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Microplastic occurrence and its potential role as a carrier for SARS-CoV-2 in health center wastewater treatment plant and surface water

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ABSTRACT

This study investigated the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNAs on the surface of microplastics (MPs) collected from a hospital wastewater treatment plant (WWTP) and the Qarasou river in Kermanshah, Iran. The MPs were characterized using stereo-, scanning electron-, and Fourier-transform infrared spectroscopies to determine their appearance, quantification, qualification, and morphology. The virus RNAs were extracted by rinsing the surface of the MPs with distilled water, followed by swapping and transferring the scratched material into viral transport medium. Identification was performed using SARS-CoV-2 molecular detection kits and real-time quantitative polymerase chain reaction (RT-qPCR). The most common type of MPs found in the wastewater acted as a vector for the virus RNA, whereas virus RNA was only identified in one sample of river water. These findings suggest that MPs can facilitate the spread of the virus through WWTPs and into receiving water bodies. Thus, research on MPs acting as carriers of COVID-19 should be emphasized as determining the presence of the coronavirus on the surface of MPs is crucial in determining a country's health strategies. Furthermore, this study shows that MPs have the potential to act as vectors for pathogens and create new microbial niches in aquatic environments.

1. Introduction

Plastic is a synthetic organic polymer that was first produced in 1940 and is extracted from oil or gas (Andrady, 2011). The plastic industry produced 367 million tons of plastic in 2020 (Franco et al., 2023), but due to population growth and increased plastic consumption, production is expected to double by 2025 and triple by 2050 (Freeman et al., 2020). Microplastics (MPs), which are particles with a size <5 mm, are the most significant category of plastics released into the environment and aquatic ecosystems. MPs have been found to have a widespread presence and accumulation in surface waters and other aquatic media, making water pollution by MPs a global concern (Frydkjær et al., 2017). MPs can persist in the marine environment for hundreds of years (Velez et al., 2018) and enter the environment through wastewater treatment plants (WWTPs), industrial activities, personal care products, cleaning products, and the breakdown of larger plastic particles (Kemper and Luchetta, 2003; Koelmans et al., 2015).

The literature has reported that the use of personal protective equipment (PPE) in health centers, hospitals, and general usage is a contributing factor to increased MPs (Lee and Kim, 2022). Due to their small size, MPs are bioavailable to organisms through the food chain, causing adverse effects such as decreased mobility, intestinal obstruction, suffocation, or reduced nutrition in aquatic organisms (Pazos et al., 2017). Additionally, the composition and relatively high surface area of MPs make them a suitable carrier for accumulating organic compounds, resistant pathogens, heavy metals, and toxic compounds (Teuten et al., 2009), posing a threat to the health of the aquatic food chain and the environment (Laganà et al., 2019). MPs have been identified as an important carrier for pollutants such as DDT (dichloro diphenyl trichloroethane), PAHs (polycyclic aromatic hydrocarbons),

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PCB (polychlorinated biphenyl), PBDE (Polybrominated diphenyl ethers), and metals (Brennecke et al., 2016; Mato et al., 2001; Rios et al., 2007). Moreover, MPs can act as a medium for the accumulation of microorganisms and bacterial and algal biofilms (Dobretsov et al., 2009; Harrison et al., 2014; Masó et al., 2003), but their potential as a carrier for toxins, heavy metals, and pathogens has received less attention compared to their presence, origin of contamination, dispersion, and accumulation (Rochman et al., 2013).

In December 2019, SARS-CoV-2, a member of the Coronaviridae family, emerged in China, leading to over 54 million infected cases and >1.3 million deaths worldwide as of November 15, 2020 (WHO). The COVID-19 pandemic has resulted in an increased demand for single-use plastic, particularly in the form of personal protective equipment, providing a unique opportunity for MPs contamination studies. Although SARS-CoV-2 is primarily a respiratory disease, studies have reported that viral RNA can be detected in the feces of infected individuals even after respiratory symptoms subside (Kitajima et al., 2020). While few studies have been conducted on transmission routes such as water and wastewater, there is considerable evidence that water and waste streams can play a role in the transmission of coronavirus (Chaudhry and Sachdeva, 2020).

Due to the disposal of waste contents of the upper respiratory system (phlegm, saliva, and runny nose) and feces of people infected with SARS-CoV-2 into WWTPs and ultimately into water sources, it is necessary to conduct studies on the routes in which SARS-CoV-2 spreads in water resources, in parallel with global research. Several studies have reported the presence of SARS-CoV-2 RNA in stool samples from infected individuals (Tang et al., 2020; To, K.K.-W, et al., 2020; Wang et al., 2020; Xiao et al., 2020). Additionally, a few studies have reported the potential for the survival of viruses, particularly SARS-CoV-2, on various surfaces, including metal, paper, wood, and plastic. The duration of survival varies from a few hours in the open air to >10 days on different surfaces, especially polymer and plastic surfaces (Hoseinzadeh et al., 2020). Researchers have investigated health center waste as a source of MPs carrying COVID-19 viruses that enter the aquatic ecosystem following the breakdown of their sources (Iheanacho et al., 2023). Although the role of MPs as a carrier has been reported in various studies, the MP potential for carrying the SARS-CoV-2 in water and wastewater has not yet been studied.

The study aims to investigate the potential of MPs to carry SARS-CoV-2 RNA in the Farabi Hospital WWTP and the Qara-sou river in Kermanshah, Iran. The Farabi Hospital WWTP is of particular interest due to the presence of MPs in different sections of the treatment plant, while the Qara-sou river is directly and indirectly affected by the discharge WWTP of hospitals and other municipal and industrial wastewaters. The research seeks to determine the presence of SARS-CoV-2 RNA on the surface of MPs in these environments, shedding light on the potential role of MPs in the transmission of the virus.

2. Materials and methods

2.1. Study area

Fig. 1 illustrates the study area and sampling locations in Kermanshah, Iran. Two sampling areas, Farabi Hospital WWTP (A) and the Qara-sou river (B), are shown. The Farabi Hospital WWTP (34.333335, 47.054488) sampling points included four locations: the pumping station, aeration tank, sedimentation tank, and effluent zone (storage tank). Farabi Hospital is the primary point of care for COVID-19 patients. The Farabi Hospital WWTP is a hybrid system of an extended activated sludge and a subsurface biofilter (Fig. 1A). The activated sludge aeration tank is in the form of plug flow, and the biofilter is filled with scoria rocks. The second sampling area is the Qara-sou river, which is the main river of Kermanshah and is fed by seasonal and permanent rivers upstream and is the receiving water body for the municipal WWTP. The sampling points on the Qara-sou river were upstream (34.353260, 47.115405) and downstream (34.308891, 47.150533) lo-

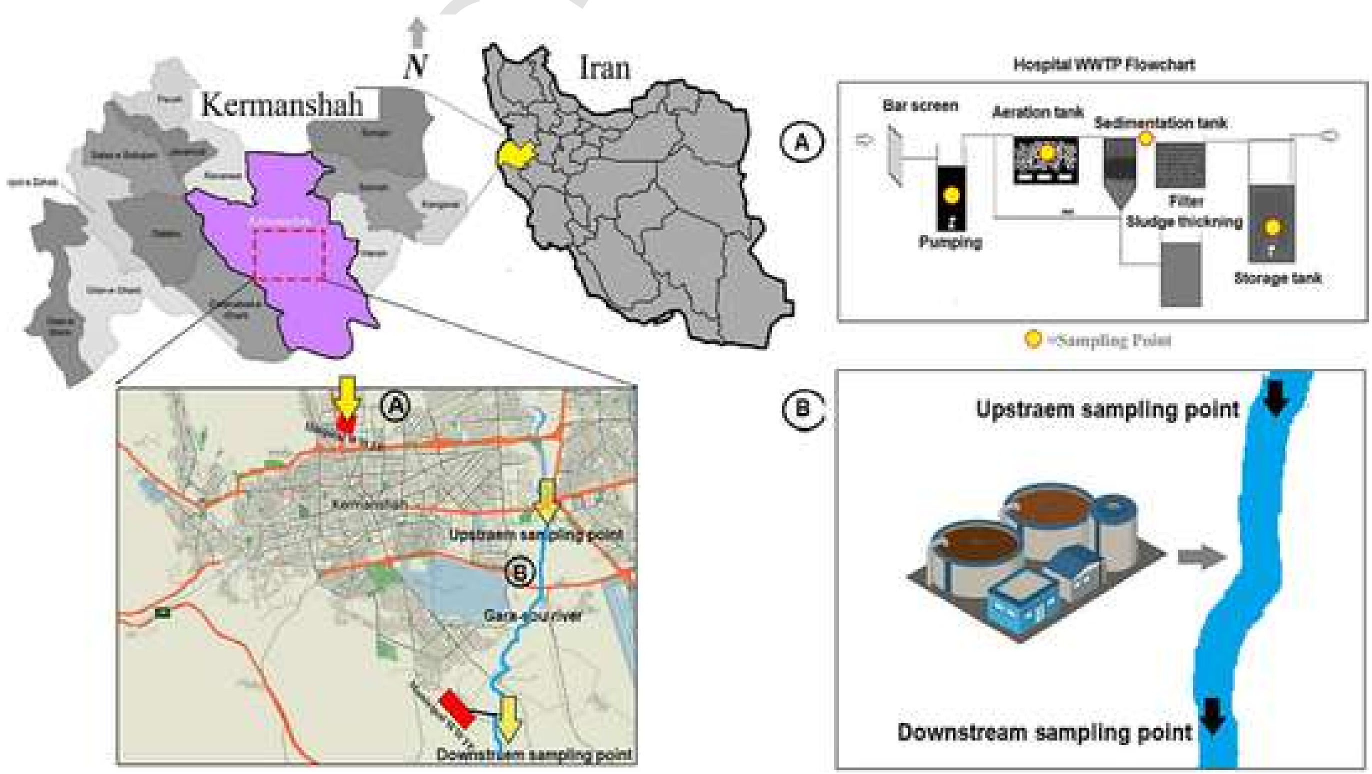

Fig. 1. Schematic of the study area and sampling locations, including a process diagram of the Farabi Hospital WWTP (A) and the Upstream and Downstream sampling points on the Qara-sou river (B).

cations of the Kermanshah municipal WWTP effluent discharge (Fig. 1.B).

2.2. Study design

To investigate the possibility of the presence of SARS-CoV-2 on the surface of MPs, the occurrence of MPs in hospital wastewater was investigated first (based on section 2.5). After identifying common MPs in the different units of Farabi WWTP (based on section 2.5 and then 2.6), it was found that they are similar to the same plastic items used in hospital wards, such as gloves, syringes, covers, etc. Then 6 pieces of plastic, which were more common, were purchased raw, cut into 5 × 5 mm dimensions, and placed in a metal net with many pores. Subsequently, the nets containing MPs were placed in different units (Fig. 1. A) of the WWTP (Sampling points mentioned in section 2.1). After 7 days, the nets were taken out from the wastewater stream and immediately transferred to the lab in sterile containers. This study was concurrent with the 4th peaks of COVID-19 in the hospital (2021, April and May). Finally, a total of 24 samples were taken from six different types of plastic (quadruple samples for each MP).

Also, the collection of plastics was done in the upstream and downstream of the Qara-sou River (Fig. 1. B), and a total of 10 pieces of plastic (five samples upstream and five samples downstream) that were in contact with the river flow were collected by manta nets. These samples were immediately transferred to the lab in the same condition as the WWTP samples.

2.3. Viral RNA sample preparation and extraction

SARS-CoV-2 RNA samples were taken from the surface of MPs after rinsing the MPs with distilled water. The rinsed MPs were then swabbed using sterile flexible swabs (Easy Swab, Komed, SungNam, Korea) and immediately dipped in viral transport medium under the fume hood. All the samples were transferred for RNA extraction. Total RNA was extracted from the samples using the Viral RNA extraction kit, the RNJia Virus Kit (Roje Technologies), based on the company's protocol. The kit uses a silica-based membrane technology in the form of a convenient spin column.The purified RNA is free of proteins, nucleases, and other contaminants or inhibitors of downstream applications. The isolated RNA can be directly used in PCR, qPCR, or other nucleic acid-based assays. Lysis is achieved by incubating the sample in BFC buffer. Ethanol is added to the lysate to achieve the appropriate conditions for RNA binding to the silica membrane. RNA is then selectively bound to the membrane, and contaminants are removed using two specific washing buffers. Finally, pure viral RNA is eluted in a rehydration buffer, and the isolated RNA is ready for downstream applications.

The quantity and purity of the extracted RNA were assessed by measuring absorbance at 230, 260, and 280 nm using a NanoDrop spectrophotometer (Thermo Scientific, USA).

2.4. Real-time polymerase chain reaction (RT-qPCR)

The RT-qPCR was performed using the Roche LightCycler 96 System by 10 μL of RNA in each PCR reaction in a final volume of 20 μL (Roche, Germany). Each PCR reaction contained 1 μL of primers- probe and 9 μL FastStart SYBR Green Master (Pishtaz Teb Azma, Iran). The kit's probe and primer mixture is designed using the gene target-dual method, which simultaneously detects the protected genomic sequences of the RdRp region and the N protein nucleocapsid. The provided PCR reaction solution in the kit allows for qualitative reproduction of this pattern and increased fluorescence signal through the devices for Real-Time PCR measurement. This diagnostic kit includes a solution containing a probe and an internal control primer. The inclusion of RNase P as a control increases the accuracy of the sampling process and extraction is performed to avoid false negative results. RT- PCR amplifications were performed by the company's programs for cDNA synthesis, extension, and fluorescence measurement. Three channels were used in the RT-qPCR reaction (RdRp, N gene from SARS-CoV-2 genome and Ransep as internal control). Signals for FAM, HEX, and Texas Red were measured in each channel.

2.5. Microplastic sample preparation

Wastewater samples were collected from different hospital WWTP units (Fig. 1. A) in 1-l containers and transported to the laboratory under safe conditions using a cool box. The samples were then treated with 35% hydrogen peroxide (H_2O_2) (Heshmati et al., 2021; Makhdoumi et al., 2021) to oxidize the organic matter under a fume hood. After 48 h of exposure to H_2O_2, the samples were passed through a Buchner funnel with a glass fiber filter (Grade 50/A, 1.2 μm pore, 47 mm diameter) and placed in clean, covered Petri dishes to dry completely before further examination.

2.6. Microplastics characterization

After preparing the samples, morphological characterization and quantitative detection of MPs were determined using a stereomicroscope (NOVEL NSZ-810, Ningbo Yongxin Optics Co., Ltd., Zhejiang, China). Accordingly, MPs were classified based on their size (0.001–0.5, 0.5–1, 1–2, 2–3, 3–4, and 4–5 mm) using Feret's diameter measurement. Additionally, MPs were categorized based on their color (black, red, white/transparent, blue, and green) and shape (film, fiber, and fragment). A hot needle was used to correctly distinguish MP particles from other suspected crystal structures such as chemical particles and salt. By approaching the hot needle, the MP particles quickly curl or melt, while other non-plastic materials will not (Roch and Brinker, 2017).

The surface features of the MP particles were conducted using a scanning electron microscope (SEM) (Hitachi SU3500, Bruker Nano GmbH Berlin, Germany). Furthermore, FTIR (Fourier-transform infrared spectroscopy) was employed to detect the types of polymers (24 samples) in the infrared range of 4000 cm^{-1} to 400 cm^{-1} (Shimadzu, Japan). After comparing the obtained spectra with previous studies data (Jung et al., 2018), the types of polymers were determined.

2.7. Quality assurance and control

To eliminate and prevent possible contamination, all laboratory equipment was washed twice with distilled water, transferred under a fume hood for drying, and immediately covered with aluminum foil. Sterile gloves were used to avoid direct hand contact in all steps of preparing the viral transport medium samples. Additionally, before and after each procedure for each specimen, all work surfaces and instruments were cleaned with 70% ethanol. Furthermore, a blank sample of 200 ml distilled water was placed in an open-mouthed glass container and positioned alongside the laboratory equipment during the analysis process of wastewater samples. The purpose of this blank sample was to catch any airborne MP particles that may have been present in the laboratory environment and affect the accuracy of the tests. After filtering the distilled water and examining it under a stereomicroscope (section 2.5 and 2.6), no MP particles were observed on its surface.

3. Results and discussion

3.1. MPs quantification and qualification in the hospital wastewater

The wastewater samples were analyzed to determine the characteristics of the MPs from all the units (including pump station, aeration tank, sedimentation and effluent), and the results can be seen in Fig. 2. According to Fig. 2. a-d, there are photographic images of MPs deter-

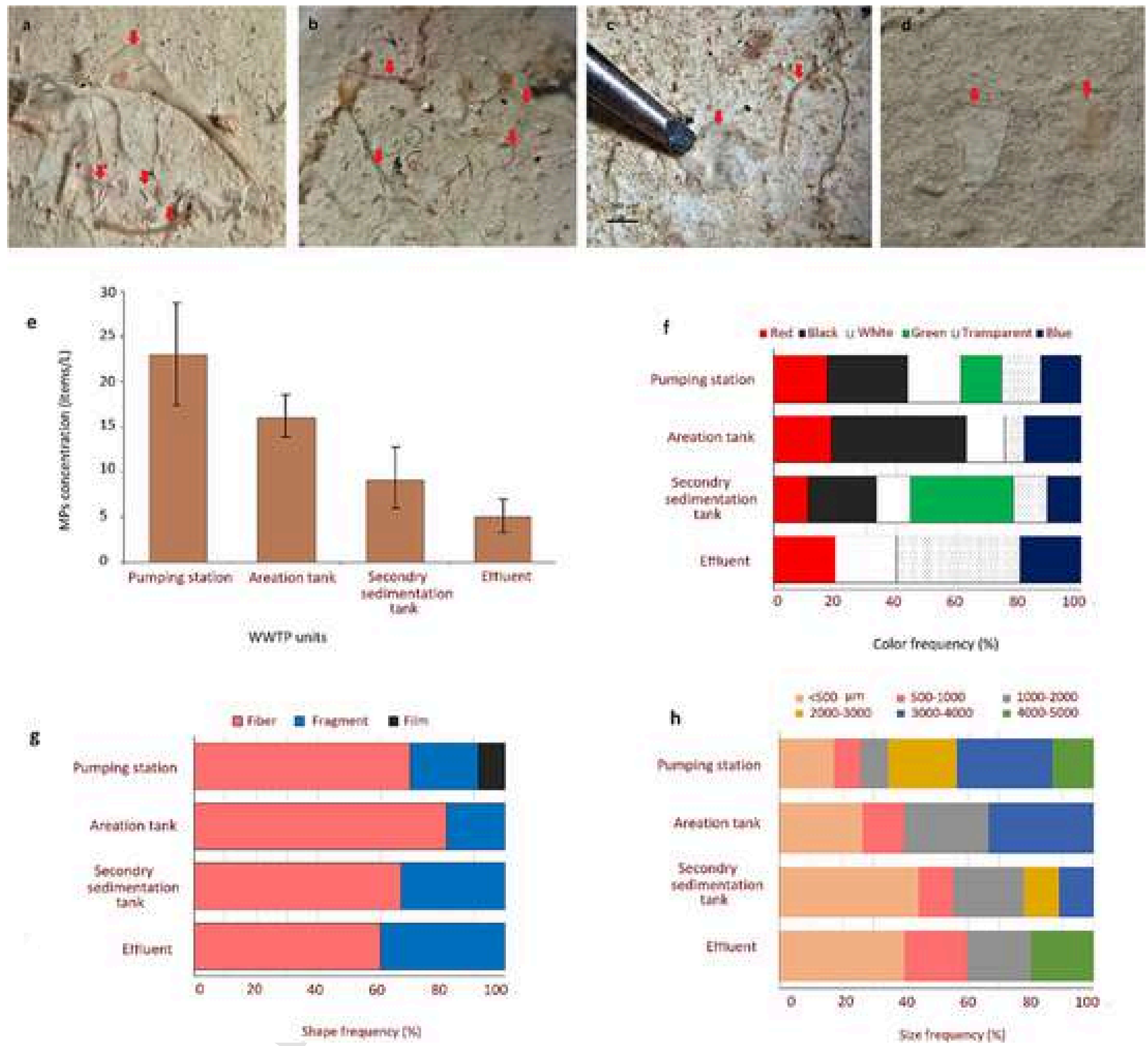

Fig. 2. Photographic images of detected MPs (a-d) and the qualification and quantification of MPs (e-h).

mined under visual observation in the form of fibers, films, and fragments. The findings indicate that the MPs concentrations are attenuated during the WWTP process. The mean concentration of MPs in influent samples was 23 particles per liter (Fig. 2. e), while the effluent sample contained 5 particles per liter, accounting for a removal rate of 78%. Fig. 2. f and g illustrate the frequency of colors and shapes among the detected MPs. The black type of MPs was dominant in the wastewater treatment units, but no observations in the effluent. Fiber was the MP shape with highest frequency followed by fragment shape then film shape (Fig. 2. g). Although all sizes of MPs were observed in the samples, the smaller sizes (< 0.5 mm) were dominant in the WWTP units increasing in frequency with each treatment step (Fig. 2. h).

FTIR analysis was used to identify the main types of polymer types collected in the WWTP of Farabi Hospital. Accordingly, the MP particles were classified into seven types of polymers: polypropylene (PP), polyethylene (PE), latex, polyurethane (PU), polystyrene (PS), polyamide/nylon (PA), and high-density polyethylene (HDPE). The occurrence of these types is likely due to their use in different hospital wards that entered into the wastewater. In the second step of the study, to have a similar condition of surface area, type, and number of MPs, all types that were detected in the WWTP, including PE, PP, latex, PU, PS, and PA, were located in the WWTP units to trace the RNA of COVID-19 (Figs. 3 and 4).

3.2. Trace of viral RNA in the WWTP samples

Cut-out plastics were introduced into different units of the WWTP, including the pumping station, aeration tank, secondary sedimentation tank, and effluent, with their typical appearances represented in Fig. 3 a-c. Fig. 3 d-f shows the SEM of the MPs recovered after 7 days of exposure to wastewater containing COVID-19 viruses. Accordingly, the effects of environmental conditions and stresses are visible, including aging, deposition, fracture, crushing, and crumpling. These changes may enhance the accumulation of SARS-CoV-2 RNA.

At this step, the most frequently occurring polymers were placed in the WWTP treatment units. The presence of SARS-CoV-2 RNAs for each

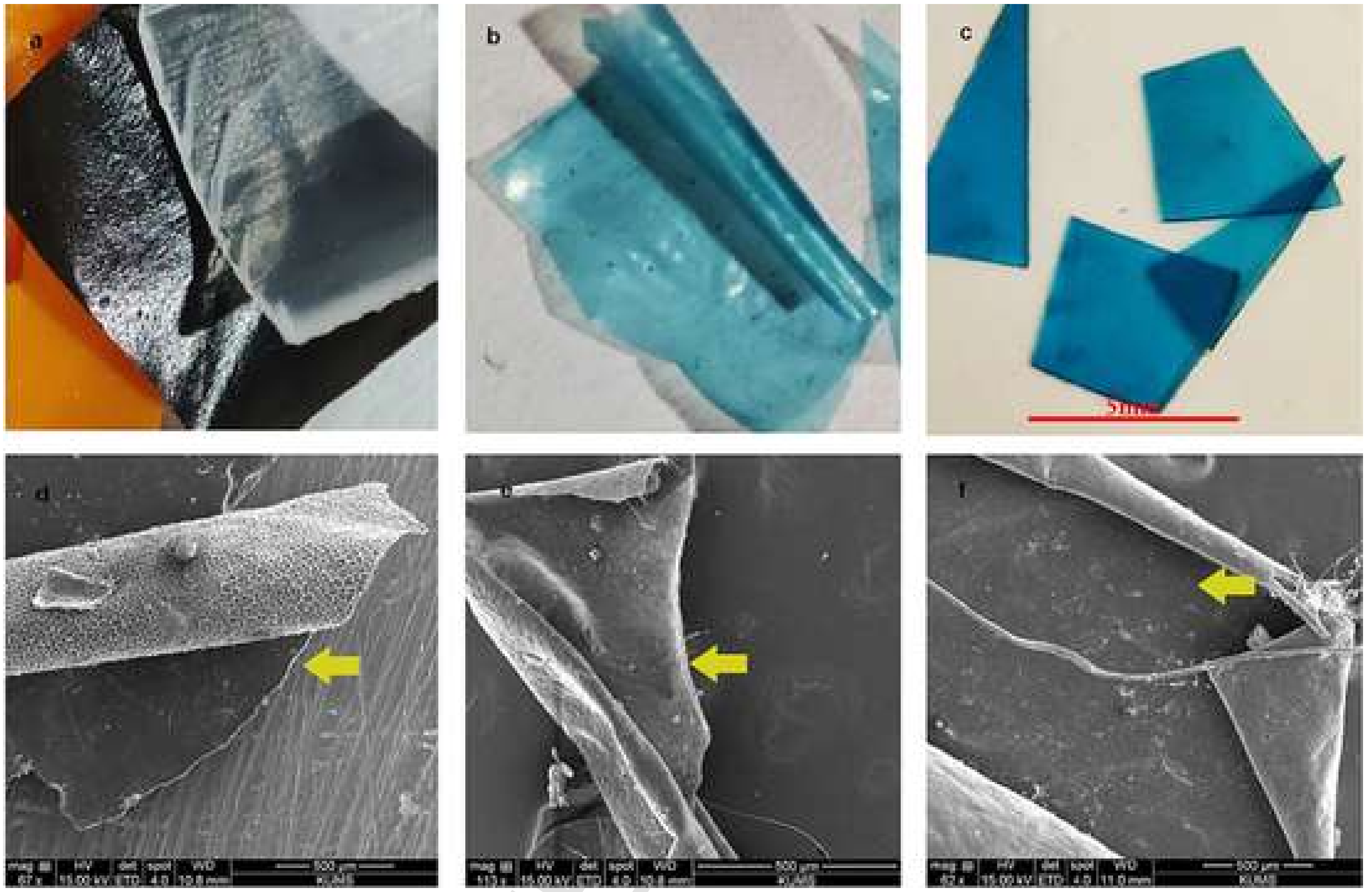

Fig. 3. Photographic images of MPs before entering the WWTP (PA: a, PP: b, and PE: c) and SEM images of MPs particles after 7 days (d, e, and f; The yellow arrow shows the surface of MPs). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

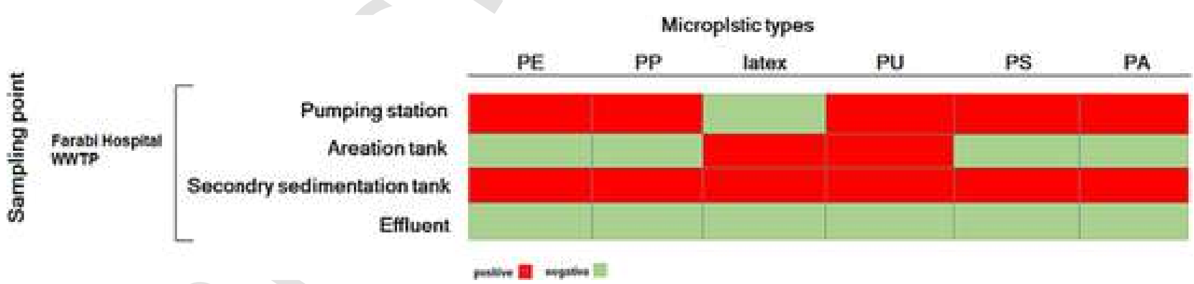

Fig. 4. SARS-CoV-2 RNAs tracing points of WWTP of Farabi Hospital.

type of polymer after a week of contact is shown in Fig. 4. Accordingly, at the pumping station, the first point of sampling, only one MP sample (latex) did not contain viral RNA. At the second point, the aeration tank, SARS-CoV-2 RNA was observed on latex and PU samples. Although viral RNA was present on all MP surfaces in the secondary sedimentation tank, no positive viral RNA footprints was detected in the final effluent. The fate of the novel coronavirus (SARS-CoV-2) in wastewater is not fully understood, but it may be influenced by environmental conditions such as temperature, pH, viral structure, and wastewater characteristics (Amoah et al., 2020). Operational factors such as hydraulic retention time (HRT) and mixing can also facilitate the accumulation of viral RNA onto MP surfaces, with higher mixing and aeration types may playing an important role. Due to this fact, the frequency of MPs in the aeration tank is lower compared to the MPs in the pumping station and secondary settling. According to the results, the frequency of MPs containing SARS-CoV-2 RNA in the aeration tank is lower than that of the pumping station and secondary sedimentation (Fig. 4). According to the literature, physical, biological, and chemical methods are potentially able to remove enteric viruses. However, sedimentation mechanisms alone for viral removal are low (Bogler et al., 2020), so the residues are still present in the wastewater matrix. Thus, adsorption of viral RNA may have occurred onto MPs, resulting in a higher number of positive samples. The secondary treatment, a combination of the aeration tank with secondary sedimentation, can retain a larger portion of entered viruses by sorption of viruses into activated flocs or organic particulates, followed by settling in the sedimentation tank. There is therefore a high competition between adsorption by organic particles or flocs with MPs, resulting in a higher number of negative samples. Al-Duroobi et al. (2023) reported that enhanced viral particle adsorption occurs with a higher number of suspended solids and biosolids.

In the secondary settling tank, the presence of unabsorbed viruses and a laminar flow, along with a relatively long time, has provided suitable conditions for accumulation of viruses onto the surface of MPs. Abu Ali et al. (2021) recommended that after secondary clarification,

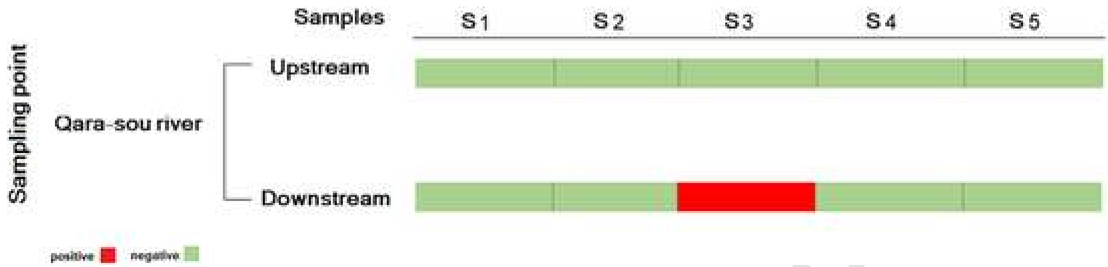

Fig. 5. SARS-CoV-2 RNAs tracing on MPs detected in the Qara-sou river.

due to the hydrophobic envelope of the virus, which reduces the solubility of SARS-CoV-2 in water, there is higher adsorption of viral RNA onto MPs surfaces. The researchers also reported that removal efficiency reached 100% in the effluent of tertiary treatment prior to disinfection. A similar pattern may have occurred because no positive RNA detection was found in the effluent storage tank. On the other hand, SARS virus types are sensitive to disinfectants such as chlorine compounds used in WWTPs. Research has recommended that disinfection methods can be used for inactivation of SARS-CoV-2 in hospital wastewater. Wang et al. (2005) showed that inactivation of SARS-CoV in hospital wastewater could be achieved after 30 min of disinfection with > 10 mg/L chlorine (0.4 mg/L residual free chlorine) or 40 mg/L chlorine dioxide (2.19 mg per liter free residual chlorine). Research has shown that the inactivation of enveloped viruses (influenza and coronaviruses) in aqueous media varies between 24 min and 117 days (T90%) (Mohan et al., 2021). These differences in inactivation are associated with environmental conditions such as temperature, pH, and the composition of aqueous media. For example, coronaviruses are more susceptible to inactivation at higher temperatures of wastewater or persistence of disinfectants than other viruses.

3.3. *Trace of viral RNA in the river samples*

The most common types of MPs found in river samples were PP, PU, PS, and PA in upstream locations, and HDPE, PU, PS, and PA in downstream locations, based on FTIR analyses (data not shown). Fig. 5 shows that SARS-CoV-2 RNAs were not observed in the 5 sampling points upstream of the river, but were found in one of the downstream samples (S_3). Kitajima et al. (2020) conducted surveillance for the presence of SARS-CoV-2 RNA using quantitative and nested PCR assays in a WWTP and river from Yamanashi Prefecture, Japan. The authors reported that viral RNA was detected in secondary-treated wastewater samples (2.4×10^3 copies/L), whereas no river samples tested positive for SARS-CoV-2 RNA. Similarly, Rimoldi et al. (2020) investigated raw wastewater and treated samples from Milano and Monza e Brianza Vettabbia Canal WWTPs and Lambro Meridionale River in Italy for SARS-CoV-2 RNA using RT-PCR and infectivity tests. SARS-CoV-2 footprint was detected only in raw wastewater samples, while inspection of viruses in environmental samples like water relies strongly on PCR-based methods that require reference sequences for primer design. This approach can accurately detect known viruses, but it cannot identify new genotypes or emerging and invasive viral species (Adriaenssens et al., 2018). The analysis of virus diversity in the Ile-Balkhash basin, Kazakhstan, was evaluated by Alexyuk et al. (2017), resulting in the appearance of a wide variety of autochthonous viruses from different families such as *Coronaviridae, Herpesviridae and Reoviridae* in surface water, including river, water reservoir, and lake samples. These findings were related to pollution of the water bodies from sewage discharges. Guerrero-Latorre et al. (2020) reported the presence of SARS-CoV-2 in urban streams (river water) exposed to sewage discharges. The results showed that the SARS-CoV-2 gene was present in all the locations collected from the Machángara river (Quito, Ecuador) with concentrations ranging from 2.07×10^5 to 3.19×10^6 GC/L. This confirms the presence of SARS-CoV-2 in the Qara-sou river influenced by the Kermanshah city WWTP.

The literature reports another concern about the time required for virus inactivation in pure water and treated sewage, which can take several days at ambient temperatures, resulting in SARS-CoV-2 transmission (Mohan et al., 2021). The estimated SARS-CoV-2 risk analysis of Zaneti et al. (2021) for workers in wastewater disclosed that the aggressive and extreme risk scenarios are higher than the tolerable infection for SARS-CoV-2, indicating that sewage systems could be a possible transmission pathway for the virus. This fact is very important when considering that SARS-CoV-2 can accumulate on MPs surfaces and can easily be transmitted through water flow. A single MP particle can travel hundreds of kilometers through a river during inactivation time, creating a new route for microplastic-mediated transmission.

4. Conclusion

This study is the first to investigate the presence of SARS-CoV-2 RNA on MPs particles released into hospital WWTP and the receiving surface water. The results of the study demonstrated that the hospital WWTP, which has a hybrid system of an extended activated sludge and a sub-surface biofilter, has a relatively good (78%) MP removal efficiency. Furthermore, the study found that the most common type of MPs in the wastewater acted as a vector for the virus RNA. Specifically, polymers such as PE, PP, latex, PU, PS, HDPE, and PA were identified in all the sampling points of the hospital WWTP as vectors for MPs, whereas virus RNA was only identified in one plastic sample of river water. These findings suggest that MPs can facilitate the spread of the virus through WWTPs and into receiving water bodies. Consequently, the presence of SARS-CoV-2 RNA on the surface of MPs in WWTPs and rivers should be considered in the design and operation of these facilities. Appropriate treatment technologies that can remove MPs and associated contaminants from wastewater should be implemented, and further research on this issue is recommended in current international surveillance programs to assess the role of MPs as carriers of the coronavirus in disease transmission.

Ethical consideration

The authors have observed all the ethical issues throughout the experiment (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, have been completely observed by the authors. The Ethics code of this study was IR.KUMS.REC.1401.191.

CRediT authorship contribution statement

Tooraj Massahi: Methodology, Writing – original draft. **Abdulfattah A. Amin:** Writing – review & editing. **Ronak Abdulazeez Meshabaz:** Writing – review & editing. **Meghdad Pirsaheb:** Validation, Writing – review & editing. **Leigh Terry:** Writing – review & editing. **Pouran Makhdoumi:** Writing – original draft, Writing – review & editing. **Sara Kianpour:** Writing – original draft. **Fatemeh Zamani:**

Methodology, Writing – review & editing. **Hooshyar Hossini:** Conceptualization, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Uncited reference

WHO, 2020

Declaration of competing interest

The authors report no conflicts of interest.

Data availability

Data will be made available on request. All data used in this work are present in the paper.

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