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Removal capacities of wastewater treatment plants to minimize spread of enteroviruses

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ABSTRACT

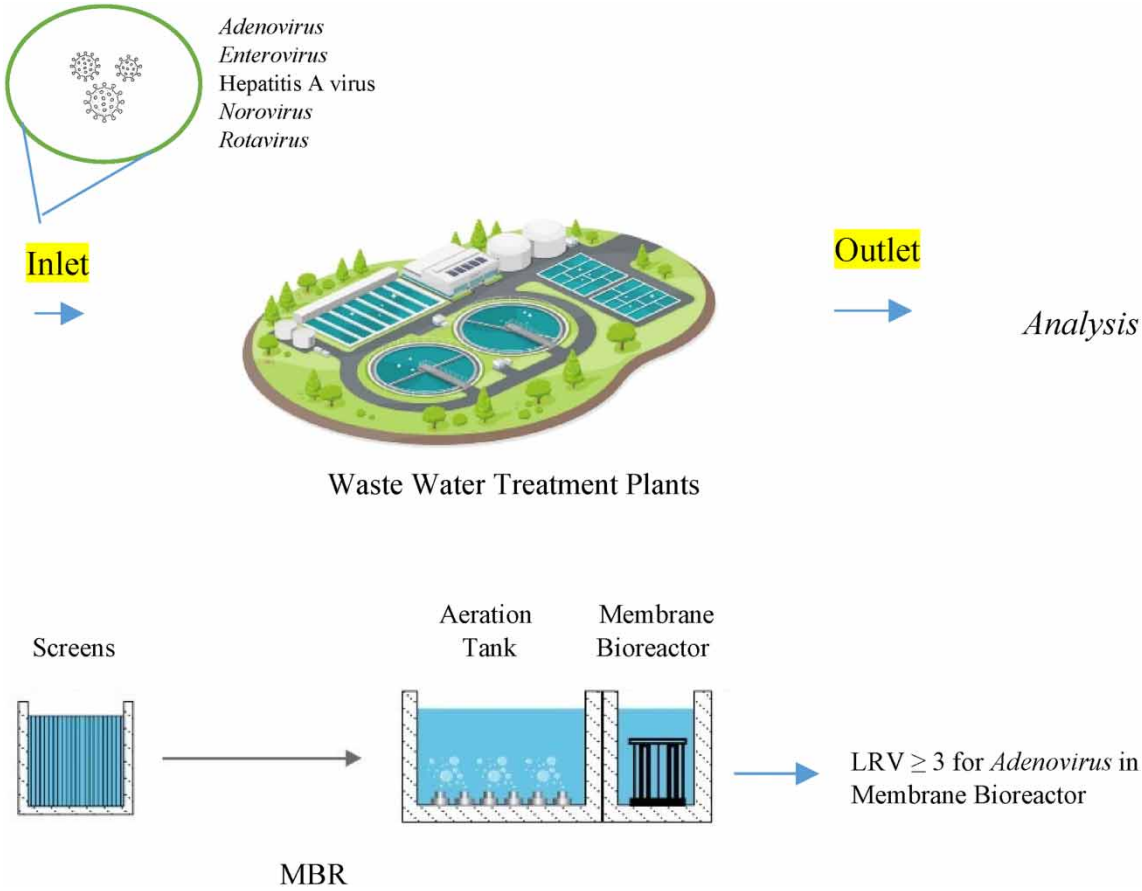
The enteroviruses such as *Adenovirus*, *Enterovirus*, Hepatitis A virus, *Norovirus*, and *Rotavirus* are mostly transmitted via water and are commonly seen in Turkey. Due to lack of regulations for monitoring these viruses at the discharge points of wastewater treatment plants (WWTPs), the discharges reach to surface waters causing water-borne diseases. This emphasizes the importance of WWTPs' removal capacities to minimize the spread of enteroviruses. To evaluate the removal capacities of enteroviruses, five different types of WWTPs from Ankara-Turkey were investigated over a year. Seasonal abundances of enterovirus-specific genes in both influents and effluents from each WWTP were determined by quantitative polymerase chain reaction (qPCR). The reduction of enteroviral nucleic acids in the effluent wastewater samples compared to the influent wastewater samples was assessed as the removal capacity in $\log^{10}$ reduction values (LRVs). The qPCR results revealed that among five different types of WWTPs tested, membrane bioreactors effectively removed *Adenovirus* alone with LRVs more than 3. The removal of *Enterovirus*, Hepatitis A virus, *Norovirus*, and *Rotavirus* in WWTPs with varying processes was below LRV 3.

Key words: *Enterovirus* removal, hepatitis a virus, *Norovirus*, *Rotavirus*, wastewater treatment plants

HIGHLIGHTS

- Five different types of WWTPs were investigated to evaluate *Enterovirus* removal capacities.
- *Enteroviruses*-specific genes in influents and effluents were determined by qPCR.
- Among five different types of WWTPs tested, MBR effectively removed *Adenovirus* only.

GRAPHICAL ABSTRACT



INTRODUCTION

The enteroviruses *Adenovirus*, *Enterovirus*, Hepatitis A virus, *Norovirus,* and *Rotavirus* are mostly transmitted via water and are commonly seen in Turkey (Alp & Kuleaşan 2018; Dağ Güzel *et al*. 2023). Among them, *Adenovirus*es are non-enveloped, double-stranded DNA viruses with a diameter of 90–100 nm. The individuals infected with *Adenovirus* are extremely contagious during the incubation period. *Adenovirus*es may cause respiratory infection epidemics (Crenshaw *et al.* 2019). *Enterovirus*es are single-stranded, non-enveloped RNA viruses, and have a size of ~ 30 nm in diameter. Beside respiratory illnesses and gastroenteritis, *Enterovirus* may cause meningitis and encephalitis (Chen *et al.* 2020). Hepatitis A virus is a non-enveloped, single-stranded RNA virus, and has a size of 27–32 nm in diameter. This virus cause liver infection known as hepatitis A (Sridhar 2023). *Norovirus*es are non-enveloped, single-stranded RNA viruses with a diameter of 29–35 nm. *Norovirus*es are highly contagious and cause acute gastroenteritis (Boussettine *et al.* 2020). *Rotavirus*es are non-enveloped, double-stranded RNA viruses with a diameter of ~70 nm. *Rotavirus* is the most common cause of childhood acute gastroenteritis worldwide (LeClair & McConnell 2023). Viral infections yield a high number of virions that lead to a variety of diseases. Among them, enteric viruses appear in large numbers in fecally-contaminated waters and in vegetables exposed to them. Once infected, enteric viruses replicate in intestinal systems causing gastroenteritis and water-borne epidemics worldwide. Therefore, they are mostly transmitted by the fecal–oral route or through direct human contact (Haramoto *et al.* 2018). Due to excretion by infected individuals they end up in sewage systems. Apart from pathogenic viruses, water-borne viruses originating from food industry, animal husbandry, seasonal runoffs, and other sources also appear in wastewaters. Enteric viruses, especially, spread over raw/treated wastewaters and receiving water bodies. The water sources, on the other hand, can be used for agriculture, animal husbandry, potable water production, and aquifer feeding. Sludge from wastewater treatment plants (WWTPs) is also used as a soil conditioner or fertilizer (Corpuz *et al*. 2020).

Although wastewater treatment involves physical, biological, and chemical processes these may not be sufficient to remove viruses since WTTPs are mostly not designed for the efficient removal of viruses. Additionally, WWTP discharges carrying pathogenic viruses are of great public concern. However, there is scarce information about the removal of viruses in WWTPs with varying processes. Because traditional WWTPs are primarily designed for the removal of bacteria, organic matter and chemicals, they are not very effective for the removal of viruses. Moreover, viruses, smaller than bacteria, and other microorganisms require specialized methods to eliminate them. There is a growing need to understand the fate of viruses in treatment processes (Ahmad *et al.* 2021). For this reason, in this study, conventional activated sludge (CAS), biological nutrient removal (BNR), sequencing batch reactor (SBR), a WWTP with coagulation–flocculation and UV disinfection unit (CoFlUV), and membrane bioreactor (MBR) types of WWTPs were analyzed for their removal efficiency of enteric viruses, namely *Adenovirus*, *Enterovirus*, Hepatitis A virus, *Norovirus*, and *Rotavirus*.

METHODOLOGY

Sampling of WWTPs

In this study, five WWTPs with varying processes such as CAS, BNR, SBR, CoFlUV units, and MBR located in Ankara-Turkey were sampled (Figure 1). Samples were collected from influents and effluents of each facility (Figure 2). Additionally, sludge samples were only taken from CAS. Average operational parameters and influent and effluent water qualities of the WWTPs tested are given in Tables 1 and 2. The CAS system sampled included preliminary treatment, primary treatment, secondary treatment, and anaerobic sludge digester. The BNR process consisted of an A^2O system with consecutive anaerobic, anoxic, and aerobic tanks. The SBR system included aeration, mixing, and settling steps. The WWTP with CoFlUV units was located in the industrial zone of Ankara and it treated both municipal and industrial wastewater. This system consisted of screening, aerated grit chamber, equalization basin, consecutive coagulation and flocculation tanks, final clarifier, and an UV disinfection unit. The MBR system contained membranes with pore size of 0.038 μm.

Water/sludge sampling and fixation

Between the years 2021 and 2022, each WWTP was seasonally sampled by taking 1 L of water and sludge in triplicate and placing them in sterilized bottles. For sample fixation and viral RNA and DNA extraction, the collected samples were delivered to the laboratory in a cooling box within 2 h. For each season, 33 samples were collected, and a total of 132 samples, including influent, effluent, and sludge samples, were analyzed for all the enteric viruses tested. Sample fixations were done within 24 h of sampling. The collected water samples were concentrated by polyethylene glycol (PEG) precipitation method (Beyer *et al.* 2020). In this method, 80 g of PEG 6000 (Sigma-Aldrich) and 17.5 g of NaCl (BioShop) were added to 1 L of the

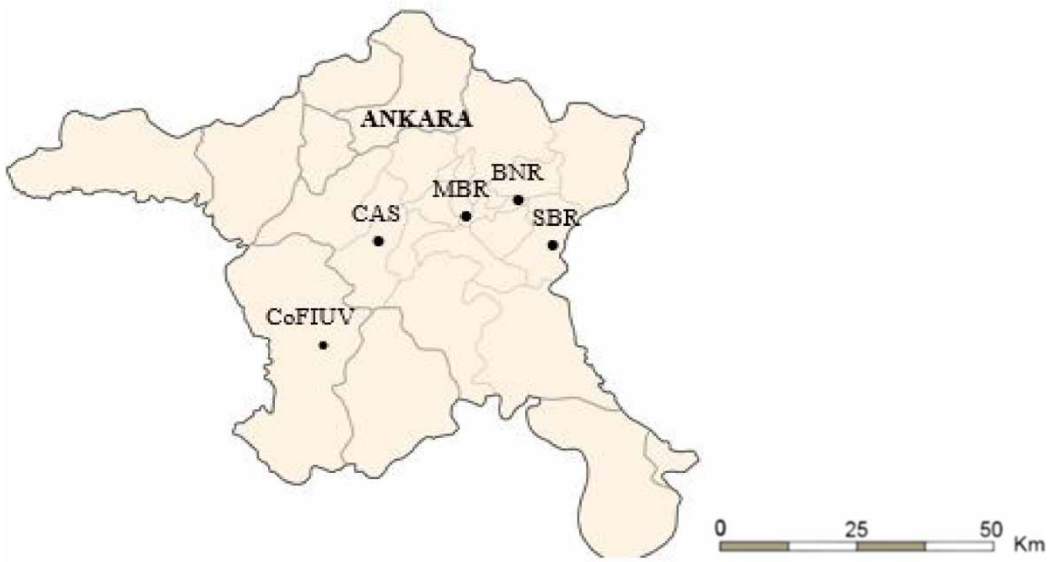


Figure 1 | Locations of WWTPs tested in Ankara-Turkey.

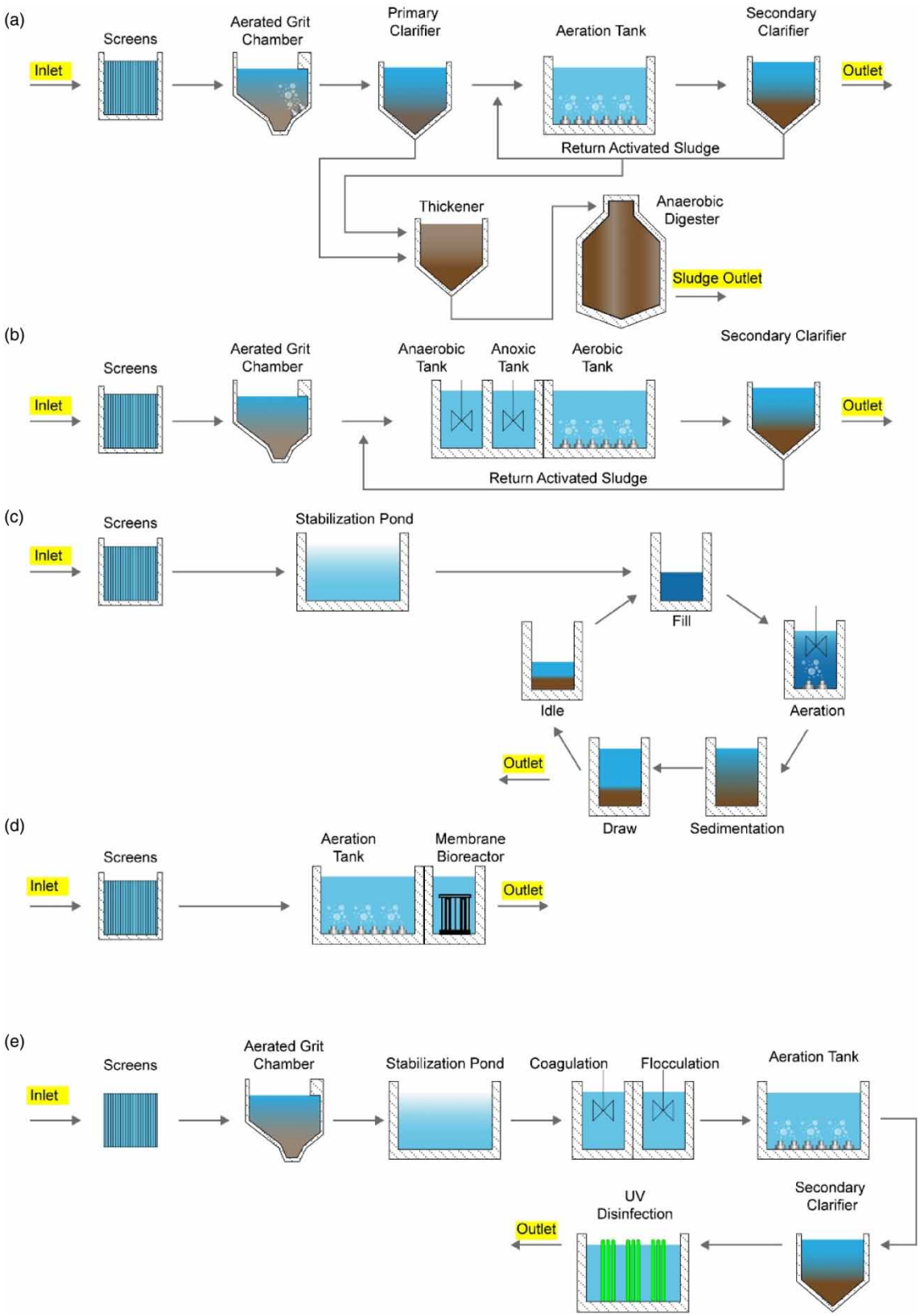


Figure 2 | Sampling points of wastewater treatment plants. (a) Conventional activated sludge (CAS); (b) biological nutrient removal (BNR); (c) sequencing batch reactor (SBR); (d) membrane bioreactor (MBR); (e) WWTP with coagulation–flocculation and UV disinfection unit (CoFlUV) (Onursal & Icgen 2023).

Table 1 | Average influent and effluent water qualities of the WWTPs sampled

Water quality	pH		TSS (mg/L)		COD (mg/L)		BOD (mg/L)		Total N (mg/L)		PO_4-P (mg/L)	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
CAS	7.7	8.0	297	24	402	52	240	19	56	39.0	5.0	1.2
BNR	7.7	8.0	207	18	334	27	183	15	28.8	9.0	5.8	2.0
SBR	8.0	7.6	177	24	352	49	201	19	na	na	na	na
CoFlUV	8.5	6.0	600	200	2,500	400	800	100	na	na	na	na
MBR	7.3	7.7	na	na	426	19	250	Nd	na	na	na	na

TSS, total suspended solids; COD, chemical oxygen demand; BOD, biochemical oxygen demand; nd, non-detected; na, data not available.
Coagulant: $FeCl_3$; UV dosage: 15 mJ/cm^2.

Table 2 | Average operational parameters of the WWTPs sampled

WWTP			Operating parameters			
	Flow rate (m^3/d)	MLSS (mg/L)	HRT (h)	SRT (d)	RAS (%)	WW source
CAS	765,000	1,730	12	3	60	Dom
BNR	41,818	4,125	28	16	85	Dom
SBR	3,000	5,216	8	na	–	Dom
CoFlUV	10,000	400	27	27	100	30% Dom
MBR	15,000	4,180	18	na	na	Dom

MLSS, mixed-liquor suspended solids; HRT, hydraulic retention time; SRT, solids retention time; RAS, return activated sludge; WW, wastewater; h, hour, Dom, domestic; na, data not available; nd, non-detected.

wastewater sample and mixed using a magnetic stirrer for 1 h and then stored at 4 °C overnight. Samples were centrifuged for 1 h at 12,200 g with a Thermo Scientific SL 16R device and pellets were collected. The collected pellets were suspended in 15 mL of 10× Phosphate-Buffered Saline (PBS) (BioShop), 150 μL of Tween-80 (BioShop), and 15 mL of chloroform (Merck). Then, the samples were centrifuged for 15 min at 1,400 g and the upper phase was taken to remove the chloroform in the lower phase. The remaining pellets were suspended with 0.05 mol/L of glycine (pH = 7.2) and 3% beef extract. Finally, the centrifugation process was repeated and the supernatants were combined. Concentrated samples were stored at −20 °C until nucleic acid extraction. Sludge samples were prepared as in the study by Prado *et al.* (2014). The collected sludge samples were mixed using a magnetic stirrer at room temperature for 20 min after adding 10% beef extract in a volume of 1:10. Then, the supernatant of the samples was centrifuged at 4,000 g for 30 min and cleared of contamination by passing through a low-protein binding filter (0.22 μm) (Merck Millipore vacuum filter and pump, Whatman® polycarbonate filter discs 0.2 μm). Concentrated samples were stored at −20 °C for a maximum of 3 months until nucleic acid extraction.

Nucleic acid extraction

Nucleic acid extraction of water and sludge samples was performed using the viral RNA/DNA isolation kit (EURX GeneMATRIX, Poland). Isolated viral nucleic acids were stored at −20 °C for a maximum of 3 months for further analysis. The quality and concentration of extracted DNA were determined by Nano drop (Colibri, Berthold, Germany) microvolumetric light spectroscopy and 2.5% agarose gel electrophoresis (Bio-Rad, USA) (Wen *et al.* 2019). The quality and quantity of nucleic acid samples were examined by looking at their absorbance at 260/280 nm and 260/230 nm wavelengths ratios on Nano drop. The absorbance at 260/280 ratio of ~1.8 is generally accepted as pure for DNA; a ratio of ~2.0 is generally accepted as pure for RNA. Expected 260/230 values should be in the range of 2.0–2.2 (Mathieson & Thomas 2013). DNA and RNA samples were selected in accordance with these ratios. Agarose gel electrophoresis analyses were used as an electrical conducting agent. Agarose gel was prepared as follows: 10× Tris-Borate-EDTA (TBE) buffer containing 108 g/L of Tris (BDH, UAE), 55 g/L of boric acid (BioShop, Canada) and 40 mL/L of EDTA (Sigma-Aldrich, Germany) (pH 8) were mixed and diluted to 1×. To prepare 2.5% concentration of Agarose gel, 20 g of Agarose was added to 1 L 1× of TBE Buffer. Then, 40 mL of this solution was used to make the Agarose gel. 2.5 μL RedSafe™ dye (INtRON Biotechnology, South Korea) was used to dye the gel (Wen *et al.* 2019).

Reverse transcription of viral RNA to cDNA

Since *Enterovirus*, Hepatitis A virus, *Norovirus*, and *Rotavirus* have RNA as the genetic material, complementary DNAs (cDNA) of these viruses were obtained through reverse transcription (RT). The RT process used was based on the protocol published by Osuolale & Okoh (2015). 20 μL of the sample obtained from RNA extraction was taken, 2 μL of random hexamer primer, 2 μL of dNTP mixture, 4 μL of diethylpyrocarbonate-treated (DNAse-, RNAse-, and Nuclease-free) water, 8 μL of 5× RT buffer solution, 1 μL of reverse transcriptase (EURx, smART) were added. Then, the mixture was heated for 10 min at 25 °C, 30 min at 60 °C, and 5 min at 85 °C (Osuolale & Okoh 2015). To increase RT efficiency, an RNase inhibitor was also used which provided the RT enzyme. The enzyme had reduced RNase H activity that cleaves the RNA of RNA/DNA hybrids. For higher RT efficiency, random hexamer primers were used, due to the ability of random primers to transcribe 5′ ends of viral genes. The amount and quality of cDNA contained in the sample obtained as a result of the reaction was determined by

observing the absorbance values at 260- and 280-nm wavelengths and then diluted 10 times before applying polymerase chain reaction (PCR) by using a T100 Thermal Cycler (Bio-Rad, USA) (La Rosa & Muscillo 2013).

Qualitative analyses of viral DNA and cDNA

The PCR reactions were performed in 25 μL of reaction mixtures. 5 μL (100 ng/μL) of DNA was added to 20 μL of PCR mixture. The mixture contains 2 mM of reaction buffer solution (Solis BioDyne, Estonia), 2 mM of $MgCl_2$ solution (Solis BioDyne, Estonia), dNTP mix (NewEngland BioLabs), forward, and reverse primer sets specific for each enteric virus (Table 3) and 0.2 μL of Taq DNA polymerase (FIREPol) and ultrapure water. Negative controls were used in each PCR reaction, and reproducibility was confirmed through duplicate reactions. The amplicons obtained as a result of PCR were run on 2.5% agarose gel electrophoresis at 90 V (BIO RAD PowerPac Basic device). The molecular weight of the amplicons was calculated using a 50-bp DNA ladder (EUR X, Perfect Plus) which was loaded in each agarose gel.

Quantitative analyses of viral DNA and cDNA

The quantitative PCR (qPCR) process was performed for the quantitative analyses of viruses. A BioRad CFX Connect 96 (USA) instrument was used for the qPCR analyses. The amount of 20 μL reaction mixture was prepared for qPCR analysis, including 3 μL of template DNA, 10 μL of Promega GoTaq® qPCR Master Mix (USA), and forward and reverse primers. Negative controls were included in each qPCR to assess non-specific amplifications. Purified PCR products were used to construct standard curves required for quantification (Osuolale & Okoh 2017). These PCR products were obtained by conventional PCR for each virus, using the primer pairs specified in Table 3. PCR products were run on the agarose gel and then were extracted and purified using the NucleoSpin® Gel and PCR Clean-up kit. The concentrations of purified PCR products were measured by microvolumetric light spectrophotometry. The copy numbers were calculated using the following Equation (1) as previously stated by Mijatovic-Rustempasic *et al.* (2013).

$$\frac{\text{Copy number of DNA}}{\mu\text{L}} = \frac{b*c}{L*a*10^{12}} \tag{1}$$

In the equation, the letters *a, b, c,* and L represent the weight of kb DNA per pmol (1 kb DNA = 0.66 μg/pmol), Avogadro number (6.022×10^{23}/mol), the concentration of template in μg/μL and length of template containing the target gene, respectively. A dilution series was prepared from the template of known quantity with a dilution coefficient of 1/10. Dilution series, calculated separately for each gene and with known gene copy numbers, were used as template DNA to create standard curves for quantification in the qPCR. The qPCR assay was performed according to the following steps: initial denaturation at 95 °C for 5 min, following 45 cycles of denaturation at 95 °C for 15 s, annealing for 30 s, and elongation at 72 °C for 30 s. To

Table 3 | Primers used in the study

	Primer name	Sequence (5′ → 3′)	Amplicon size (bp)	Annealing temperature (°C)	References
Adenovirus	JTVX-F	F- GGACGCCTCGGAG TACCTGAG	96	55	Osuolale & Okoh (2015)
	JTVX-R	R- ACIGTGGGGTTTCTGAACTTGTT			
Enterovirus	EQ-1	F- ACATGGTGTGAAGAGT CTATTGAGCT	142	58–60	Connell *et al.* (2012)
	EQ-2	R- CCAAAGTAGTCGGTTCCGC			
Hepatitis A virus	HAV68 HAV240	F- TCACCGCCGTTTGCCTAG R- GGAGAGCCCTGGAAGAAAG	191	60	Cigor & Okoh (2012)
Norovirus	COG2F	F-CARGARBCNATGTTYAGR TGGATGAG	98	54	Xue *et al.* (2013)
	COG2R	R- TCGACGCCATCTTCATTCACA			
Rotavirus	NVP3-F NVP3-R	F- ACCATCTWCACRTRACCCTC R- GGTCACATAACGCCCCTATA	87	60	Mijatovic-Rustempasic *et al.* (2013)

generate the melting curves at the end of the cycles, the tubes were gradually heated from 65 to 95 °C. The DNA samples were analyzed in triplicate. The specificity of the products was checked with R^2 values and melting curves. For all the standard curves, R^2 values were higher than 0.95.

Statistical analyses

Virus removal efficiencies for each different wastewater treatment plant were measured via logarithmic removal values (LRV). These values were determined by taking the logarithm of the ratio of virus concentrations (C) at the C_{inlet} and C_{outlet}

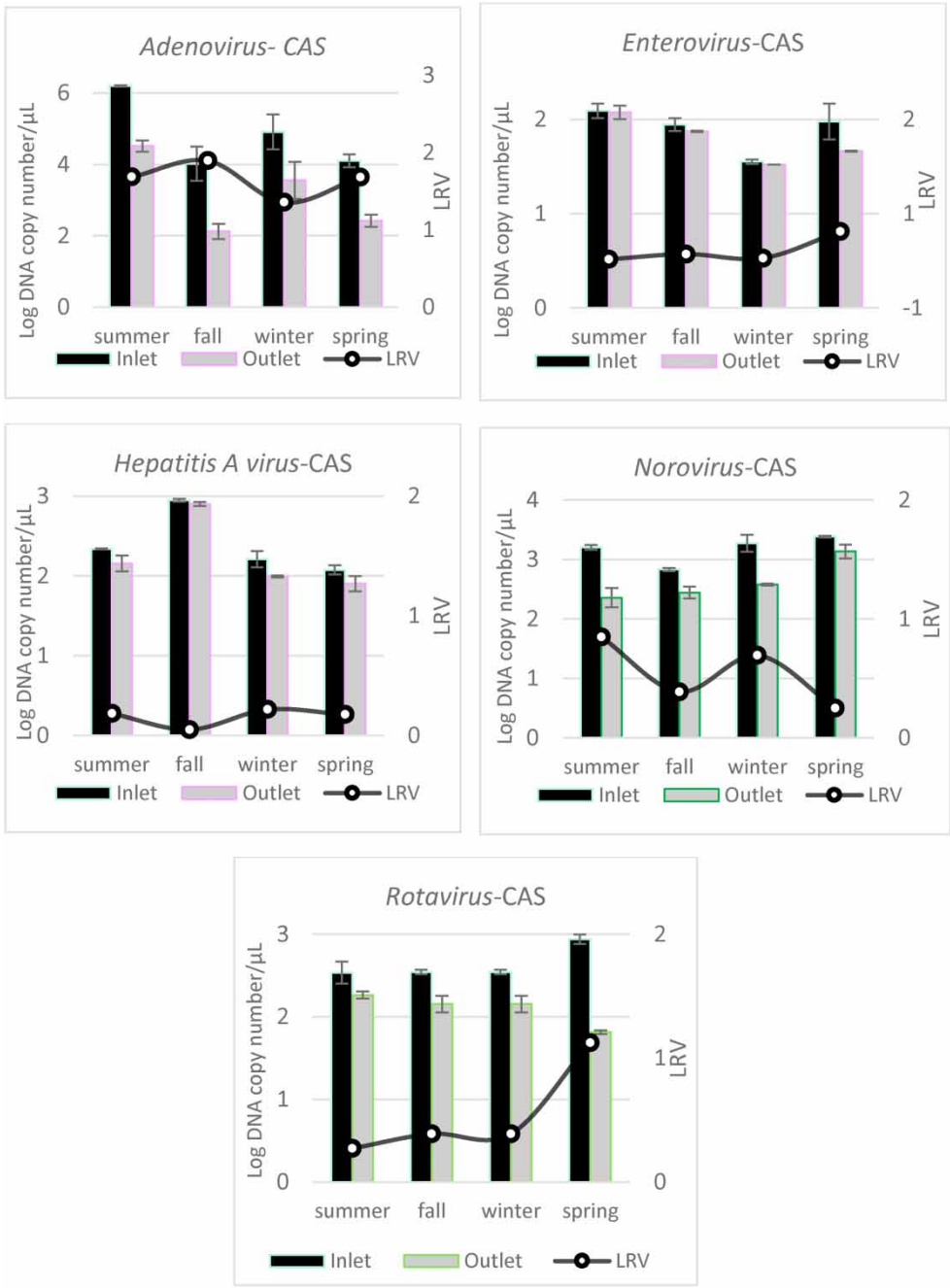


Figure 3 | Seasonal LRVs for enteric viruses in the CAS. Error bars correspond to the standard deviations.

of the wastewater treatment plant, as given in Equation (2).

$$\mathrm{LRV} = \log_{10}(C_{\mathrm{inlet}}/C_{\mathrm{outlet}}) \tag{2}$$

LRVs 1, 2, 3, 4, and 5 correspond to 90, 99, 99.9, 99.99, and 99.999% of target virus removal efficiencies, respectively. This pattern is followed as LRVs grow (Von Sperling *et al.* 2020). The World Health Organization (WHO) recommends 2–3 LRVs for reclaimed wastewater used in restricted irrigation and 6–7 LRVs for unrestricted irrigation (Sano *et al.* 2016). In Turkey, there are no current regulations regarding viral removal, therefore, LRV 3 was selected as a baseline for efficient removal in this study. WWTPs with an LRV below 3 were assumed to be not fully effective and release viruses at discharge points. Seasonal removal variations of viruses were also evaluated by the one-way analysis of variance (ANOVA) and Tukey's post hoc test (SPSS Statics for Windows v.24.0; IBM Corp., Armonk, NY) at a significance level of $p < 0.05$. The LOD value for each

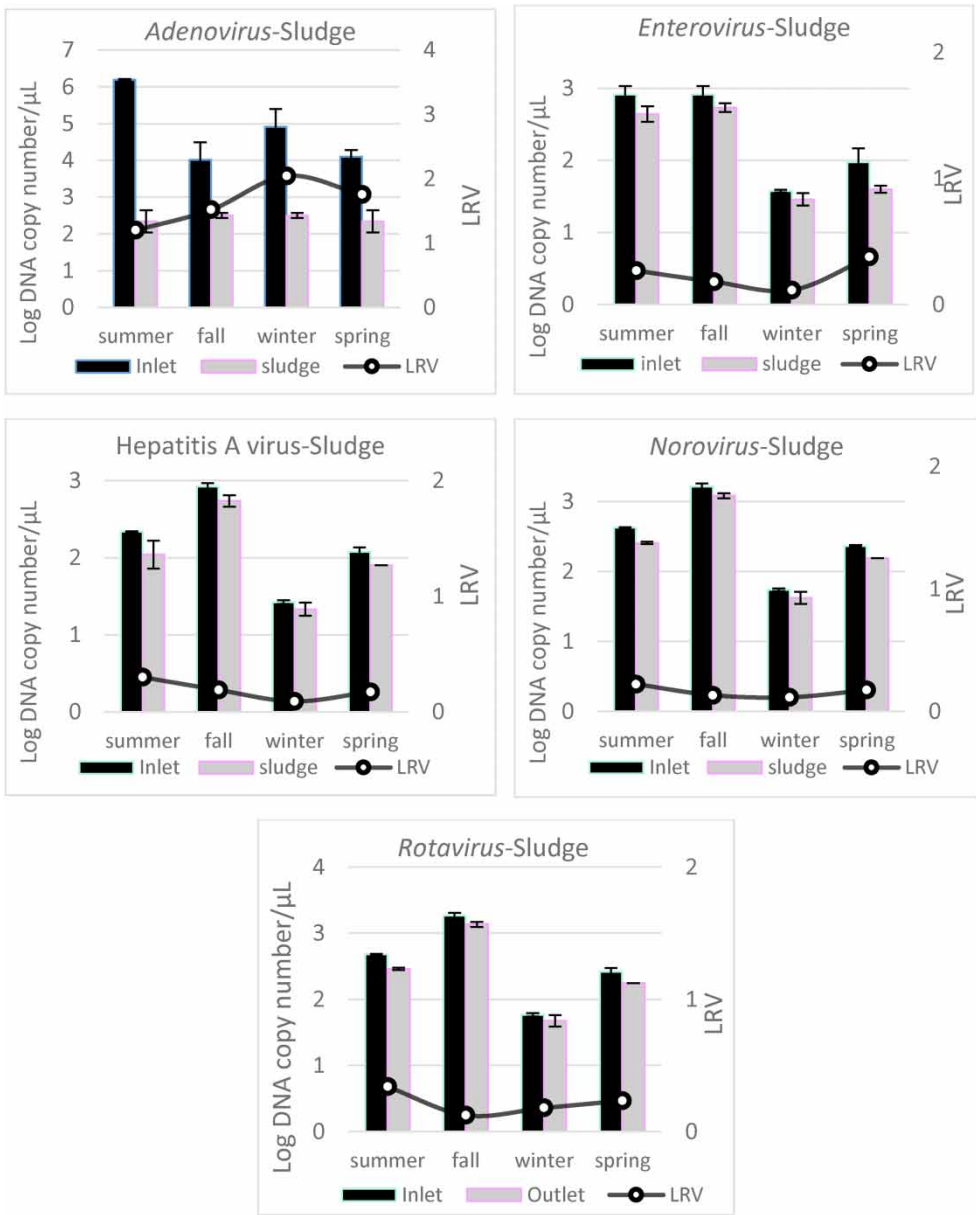


Figure 4 | Seasonal LRVs for enteric viruses in the sludge of the CAS. Error bars correspond to the standard deviation.

qPCR assay was determined as the lowest measurement value. Values of sample LRVs below the LOD were calculated as zero. The limit of quantification (LOQ) value was also determined as the highest measurement for each qPCR analysis. Values below the LOQ but above the limit of detection were set to the mean of these limits (Klymus *et al*. 2019). In the current study, the LOD value for *Adenovirus* was 3.97, for *Enterovirus* 5.98, for Hepatitis A virus 5.92, for *Norovirus* 4.85, for *Rotavirus* 6.08 log DNA copy number/L.

RESULTS AND DISCUSSION

Removal of enteric viruses in the CAS

The sampled CAS system receives wastewater from Central Ankara and has a capacity of 765,000 m^3 /day. In this CAS system, the highest LRVs for *Adenovirus* and *Rotavirus* were 1.89 and 1.13, respectively. *Enterovirus*, Hepatitis A virus,

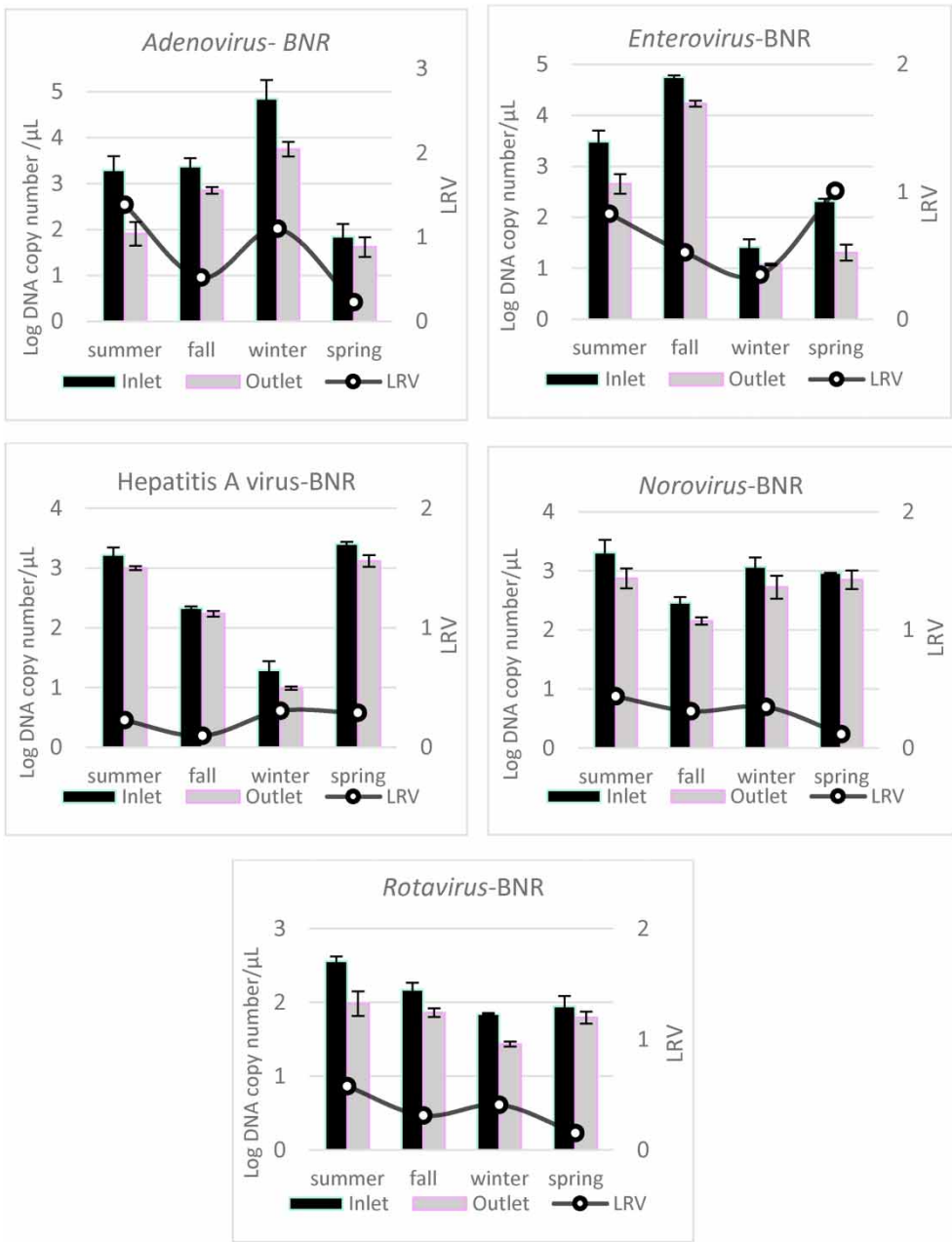


Figure 5 | Seasonal LRVs for enteric viruses in the BNR. Error bars correspond to the standard deviations.

and *Norovirus*' LRVs were below 1 (Figure 3). Influent and effluent concentrations of viruses were given in Supplementary material, Table A1. Seasonal variations were only significant for the removal efficiencies of *Norovirus* and *Rotavirus* ($p < 0.05$). These results were compatible with previous studies (Schmitz *et al.* 2016; Sidhu *et al.* 2017; Kaliakatsos *et al.* 2019; Jian-Ju *et al.* 2022). In CAS, virus removal mostly takes place over adsorption of viral particles to the sludge. However, non-enveloped enteric viruses are commonly found in the liquid fraction of wastewater as indicated by Wang *et al.* (2022). This might explain the lower LRVs obtained in the current study, too. Therefore, current findings also support the idea of additional treatments, with CAS as CAS + UV or CAS + chlorination (Kaliakatsos *et al.* 2019).

Antiviral agents from microorganisms inactivate viruses in the supernatant part of sludge. Additionally, adsorption onto the solid is another method of virus removal in activated sludge. In the sludge treatment of CAS, the highest LRV of *Adenovirus* was 2.04. LRVs of *Enterovirus,* Hepatitis a virus, *Norovirus,* and *Rotavirus* were below 0.4 (Figure 4).

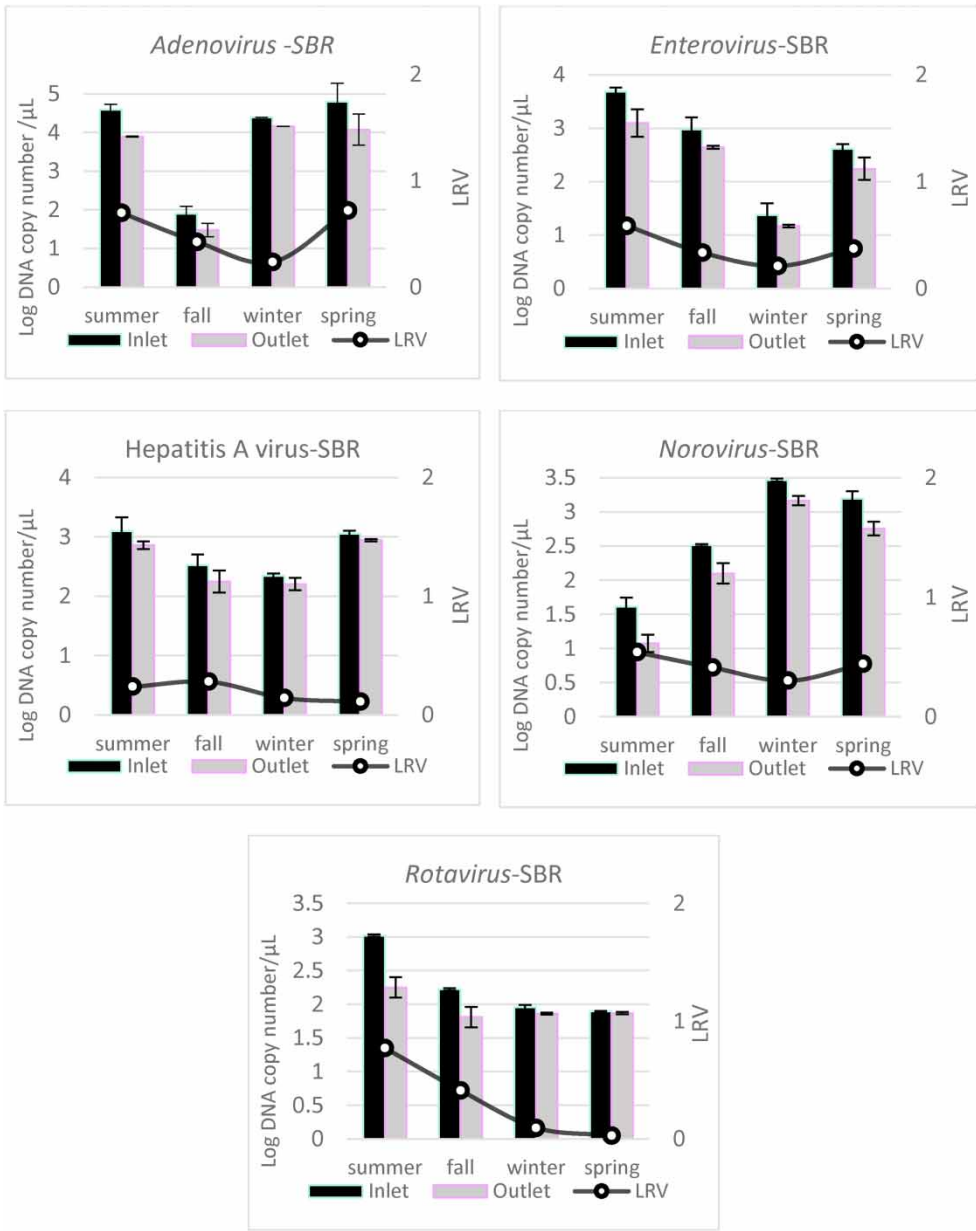


Figure 6 | Seasonal LRVs for enteric viruses in the SBR. Error bars correspond to the standard deviations.

Removal of enteric viruses in the BNR

The BNR system sampled showed the highest LRVs for *Adenovirus* and *Enterovirus* that were 1.38 and 1, respectively. However, the LRVs for Hepatitis A virus, *Norovirus,* and *Rotavirus* were below 1 (Figure 5). Seasonal variations were only significant for *Adenovirus* and *Enterovirus* ($p < 0.05$). (Influent and effluent concentrations of viruses were given in Supplementary material, Table A2.) However, the study done for Wellington Water Porirua WWTP (2020) displayed an average of 2.89 LRVs for *Adenovirus* in the BNR process. The current study, on the other hand, revealed that BNR was not effective for enteric viruses' removal.

Removal of enteric viruses in the SBR

The SBR system sampled in the study yielded LRVs below 1 for all the enteric viruses tested (Figure 6). Seasonal variations were only significant for the removal of *Rotavirus* ($p < 0.05$). (Influent and effluent concentrations of viruses were given in

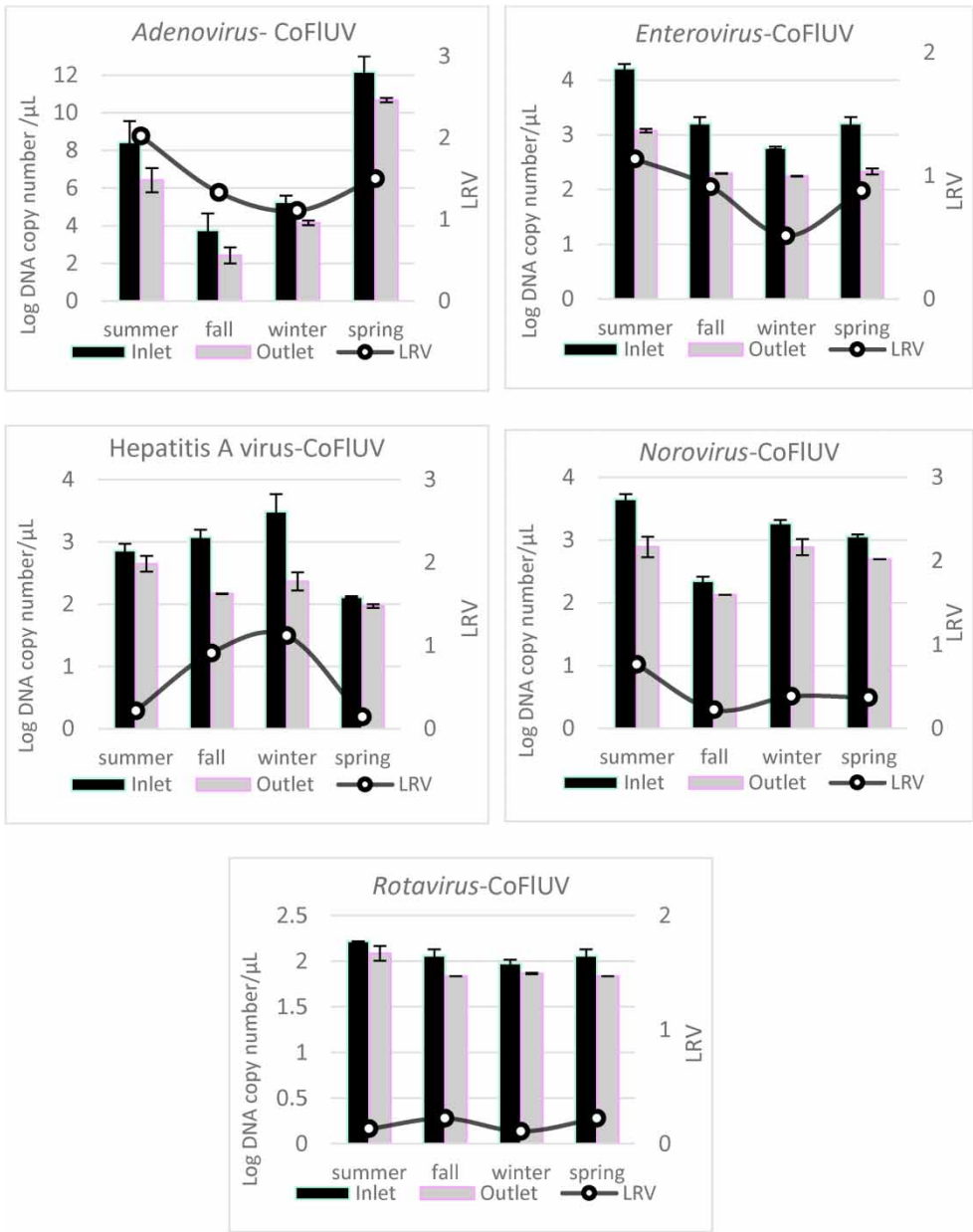


Figure 7 | Seasonal LRVs for enteric viruses in the CoFlUV unit. Error bars correspond to the standard deviations.

Supplementary material, Table A3.) None of the studies in the literature indicate removal efficiencies of enteric viruses observed with the SBR. Thus, the SBR was found not to be an efficient process to remove enteric viruses.

Removal of enteric viruses in WWTPs with the CoFlUV unit

The highest LRVs for *Adenovirus*, *Enterovirus,* and Hepatitis A virus were observed as 2.02, 1.14, 1.12 in the CoFlUV system sampled in the study, respectively (Figure 7). The LRVs were below 1 for *Norovirus* and *Rotavirus*. Seasonal variations were only significant for *Enterovirus*, Hepatitis A virus, and *Norovirus* ($p < 0.05$). (Influent and effluent concentrations of viruses were given in Supplementary material, Table A1.)

In the literature, no enteric virus removal record was observed for the CoFlUV unit. It seemed that UV irradiation alone was not an efficient method for especially non-enveloped viruses. UV irradiation can damage nucleic acids (DNA or RNA) of viruses, primarily through the formation of pyrimidine dimers. However, this damage can be overcome through repaired mechanisms. (Tortora *et al.* 2016). Moreover, the turbidity of the treated water, UV exposure duration, UV lamp quality, and UV dose all affect the efficiency of disinfection (Gullian *et al.* 2011). As suggested by Kaliakatsos *et al.* (2019), results

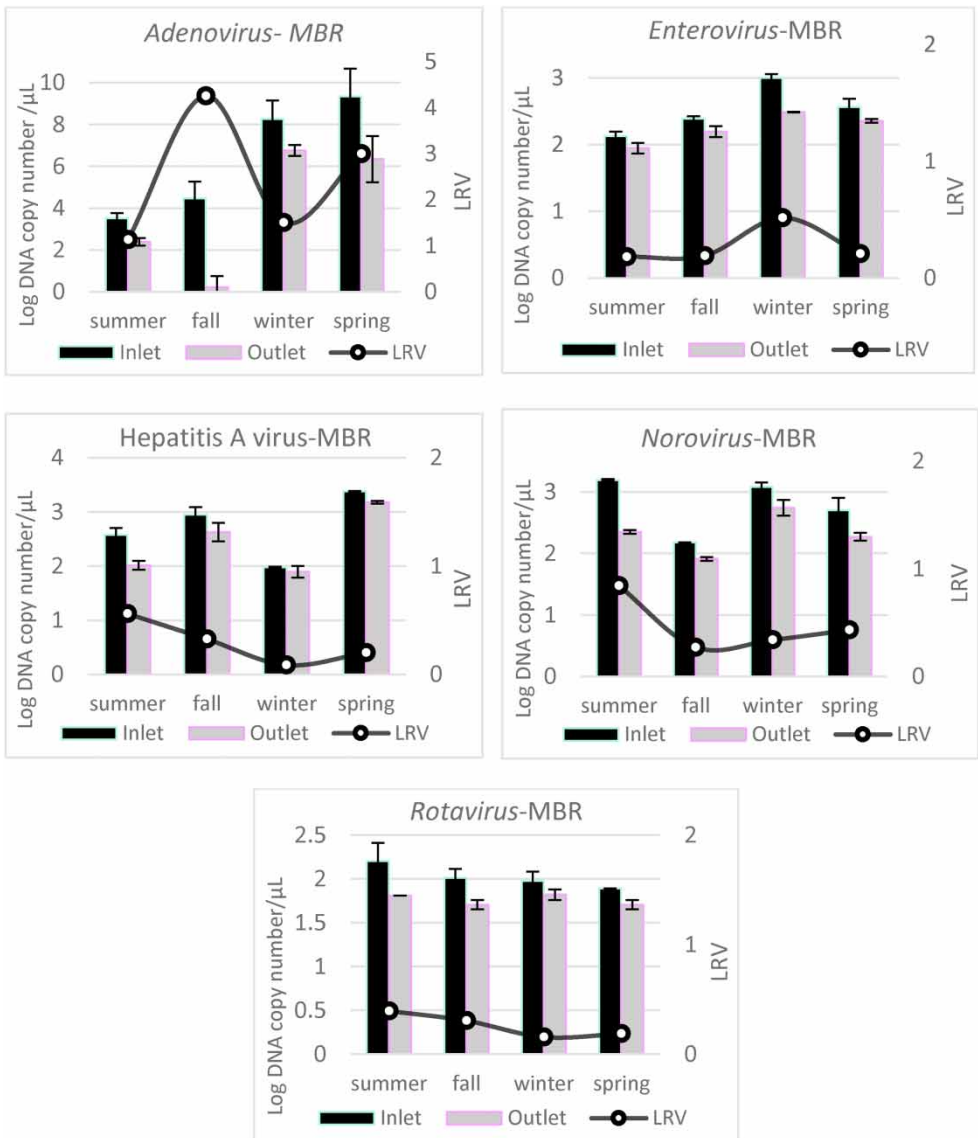


Figure 8 | Seasonal LRVs for enteric viruses in the MBR. Error bars correspond to the standard deviations.

of the current study also supported the idea that combining UV with other processes like MBR or CAS might provide better removal efficiencies for enteric viruses.

Removal of enteric viruses in the MBR

Viruses are small in size compared to other microorganisms. *Enterovirus*, Hepatitis A virus, and *Norovirus* are approximately 30 nm in diameter, while *Adenovirus* is ~100 nm (Chan *et al.* 2017; Baggen *et al.* 2018; Crenshaw *et al.* 2019; Sridhar 2023). The pore size of the membranes used in the MBR system sampled in the study was approximately 38 nm. This pore size led to high LRVs of 3 and 4.2 for *Adenovirus* (Figure 8). However, the LRVs for *Enterovirus*, Hepatitis A virus, *Norovirus,* and *Rotavirus* were below 1. This could mainly be explained by the sizes of enteric viruses. Due to the fact that the size of *Adenovirus* is ~100 nm, it could be efficiently trapped by the MBR. No significant seasonal variation was observed in the removal efficiencies of the enteric viruses tested in the MBR. (Influent and effluent concentrations of viruses were given in Supplementary material, Table A5.) The studies conducted by Jian-Ju *et al.* (2022), Chen *et al.* (2021), Kaliakatsos *et al.* (2019), and Purnell *et al.* (2016) also showed the efficient removal of *Adenovirus*'es in the MBR. Miura *et al.* (2018) reported 2 LRVs for *Rotavirus* in the MBR. In the MBR, Kaliakatsos *et al.* (2019) showed 2.3 LRVs for *Norovirus* and 4.9 LRVs for *Enterovirus*. Prado *et al.* (2019), on the other hand, underlined higher LRVs in the case of combining MBR and reverse osmosis process together.

In the MBR process, the most important parameter is pore size. Viruses that are larger than the pore size cannot pass through pores, physically. On the other hand, viruses can form flocs and become larger. In this case, they cannot pass through the pores. Fouling formed around the membrane filters affects the removal efficiency, too. Thus, backflushing and refreshing filters are required to enhance the filter activity (Metcalf & Eddy 2014).

The viruses used in the current study were non-enveloped viruses without the lipid layer. The capsid structure of these viruses enhances resistance to treatment (Corpuz *et al.* 2020). Thus, they are resistant to extreme pH, heat, dryness, UV, and simple disinfectants. The limits of common disinfection techniques for the destruction of human enteric viruses have led some researchers to focus on physical methods for the destruction of viral particles. Membrane ultrafiltration and nanofiltration are the methods that were used for this purpose. Moreover, combining the MBR with other processes such as reverse osmosis or UV provides improved results (Lanrewaju *et al.* 2022).

CONCLUSIONS

In this study, CAS, BNR, SBR, CoFlUV, and MBR were tested in terms of their enteric virus removal efficiencies. Among them, MBRs seemed to be the only promising process with higher *Adenovirus* removal efficiencies. By using MBR systems with microfiltration, ultrafiltration, nanofiltration, and reverse osmosis current WWTPs can be upgraded to remove enteric viruses efficiently. The removal efficiency of enteric viruses in WWTPs can also be enhanced by combining individual processes to minimize the potential risk in discharges.

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DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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