noury[7] · Parisa Eydi[4] · Sajjad Sayyari Sis[4] · Nasimolzahra Toghyanian[8] · Reza Yegani[4]

Received: 17 June 2022 / Accepted: 7 December 2022 / Published online: 17 December 2022

Abstract

It is crucial to have access to clean water resources during the COVID-19 pandemic for hygiene, since virus infection through wastewater leaks in metropolitan areas can be a threat. Accurate monitoring of urban water resources during the pandemic seems to be the only way to confirm safe and infected resources. Here, in this study, the amount of Severe Acute Respiratory Syndrome Coronavirus 2's Ribonucleic Acid (SARS-CoV-2 RNA) in the Tabriz urban water network located in the northwest of Iran was investigated by an extensive sampling of the city's water sources at a severe peak of the COVID-19 pandemic. The sampling process comprised a range of water sources, including wells, qanats, water treatment facilities, dams, and reservoirs. For each sample, a combination of polyethylene glycol (PEG) and sodium chloride (NaCl) was used for concentration and a laboratory RNA-based method was conducted for quantification. Before applying the extraction and quantification procedure to real samples, the proposed concentration method was verified with synthetic serum samples for the first time. After the concentration, RNA extraction was done by the BehPrep extraction column method, and Reverse Transcription Polymerase Chain Reaction (RT-PCR) detection of the virus was done by Covitech COVID-19 RT-PCR kit. In none of the water supply resources, SARS-COV-2 RNA has been detected except in a sample grabbed from a well adjacent to an urban wastewater discharge point downstream. The results of molecular analysis for the positive sample showed that the CT value and concentration of the virus genome were equal to 32.57 and 5720 copies/L, respectively. Quantitative analysis of real samples shows that the city's water network was safe at the time of the study. However, given that the positive sample was exposed to wastewater leakage, periodic sampling from wells and qanats is suggested during the pandemic until it can be proven that the leakage to these water sources is impossible.

Keywords SARS-CoV-2 RNA · COVID-19 pandemic · Virus concentration · Virus quantification · Water resources · Tabriz

✉ Reza Yegani
Ryegani@sut.ac.ir

[1] Department of Water Engineering, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

[2] Drug Applied Research Center, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

[3] Water Research Institute, Tehran, Iran

[4] Membrane Technology Research Center, Faculty of Chemical Engineering, Sahand University of Technology, PO. BOX 51335/1996, Tabriz, Iran

[5] Health and Environment Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[6] Department of Statistics and Epidemiology, Faculty of Health, Tabriz University of Medical Sciences, Tabriz, Iran

[7] Iranian Water Resources Management Company, Tehran, Iran

[8] Parks and Green Space Organization of Tabriz Municipality, Tabriz, Iran

Introduction

The COVID-19 pandemic is an ongoing global pandemic of coronavirus disease caused by severe respiratory syndrome coronavirus 2 (SARS-CoV-2) (Najafi et al. 2022; Khodadadi et al. 2020). Sustainable water supply plays an important role in ensuring public health, during the COVID-19 pandemic where there is an increased water demand for hygiene (Kalbusch et al. 2020). The worst-case scenario may happen when the water itself becomes the source of infection. This scenario is thought to occur if the COVID-19 patients' excreta enters drinking water resources due to the sewage network malfunction or lack of a waste management system. Also, if rainwater helps the viruses absorbed in household waste to enter the runoff (Wang et al. 2020a, 2021; La Rosa et al. 2020). Viral contamination of surface water, as well as drainage channels, can lead to the sources of contamination of groundwater sources, especially wells that are the source of drinking water supply in cities (Salem et al. 2021). On the other side, the SARS-CoV-2 virus has been reported to persist in aqueous media from a few hours to a few weeks, though their viability and infectivity strongly depend on several factors (Buonerba et al. 2021). Considering the different characteristics of the water resources, intermittent sampling seems to be the only way to answer the upcoming important question about the persistence of the SARS-CoV-2 virus in these hydrological assets.

SARS-CoV-2 is a member of the Coronaviridae family, which comprises enveloped and single-stranded ribonucleic acid (RNA) viruses with sizes ranging from 60 to 220 nm (Corpuz et al. 2020). RNA molecules or their fragments can be detected from damaged, and thus inactive, viral particles, although it does not necessarily imply infectivity of that specimen. Analytical kits developed for the rapid assay based on Reverse Transcription Polymerase Chain Reaction (RT-PCR) are also available for SARS-CoV-1, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and the novel SARS-CoV-2 virus (Buonerba et al. 2021). The infectivity of a virus can be determined by plaque assay and cell cultures process (Baer and Kehn-Hall 2014) which is specifically described for the SARS-CoV-2 virus in the references (Harcourt et al. 2020; Hoehl et al. 2020). The SARS-CoV-2 RNA has been detected worldwide in wastewater but with no viable virions indicating very limited stability of this virus in aquatic medium, while proximal viruses to SARS-CoV-2 showed stability within 2 and 14 days in wastewater (Buonerba et al. 2021). Unconcentrated viruses in wastewater and surface water results below the detection limit of the analyzing methods have been strongly recommended by researchers as a mandatory step to detect viruses in aqueous media (La Rosa et al. 2020). Despite a variable number of viral particles that can be lost by applying each method, concentrated methods, such as centrifugation/ultracentrifugation, virus adsorption-elution (VIRADEL), membrane (electropositive or electronegative) filtration, centrifugal ultrafiltration, or precipitation with suitable coagulating agents, have been also recommended (Buonerba et al. 2021). Due to uncertainties in terms of the virus's existence in aqueous media, preliminary precautions have been recommended with chlorine disinfection (Wang et al. 2020b). Nevertheless, there is no extensive literature on the effectiveness of water treatment processes that ensure the correct elimination of SARS-CoV-2 (García-Ávila et al. 2020). Not only the amount of Chlorine content in water but also variations in other characteristics such as temperature, salinity, and pH are important factors modulating viral presence in a strain-dependent manner (Labadie et al. 2018).

Changes in human water consumption can affect the quality of freshwater resources (Rezaei et al 2019 and 2020). A group of COVID-19-related studies in the water sector found the footprint of the virus in the wastewater resources using the direct measurement of the virus content in the samples (Farkas et al. 2021; Haramoto et al. 2020; Hasan et al. 2021; Mlejnkova et al. 2020; Panchal et al. 2021; Perez-Cataluña et al. 2021). A study in Palestine alarmed for the risk of contamination in urban water supplies—caused by leakage from existing cesspits (Anayah et al. 2021). In Iran, research reported that the pandemic has increased the pressure on the already strained water resources in a studied megacity (Feizizadeh et al. 2021). In Brazil, it was proved that the effect of the COVID-19 pandemic and the subsequential lockdown commands on the water consumption trend is not the same for all types of uses, so it was decreasing for commercial, industrial, and public categories while increasing for residential category (Kalbusch et al. 2020). Although the issue is still unknown in terms of the virus concentration, but positive evidence was found showing an unintended while the promising trend for the restoration of other water quality indicators in India until now (Chakraborty et al. 2021). Additionally, some studies discovered the association of the hydrometeorological parameters with the infection rate (Wang et al. 2021; Pal and Masum 2021; Sobral et al. 2020), while others suggested the consideration of the pandemic in climate change (Armitage and Nellums 2020) and water scarcity (Boretti 2020) issues.

Due to the lack of laboratory data from earlier studies demonstrating the presence of SARS-CoV-2 RNA in a city's water resources, the aim of this study was to look for the sampled resources contaminated by SARS-CoV-2 RNA. The sampling process comprised a range of water sources, including wells, qanats, water treatment facilities, dams, and reservoirs to conduct a thorough assessment of the infection situation in the urban water network. Samples were taken

during a pandemic's peak and were analyzed using an RNA-based concentrating and precipitating method. Among RNA-based methods, the concentration approach tries to quantify the viral RNA through RT-PCR and Quantitative Reverse Transcription PCR (RT-qPCR) methods, while sequencing methods study different strains of the virus (Bofill-Mas et al. 2020; Feng et al. 2020). Despite the development of non-RNA-based methods due to the need for significant levels of amplification in the PCR-based methods (Barceló 2020), RNA-based methods are still used widely for identifying SARS-CoV-2 in water media. The proposed concentration approach in this study was initially validated using several synthetic samples before implementing viral extraction and quantification on real water samples. The proposed sampling and their concentration steps can be used for conducting other similar research and to complement the technical knowledge in the field of urban health management during pandemics.

Materials and methods

Study area

Tabriz is the fourth largest city in Iran having almost 2 million population and covering an area of about 245 square kilometers with industrial and ancient cultural characteristics located at 1321 m elevation above sea level. The city has a semi-arid climate prone to water scarcity crises induced by climate change, agricultural and industrial activities, and population growth. The city has experienced several waves of the COVID-19 pandemic since early 2020 and home quarantine began in Tabriz at that time causing a significant increase in domestic water consumption and changes in water consumption patterns (Feizizadeh et al. 2021). The portion of water allocated to sanitation and disinfection was more than it used to be consumed before, while it has always been a question if the water used for cleaning is the source of the infection itself. The annual production of water by the Water and Wastewater Company of East Azerbaijan Province (WWC-EAP) is 136 million cubic meters. Tabriz drinking water is supplied from four types of resources including a river, wells (wells distributed in the northern slopes of Sahand Mountain and east of Tabriz), a dam, and qanats with a share of 55, 33, 12, and 1%, respectively (See Table 1). Drilling new wells, restoration of abandoned, wells, and increasing the discharge of dedicated water from the Nahand Dam are medium-term solutions to solve the problem of drinking water shortage in Tabriz (WWC-EAP: water and wastewater company of East Azerbaijan Province, 2021). Figure 1 shows the outline of the Tabriz urban water and wastewater network.

Sampling and laboratory measures

Since the PEG concentration and deposition method have been widely used in RNA experiments of SARS-CoV-2 in wastewater and other environmental samples such as water sources, in this study, the same approach was taken for quantitative analysis of samples from the city's conventional water resources. Here, the viral concentration was carried out using the polyethylene glycol (PEG) precipitation followed by RNA extraction and detection by real-time-qPCR to target the E and S gene segments. Figures 2 and 3 show the sequence of steps taken for this study in phases one and two of the project.

Phase one: accuracy assessment of concentration methods using synthetic samples

Concentration method using PEG and NaCl

A brief description of the steps taken for concentrating the samples is as follows: 100 ml of physiological serum is poured into 250 ml of sterile laboratory bottles. Four concentrations (3 ± 5, 30 ± 10, 300 ± 100, and 3000 ± 1000 copies/milliliters) of viral culture medium are injected into the laboratory bottles. The resulting solution is stirred at 4 °C at 100 revolutions per minute (rpm) for 10–30 min to homogenize the density of the virus throughout the volume of the solution. pH is adjusted to 7–7.5 for the solution using 5 Molarity (M) hydrogen chloride (HCl) and 0.5 M sodium hydroxide (NaOH) if necessary. In a ratio of 1: 3 of the solution, 40% polyethylene glycol (PEG) 8000 and 8% sodium chloride (NaCl), were added to serum samples and poured into 50 ml centrifuge tubes, and gently shaken to

Table 1 The share of water resources in Tabriz drinking water supply (WWC-EAP: water and wastewater company of East Azerbaijan Province, 2021)

No	Resource	Share	% from the total volume
1	Zarrineh River	About 2500 L per second (75 million cubic meters per year)	55%
2	Wells	About 1500 L per second (47 million cubic meters per year)	32%
3	Nahand Dam	About 550 L per second (5.17 million cubic meters per year)	12%
4	Qanats	–	1%

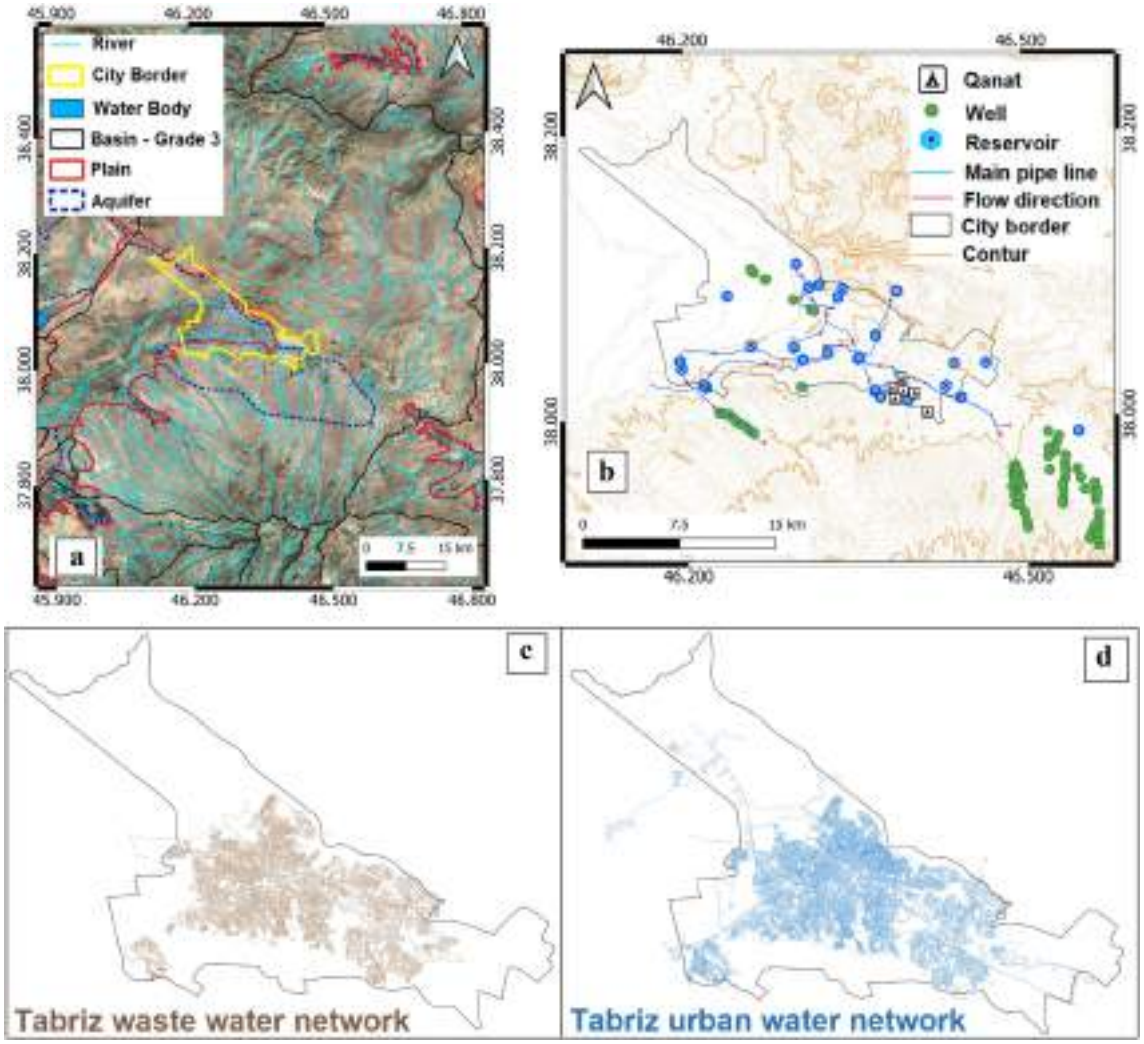

Fig. 1 Study area: **a** Geographical location of Tabriz city, **b** Tabriz water resources, **c** Tabriz wastewater network, and **d** Tabriz urban water network

mix. Tubes containing 20 ml of the mixture are incubated at 4 °C for 24 h. The centrifuge used has a fixed angle rotor with 6 positions, with an acceleration of 9 and a break speed of 6, which is set to centrifuge the samples under a force of 12,000 g for 30 min at a temperature of 4 °C. After centrifugation, the supernatant in each tube was slowly overflowed and the resulting pellet was resuspended with 0.5 ml of saline phosphate buffer (PBS), and finally, 1.5 ml of the eluted pellet was transferred for RT-PCR analysis or stored (at 4 °C for 3 days, − 20 °C for 1 week or -80 °C for a long time) until the process of molecular analysis is conducted. Since the plan was to quantify RNA in three repetitions, three samples of each concentration were prepared for the next step.

Extraction of RNA

The RNA extraction method was performed using a demonstrated protocol (Fathizadeh et al. 2020) and BehPrep viral RNA extraction kit according to the manufacturer's instructions. Briefly, 200 µL of the concentrated sample along with 300 µl (µL) of the lysis buffer containing carrier RNA was added to the tube and then incubated for 10 min at room temperature. 250 µL of ethanol was added to the sample, and after 15 s of vortex, the whole sample was centrifuged at 8000 rpm for 1 min. 500 µL of washer buffer was added to the column and was centrifuged at 8000 rpm for 1 min at room temperature. This step was repeated twice. The column was centrifuged at

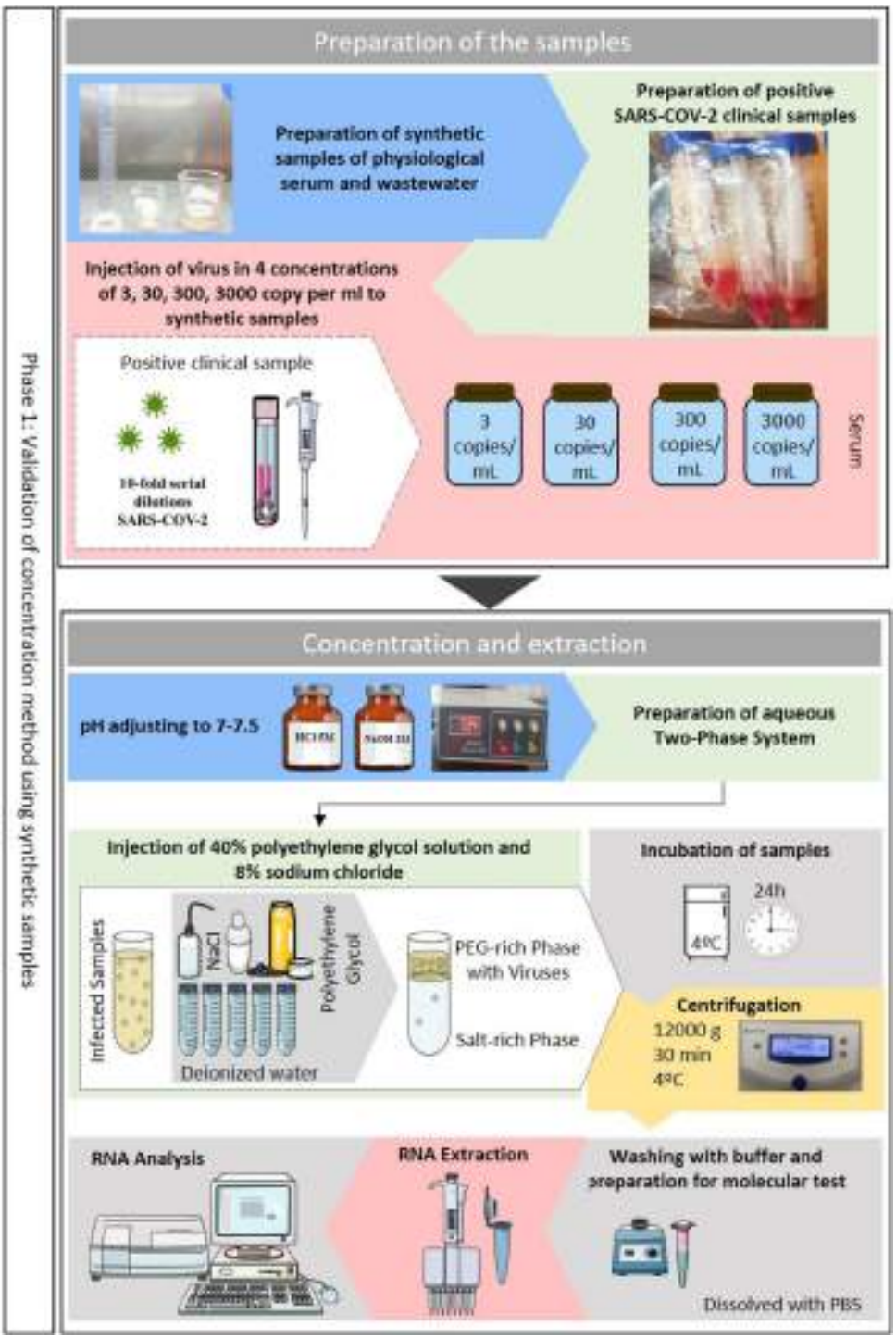

Fig. 2 Flowchart of phase one: analysis of synthetic samples

13,500 rpm for 3 min to dry. Then, 100 µL of elution buffer was added to the membrane. After incubation at room temperature, centrifuged at 13,500 rpm for 1 min, and then, the extracted RNA was stored for further analysis at − 80 ºC freezer.

Real-time PCR

Real-time PCR for detection of COVID-19 was done by Covitech COVID-19 kit (by CPVITECH, Iran). According to the manufacturer's instruction, 10 µL of each sample

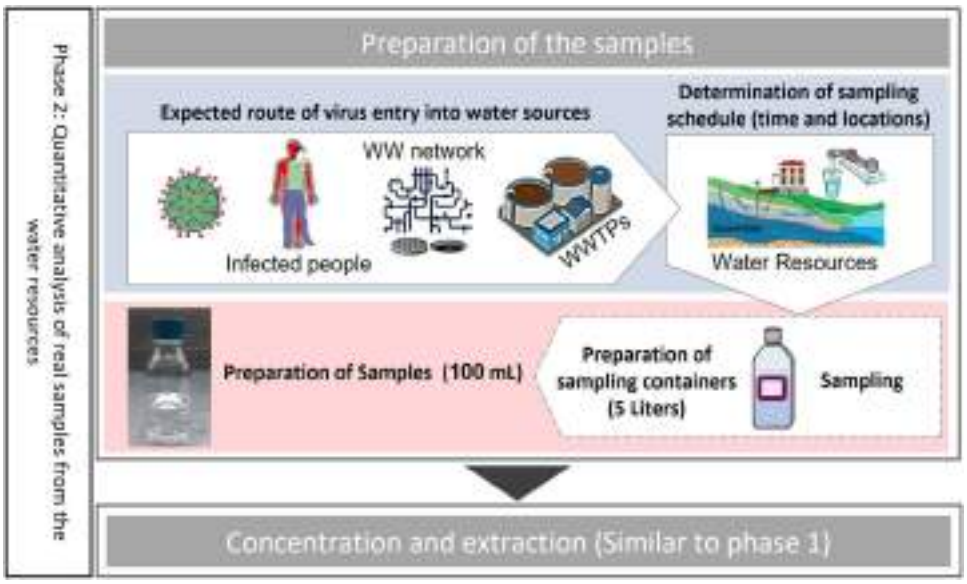

Fig. 3 Flowchart of phase two: analysis of real samples

was added to 10 µL of master mix (containing 1 µL primer with 10 µL master mix in the total volume of 20 µL) and the program was (55 ºC initial cDNA synthesis step for 20 min, 95 ºC initial denaturation for 3 min, and 55 cycles of 95 ºC for 15 s and 60 ºC for 40 s). E gene was detected in FAM channel (green), the S gene was detected in the ROX channel (red), and endogenous control was detected in the HEX channel (orange). Positive and negative control were used as provided by the manufacturer. A Mic-Biomolecular thermal cycler was used for amplification. For quantification standard containing 200,000, 20,000, 2000, 200, and 20 copy virus/µL was used for absolute quantification, and quantification was done by a standard curve which provided by Magnetic Induction Cycler Polymerase Chain Reaction software (MicPCR) according to provided standards. Equation 1 was used to calculate the number of viruses per milliliter of samples

$$\text{Viral load} = \frac{\frac{viral\ gene\ copies}{\mu L\ eluted\ RNA} * 60\mu L(total\ volume\ of\ eluted\ RNA)}{100\,mL(initial\ volume\ of\ concentrated\ sourfacewater)}. \quad (1)$$

Phase two: detection tests on real samples

This step aims to identify the virus in real samples obtained from water sources and water supply facilities and to quantify the number of viral particles in the samples. The step was taken in two phases including water sources sampling according to the time schedule agreed upon with the biostatistics and epidemiology team and monitoring and testing of the samples according to the selected instructions.

Sampling schedule

Here, in this study, selection of the sampling sites was done by the expert panel including members from the city's Water and Wastewater Company (WWC), the city's municipality, Provincial Headquarters Against Corona (PHAC), and the research team. During the sequence of meetings, a variety of factors were evaluated for the water recourses in terms of their type, geographical location, spatial distribution, resource contribution in water supply, vicinity to the sites that probably produce infectious wastewater, and hydraulic connection and flow direction. The rationale for sampling is that it should include a variety of water supply resources such as drinking water wells, qanats, water treatment plants, dams, reservoirs, and wells for green space and agricultural irrigation. In terms of wells, both out-of-town and in-town wells that supply urban water were sampled for which an appropriate spatial distribution of samples was observed. Attempts were made to sample all the wells along the river crossing from the middle of the city (Mehranehrood River). Since the river is the main drain of the city, wastewater from the upstream and also from some parts of the city that are not equipped with a wastewater network is discharged directly or can probably leak into this river, while in the water shortage periods, water is injected from wells around the river into the urban water network. Regarding reservoirs, instead of focusing on the spatial distribution of sampling, the importance of the reservoirs' role in the water supply of the city was considered as a determining factor in selection. Since 55% of Tabriz water is supplied from the Zarrineh River, the in-line reservoirs of Shahrake Imam, Manzariyeh, and Karjan are the representative of it. 12% of Tabriz water

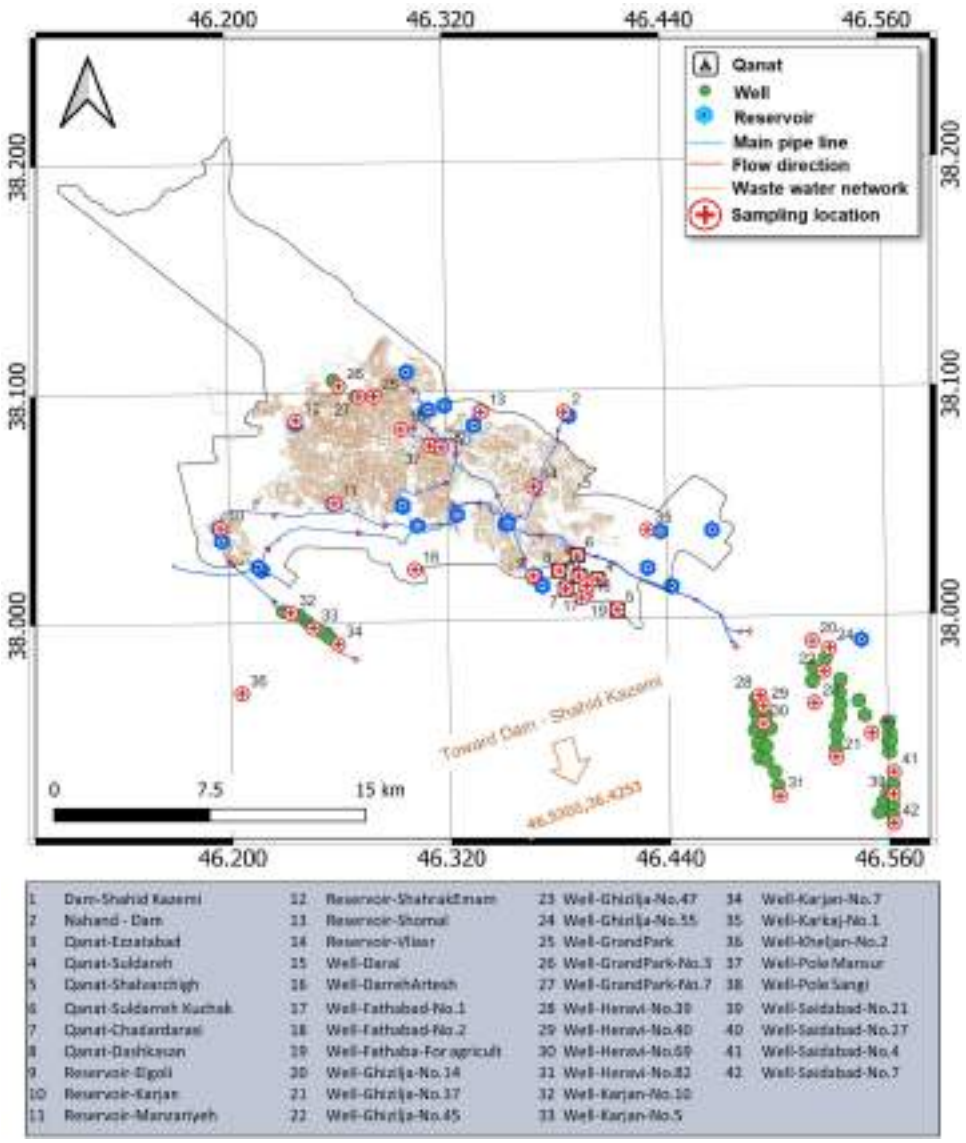

Fig. 4 Location and name of selected sites for sampling. Samples No. 38, 40, and 41 have been taken from Shahid Kazemi Dam (located outside the map from its southern side), which supplies the most portion of the urban water requirement of Tabriz

supply is also from Nahand dam, for which Valiasr reservoir is the representative of this dam and also is the representative of its upstream reservoir according to the flow direction and hydraulic connection of these two reservoirs. Therefore, if the water of the Valiasr reservoir is healthy, it can ensure that the water of the upstream reservoir is safe. Since the reservoirs of Tabriz have no leakage connection with subsurface water and groundwater, and on the other hand, sampling from upstream treatment plants was on the agenda, so sampling of all reservoirs was not necessary. However, a number of important reservoirs in the city, which covered a large population, were sampled to ensure the safety of Tabriz's domestic water. Regarding qanats, samples were grabbed from almost all of the main qanats in the city. Figure 4 shows the spatial distribution of the selected sites for sampling. In this study, 55 Tabriz's water resource locations were sampled over the course of 5 days (2021 July 22 to 2022 January 7) during the peak of the pandemic (Worldometers.info, 2022).

Sampling instructions

Plastic containers with a volume of 5 L were used for sampling, since the containers were temperature sensitive and therefore sterilized using the Environmental Protection Agency (EPA) method (Fout et al. 2016) instead of the autoclave. The disinfection process of these containers was done using distilled water and 0.5% sodium hypochlorite which were kept in each container for 30 min to remove contaminants. Since the presence of sodium hypochlorite in the container has a decreasing effect on the number and concentration of viruses, after the disinfection process, all containers were washed three times with distilled water to remove it. Also, to achieve complete elimination of chlorine,

50 ml of 1 M sodium thiosulfate was added to the containers and then washed. Washing with distilled water was repeated several times to remove residues of the materials used in the previous steps, and then, the aluminum foil lid was used to prevent the entry of pollutants.

In sampling water for virological testing, care must be taken to ensure that the grabbed sample represents the water that must be judged. Representative samples must contain information, such as location, date, time of sampling, and name of the sampler. This information was completed and implemented in accordance with the standards 2348-2347-7960-7961 (Fout et al. 2016). Each sample was taken instantly (grabbed) by the sterile plastic containers and transferred to the Applied Pharmaceutical Research Center of Tabriz University of Medical Sciences. It was best to start the test immediately after sampling as such a condition was not possible in practice, especially in rural areas. Therefore, to control the desired conditions and preserve the majority of possible viruses remaining in the samples, the samples were stored in refrigerated containers filled with dry ice and packages containing ordinary ice at 4 °C while transferring. Under no circumstances did the sampling interval before the test exceed 12 h. Figure 5 shows the equipment used for observing safety at work, sampling, and testing at the research center.

Fig. 5 Security measures, equipment used for sampling/testing, and the center where the tests have been conducted

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp; 2016. The normal distribution of continuous variables was determined using the Shapiro–Wilk test (Shapiro and Wilk 1965). Data were summarized using mean (± SD) and frequency (percent) for numerical and categorical variables, respectively. The linear regression model was used to determine the effect of virus concentration on recovery. All tests were two-sided and the significance level was set at 0.05.

Results

The results of this research are presented in two parts: analysis of synthetic and real samples.

Quantitative analysis of synthetic samples

To evaluate the efficiency of the PEG/NaCl precipitation method with Covitech quantification kits, the SARS-COV-2 virus was seeded into each serum sample ($n = 12$) to a final concentration of 3–3000 (copie /ml). The mean virus recovery rate was calculated as 30.41 ± 12.08% (ranging from 1% to 69.33%). According to the simple linear regression test, there was a significant difference between the tested precipitation method with the COVITECH kit and the calculated concentration of viruses ($p < 0.05$). With increasing each unit of virus concentration (copies/µL), the average recovery has increased by 0.02% ($\beta = 0.02$), which is statistically significant (p value = 0.048). There was no inhibitor in the present experiment. The result of the real-time PCR and quantification test for concentrate samples taken from the PEG/NaCl precipitation has been shown in Fig. 6.

Quantitative analysis of real samples (from water sources)

As shown in Table 2, of all the samples examined, only sample No. 27 was positive for the SARS-COV-2 virus. This sample was taken from well No. 7 of Tabriz Grand Park. The geographical coordinate of the well is E:

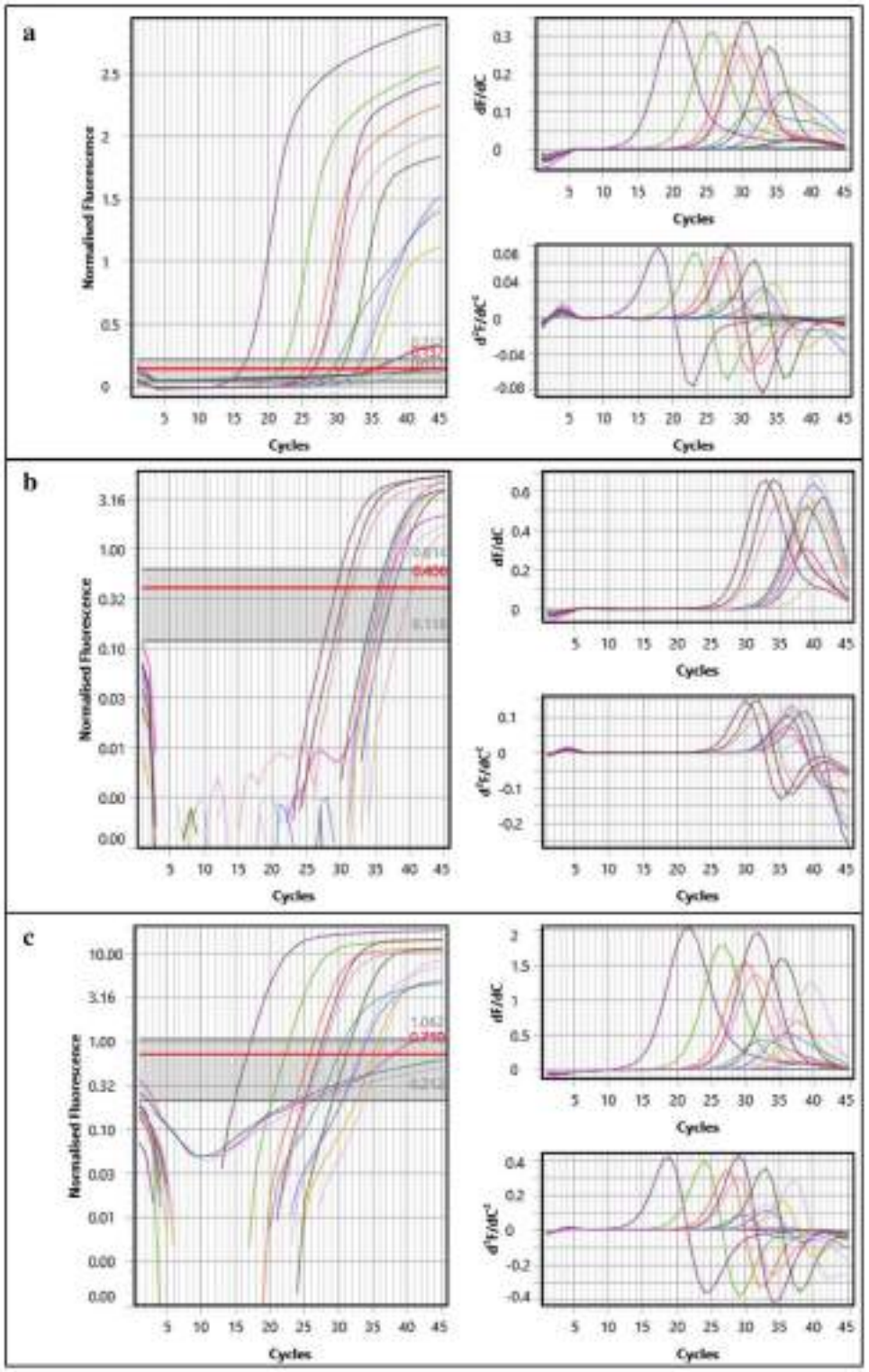

Fig. 6 Amplification curves of the SARS-CoV-2 identification by PEG/NaCl precipitation and COVITECH kits: **a** E gene (in the green channel), **b** S gene (in red channel), and **c** endogenous control (in orange channel). These curves show the accuracy and sensitivity of the identification of target genes

46.27208, N: 38.09812 which is located at the site where a part of urban wastewater is discharged to Mehranehrood River. The results of molecular analysis for the positive sample showed that the CT value and concentration of the virus genome were equal to 32.57 and 5720 copies/L, respectively. Quantitative analysis of real samples shows that the city's urban water network currently is not the cause of the SARS-CoV-2 outbreak, but this is not a permit for stopping the SARS-CoV-2 detection projects in this network. Mehranehrood River has never been the

Table 2 Specifications of samples obtained from water resources in Tabriz city

No.	Name		Result	Temperature of the sample (C°)	Temperature of the environment (C°)	pH	Cl (mg/L)	Sampling time (24 h)	CT[a] Values	Concentration (copies/L)
1	Dam-Shahid Kazemi	Entrance	Neg.	20.4	34	7.9	Raw	16:30	0	0
		Entrance (without Bukan discharge)	Neg.	29.5	34	7.98	Raw	17:20	0	0
		Reservoir	Neg.	26.9	38	7.85	0.8	10:05	0	0
		Reservoir	Neg.	24.3	28	7.48	0.8	10:35	0	0
		Reservoir	Neg.	26.7	29	7.7	0.9	11:2	0	0
		Outlet of WTP	Neg.	26.6	31	7.67	0.9	12:50	0	0
		Entrance-WTP-Behind the filter	Neg.	27.9	31	8.17	Raw	12:30	0	0
		Intake of WTP	Neg.	29.8	32	8.29	Raw	13:40	0	0
2	Nahand Dam	Reservoir	Neg.	23.3	30	8.15	Raw	12:50	0	0
		Entrance-River	Neg.	24.1	31	7.99	Raw	13:30	0	0
		Outlet of dam	Neg.	19.7	31	8.24	Raw	13:05	0	0
		Entrance of WTP	Neg.	14	29	7.95	Raw	11:50	0	0
		Outlet of WTP	Neg.	20.3	29	7.96	0.8	11:20	0	0
3	Qanat-Ezzatabad		Neg.	14.3	32	8.68	Raw	12:15	0	0
4	Qanat-Suldareh		Neg.	17	27	8.85	Raw	10:40	0	0
5	Qanat-Shalvarchigh		Neg.	14.6	32	9.31	Raw	12:05	0	0
6	Qanat-Suldarreh Kuchak		Neg.	18.7	30	8.48	Raw	11.5	0	0
7	Qanat-Chadardarasi		Neg.	25.3	33	8.15	Raw	12.25	0	0
8	Qanat-Dashkasan		Neg.	18.5	33	8.63	Raw	12.35	0	0
9	Reservoir-Elgoli		Neg.	21.2	33	7.35	0.8	13:33	0	0
10	Reservoir-Karjan		Neg.	28.4	35	7.69	0.8	15:15	0	0
11	Reservoir-Manzariyeh		Neg.	27	34	8.33	0.8	14:00	0	0
12	Reservoir-ShahrakEmam		Neg.	27	34	8.55	0.8	14:30	0	0
13	Reservoir-Shomal		Neg.	26.6	34	7.76	0.8	15:00	0	0
14	Reservoir-Vliasr		Neg.	19.7	36	7.67	0.8	15:20	0	0
15	Well-Darai		Neg.	16.7	27	7.09	Raw	9:55	0	0
16	Well-DarrehArtesh		Neg.	20.7	34	6.95	Raw	12:27	0	0
17	Well-Fathabad-No. 1		Neg.	15.5	27	7.87	Raw	10:15	0	0
18	Well-Fathabad-No. 2		Neg.	16.1	27	7.87	0.8	10:00	0	0
19	Well-Fathabad-For agricult		Neg.	14.7	27	7.82	Raw	10:24	0	0
20	Well-Ghizilja-No. 14		Neg.	22.3	32	7.08	Raw	11:15	0	0
21	Well-Ghizilja-No. 37		Neg.	15.7	33	7.35	Raw	12:15	0	0
22	Well-Ghizilja-No. 45		Neg.	18.3	32	7.36	Raw	11:50	0	0
23	Well-Ghizilja-No. 47		Neg.	16.1	32	7.33	Raw	11:35	0	0

Table 2 (continued)

No.	Name	Result	Temperature of the sample (C°)	Temperature of the environment (C°)	pH	Cl (mg/L)	Sampling time (24 h)	CT[a] Values	Concentration (copies/L)
24	Well-Ghizilja-No. 55	Neg.	23.8	33	7.12	Raw	12:50	0	0
25	Well-GrandPark	No. 5	Neg.	15.3	30	7.04	Raw	10:45	0
		No. 4	Neg.	15.7	30	7.04	Raw	10:35	0
		No. 1	Neg.	15.8	27	6.99	Raw	9:45	0
26	Well-GrandPark-No. 3	Neg.	15.9	29	7.05	Raw	10:20	0	0
27	**Well-GrandPark-No. 7**	**Pos.**	**17**	**27**	**7.06**	**Raw**	**9:32**	**– 32.57**	**5720**
28	Well-Heravi-No. 39	Neg.	20.3	31	6.96	Raw	10:15	0	0
29	Well-Heravi-No. 40	Neg.	19.4	36	6.97	Raw	15:00	0	0
30	Well-Heravi-No. 69	Neg.	19.8	29	6.8	Raw	9:20	0	0
31	Well-Heravi-No. 82	Neg.	21.5	29	6.73	Raw	9:55	0	0
32	Well-Karjan-No. 10	Neg.	23.9	34	7.19	Raw	13:40	0	0
33	Well-Karjan-No. 5	Neg.	23.4	35	7.13	Raw	13:55	0	0
34	Well-Karjan-No. 7	Neg.	25.3	35	7	Raw	14:43	0	0
35	Well-Karkaj-No. 1	Neg.	22.5	28	6.76	Raw	10:56	0	0
36	Well-Kheljan-No. 2	Neg.	19.5	33	6.82	0.8	12:20	0	0
37	Well-Pole Mansur	Neg.	17.6	33	7.07	Raw	11:40	0	0
38	Well-Pole Sangi	Neg.	16.8	33	7.06	Raw	11:25	0	0
39	Well-Saidabad-No. 21	Neg.	15.4	34	7.28	Raw	13:35	0	0
40	Well-Saidabad-No. 27	Neg.	17.6	36	7.3	Raw	14:1	0	0
41	Well-Saidabad-No. 4	Neg.	14.3	36	7.35	Raw	13:50	0	0
42	Well-Saidabad-No. 7	Neg.	16.6	34	7.3	Raw	13:20	0	0

[a]CT: Cycling threshold is equal with the threshold which fluorescent was detected by the machine

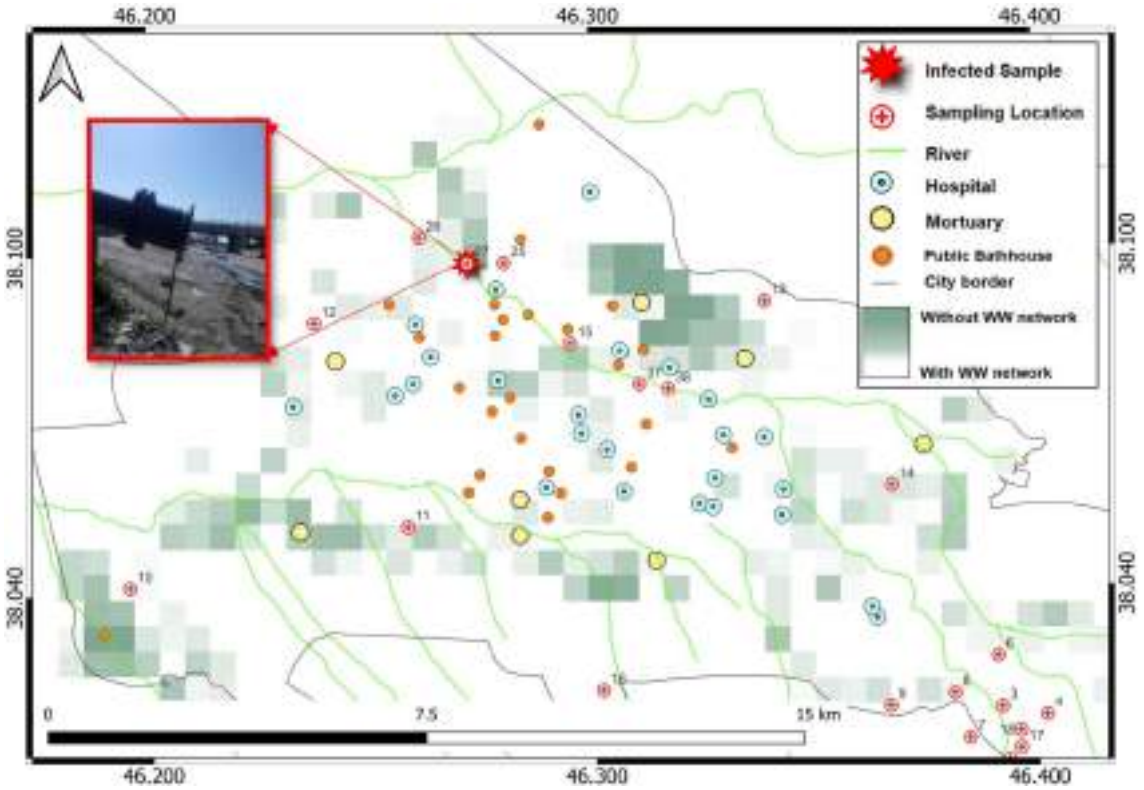

Fig. 7 Location of the positive sample and the spatial distribution of potentially infected elements such as mortuaries, hospitals, public bathhouses, and areas that have not been equipped with the wastewater (WW) network. The location of the positive sample was adjacent to a place where a part of urban wastewater is discharged to the Mehranehrood River

drinking water supplier of the city, so identification of positive samples downstream of this river does not directly threaten the safety level of the city's drinking water. As the main drain, the wastewater discharge from the unequipped areas still joins the Mehranehrood River, and the adjacency of the positive sample to this river can ring the bell for upstream adjacent wells. However, at the time of this research, the samples taken from these wells were all negative. Figure 7 shows the location of the detected positive sample among the spatial distribution of suspected elements that probably could transfer huge amounts of the virus to the urban water network (such as mortuaries, hospitals, public bathhouses, and the areas without wastewater network). Although some areas in the city (almost 30% of the area) are not yet equipped with a wastewater network and despite the relatively wide spatial distribution of suspicious elements, the city's water supply sources were fortunately safe at the time of this study. However, the threat of leaking makes the continuous monitoring of the resources such as wells and qanats essential until the time it can certainly be proved that wastewater leakage to these water resources is impossible.

Discussion

Influenza and SARS-CoV-2 virus transmissibility has been associated with meteorological and hydrological variables in the literature (Jaakkola et al. 2014; Wang et al. 2020c). According to the World Health Organization (WHO), the SARS-CoV-2 virus has not been observed in drinking water; however, in countries where water treatment is not equipped to remove viruses, the presence of the virus is unknown (Langone et al. 2021). In reality, no cases of transmission by transmission via contact with sewage or contaminated water have been documented yet and the few investigations of aqueous matrices have not detected infectious viruses. On the other hand, studies are showing that SARS-CoV-2 can remain viable, i.e., infectious, for up to 4.3 and 6 days in sewage and water, respectively. These are strong pieces of evidence that the contamination mediated by contact with sewage or contaminated water cannot be ruled out (Giacobbo, et al 2021). Since previous studies have not demonstrated that SARS-CoV-2 RNA might exist in a city's drinking water, a validated concentration and

quantification approach was used to look for SARS-CoV-2 RNA in the city's municipal water resources.

Due to the different conditions in various types of water resources, the persistence of viruses is not supposed to be similar. Rivers usually provide an unstable substrate for viruses due to flow disturbances, where the formation of viral aerosols is more probable than in lakes (Wigginton and Boehm 2020). The influence of physical, chemical, and biological factors in the lake environment may help to inactivate the viruses. For example, bacteria may inactivate viruses by secreting proteases and destroying the capsid of the virus (Callan et al. 1997). Compared to river water, lake water provides an unsuitable environment for viruses, in which hydroxyl radicals with strong standard oxidation—reduction potential can inactivate viruses efficiently (Xu et al. 2020). This study showed that SARS-COV-2 RNA can exist in the environmental conditions of a water well, considering the fact that this well has been continuously exposed to leakage from a contaminated source.

Given that the water temperature, ambient temperature, and pH of the detected positive sample in this research at the time of sampling were equal to 17 C^o, 27 C^o, and 7.06, respectively, it can be stated that in the normal condition, SARS-COV-2 RNA could persist in a water matrix adjacent to an infected wastewater discharge point. However, it can be expected that by cutting the infected wastewater discharge, the virus becomes inactivated, since literature shows the inactivation of 99.9% of the Coronaviruses in tap water at 23 Co and 4 Co within 10 and more than 100 days, respectively (Gundy et al. 2009). High temperature is reported to slow the spread of COVID-19 (Sarkodie and Owusu 2020). Since the sampling was carried out in the warm seasons and the temperature of the analyzed wells ranged between 17 and 27 C^o; therefore, the negative results of other analyzed wells could be due to the high temperature of the wells' water. According to the WHO reports, Coronavirus was not detected in the drinking water after the addition of Chlorine (World Health Organization 2020). Since, in the current work, no Chlorine was added to the analyzing wells, it can be concluded that the probable viability of Coronavirus in the conventional water resources is very low. Regarding the impact of pH on the Coronavirus viability, different evidence was reported. It has been stated that the pH is one of the most important influential parameters on Coronavirus viability in an aqueous medium (Tubatsi and Kebaabetswe 2022). Although the outer membrane of the SARS-COV-2 virus is stable in a wide range of pH, indicating an insignificant effect of pH on the entry of coronaviruses into host cells (Aydin et al. 2014), however, the behavior of the virus against pH under acidic, alkaline, and neutral conditions in water environments will be different. Chin et al. showed that Coronavirus has strong resistance in pH ranging from 3 to 10 at 25 C^o (Chin et al. 2020). Further increase in pH can damage the virus viability. In the current work, since the pH of all wells ranged between 6.5 and 7.6, therefore, pH plays a minor role in the present research.

The infected resource adjacent to municipal wastewater discharge shows that the leaks from the sewage collectors must be taken as a serious issue for the city's water and wastewater organization during the pandemic. It can be concluded that both unconventional water sources and their adjacent conventional water resources should periodically be investigated for detecting infected spots and the virus transfer routes to the water supply network due to probable leakages and failures in the wastewater systems. This requires a continuous research plan corresponding to the peaks and declines of the pandemic, so that the causes and behavior of water resource contamination can be pathologically examined. None of the samples taken after the chlorine station were positive for the SARS-COV-2 virus, although the results of this study cannot conclusively confirm the effect of chlorine in disinfecting this type of virus.

Conclusion and recommendations

The first objective of this study was to test a concentration method for the detection of the virus RNA in water matrices, which has been carried out with the appropriate number of repetitions and with the protocol explained according to the methods approved by valid references. A combination of polyethylene glycol (PEG) and sodium chloride (NaCl) was developed and validated as a useful method for concentrating real water samples taken from urban water resources and preparing them for the quantification procedure. This method is proposed to be tested and used for future studies that need concentration steps prior to SARS-COV-2 RNA extraction. The second goal was the spatial investigation of the SARS-COV-2 RNA in the water resources of Tabriz city. The logic is that if this SARS-COV-2 RNA is detected even in a short time window, then it can be stated that the city's urban water system is at risk of contamination during the pandemic and the need for monitoring can be suggested. Because the research was able to find SARS-COV-2 RNA in one of the water supplies within a time frame in a pandemic's peak, monitoring of other time periods is recommended for future studies with a broader scope of purpose, such as leakage tracking. The results of this study could not comment on the persistence of the SARS-COV-2 RNA after cutting off the discharge of sewage to the section of the river where the well with a positive sample was adjacent to it. Further research with a different design framework is needed to determine how long SARS-COV-2 RNA can persist around the wastewater discharge site and how far it can be transferred from the site by leakage or water flow. This study considered the relationship between the upstream and

downstream reservoirs and the resource's vulnerability to leakage in distributing the location of the water samples, so that compared to the closed reservoirs, more samples were allocated to wells and aqueducts. Considerations from the current study may be utilized in similar future studies to choose the location of water samples, so that they are the best representative of all sorts of water resources and the entire urban water system.

Authors contributions Hamed Sabzchi-Dehkharghani, Hossein Samadi Kafil, and Rana Naderi-Ahranjani contributed to the analysis of the results, to process the experimental data, and to the writing of the manuscript. Reza Yegani, Abolfazl Majnooni-Heris, and Ahmad Fakherifard were involved in planning and supervising the work. Abbas Akbarzadeh, Mohammad Mosaferi, Neda Gilani, Mojtaba Noury, and Nasimolzahra Toghyanian discussed the results and commented on the manuscript. Rana Naderi-Ahranjani, Parisa Eydi, and Sajjad Sayyari Sis contributed to sampling and laboratory measures.

Funding This study was funded by Iran Water Resources Management Company.

Availability of data and materials The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

References

Anayah F, Al-Khatib IA, Hejaz B (2021) Assessment of water and sanitation systems at Palestinian healthcare facilities: pre-and post-COVID-19. Environ Monit Assess 193:1–22. https://doi.org/10.1007/s10661-020-08791-4

Armitage R, Nellums LB (2020) Water, climate change, and COVID-19: prioritising those in water-stressed settings. Lancet Planetary Health 4:e175. https://doi.org/10.1016/S2542-5196(20)30084-X

Aydin H, Al-Khooly D, Lee JE (2014) Influence of hydrophobic and electrostatic residues on SARS-coronavirus S2 protein stability: Insights into mechanisms of general viral fusion and inhibitor design. Protein Sci 23(5):603–617. https://doi.org/10.1002/pro.2442

Baer A, Kehn-Hall K (2014) Viral concentration determination through plaque assays: using traditional and novel overlay systems. J vis Exp. https://doi.org/10.3791/52065

Barceló D (2020) Wastewater-based epidemiology to monitor COVID-19 outbreak: present and future diagnostic methods to be in your radar. Case Stud Chem Environ Eng 2:100042. https://doi.org/10.1016/j.cscee.2020.100042

Bofill-Mas S, Rusiñol M (2020) Recent trends on methods for the concentration of viruses from water samples. Curr Opin Environ Sci Health. 16:7–13. https://doi.org/10.1016/j.coesh.2020.01.006

Boretti AJG (2020) Covid19 pandemic as a further driver of water scarcity in Africa. Geo J 87:787–814. https://doi.org/10.1007/s10708-020-10280-7

Buonerba A, Corpuz MVA, Ballesteros F, Choo K-H, HASAN SW, Korshin GV, Belgiorno V, Barceló D, Naddeo V (2021) Coronavirus in water media: Analysis, fate, disinfection and epidemiological applications. J Hazard Mater 415:125580. https://doi.org/10.1016/j.jhazmat.2021.125580

Callan RJ, Hartmann FA, West S, Hinshaw VS (1997) Cleavage of influenza A virus H1 hemagglutinin by swine respiratory bacterial proteases. J Virol 71:7579–7585. https://doi.org/10.1128/jvi.71.10.7579-7585.1997

Chakraborty B, Roy S, Bera A, Adhikary PP, Bera B, Sengupta D, Bhunia GS, Shit PK (2021) Cleaning the river Damodar (India): impact of COVID-19 lockdown on water quality and future rejuvenation strategies. Environ Dev Sustain 23:11975–11989. https://doi.org/10.1007/s10668-020-01152-8

Chin AW, Chu JT, Perera MR, Hui KP, Yen HL, Chan MC, Peiris M, Poon LL (2020) Stability of SARS-CoV-2 in different environmental conditions. Lancet Microbe 1(1):e10. https://doi.org/10.1016/S2666-5247(20)30003-3

Corpuz MVA, Buonerba A, Vigliotta G, Zarra T, Ballesteros JRF, Campiglia P, Belgiorno V, Korshin G, Naddeo V (2020) Viruses in wastewater: occurrence, abundance and detection methods. Sci Tot Environ 745:140910. https://doi.org/10.1016/j.scitotenv.2020.140910

Farkas K, Hillary LS, Thorpe J, Walker DI, Lowther JA, Mcdonald JE, Malham SK, Jones DL (2021) Concentration and quantification of SARS-CoV-2 RNA in wastewater using polyethylene glycol-based concentration and qRT-PCR. Method Protoc 4:17. https://doi.org/10.3390/mps4010017

Fathizadeh H, Taghizadeh S, Safari R, Khiabani SS, Babak B, Hamzavi F, Ganbarov K, Esposito S, Zeinalzadeh E, Dao S (2020) Study presence of COVID-19 (SARS-CoV-2) in the sweat of patients infected with Covid-19. Microbial Pathogen 149:104556. https://doi.org/10.1016/j.micpath.2020.104556

Feizizadeh B, Omarzadeh D, Ronagh Z, Sharifi A, Blaschke T, Lakes TJ (2021) A scenario-based approach for urban water management in the context of the COVID-19 pandemic and a case study for the Tabriz metropolitan area. Iran 790:148272. https://doi.org/10.1016/j.scitotenv.2021.148272

Feng W, Newbigging AM, Le C, Pang B, Peng H, Cao Y, Wu J, Abbas G, Song J, Wang D-B (2020) Molecular diagnosis of COVID-19: challenges and research needs. Anal Chem 92:10196–10209. https://doi.org/10.1021/acs.analchem.0c02060

Fout GS, Cashdollar JL, Griffin SM, Brinkman NE, Varughese EA, Parshionikar SU (2016) EPA Method 1615. Measurement of enterovirus and norovirus occurrence in water by culture and RT-qPCR. Part III. Virus detection by RT-qPCR. J vis Exp 16:e52646. https://doi.org/10.3791/52646

García-Ávila F, Valdiviezo-gonzales L, Cadme-Galabay M, Gutierrez-Ortega H, Altamirano-Cárdenas L, Zhindón-Arevalo C, Del pino LF (2020) Considerations on water quality and the use of chlorine in times of SARS-CoV-2 (COVID-19) pandemic in the community. Case Stud Chem Environ Eng. 2:100049. https://doi.org/10.1016/j.cscee.2020.100049

Giacobbo A, Rodrigues MAS, Ferreira JZ, Bernardes AM, de Pinho MN (2021) A critical review on SARS-CoV-2 infectivity in water and wastewater. What do we know? Sci Total Environ 774:145721. https://doi.org/10.1016/j.scitotenv.2021.145721

Gundy PM, Gerba CP, Pepper IL (2009) Survival of coronaviruses in water and wastewater. Food Environ Virol 1(1):10–14. https://doi.org/10.1007/s12560-008-9001-6

Haramoto E, Malla B, Thakali O, Kitajima M (2020) First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. Sci Tot Environ 737:140405. https://doi.org/10.1016/j.scitotenv.2020.140405

Harcourt J, Tamin A, Lu X, Kamili S, Sakthivel SK, Murray J, Queen K, Tao Y, Paden CR, Zhang J (2020) Severe acute respiratory syndrome coronavirus 2 from patient with coronavirus disease United States. Sci Total Environ 26:1266. https://doi.org/10.3201/eid2606.200516

Hasan SW, Ibrahim Y, Daou M, Kannout H, Jan N, Lopes A, Alsafar H, Yousef AF (2021) Detection and quantification of SARS-CoV-2 RNA in wastewater and treated effluents: Surveillance of COVID-19 epidemic in the United Arab Emirates. Sci Total Environ 764:142929. https://doi.org/10.1016/j.scitotenv.2020.142929

Hoehl S, Rabenau H, Berger A, Kortenbusch M, Cinatl J, Bojkova D, Behrens P, Böddinghaus B, Götsch U, Naujoks F (2020) Evidence of SARS-CoV-2 infection in returning travelers from Wuhan, China. N Engl J Med 382:1278–1280. https://doi.org/10.1056/nejmc2001899

Jaakkola K, Saukkoriipi A, Jokelainen J, Juvonen R, Kauppila J, Vainio O, Ziegler T, Rönkkö E, Jaakkola JJ, Ikäheimo TM (2014) Decline in temperature and humidity increases the occurrence of influenza in cold climate. Environ Health 13:1–8. https://doi.org/10.1186/1476-069X-13-22

Kalbusch A, Henning E, Brikalski De Luca MPFV, Konrath AC (2020) Impact of coronavirus (COVID-19) spread-prevention actions on urban water consumption. Resour Conservat Recycl 163:105098. https://doi.org/10.1016/j.resconrec.2020.105098

Khodadadi E, Maroufi P, Khodadadi E, Esposito I, Ganbarov K, Espsoito S, Yousefi M, Zeinalzadeh E, Kafil HS (2020) Study of combining virtual screening and antiviral treatments of the Sars-CoV-2 (Covid-19). Microb Pathog. https://doi.org/10.1016/j.micpath.2020.104241

La Rosa G, Bonadonna L, Lucentini L, Kenmoe S, Suffredini E (2020) Coronavirus in water environments: Occurrence, persistence and concentration methods-A scoping review. Water Res 179:115899. https://doi.org/10.1016/j.watres.2020.115899

Labadie T, Batejat C, Manuguerra J-C (2018) Influenza Virus Segment Composition Influences Viral Stability in the Environment. Front Microbiol 9:1496. https://doi.org/10.3389/fmicb.2018.01496

Langone M, Petta L, Cellamare C, Ferraris M, Guzzinati R, Mattioli D, Sabia G (2021) SARS-CoV-2 in water services: Presence and impacts. Environment Pollut 268:115806. https://doi.org/10.1016/j.envpol.2020.115806

McDonald RI, Weber K, Padowski J, FLörke M, Schneider C, Green PA, Gleeson T, Eckman S, LEHNER B, Balk D (2014) Water on an urban planet: Urbanization and the reach of urban water infrastructure. Global Environ Change 27:96–105. https://doi.org/10.1016/j.gloenvcha.2014.04.022

Mlejnkova H, Sovova K, Vasickova P, Ocenaskova V, Jasikova L, Juranova E (2020) Preliminary study of SARS-CoV-2 occurrence in wastewater in the Czech Republic. IJERPH 17:5508. https://doi.org/10.3390/ijerph17155508

Najafi K, Maroufi P, Khodadadi E, Zeinalzadeh E, Ganbarov K, Asgharzadeh M, Kafil HS (2022) SARS-CoV-2 receptor ACE2 and molecular pathway to enter target cells during infection. Reviews in Medical Microbiology. https://doi.org/10.1097/MRM.0000000000000237

PAL S, MASUM MH (2021) Effects of meteorological parameters on COVID-19 transmission trends in Bangladesh. Environmental Sustain 4:559–568. https://doi.org/10.1007/s42398-021-00195-5

Panchal D, PRAKASH O, BOBDE P, PAL S (2021) SARS-CoV-2: sewage surveillance as an early warning system and challenges in developing countries. Environ Sci Pollut Res Int 28(18):22221–22240. https://doi.org/10.1007/s11356-021-13170-8

Perez-Cataluña A, Cuevas-Ferrando E, Randazzo W, Falcó I, Allende A, Sánchez G (2021) Comparing analytical methods to detect SARS-CoV-2 in wastewater. Sci Total Environ 758:143870. https://doi.org/10.1016/j.scitotenv.2020.143870

Rezaei A, Hassani H, Hassani S, Jabbari N, Mousavi SBF, Rezaei S (2019) Evaluation of groundwater quality and heavy metal pollution indices in Bazman basin, southeastern Iran. Groundw Sustain Dev 9:100245. https://doi.org/10.1016/j.gsd.2019.100245

Rezaei A, Hassani H, Tziritis E, Mousavi SBF, Jabbari N (2020) Hydrochemical characterization and evaluation of groundwater quality in Dalgan basin SE Iran. Groundw Sustain Dev 10:100353. https://doi.org/10.1016/j.gsd.2020.100353

Salem HSA, Shams MY, Hassanien AE, Nosair AM (2021) COVID-19 and Water Resources Nexus: Potential Routes for Virus Spread and Management Using Artificial Intelligence Techniques. The Global Environmental Effects During and Beyond COVID-19. Springer. Doi: https://doi.org/10.1007/978-3-030-72933-2_2

Sarkodie SA, Owusu PA (2020) Impact of meteorological factors on COVID-19 pandemic: Evidence from top 20 countries with confirmed cases. Environ Res 191:110101. https://doi.org/10.1016/j.envres.2020.110101

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). Biometrika 52(3/4):591–611. https://doi.org/10.2307/2333709

Sobral MFF, Duarte GB, DA PENHA SOBRAL AIG, Marinho MLM, De Souza Melo A (2020) Association between climate variables and global transmission oF SARS-CoV-2. Sci Total Environ 729:138997. https://doi.org/10.1016/j.scitotenv.2020.138997

Tubatsi G, Kebaabetswe LP (2022) Detection of Enteric Viruses from Wastewater and River Water in Botswana. Food Environ Virol. https://doi.org/10.1007/s12560-022-09513-4

Wang J, Feng H, Zhang S, Ni Z, Ni L, Chen Y, Zhuo L, Zhong Z, Qu T (2020a) SARS-CoV-2 RNA detection of hospital isolation wards hygiene monitoring during the Coronavirus Disease 2019 outbreak in a Chinese hospital. Int J Infect Dic 94:103–106. https://doi.org/10.1016/j.ijid.2020a.04.024

Wang J, Shen J, Ye D, Yan X, Zhang Y, Yang W, Li X, Wang J, Zhang L, Pan LJEP (2020b) Disinfection technology of hospital wastes and wastewater: Suggestions for disinfection strategy during coronavirus Disease 2019 (COVID-19) pandemic in China. Environ Pollution 262:114665. https://doi.org/10.1016/j.envpol.2020b.114665

Wang J, Li W, Yang B, Cheng X, Tian Z, Guo H (2021) Impact of hydrological factors on the dynamic of COVID-19 epidemic: A multi-region study in China. Environ Res 198:110474. https://doi.org/10.1016/j.envres.2020.110474

Wang M, Jiang A, Gong L, Luo L, Guo W, Li C, Zheng J, Li C, Yang B, Zeng JJM (2020c). Temperature significant change COVID-19 Transmission in 429 cities. doi:https://doi.org/10.1101/2020c.02.22.20025791

Wigginton KR, Boehm AB (2020) Environmental engineers and scientists have important roles to play in stemming outbreaks and pandemics caused by enveloped viruses. ACS Publications. https://doi.org/10.1021/acs.est.0c01476

World Health Organization (2020) Water, sanitation, hygiene and waste management for COVID-19: technical brief, 03 March 2020 (No. WHO/2019-NcOV/IPC_WASH/2020.1). World Health Organization.

WORLDMETERS.INFO. 2022. Coronavirus Cases in Iran. Available at: <http://www.worldometers.info/world-population/> [Accessed 26 Jan 2022].

WWC-EAP: water & waste water company of East Azerbaijan Province, 2021. Aannual water consumption report of East Azerbaijan Province's cities. https://en. abfaazarbaijan.ir.

Xu H, Li Y, Liu J, Du H, Du Y, Su Y, Jiang HJ (2020) Photogeneration and steady-state concentration of hydroxyl radical in river and

lake waters along middle-lower Yangtze region, China. Water Res. https://doi.org/10.1016/j.watres.2020.115774