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Wastewater discharge and surface water contamination pre- and post- COVID 19—global case studies

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9.1 Introduction

There has been an increase in novel viruses in the past few decades like SARS-CoV-1 [2003], MERS-CoV [2012–2015], Influenza Virus [2009–2010], and now the deadly SARS-CoV-2 [2019–]. This global pandemic of SARS-CoV-2 has resulted into 52.6 million infections and close to 1.2 million deaths up to date November 12, 2020. The new deadly virus is the third of the coronavirus family to cross the barrier of animal and humans after SARS-CoV-1 and MERS (Drosten et al., 2003; Huang et al., 2020; Zaki et al., 2012). Symptoms of the pandemic disease includes fever, body fatigue, shortness of breath and dry cough (Guan et al., 2020; Huang et al., 2020). The fatality rate, however, is an average of 6.85% (despite having a higher contagious nature), which is quite less than there precursors SARS-CoV-1 and MERS-CoV, having fatality rate of >=50% (Wang et al., 2020). The reproduction number (number of individuals prone to infection per infected person) range from 1.4 to 6.5, which is somewhat equal to reproduction number of SARS-CoV-1.

SARS-CoV-2 transmission, human to human, mainly depended on the intake of virally contaminated aerosols and droplets recent studies have also suggested the probable fecal oral route of contamination, as the viral RNA was detected in the stool of certain percentage of the infected patients (Zhang et al., 2020; Lodder and de Roda Husman, 2020). The virally RNA gene getting detected in the stool test, initiated the researches to find its trail in wastewater treatment plants. Several countries have started the wastewater surveillance for the detection of viral SARS-CoV-2 RNA in WWTPs around the world in order to track the future probable cases at a community scale (Bivins et al., 2020). This will also help the policyholders to have a better understanding of the upcoming situations in near future. However, the fecal shedding in wastewater could be especially relevant in low sanitation countries where insufficient or nonexistent treatments of wastewater are applied.

Wastewater-based epidemiology (WBE) was coined by (Daughton and Jones-Lepp, 2001) and then was used as first for tracking drugs in wastewater (Zuccato et al., 2008). The approach fundamentally relies on the concept of stability of any substance in the wastewater under ambient condition, and how its concentration can be used to detect the infectious rate in a group of person in a hospital or at a community level. Therefore, various countries on the verge of this methodology have used WBE to be the approach for early prediction of COVID 19 infectious individuals in a society (Kumar et al., 2020b). WBE also helps in determination of the changes in genetic sequence noticed over a period of time and study different viral strains when studied over temporal and spatial scale (Hart and Halden, 2020a). WBE is beneficial in the cases where there are individuals with mild or no symptoms and goes unreported in the system, and ends up infecting others, predicting an actual picture of infectious rate at a community scale (Polo et al., 2020).
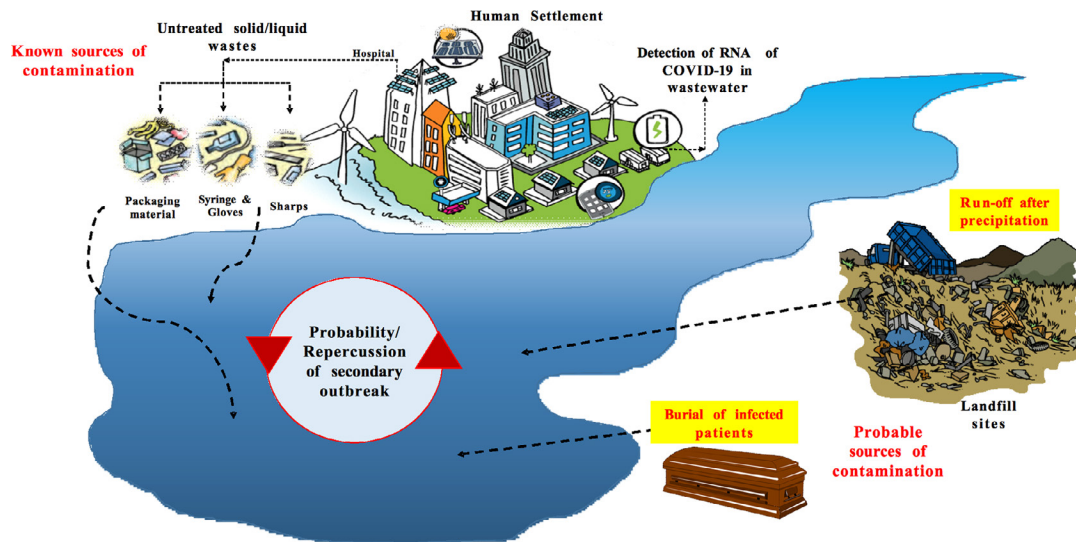
FIG. 9.1   Presence of viruses in water environment from different sources.

9.2 Presence in aquatic environment

The entrance pathways of the SARS-CoV-2 RNA can be through many sources as shown in figure (Fig. 9.1). All these known and probable sources of contamination tends toward the potential COVID transmission in the aquatic environment (Kumar et al., 2020a). The contamination of surface water systems with the partially treated effluent may be responsible for the future outbreaks. SARS-CoV-2 detection in the fecal samples of infected people made it very clear to be present in the wastewater as well. The instances of detection of viral RNA in the sewage were reported in (a) Netherland (Medema et al., 2020), (b) Australia (Ahmed et al., 2020), (c) Italy (La Rosa et al., 2020), (d) USA (Wu et al., 2020), (e) France (Wurtzer et al., 2020), and (f) India (Kumar et al., 2020b). The viral RNA concentration detected in the wastewater ranged to as high as 19,000 copies/L in one of the location in Wuhan, admitting COVID 19 patients in the nearby area (Zhang et al., 2020). These presence of SARS-CoV-2 RNA in wastewater samples across the wastewater has helped in predicting the infectious rate within the community, including the ones with mild or asymptomatic infected individuals. These results having advantages also arises the concern of future outbreak, if the virus survives longer in the environment even after certain disinfection procedures (Mallapaty, 2020).

9.2.1 Comparison to other viruses (enveloped/nonenveloped) detected in water

SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome) are enveloped viruses requiring more favorable conditions for their survival rate. Enveloped viruses are assumed to be not persistent in the However, the results showed that T_{90} value of human Coronavirus (hCoV-229E) changes between 200 and 400 days at 4°C in buffer, surface water, groundwater, and tap water while this value was 20–40 days for the enveloped viruses in wastewater at 4°C (Ye, 2018). Brainard et al. (2017) showed even wastewater temperature increased to 20°C, SARS-CoV has T_{90} value greater than 3 days (Tables 9.1 and 9.2).

The genome sequencing is more than 80% similar of SARS-CoV-2 with its precursor SARS-CoV, which were known for severe respiratory and enteric symptoms (Chan et al., 2020). The SARS-CoV virus can survive up to 14 days at 4°C, 2 days at 20°C in sewage, howbeit it's RNA survives for even extended periods (Lodder and de Roda Husman, 2020). Another similarity between SARS-CoV-1 and SARS-CoV-2 (both of them has envelope) is its survival ability ranging from 10^{5-6} genome copies/swab, hinting toward fecal–oral route of contaminations contamination with the viruses are common (Woelfel et al., 2020). Previous reported the surrogate coronaviruses survival ability from few days to several weeks in water and sewages (Casanova et al., 2009). These CoVs survival ability can range from hours to years, infectious nature not considered (Mullis et al., 2012). The surface water in the developing countries remains more probable for the contamination with WWTP effluents in the developing countries. This also paves the way for contamination in the zones of surface and groundwater interactions. The enteric viruses with and average size of 60 nm, having an easy pass-through aquifers (Borchardt et al., 2003). On the contrary, SARS-CoV-2 are with average 100 nm size has somewhat more resistivity issue while infiltration. The entero and

TABLE 9.1 Different methods for wastewater quantification for detection of viral RNA gene.

Countries	Concentration	Target gene				Genome copies/mL	References
		ORF1ab	S	N	E		
Italy	PEG precipitation of centrifuged supernatant	√	√	-	-	-	La Rosa et al., 2020
Netherland	Centricon ultrafiltration of centrifuged supernatant	-	-	√	√	-	Medema et al., 2020
Spain	Al flocculation—beef extract precipitation	-	-	√	-	10^{4-5}	Randazzo et al., 2020
Australia	pH → (-4) Electronegative filtration	-	-	√	-	12	Ahmed et al., 2020
China	PEG precipitation of centrifuged supernatant	√	-	√	-	-	Zhang et al., 2020
France	Ultracentrifugation	-	-	-	√	10^{3-6}	Wurtzer et al., 2020
Israel	PEG precipitation of centrifuged supernatant	-	-	-	√	-	Or et al., 2020
USA	PEG precipitation of filtered sample	-	-	√	-	10^{3-4}	Wu et al., 2020
Turkey	PEG precipitation of centrifuged supernatant	-	-	-	-	10^{2-5}	Kocamemi et al., 2020

Adapted from Farkas et al. (2020).

TABLE 9.2 Presence of different genes of SARS-CoV-2 in surface water.

Countries	Wastewater	Surface water	Presence of gene			Reference
			Orflab	N	E	
Italy	√		− (treated)	− (treated)	− (treated)	Rimoldi et al., 2020
	√		+ (raw)	+ (raw)	+ (raw)	
		√	+ (raw)	+ (raw)	− (treated)	

adeno viruses which have been detected so far in groundwater are polio, echo, coxsackie, noro, rota, reo, calici, and HAV (Borchardt et al., 2003). These viruses have a strong perspective of contaminating the environmental components including water cycle (Kumar et al., 2020a). It should be noted that the studies about enveloped virus survival in groundwater is limited.

In last 2 to 3 decades, the migration of virus to aquatic environment has been mainly focused on the nonenveloped viruses, as these viruses do not have susceptibility issues and most of them survives a certain number of inactivation mechanism (Bosch et al., 2006). On the other hand, envelope viruses are more fragile in nature and susceptible to elements like heat, UV, pH, which damages their outer envelope and make it loose its infectious nature (Polo et al., 2020). Some of the other studies, on contrary, have started detecting envelope virus like CoVs in the sewage network too, despite going through a long sewer time, and that too under harsh ambient conditions (Kumar et al., 2020c, 2020d).

9.3 Persistence and removal

A study revealed the actual disinfection procedure needed for the complete removal of viral RNA (Zhang et al., 2020). The presence of RNA with an average concentration of 9.6×10^6 copies/L even after the chloride-based disinfection (NaClO). The author also asks for the reconsideration of WHO and CDC prescribed disinfection measures and the amount of byproduct generated causes ecological risks (if used in higher concentration). (Adelodun et al. 2020) suggest the use of decentralization treatment facilities for the purpose of eradicating the SARS-CoV-2 like

viruses. The usual WWTPs set up for the purpose of treating community sewage should not be used in parallel with the effluents from health care facilities. Many times improper disposal of infirmary wastes can cause risk of infection (Wang et al., 2020). These types of issues are mostly seen in the low income countries where communities depend on stream and open wells for their daily water use. One of the other measure that can be taken is the community wide testing in the developing countries as well, there has been quite a difference noticed in the testing rate of countries, which somewhat hides the real picture of total infection in the country. WBE is one of such method with which a prior knowledge about the future outbreak can be obtained. There has been a wide scale lack of adequate water quality for the human consumption and sanitation which gives rise to many water borne diseases. There was further trend noticed since the start of the decade where the point of use devices was used for the remediation of these viral pathogens at the sources. These included ZVI filters, bio sand filters, cellulose-based filters, and gravity-based ultrafiltrations. Other inactivation mechanism includes the use of Chlorine, alcohol, UV and sunlight-based mitigations.

9.4 Wastewater-based epidemiology

The wastewater hosts a great number of pathogenic viruses (Adriaenssens et al., 2018). Wastewater is already known to host enteric viruses which follows fecal–oral route of transmission and causes gastrointestinal illness. The contaminated wastewater once released into the river system can degrade the environmental health by altering the food chain. The monitoring of wastewater has proved to be beneficial in the past few years. This method has been used to detect the presence of any biological sample of living beings including chemicals like antibiotic drugs, ARGs, MGEs and viral titters (Randazzo et al., 2020). The methodology followed for WBE is shown in figure (Fig. 9.2). The current pandemic has highlighted issues related to the current pace of clinical testing, as there has been lack in the access in most of the economic fragile countries. The analysis of clinical samples at current rate cannot provide a bigger picture of the infectious rate within the community. Therefore, wastewater surveillance at a community scale can be a critical tool for the SARS-CoV-2 infectivity prediction (Polo et al., 2020).

WBE has already been proven as the useful surveillance too for the diseases like poliovirus, norovirus and hepatitis A virus (Asghar et al., 2014; Hellmer et al., 2014). Therefore, it can used for the prediction of community scale infectious rate in the current COVID 19 pandemic too (Hart and Halden, 2020a). WBE is effective to predict the future outbreak of disease. The viral shedding in the stool of the infected patients goes up to 3–4 weeks after the first symptoms appears. Another important discussion involves the increase in the RNA concentration in third and
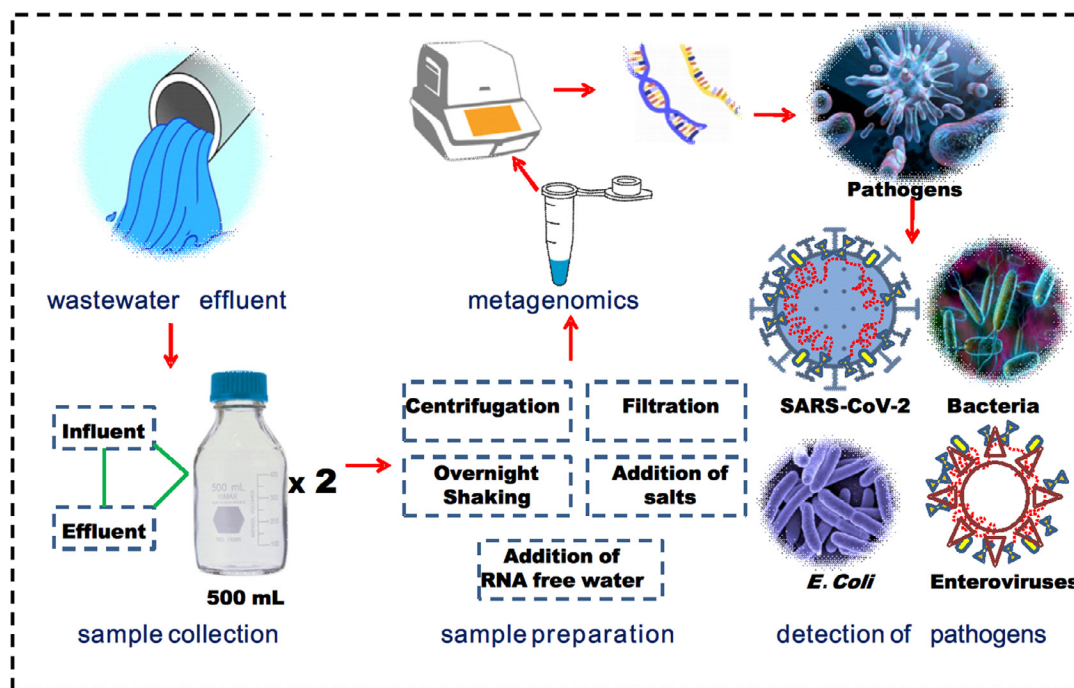

FIG. 9.2   Wastewater-based epidemiology for detection of pathogens.

fourth week than the first and second week (Zheng et al., 2020). A better WBE approach can help in detecting the presence of infection in an entire community population. These can help us in reducing the economic damage due to the pandemic time lockdown in different nations and lift the same in some section of the city where the infection is not so prevalent (Hart and Halden, 2020a). The current WBE methodology detects the viral RNA from wastewater sample and helps us to detect the infection prevalence, diverse in the genetics of the virus and geographical effected area (Xagoraraki and O'Brien, 2020). Therefore, to have a correct estimation, the methodology must be sensitive, reproducible and reliable across all the laboratories around the world (Lu et al., 2020). Another advantage of WBE remains the detection of asymptomatic and mild-symptomatic infected individual at community scale and therefore, it is easier to estimate the actual degree of virus circulation.

There are many critical step which are involved in the procedure of WBE mentioned below (Polo et al., 2020) (a) *Sampling method*: Inside this particular category, timing is more important, as in case of large decentralized plants, the sewage may take 24 hours to reach plant from household, and the amount of viral RNA analyzed may vary according to the time of sampling and will reduce down as the envelope viruses are more susceptible to inactivation. The temperature effect may also incorporate it, as the results may show a good amount of concentration when measured during colder months rather than summer months (Hart and Halden, 2020a, 2020b). (b) *Concentration and recovery of the viral titers*: two of the strategy were suggested as the best in order to have accurate quantification of the virus (Lu et al., 2020) prefiltration → salt addition → electro(-)ve membrane filtration and/or prefiltration → PEG-based separation → overnight standing, the significant concentration method is critically important as there are very few viral load in large volume of wastewater. (c) *Quantification* is another step needed to be taken care of as most of the time the viral concentration gets effected by the presence of fats, proteins and humic substances (Gibson et al., 2012).

9.5 Case studies

There are several countries which have confirmed the presence of viral RNA in surface and wastewater. The suspected fecal oral route of transmission of the virus like the Ebola, HAV, and HEV still remains the hypothetical, but is critically important when more than half of the global population lacks access o proper sanitation (Heller et al., 2020; WHO, 2019). Feces of the COVID 19 infected people reaches the sewer system and undergoes dilution and gets affected by the conditions of pH, temperature, and other substances. Being an envelope virus, they are more prone to losing their infectious nature and envelope than the nonenvelop virus. These factors also results in decrease in their concentration (Foladori et al., 2020).

Quito (Ecuador) as in many other cities worldwide, wastewater is directly discharged into natural waters (Guerrero-Latorre et al., 2020). The river samples were taken during the cases were on peak of the pandemic. The samples were concentrated for the detection of N1 and N2 target regions, where analyzed samples were in the range of 2.91 to 3.19×10^5 gc/L for N1 and 2.07×10^5 to 2.22×10^6 gc/L for N2. These results do have an environmental and health impact in the low sanitation lower GDP countries. Arslan et al. (2020) raises serious concern of potential risk of viral disease to the low-income countries. Many of these developing countries lack a sound and reliable health facility, which puts burden on few facilities present there and thereby increasing the load of viral titer in the wastewater facility. The study reported countries like Pakistan and Nigeria, having not a single functional WWTP in its soil, thereby again putting the burden locally available treatment ponds. This untreated/partially treated wastewater then ends up contaminating the local water streams, increasing the chances of re-infection (Afzal et al., 2019; Omole et al., 2019).

Even the developed European nation like Italy was not excluded from the pandemic reaching its surface water. River samples from three sites showed positive result for the presence of viral RNA when tested by RT-PCR. This may be attributed to ineffectively treated sewage discharges to natural water systems (Rimoldi et al., 2020). Considering WBE strategy, detection of N3 was noticed at least 6 days before the actual COVID case arrived in Amersfoort, Netherland, when sample in one of the WWTPs (Medema et al., 2020). Other instances of the first few studies are the detection of viral particles in the sewage with an observed value of around 10 copies/mL (Wu et al., 2020). Wurtzer et al. (2020) studied the variation of increase in infected individuals with the increase in viral titer reported in the wastewater samples in range of 50–3000 copies/mL. Randazzo et al. (2020) detected the first traces of SARS-CoV-2 RNA in wastewater of Spain with the concentration of 250 copies/mL.

There has been a variation in the amount of gene copies found per mL of the sample. Ahmed et al. (2020) detects only 0.019 to 0.12 genome copies/mL in Australia, while Wu et al. (2020) detects 10–240 genome copies/mL. The

former study validated the amount of viral RNA detected in sewage matched with the prevalence of the infected cases in the city. The later study implies that less number of cases were reported than the predicted after wastewater surveillance. This paves the way for the further improvement in the surveillance like studying the relative changes in the concentration of viral RNA at the WWTP inlet (on daily basis) can help us in predicting the increase in COVID circulation in the community (Medema et al., 2020).

9.6 Environmental implications and policies

Owing to the highly contagious nature of human pathogenic viruses, the screening at individual level in some of the highest populous country becomes extremely difficult (Farkas et al., 2020). The less known cases generally go unnoticed due to the individuals having mild symptoms. These mild or no symptoms cases causes' error in the modeling and assessment of the pandemic model at a global scale. Therefore, creating an urgent need for the prior detection of outbreaks in a community scale and thus provide enough time to come up with a proper protocol to be followed for its mitigation.

There are number of studies which have so far detected the presence of viral titers in the treated effluent of from the wastewater treatment plants (WWTPs). The concentration of COVID RNA present was up to 10^4 genome copies/100 mL in the wastewater effluent. The reduction noticed in WWTPs are in the range of 1–2 log removal (Zhang et al., 2020; Randazzo et al., 2020; Wurtzer et al., 2020). The repercussion of having highly contagious virus in the water environment is still unknown. The ability of the newly pandemic virus to infect the marine animals is reported in some of the studies (Kim et al., 2020). The animals which are living readily close to these effluent outlets are more prone to the viral RNA of this pandemic virus. The next thing which should be looked upon is the ability of these virus remaining infectious still remains doubtful. Further researches regarding the infectious nature of the virus in wastewater should be properly investigated. Most of the time, the viral titers has no effect as when the wastewater gets discharged into surface water, its dilution level is very high.

The WBE-based approach is again necessary and need for the hour for at least a year now in order to trace the early warning of the probable infected cases a week later. The issue with COVID 19 is the largest share of the pandemic are asymptomatic individuals, who don't get easily tracked down and thus ends up infecting other individuals as well (Larsen and Wigginton 2020). WBE can also be thought of as a farsighted technique apart from COVID 19 surveillance as the method can help us track the other pathogen which can be possibly occurring in near future. The correlation between the genome copies detected per L of the wastewater and the number of infected people still remains doubtful. This is due to the fact of its dependency on several other factors, as the most of the cases remain untraceable.

9.7 Conclusion

There has been numerous studies which conclude about the viability of the WBE study for planning a better framework for the study of future virus outbreaks. There are several methods for concentrating the wastewater samples in order to further process the extract with the help of PCR methods. The process of real time detection of viral RNA in wastewater and surface water should be further researched. The WBE has proven to be beneficial for having an early idea about the future probable cases. If optimized even further can be applied for the new or old viruses too. The WBE approach can also be made portable, and the current WWTPs can be made to accommodate the quantification and analytical technique in order to have a clearer picture on daily basis, therefore stating the probable peak of any endemic. This approach can also be useful for the other pathogens and emerging contaminants.

The biomarkers contained in the wastewater at any WWTP in a city are very crucial for the surveillance system, needed for prediction of infectious rate in a community. The surveillance tells us not only about the societal health, but also accounts for the asymptomatic carriers as they mostly go untested. Although, it is difficult to pinpoint the location of infected individuals, it gives a broader and clear picture of penetration of disease in the society. WBE has ability to be implemented in all categories of nation, be it developing or developed. The effectiveness of the technique has already proven useful for illicit drugs, pathogens detection and therefore can be implemented in any setting trying to monitor the health of community in terms of antibiotic resistance, virus circulation, or any other disease prevalence.

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