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QUANTITATIVE ASSESSMENT OF SARS-COV-2 IN MUNICIPAL WASTEWATER IN COMPARISON TO DAILY EPIDEMIOLOGICAL REPORTS ON THE INCIDENCE OF COVID-19 IN THE REGION

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Purpose: in this article, virological analysis was used to detect SARS-Cov-2 RNA in municipal sewage. As part of the work obtained comparatively with current epidemiological data to assess, or in terms of the indicator of the impact of COVID-19, municipal sewage can be a representative medium for assessing the health status of residents, sewage catchment.
Methodology: The research used the physicochemical analysis method using a set of research techniques used in chemistry and related sciences, which allow for determining the physical and chemical properties of substances.
Result: based on the obtained results of physicochemical parameters of wastewater, it can be concluded that the quality of wastewater has a significant impact on virus RNA concentration, which directly affects the sensitivity, viability and reproducibility of the SARs-CoV-2 virus in the same volume of analyzed wastewater**.**
Originality: research groups around the world have begun analyzing wastewater for a new indicator that can estimate the total number of infections in a given community. Scientists have already shown that there is a correlation between the concentration of SARS-CoV-2 RNA in wastewater and the number of identified clinical cases of COVID-19. They also suggest that monitoring RNA concentrations can provide information about cases of infection 4-7 days in advance, and thus provide an early warning system or identify sources of serious infections.
Keywords: sars-cov-2, municipal sewage, region.

1. Introduction

The SARS-CoV-2 virus originally known as 2019-nCoV (novel coronavirus) was discovered in late 2019 in Wuhan, central China (Hubei Province). SARS-CoV-2 is the seventh identified human coronavirus that has proven to be extremely infectious and highly virulent. The rapid spread of the coronavirus resulted in January 2020 WHO's declaration of a public health emergency and respectively a pandemic state in March 2021 (https://www.who.int/..., 2021). Currently (8.01.2022), there are over 298 915 721 confirmed cases of COVID-19 infection worldwide (Americas:108 806 129; Europe: 108 040 601; South-East Asia: 45 406 695; Eastern Mediterranean: 17 277 716; Western Pacific: 11 841 165; Africa: 7 542 653). Since its beginning, the pandemic has caused more than 5,46 million deaths (https://covid19.who.int/, 2022). In Poland, according to information presented by the Ministry of Health from March 2020 (4.03.202) up to date (8.01.2022), more than 4,20 million infections have been reported in Poland, with over 99,727 cases resulting in death (https://www.gov.pl/web/koronawirus, 2022). Coronavirus infections occur mainly by droplet infection through direct contact with an infected person. However, because of the survival of the virus on various types of abiotic surfaces, infection by indirect transmission through touch from contaminated surfaces and materials (Tan, Chenshan, Zhou, Van Driel, Ye, Zhong, 2020; Chin, Chu, Perera, Hui, Yen, Chan, Peiris, Poon, 2020). Although the main mechanism of SARS-CoV-2 transmission is based on the airborne transmission of the so-called respiratory drop-lets and aerosols (Meselson, 2020) the presence of the virus in the faeces and urine of infected individuals is particularly important in terms of monitoring virus transmission by testing environmental samples.

Literature data confirm that the amount of SARS-CoV-2 virus RNA present in the sputum and feces of patients with mild disease symptoms are similar to those found in the upper respiratory tract of COVID-19 patients (Wölfer, Corman, Guggemos, Seilmaier, Zange, Müller, Niemeyer, Jones, Vollmar, Rothe, Hoelscher, Bleicker, Brunink, Schneider, Ehmann, Qu, Kuang; Yang, Li, Dai, Liu, Li, Jie, 2020). The presence of SARS-CoV-2 virus in the gastrointestinal tract, feces, and urine of COVID-19 infected individuals has also been confirmed in studies conducted by Wu and Singer (Wu, Xiao, Zhang, Gu, Lee, Kauffman, 2020; Singer, Wray, 2020). Thus, the presence of RNA virus in urine and feces at concentrations allowing its quantitative and qualitative identification allows to use WBE approach (Wastewater-Based Epidemiology) to assess the current course and predict the direction of the pandemic (Douhgton, 2020). Application of the WBE approach, based on virological analysis of municipal wastewater, allows assessing of the real scale of pandemic development including identification of new disease outbreaks. Moreover, the approach makes it possible to obtain the data and information necessary for decision-makers to take actions related to the allocation of resources during emergencies (prioritization of needs), to target response and prevention actions as well as to lift restrictions and bans in areas with low incidence (Douhgton, 2020; Ahmed, Angel, Edson, Bibby, Bivins, O'Brien, Choi, Kitajima, Simpson, Li, Tscharke, Vergahen, Smith, Zaugg, Dierens, Hugenholtz, Thomas, Mueller, 2020; Lorenzo, Picó, 2019). According to the assumption of the WBE model, when pathogenic organisms are excreted with the feces or urine of an infected person, this tool can be used to assess the concentration of pathogens in collective sewage systems.

The results obtained during analyses can be used to estimate the health status of the population living in the sewage catchment area. Thus, in times of a COVID-19 pandemic, the WEB model provides an alternative tool for predicting pandemic status in a region that can be used when analytical and diagnostic capabilities are limited or when there is no basis for testing so-called asymptomatic patients. A conceptual diagram of the application of the WBE model to a sewer catchment is shown in Fig. 1.

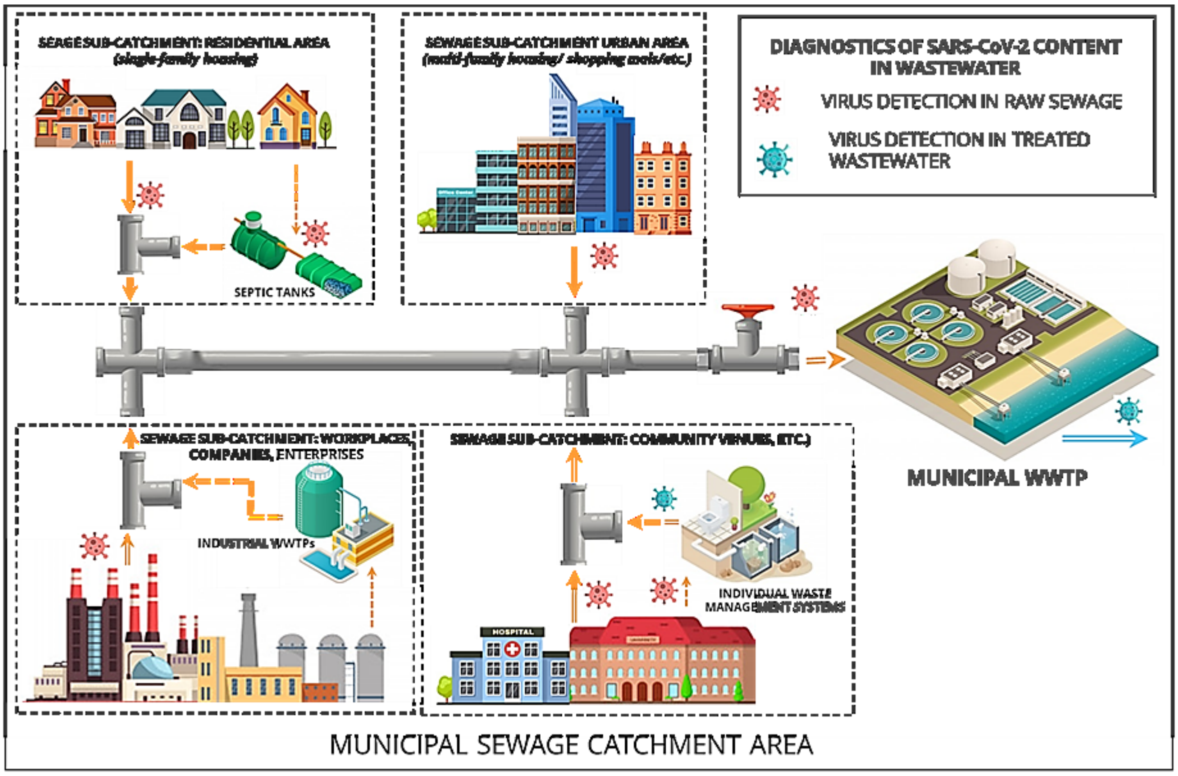

Figure 1. Municipal sewerage catchment area with delineated areas of potential sub-catchments.

The paper presents the results of microbiological analysis for the detection of SARS-CoV-2 carried out in the period of the observed increase in the number of new COVID-19 cases in the country and region. Due to the lack of possibility to obtain detailed data showing the actual number of sick inhabitants of the sewage catchment area (no available data at a local level), the results of virological analyses were compared with epidemiological data provided by sanitary services in the form of daily reports. Thus, the government data presented by the Ministry of Health and provincial sanitary and epidemiological units were used.

2. Materials and Methods

2.1. Sewerage catchment characteristics

The analyzed area is located in the south of Poland in Śląskie Voivodship. The WWP is located in the city which belongs to the Upper Silesian and Zagłębie Metropolis (GZM). According to hydrological data the WWTP is located in the Kłodnica river basin (Odra River tributary). The WWTP is one of 2 facilities serving a city with a population of 40 000 and has been designed as a mechanical-biological treatment plant with a target capacity of 8500 m^3/day. The average volume of wastewater treated is 1 755 thousand m3 per year. The average inflow of wastewater on rainy and dry days is 5,05 m3 and 3,01 m3 per day respectively. The sewerage catchment area served by WWTP includes the city center and adjacent residential areas. Over 95% of the wastewater delivered to the facility by the combined sewer system is municipal wastewater.

The research process was designed in such a way which would facilitate to assess the possibility of applying the SEM/EDS technique to qualitatively identify the silica marker as well as to assess the applicability of this method in the case of different types of abiotic surfaces of various structure, porosity and shape. The possibility of applying the silica marker as a potential marker of virus pathogens, along with the option of its further identification on different types of carriers, constitutes a significant issue concerning simulation tests under laboratory and real conditions in terms of the pathogen spread as well as its presence on different types of surfaces. Considering the above, the idea of this research study was based on the assumption that the examined solutions would be sprayed by means of an atomizer on different types of carriers in a manner simulating the direct transmission route of the coronavirus (i.e., cough and sneezing) and next the carriers with the sedimented material would be subject to microscopic analyses using the SEM/EDS technique.

Two types of solutions were used in this study as research material; namely, the control solution (physiological saline solution constituting the equivalent of human saliva) and the silica marker solution (constituting the surrogate of the SARS-CoV-2 virus). The following three types of abiotic carriers were employed in the research: a membrane filter SKC, a sponge filter CIP and a graphite disc. The solutions were sprayed onto the carriers under laboratory conditions in the form of aerosols produced by means of a Turn'n'Spray atomizer (Bürkle, GmbH). The experiment was conducted within the framework of two research cycles for each type of the carrier. Each cycle consisted of three series during which the two solutions were applied on each of the carriers; the control solution (cycle 1) and the silica marker (cycle 2). In the next step, the carriers were subject to the SEM/EDS microscopic analysis.

2.2. Sampling

2.2.1. Wastewater sampling site characteristics

Municipal wastewater samples were taken directly from the raw inflow to the WWTP. The sewage sampling point was located in the mechanical part of the technological WWTP system (Fig. 2).

(a) (b) (c) (d)

Figure 2. Urban waste water sampling location (a)(b) Mechanical grating station and wastewater inlet to biological reactors; (c)(d) Grating stand - mechanical part of the treatment plant.

Each time sampling was carried out after the grating station, directly before the equalization tank and the wastewater inlet to the biological reactor The locations of the wastewater collection point are shown in the figures below.

2.2.2. Frequency and method of sampling

Municipal wastewater samples were collected by employees of the Department of Water Protection and Department of Underground Research and Surface Maintenance of the Central Mining Institute. The samplers have undergone training and have knowledge and experience in environmental sampling, confirmed by an appropriate certificate. In order to minimize the risk of samples contamination including pathogens such as SARS-CoV-2, wastewater sampling was conducted under sanitary regime conditions. A total of 6 effluent samples were collected for analysis for the presence of SARS-CoV-2 in municipal wastewater. The collected samples were subjected to quantitative and qualitative analysis including evaluation of the concentration of isolated RNA of SARS-CoV-2 virus. Sampling was conducted during the period of increased incidence of COVID-19 in the region where the catchment is located. The sampling campaign was conducted from 6th to 17st December, 2021 (6.12.2021; 0.12.2021; 10.12.2021; 13.12.2021; 15.12.2021; 17.12.2021). Samples were taken at the treatment plant during the observed so-called morning peak. Sewage in the amount of 3x500 mL was collected with a hand scoop according to the method EN ISO 5667-10:2020 (ENISO5667-10:2020. Water quality…). Immediately after collection, wastewater samples were placed in a thermal transporter. Samples in volumes of 2x500 ml were transported to the microbiological laboratory for SARS-CoV-2 analysis. Samples delivered to the laboratory were frozen at -80°C. Virological analyses were performed simultaneously for all tested batches. At the same time, a batch of wastewater in value of 500 mL was transported to the Laboratory of Environmental Analysis of Central Mining Institute and was subjected to physicochemical analysis for selected contaminant indices.

2.3. Physicochemical analysis

2.3.1. Scope of analysis

The range of physical and chemical analyses included determination of the following parameters: temperature, pH, concentration of total suspended solids (TSS), phosphate phosphorus concentration (P-PO4), concentration of ammoniacal nitrogen (N-NH4), concentration of nitrate nitrogen (N-NO3), concentration of nitric nitrogen (N-NO2), chemical oxygen demand (COD).

2.3.2. Methodology

All selected physicochemical parameters were analyzed immediately after sampling. All tests were performed following the EN, or ISO standard protocols. If this wasn't possible, the wastewater samples were chemically stabilized and then measured within the approved timeline of appropriate standard protocols. The pH, temperature and conductivity were measured using an automatic field meter WTW MulitiLine® IDS instrument (EN ISO 10523; EN 27888: 1993-11). For other physicochemical and chemical parameter, the following test procedures were used: phosphate phosphorus concentration (P-PO4) - Analytical Method Spectroquant® Test Kits of Merck KGaA, № 1.114848; ammoniacal nitrogen (N-NH4) - Analytical Meth-od Spectroquant® Test Kits of Merck KGaA, № 1.4739, 1.1475 EN ISO 10523 2, № 1.14544, № 1.00683, № 1.14559; nitrate nitrogen (N-NO3) - Analytical Method Spectroquant® Test Kits of Merck KGaA, № 1. 109713, nitric nitrogen (N-NO2) - Analytical Method Spectroquant® Test Kits of Merck KGaA, № 1.14547, № 1.14776, № 1.00609; chemical oxygen demand (COD) - Analytical Method Spectroquant® Test Kits of Merck KGaA, № 1.114691.

2.4. Microbiological (virological) analysis

2.4.1. Sample processing for isolation and quantification of viral RNA

Previously frozen municipal wastewater samples were thawed at 4°C. For each batch of wastewater, three samples with_ a total value of 150 ml were further processed (3 x 50 ml). First, wastewater samples were centrifuged at 4°C, for 10 min at 600×g without break (Eppendorf® centrifuge 5801R), and the clear supernatant was harvested. In the next step, the samples were filtrated using a sterile syringe filter with a diameter 0,22 µm (PES/sterile). Preliminarily purified wastewater samples were then concentrated using Amicon® centrifugal ultrafiltration system (Amicon® Ultra-15 Centrifugal Filter Unit, Sigma). Each time during concentration, 15 mL of wastewater was added to the filter unit and centrifuged at 4°C for 10 min, at 6000×g, and the concentrated supernatant was harvested. The process was repeated until 45 ml of wastewater samples were completely concentrated (final volume of concentrated supernatant approximately 450 µl) and used for further RNA extraction, and the mass was determined (approx. 150 mg in influent and 5-10 mg in effluent). Contaminated equipment (e.g., filter units, tubes, reaction tubes) were stored in special waste containers and autoclaved according to the daily cleaning program in the microbiology laboratory.

2.4.2 RNA of SARS-CoV-2 virus extraction

RNA was isolated using the Viral DNA/RNA Virus kit A&A Biotechnology (34-200). According to the RNA isolation protocol included in the kit, 150 µl supernatant was mixed with lysis buffer supplemented with carrier RNA. After binding on silica membranes, samples were washed several times and eluted in total value of 50 µl RNase-free water. Isolated RNA was subjected to an RT-qPCR reaction.

2.4.3 SARS-CoV-2 specific quantitative RT-qPCR

RNA was analysed by One-step RT-qPCR using Luna Universal Probe One-Step RT-qPCR Kit (New England Biolabs) or LightCycler® Multiplex RNA Virus Master (Roche) and the CFX96 Real-Time System, with a C1000 Touch Thermal Cycler (Bio-Rad). Initial denaturation was performed for 1 min at 95°C followed by 45 cycles of denaturation for 10 s and combined annealing and extension for 30 s at 60°C. Reverse transcription was performed at 55°C for 10 min. Initial denaturation was allowed for 30 s at 95°C followed by 45 cycles of denaturation for 5 s, extension for 30 s at 60°C and final cool-down to 40°C for 30 s. The PCR runs were analysed with Bio-Rad CFX Manager software version 3.1 (Bio-Rad Laboratories). For quantifications, standard curves using plasmid DNA (RdRP) or in vitro transcribed RNA (M-gene) were used as described previously. To calculate from gene equivalents per reaction back to copies per mL, a PCR correction factor (cf) was determined with Luna Universal Probe One-Step RT-qPCR Kit (cf = 0.0037) and LightCycler® Multi-plex RNA Virus Master (cf = 0.0015), respectively. To control PCR, viral RNA of SARS-CoV-2 (isolate SARS-CoV-2-FFM1) was used as a positive control and water as a negative control.

3. Results & Discussion

During the analyzed period, the COD value in raw wastewater was in the range from 279,7 mgO_2/L to 298,6 mgO_2/L. The pH of the wastewater ranged from 6,56 to 6,89 with an average of 6,69. The maximum concentrations of ammonium nitrogen and nitrate nitrogen in the wastewater samples were 37,2 mg $N\text{-}NH_4/L$ and respectively 22,1 mg $N\text{-}NO_3/L$. The maximum recorded phosphorus concentration was 4,4 $mgP\text{-}PO_4/L$. Detailed data on the physicochemical parameters of wastewater are presented in Table 1.

Table 1.
Physical and chemical parameters of raw municipal wastewater

Sampling date	Samples ID	Physical and chemical parameters						
		Temp. [°C]	pH [-]	COD [mg/L]	$N\text{-}NH_4$ [mg/L]	$N\text{-}NO_3$ [mg /L]	$N\text{-}NO_2$ [mg/L]	$P\text{-}PO_4$ [mg/L]
06.12.2021	0071309/21	11	6,78	314,2	31,2	19,4	1,3	2,3
08.12.2021	0071508/21	12,5	6,56	310,2	29,4	15,4	1,4	2,2
10.12.2021	0072043/21	10,4	6,89	279,7	26,7	16,1	1	1,8
13.12.2021	0072365/21	10,2	6,44	384,5	35,8	17,5	2,1	4,3
15.12.2021	0073621/21	11,3	6,68	390,6	33,3	22,1	1,8	3,7
17.12.2021	0073315/21	9,6	6,79	398,6	37,2	17,4	1,5	4,4

Qualitative analysis confirmed the presence of the SARS-CoV-2 virions in each in each analysed sample. On the basis of the RT-qPCR analyses (Table 2), it was found that the content of the genetic material of the virus SARS-CoV-2 (ex-pressed as a number of copies of the gene coding nucleotide) in the raw wastewater inflow ranged from 5,4 cp/mL (5400 cp/L) to 168,90 cp/mL (168 900 cp/L). The lowest SARS-CoV-2 virus concentration was recorded on December 10th, while the highest amount of genetic material in the raw wastewater was preserved on 17th December, after the growth of SARS-CoV-2 virions observed in earlier samples (table 2).

Table 2.
Results of microbiological analysis for SARS detection in wastewater

<table>
<tr><th>Sampling date</th><th>Samples ID</th><th>Parameter</th><th>Unit</th><th>Results</th></tr>
<tr><td rowspan="3">06.12.2021</td><td rowspan="3">0071309/21</td><td>Presence of SarS-CoV-2 viruses</td><td>-</td><td>Possitive[1]</td></tr>
<tr><td>RdRP gene copy number</td><td>[cp/mL]</td><td>16,50</td></tr>
<tr><td>RdRP gene copy number</td><td>[cp/L]</td><td>16 500</td></tr>
<tr><td rowspan="3">08.12.2021</td><td rowspan="3">0071508/21</td><td>Presence of SarS-CoV-2 viruses</td><td>-</td><td>Possitive[1]</td></tr>
<tr><td>RdRP gene copy number</td><td>[cp/mL]</td><td>16,90</td></tr>
<tr><td>RdRP gene copy number</td><td>[cp/L]</td><td>16 900</td></tr>
<tr><td rowspan="3">10.12.2021</td><td rowspan="3">0072043/21</td><td>Presence of SarS-CoV-2 viruses</td><td>-</td><td>Possitive[1]</td></tr>
<tr><td>RdRP gene copy number</td><td>[cp/mL]</td><td>5,4</td></tr>
<tr><td>RdRP gene copy number</td><td>[cp/L]</td><td>5400</td></tr>
<tr><td rowspan="3">13.12.2021</td><td rowspan="3">0072365/21</td><td>Presence of SarS-CoV-2 viruses</td><td>-</td><td>Possitive[1]</td></tr>
<tr><td>RdRP gene copy number</td><td>[cp/mL]</td><td>53,5</td></tr>
<tr><td>RdRP gene copy number</td><td>[cp/L]</td><td>53 500</td></tr>
</table>

Cont. table 2.

15.12.2021	0073621/21	Presence of SarS-CoV-2 viruses	-	*Possitive*[1]
		RdRP gene copy number	[cp/mL]	53,5
		RdRP gene copy number	[cp/L]	53 500
17.12.2021	0073315/21	Presence of SarS-CoV-2 viruses	-	*Possitive*[1]
		RdRP gene copy number	[cp/mL]	168,90
		RdRP gene copy number	[cp/L]	168 900

[1]RNA of SASR-CoV-2 virus detected.

The presence of coronavirus in municipal wastewater samples has been confirmed by numerous scientific studies. So far, the presence of virus SARS-CoV-2 RNA in municipal wastewater has been confirmed by researches from Australia (Ahmed, Tscharke, Bertsch, Bibby, Bivins, Choi, Clark, Dwyer, Edison, Nguyen, Simpson, Sherman, Thomas, Verhagen, Zaugg, Muller, 2021), Brazil (Fangaro, Stoco, Souza, Grisard, Magri, Rogovski, 2019; Prado, Fumian, Mannario, Maranhão, Siqueira, Miagostovich, 2020), China (Cai, Xu, Lin, Young, Xu, Qu, 2020), the Czech Republic (Mlejnkova, Sovova, Vasickova, Ocenaskova, Jasikova, Juranova, 2020), Chile (Ampuero, Valenzuela, Valiente-Echeverria, Soto-Rifo, Barriga, Chnaiderman, 2020), France (Wurtzer, Marechal, Mouchel, Moulin, 2020), the Netherlands (Medema, Heijnen, Elsinga, Italiaander, Brouwer, 2020), India (Arora, Nag, Sethi, Rajvanshi, Saxena, Shrivastava, Gupta, 2020), Israel (Bar-On, Weil, Indenbaum, Bucrid, Bar-Illan, Elul, Levi, Aguvaeu, Cohen, Shirazi, Erster, Brown, Sofer, Mor, Mendelson, Zuckerman, 2021), Italy (La Rosa, Iaconelli, Mancini, Bonanno, Venerci, Bonadonna, Lucentini, Suffredini, 2020), United States (Wu, Xiao, Zhang, Gu, Lee, Kauffman, et.al, 2020) and Turkey (Kocamemi, Kurt, Haciogul, Yarali, Saatci, Pakdemirli, 2020). Similar results for the number of virus copies in municipal wastewater samples were obtained by Castiglioni et.al (Castiglioni, Schiarea, Pek-llegrinelli, Primache, Galli, Bubba, Manicelli, Marinelli, Ammoni, Pariani, Zuccato, Binda, 2020). In samples collected from the Milan WWTP and analyzed during March-June 2021 the concentration of_ virus copies per litre remained in the range from 13 900 cp/L to 147 000 cp/L. During the same period (March-June 2020), the detection of SARS-CoV-2 in municipal wastewater from WWTP in Lombardia confirmed the presence of the virus in the concentration range 1060-10 200 cp/L (Castiglioni, Schiarea, Pekllegrinelli, Primache, Galli, Bubba, Manicelli, Marinelli, Ammoni, Pariani, Zuccato, Binda, 2022).

The relationship between the pollutant load entered into the treatment plants and the virus concentration is shown in Figure 3. The presented data show that_ for wastewater samples with one of the highest COD values, the highest number of SARS-CoV-2 copies were observed. The maximum virus concentration (168,9 cp/mL) was recorded at the COD concentration of 398,6 mgO_2/l. Taking into account the fact that samples were taken at the same time with uniform wastewater flow, the higher COD value proves higher organic and inorganic pollutant load delivered to the sewage treatment plant by the sewer system. The higher contaminant load proves a higher concentration of fecal (faecal) and urine samples, which are the carrier of coronavirus. Presented data show that for less concentrated wastewater samples (with lower pollutant loads), lower viral RNA concentrations were obtained.

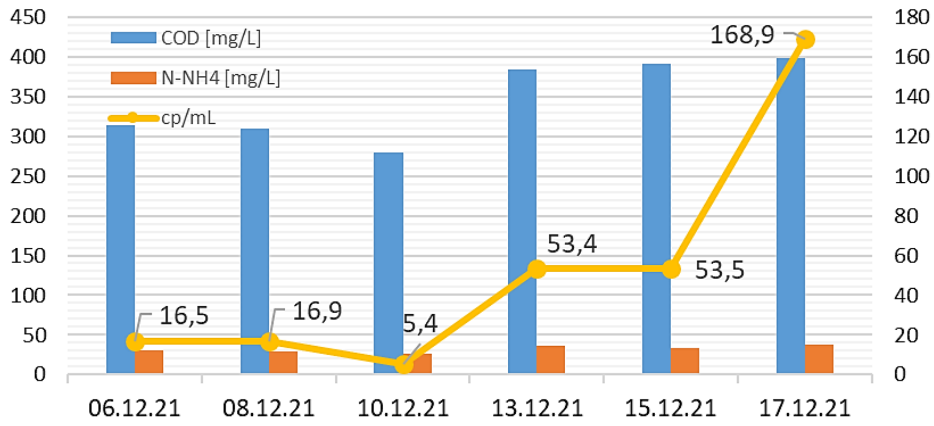

Figure 3. Number of COVID-19 infections – case study for Poland.

Based on the obtained results of physicochemical parameters of wastewater, it can be concluded that the quality of wastewater has a significant impact on virus RNA concentration, which directly affects the sensitivity, viability and reproducibility of the SARs-CoV-2 virus in the same volume of analyzed wastewater.

As part of the research, the relationship between the concentration of coronavirus in municipal wastewater and the recorded number of new COVID-19 cases in the local district in which the WWTP is located was assessed. It should be noted that epidemiological data are collected in relation to territorial units. Data from the sanitary inspection as well as medical data (number of hospitalized people), are collected and presented in reference to districts. Thus, it is not possible to obtain epidemic data on the daily number of cases in the sewage catchment area. The study referred to epidemiological data illustrating the daily number of new COVID-19 cases in the district. The table (Table 3) and graphs (Figure 5-7) show that during the 4th pandemic wave in Poland (Figure 4), the disease curve in terms of nation-al, voivodeship and local level has a similar course. A visible increase in the number of cases is recorded in days, respectively 8-9th December; 15th December; 22nd December and 29th December.

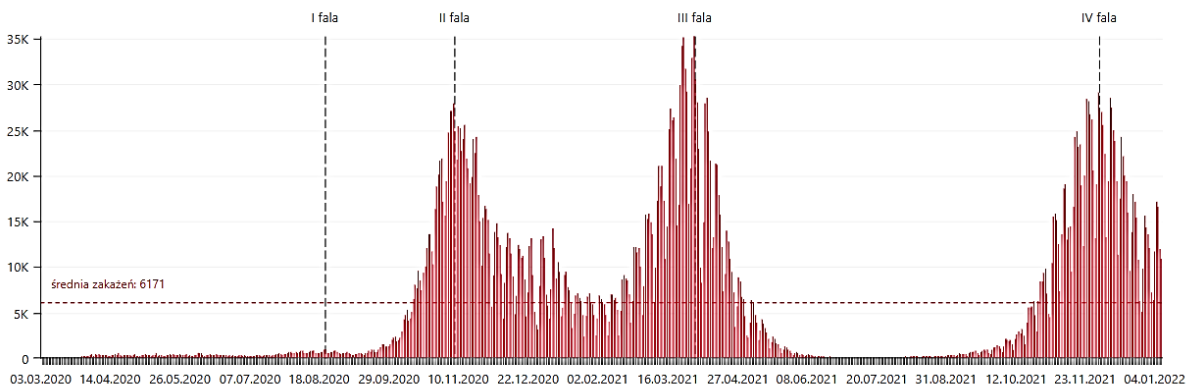

Figure 4. Number of COVID-19 infections – case study for Poland.

Table 3.
Number of new COVID-19 cases, deaths and recoveries reported in Poland, Śląskie Voivodship and ana-lysed district during December 2021

Date	Poland*			Śląskie Voivodship*		Local district*		
	Infections	Deaths	Recoveries	Infections	Deaths	Infections	Deaths	Recoveries
2021-12-01	29064	570	10077	4147	52	131	2	33
2021-12-02	27356	502	17690	3626	53	121	0	30
2021-12-03	26965	470	25041	3846	52	120	0	117
2021-12-04	25576	502	25616	3361	45	69	2	99
2021-12-05	22389	45	25390	3448	3	143	0	128
2021-12-06	13250	25	25489	1636	4	60	0	153
2021-12-07	19366	504	16922	2032	81	52	1	71
2021-12-08	28542	592	10055	4233	56	119	1	63
2021-12-09	27458	562	16827	3708	46	123	0	32
2021-12-10	24991	571	25459	3667	51	78	0	134
2021-12-11	23764	486	26546	3098	50	84	0	118
2021-12-12	19452	65	25554	3044	3	125	0	152
2021-12-13	11379	29	25126	1427	0	65	0	40
2021-12-14	17460	537	19070	1989	101	30	2	143
2021-12-15	24266	669	10909	3620	79	156	0	81
2021-12-16	22097	582	17306	2901	67	94	0	75
2021-12-17	20027	566	24693	2972	88	83	6	115
2021-12-18	19397	543	26092	2580	58	77	7	108
2021-12-19	15976	70	23670	2327	6	111	0	109
2021-12-20	9609	29	22887	1360	0	43	0	47
2021-12-21	13806	538	16527	1649	104	44	2	161
2021-12-22	18021	775	8955	2424	82	74	0	27
2021-12-23	17156	616	16218	2203	84	65	9	78
2021-12-24	15392	596	22246	2052	87	42	0	113
2021-12-25	10788	269	21012	1343	37	32	1	112
2021-12-26	6252	16	19606	853	0	14	0	85
2021-12-27	5029	38	18862	671	0	21	0	57
2021-12-28	9843	549	13618	1054	70	31	0	135
2021-12-29	15571	794	6788	2116	88	55	1	40
2021-12-30	14325	709	11588	2024	75	52	1	36
2021-12-31	13601	638	15829	1642	99	62	0	116

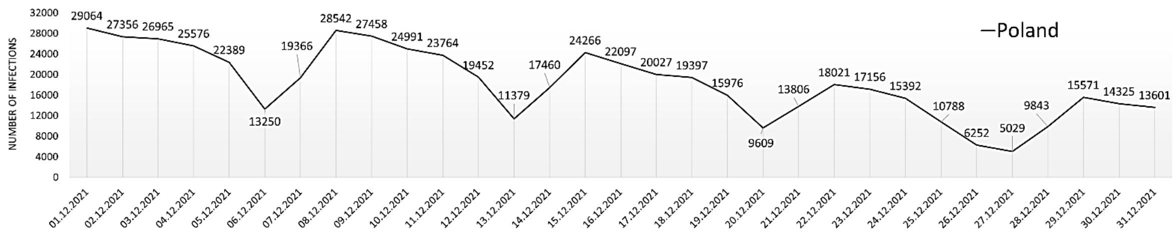

Figure 5. COVID-19 incidence curve for country during December 2021.

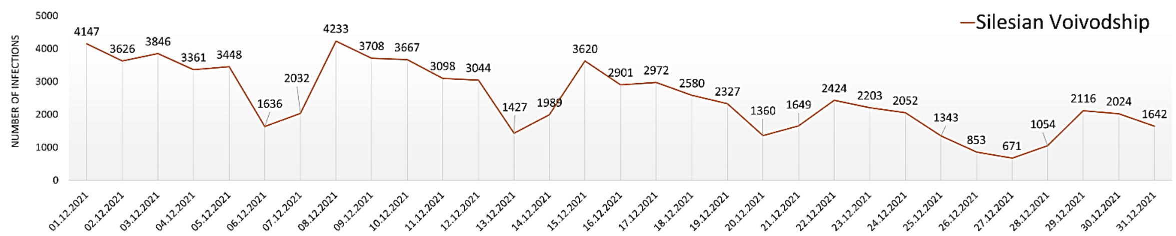

Figure 6. COVID-19 incidence curve for Śląskie Voivodship during December 2021.

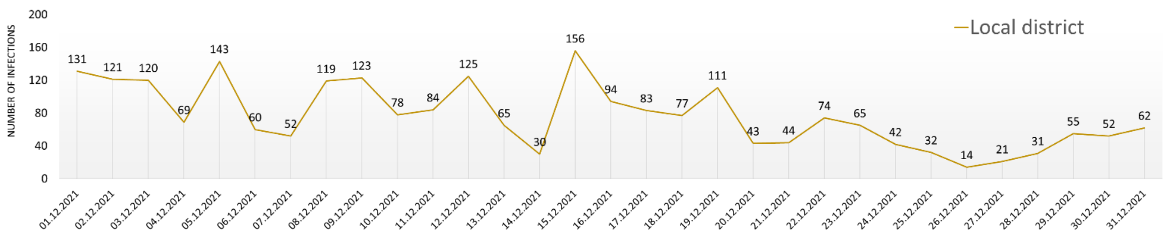

Figure 7. COVID-19 incidence curve for analysed district during December 2021.

Despite the fact that it is not possible to directly compare the results obtained in the virological analysis with epidemiological data for analyzed sewage catchment, positive results in the field of SARS-CoV-2 detection confirm the fact that the sewage reflects the health condition of the population living in the analyzed area. The results of the study indicate that raw wastewater may be an important tool for predicting COVID-19 epidemics. However, it should be noted, that the obtained data are based on a small number of repetitions and were carried out in a short time horizon. Thus, in order to obtain a full picture of the relationship, it would be necessary to extend the period of conducted analyzes and obtain more detailed epidemic data. Additionally, virus detection methods based on the RT-qPCR have shown that, depending on numerous environmental factors SARS-CoV-2 can exist in different forms: active virus, inactive virions or even inactive RNA fragments (Wurtzer, Marechal, Mouchel, Moulin, 2020). Thus, SARS-CoV-2 detection should take into account not only quantitative but also qualitative analysis of the pathogen.

4. Conclusions

Over the decades the Wastewater Based Epidemiology has become a viable and frequently used by the professional community as an alternative tool for assessing the population's consumption of chemical and pharmacological agents such as drugs, alcohol, nicotine, or caffeine. However, because of the need to rapidly assess population exposure to an-other harmful agent of microbial origin, the WEB-based methodology has found a new application in pandemic times. Although numerous studies prove its effectiveness, it should be kept in mind that the correlation between the amount of SARS-CoV-2 RNA in raw sewage and the number of new infections in the analyzed catchment allows predicting the course of the pandemic only when based on reliable data showing the actual health status of the population. The studies cited in the paper confirming the applicability of the WEB methodology for assessing the degree of pandemic development, each time were conducted for a specific and well-identified catchment for which the authors had reliable epidemiological reports of daily incidence. However, for many sewer catchments, it is not possible to obtain such detailed data showing the real incidence of COVID-19 in the population. The paper cites actual results for the amount of

SARs-CoV-2 in municipal wastewater for an urban catchment for which it is not possible to obtain such detailed epidemiological data. Obtaining detailed population morbidity data down to the city or district level is in many cases impossible. The data presented by the relevant services usually refer to the area of the voivodeship or the district, thus illustrating the health status of the community in somewhat broader terms. However, often despite the impossibility of establishing a correlation between the number of virus copies in wastewater and the number of new COVID-19 cases recorded, it must be borne in mind that microbiological characteristics of wastewater are a reflection of the health situation of the inhabitants of the catchment. However detailed data are a necessary element of the model which can be success-fully used not only to confirm the epidemiological situation but, also to track the directions of the pandemic development.

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