Wastewater an Emerging Source of Spreading COVID-19 Infections in India: A Recent Perspective

Subhendu Sekhar Bag[*a,b] and Sayantan Sinha[b]

Epidemics caused form infectious pathogens are known since the prehistoric era. The first report was around 3000 B.C. when an unknown disease was noticed amongst villagers residing in today's 'Hamim Mangha' and 'Miaoigao' located in northeastern China province. The entire village population of all age groups was locked in a house and set to fire to contain the spread of the disease. However, since then, many such cases have haunted the world population over the years. In the initial days till 1860, people did not have any idea about pathogens creating infections. It was in 1860 Luis Pasteur related the pathogenicity of puerperal fever and the pyogenic vibrio. This was the first incident when the world came to know about infectious bacteria. Thirty-two years after, in 1892, Dmitri Ivanovsk had discovered the virus for the first time. However, the first case of human infection from the virus was reported only in 1901 as a spread of Yellow fever [1], which was also the first viral pandemic reported. Therefore, viral diseases and related deaths are not new to the world community. Some of the fatal viral epidemics which have tested and threatened the world at times are Influenza (Spanish flu etc.), haemorrhagic fever, Chikungunya, Monkey pox, Small pox etc. In the last two decades, the world has also witnessed a few fatal pandemics like: Ebola viral disease, Nipah viral infections, and SARS related diseases.

Severe Acute Respiratory Syndrome (SARS) is a disease that affects human and causes infectious hemorrhagic fever. In 2002 the first case of SARS in humans was reported from infection caused due to the SARS-CoV virus in China. Followed by this, the outbreak of infections from MERS-CoV was reported in Jeddah in 2012. At present, the world is again facing the wrath of infection from a novel mutant SARS-CoV-2. Total infections counted worldwide in just five months, is over 3.2 million, with deaths accounting to over 0.2 million. Till the date, we do not yet have any vaccines/drugs to cure the disease, called COVID-19. SARS-CoV-2 is an enveloped virus with + sense single-stranded RNA.

Similar to SARS-CoV, it also belongs to the Betacoronavirus (βCoV) lineage B group of coronaviridae family. The Spiked glycoprotein (S) on the membrane of SARS-CoV-2 serves as the receptor-binding domain having two subunits S1 and S2. The S1 binds to the ACE2 receptors present on the target cell surface, followed by the HR1 and HR2 domain interaction in the S2 subunit. Such interactions help to bring the virion close to the target cell surface for fusion resulting in an infection to the host.[2] Common symptoms of infected persons include headache, fever, cough, and sneezing, which is very similar to typical flu type infections. However, with these initial symptoms, the individual experiences severe respiratory problems. It has been studied that SARS-CoV-2 can survive outside the host cells for a long time. The most common transmission route is through the direct touch of infected surfaces/objects and then touching ear, nose, eyes, and mouth. Another path for viral transmission is through the inhalation of virus-laden air droplets excreted from individuals who cough and sneeze.[3]

However, apart from this, a more alarming situation has turned up with reports that SARS-CoV-2 can survive in wastewater and faecal matter for weeks [3,4]. The outbreak of SARS in 2003 in Hong Kong could be related to a faulty sewage system in a high rise.[3,4] Also recently high concentration of CoV-2 was detected in WWTPs in Paris on 5th March, followed by 10th March when maximum death was recorded.[5] These reports alarmed that wastewater can be a potential source for the spreading of viral infections. The effect would be more in developing countries with a large population and poor personal hygiene. In developed countries with less population, modernized WWTPs, and drainage systems it is possible to treat the entire volume of wastewater generated and also maintain hygiene. India has a population of 1.35 billion, and 61754 MLD of wastewater is produced daily with a capacity of treating only 22963 MLD.[6] This data reflected that only 31% of the produced wastewater is treated, and the rest 69% of wastewater/sewage is discharged off untreated in surface water bodies.

The economy of India is mostly dependent on agriculture, which accounts 80% of total water consumption. About 135 L of water is consumed per person daily, and the rest water is consumed in offices, schools, and other places.[6] It may be even painful to provide all citizens with adequately treated drinking water. Therefore, 69% of untreated wastewater has been utilized for agriculture. About 50% of India's total population are farmers. Hence they are directly exposed to the untreated sewage during their agricultural works, cattle breeding, and other daily activities. Thus, the entire community indirectly gets exposed to wastewater through the food chain. India was under 4 consecutive lockdowns to prevent the spread of COVID-19 infection. However, for the self-sustainability and greater interest of the nation, the essential services like agriculture are going on at their usual speed. Summing up all the facts, it is clear that 1% of the infection would lead to the possibility of 1.35 crore individuals getting infected. With the available detection kits and infrastructures in India, about 37,000 individuals have been tested daily. Though, now in June the rate has been increased; however, a large no. of undetected section remains in the community. Migrant labours are coming to their home towns. Thus, at present the COVID-19 cases are increasing day by day that left a large number of infected individuals in the community or under treatment at hospitals. The wastewater generated therefore is expected to contain SARS-CoV-2 virus. The excreta from these people are expected to contain SARS-CoV-2 which gets entry in the wastewater. Moreover, wastewater is a niche of bacteria which develops resistance in presence of antibiotics comes along excreta and hospital waste. Therefore, wastewater becomes a major source of spreading SARS-CoV-2 and other bacteria-born diseases. We know, India has open drainage system. Therefore, wastewater from communities and hospitals flow through the open drains, increasing chances of virus-laden aerosol formation through evaporation and thereby increasing the chance of community spread. Additionally, the farmers using the wastewater again come in direct contact with the pathogen, increasing another severe concern of community spread.

No cost-effective system, has yet been developed to tackle the Spread of SARS related coronavirus, drug resistance microbes, and drug residues at the same time at source. Furthermore, the traditional wastewater treatment systems are costly and often function as breading places for resistant bacteria. Moreover, the classical treatment methodologies (sedimentation, filtration, chemical and membrane technologies etc.) often generate toxic secondary pollutants in the ecosystem. To deal with this situation, new STPs have to be constructed. However, fulfilling this requirement is not possible considering the present economic crisis in India. Therefore, cheap and user-friendly technology should come up from scientists and engineers.

At present, scientists in India are engaged in discovering new drugs against COVID-19. Initially, our group at IITG also started working towards the development of protease inhibitors against CoV-2. Now, we realized that the stopping spread of CoV-2 from wastewater should also be a concern. Scientists working in membrane technology can develop appropriate membrane which can restrict the passage of CoV-2 and can be fitted in portable filter systems. The development of stable and recyclable drugs that can stop viral replication can also be considered. Recently, we have conceptualized the design and synthesis of Fluorescent quantum dot (FL^sC_{QD}) coated with flavonoids extracted from False daisy that mimick heparin sulfate of heparin sulfate proteoglycan (*HSPG*) receptor. This material is expected to stop the viral cellular entry (Figure 1). Moreover, the surface flavonoid of the CQD are expected to interact with the catalytic site of the viral RdRp and prevent its activity resulting in arresting viral replication and translation mechanism (Figure 1-2).

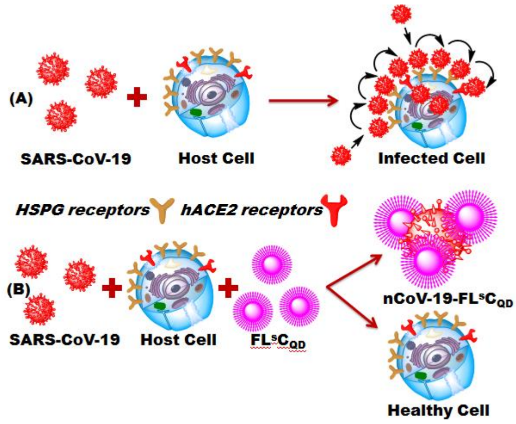

Figure1. The Proposed concept. (A) SARS-CoV-2 binds to the *HSPG* receptor on the host cell and scans the cell surface to locate its specific *hACE2* entry receptor. (B) The biogenic CQD which mimick the *HSPG* receptor of host cell would bind to the S glycoprotein of SARS-CoV-2 and thereby stops infection of a host cell.

From our literature study[7,8] we have found out that the S proteins of nCoV-19 initially interact with *HSPG* receptors to start their journey to locate specific *hACE2* receptors. Our proposed FL^SC_{QD} is expected to mimic *HSPG* receptors and, hence, be expected to bind to the SARS-CoV-2 S1 RDB. This would ultimately prevent the virus from getting attached to the HSPG receptors of the host cell. Thus the virus cannot scan the host cell surface for locating entry receptors *hACE2*. As a result, the infection is prevented.

The Bhringaraj (false daisy) is an ancient plant found in India. The leaf extract of Bhringaraj contains three potential RNA dependent RNA polymerase (RdRp) inhibitors that are wedelolactone, luteolin, and apigenin. Our initial study through molecular docking experiments (Molegro virtual docker) of these inhibitors with RdRp of SARS-CoV-2 (PDB id: 6M71) showed promising interaction with the catalytic site. The protein-ligand docking scores were found to be -113.005 kcal/mol, -113.543 kcal/mol, and -105.765 kcal/mol for the flavonoids, respectively. Wedelolactone interacts with Arg624, Asp452, Ala554, Thr556, Asp623, Lys621, Cys622, Tyr455; Luteolin interacts with Asp760, Tyr619, Lys621, Ala554, Thr556, Cys622, Arg624; and Apigenin interacts with Arg553, Ser682, Lys676, Ser681, Lys545, Arg555, Thr556, Ala554, Arg624 residues. (Figure 2).

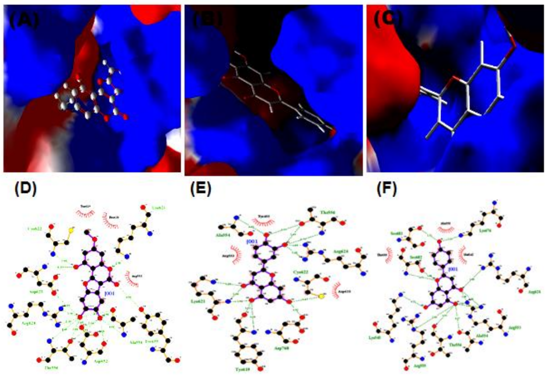

Figure2: Binding of (A) wedelolactone (B) luteolin and (C) apigenin in the catalytic site of nCoV-19 RdRp; interaction with amino acids in the active site of the catalytic pocket (D) wedelolactone, (E) luteolin and (F) apigenin.

Therefore, the docking study suggested that the False Daisy coated CQD is expected to bind to the catalytic site of viral RdRp protein. Thus, natural RdRp inhibiting molecules coated onto the material surface expected to prevent the activity of RdRp and hence would stop the viral replication and translation process leading to damage of the virus. Thus, our material could play dual role to stop the spread of virus by disinfecting the source, wastewater (Figure 1-2).

Our main focus of designing the biogenic conjugated QD[9] is to carry out both the detection and eradication of the virus by stopping the viral replication at source. The molecules present in the leaves of Bhringaraj have the potential to arrest the viral RdRp activity by binding to the active site of the viral RdRp. However, these molecules cannot signal the presence of the virus, and thus, their interaction could not be visualized or studied. To overcome this lacuna, we decided to use a fluorescent material that could help us sense the binding interaction of the flavonoids with the virus through the study of fluorescence photophysical property utilizing our "Just-Mix-&-Read Strategy".[10] Additionally, one of our primary goals is to stop the virus from locating its host cell entry receptor. Using only the screened inhibitor molecules from false daisy will not suffice this objective. Hence, the QD would serve as a platform to carry all the necessary inhibitor molecules including *HSPG* mimic to the active site of the virus.[9] Thus the false daisy coated CQD would act as a decoy to arrest the viral entry to host cell as well prevent the viral replication through binding of the RdRp inhibitors with the catalytic site of the virus. We have thus conceptualized and decided to use carbon dot because of its stability, ease of synthesis, biosafety, excellent fluorescent property, and tuneable surface property. Moreover, the CQDs or its conjugates are known to be very quickly uptaken by the cells.[9]

Furthermore, the treatment of Covid-19 has introduced the usage of various drugs and antibiotics. Exposure to even low concentration of antibiotic trigger the drug resistance to the pathogenic microbes. Therefore, hospitals/health care settings are the major and widely occurring focal source that release antibiotics and/or resistant bacteria into the environment. Removal or degradation of antibiotics/drugs at source such as Hospital or community waste water is one of the solution to the microbial resistance.

Therefore, a solar photocatalytic treatment system can also be developed for in-situ mineralization of pathogens and drugs. Such a smart system would be efficient to **degrade the un-metabolized antibiotics** thrown or come out from patients' excreta into Wastewater leading to stoppage of generation of drug resistant microbes. Thus, in the present crisis scientist and engineers should also come forward to develop a system which can **disinfect the wastewater/sludge**, a crucial sources of spreading infections from viruses and other microbes.

In conclusion, through this article, we tried to convey that wastewater can become an emerging source for community spread of COVID-19 and other pathogenic diseases in developing countries like India. We also urge scientists and engineers to make wastewater treatment their focus area at this time of emergency.

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Author Information:

* Corresponding Author: Prof. Subhendu Sekhar Bag, FRSC; email: ssbag75@iitg.ac.in

[a] Chemical Biology/Genomics Laboratory Department Chemistry, Indian Institute of Technology Guwahati, India-781039

[b] Centre For The Environment, Indian Institute of Technology Guwahati, India-781039