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Disinfection tackling the COVID-19 pandemic causes disinfection by-products (DBPs) accumulation and threatens aquatic ecosystems

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Abstract

To fight against the coronavirus infectious disease-2019 (COVID-19), chlorine-based disinfectants are extensively or even over used for water, surface and personal care decontamination. The risks of disinfection by-products (DBPs) have been alerted to cause serious secondary pollution; however, there is still lack of evidence. This study collected 110 water samples from nine lakes and two rivers in Wuhan during the COVID-19 pandemic and comprehensively analyzed the occurrence of eighteen DBPs. Trihalomethanes, halonitromethanes, halogen acetonitriles and nitrosamines had a high detection frequency and were 0.99-14.26, ND-4.62, ND-1.09 and 0.0414-0.0861 $\mu\text{g/L}$, respectively, all lower than the maximum contamination level (MCL) suggested by China and USA. Haloacetic acids were detected in all lakes and Yangtze River and ranged from 33.8 to 856.1 $\mu\text{g/L}$, much higher than the MCL. Haloacetic acids and halogen acetonitriles accounted for 74.2-95.1% of the total cytotoxicity ($0.38\text{-}3.62\times 10^5$); halonitromethanes (94.0-98.7%) contributed to the majority of genotoxicity ($0.52\text{-}5.17\times 10^4$). Dichloroacetic acid exhibited significant ecological risks to green algae in two lakes and Yangtze River (risk quotient >10), and all the other DBPs showed negligible risks (risk quotient <0.01) to fish, daphnid or green algae. Correlation and redundancy analysis identified strong correlations between total organic carbon, conductivity, $\text{NH}_3\text{-N}$, turbidity and DBPs. DBP composition and the fluorescence indices of dissolved organic matters together categorized all lakes into two types. Type-I lakes contained all DBP categories, driven by total organic carbon and secondarily formed by residual active chlorine with natural organic matters; Type-II lakes and Yangtze River only had high levels of haloacetic acids and small amounts of trihalomethanes, explained by the primary formation of DBPs in sewage. Our findings for the first time uncovered the significant accumulation and risks of DBPs in lakes and rivers of Wuhan during the COVID-19, provided the evidence of secondary pollution from intensive disinfection activities with chlorine-based disinfectants,

evaluated the potential the ecological risks of DBPs in Wuhan and along Yangtze River, and raised our re-consideration of disinfection strategy in the pandemics and post-COVID-19 era.

Keywords: COVID-19; disinfection strategy; disinfection by-products; ecological risks

1. Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has aroused over 160 million cases and 3.4 million deaths until 10th May, 2021. Fecal-oral transmission route is reported by World Health Organization (WHO) for the spread of SARS-CoV-2 ¹, and some evidences show the spillover of SARS-CoV-2 from wastewater ² or solid wastes ³. Disinfection is therefore used as the most effective strategy to prevent SARS-CoV-2 from spreading. Viable disinfectants for SARS-CoV-2 outlined by the United States Environmental Protection Agency (USEPA) include chlorine-based disinfectants, hydrogen peroxide, ethanol, octanoic acid, peroxyacetic acid and quaternary ammonium ⁴. Among them, chlorine-based disinfectants are most used because of its potency and low price ^{5,6}. Particularly, Chinese government has expended a huge amount of chlorine-based disinfectants for both indoor and outdoor spaces, and at least 2000 tons of disinfectants are estimated to use in Wuhan alone in the initial stage of the COVID-19 pandemic ⁷. These disinfectants may eventually adduct and accumulate in lakes and rivers through runoff and sewage, posing a significant threat to aquatic ecosystem and drinking water safety ⁸. However, there is still lack of evidence on the hypothetical consequence from disinfectant overuse during the COVID-19 pandemic.

Chlorine-based disinfectants can directly destroy cell walls or oxidize proteins of organisms ⁸. In addition, they can react with various kinds of organic matters, *e.g.*, natural organic matters, effluent suspended solids, microorganisms, algal toxins, and anthropogenic contaminants, to form unintended disinfection by-products (DBPs) ^{9,10}. Up to now, more than 800 DBPs have been identified, including trihalomethanes (THMs), haloacetic acids (HAAs), halogen acetonitriles (HANs), halonitromethanes (HNMs), haloacetamides (HAcAms), haloketones (HKs) and nitrosamines (NAs). Since many DBPs are reported to possess cytotoxicity, mutagenicity, genotoxicity, and/or

27 teratogenicity, increasing concerns are raised for public health ¹¹. Microorganisms, plants
28 and animals in aquatic ecosystems are highly vulnerable to environmental insult from
29 residual DBPs, which in turn affects human beings by biomagnification through the food
30 chain ¹². Yadav et al. reported the effects of DBPs on microorganisms that both microbial
31 diversity and community structure of aquatic ecosystem were altered after disinfection ¹³.
32 THM exposure leads to developmental anomalies of zebrafish, and HAAs including
33 tribromoacetic acid (TBA) and dichloroacetic acid (DCAA) are responsible for embryo
34 malformations ¹⁴. DBPs exposure occurs in three ways including inhalation, ingestion and
35 dermal absorption, leading to high levels of skin permeable and volatile DBPs in the blood
36 ^{15,16}. Several epidemiological studies have suggested that long-term exposure to DBPs
37 is related to adverse human health outcomes including higher incidence of bladder and
38 colorectal cancer ^{17,18}. Since THMs are soluble in fat and also highly volatile, they are
39 found in various food such as ice cream, juices and soft drinks. In addition, THMs are
40 reported to bioaccumulate in adipose tissue, kidneys, lungs and liver when quantity and
41 route being exposed ^{19,20}. Previous studies have suggested that many DBPs are
42 intermediate compounds that can transform into other end products by hydrolysis or
43 chlorination ²¹. For example, HANs are susceptible to further convert to haloacetic acids,
44 especially trihaloacetonitriles ²². Accordingly, many DBPs have been classified as
45 potential carcinogenic compounds to humans by International Agency for Research on
46 Cancer (IARC) ¹⁵.

47 Several regulatory guidelines are established by authorities like WHO, USEPA and
48 Bureau of China Standard for DBPs in drinking water. The maximum contamination level
49 (MCL) is 80 µg/L for THMs and 60 µg/L for HAAs in USA ²³. In China, it is 60 µg/L for
50 trichloromethane (TCM), 60 µg/L for monobromodichloromethane (BDCM), 100 µg/L for
51 dibromochloromethane (DBCM), 100 µg/L for tribromomethane (TBM), 50 µg/L for
52 dichloroacetic acid (DCAA) and 100 µg/L for trichloroacetic acid (TCAA) ²⁴. These

guidelines mainly focus on drinking water or swimming pool; however, limited studies address DBPs in natural waters, particularly after the intensive disinfection activities in the pandemics like COVID-19, when excessive DBPs in chlorinated sewage effluents and surrounding soils eventually extravasate into the receiving surface waters and contaminate the drinking water sources. As COVID-19 continues to spread across the world, the increasing consumption of chlorine-based disinfectants and production of DBPs might result in a global secondary disaster in aquatic ecosystems⁷. Therefore, it is of great significance to assess the occurrence, distribution and impacts of DBPs in aquatic ecosystems.

Till now, only two studies reported the occurrence and distribution of DBPs in several rivers and lakes during the COVID-19 pandemic in China^{25,26} and did not find a significant increase. There is still lack of a comprehensive survey targeting a broad range of water sources in megacities. This work studied the levels and compositions of DBPs in 110 water samples from nine lakes and two rivers in Wuhan in the initial stage of the COVID-2019, and for the first time found a significant accumulation and unneglected risks of HAAs in natural waters. We aimed to evaluate the levels of DBPs in lakes and rivers, assess their toxicities and ecological impacts, and explain the formation mechanisms of DBPs by exploring the organic matter compositions in these aquatic systems. Our findings highlight the secondary environmental risks after the intensive disinfection activities in the pandemics, and offer suggestions for disinfection strategies and DBPs risk management in natural waters.

2. Results

2.1 Levels of key DBPs in lakes and rivers

Mean concentrations and compositions of key DBPs in 110 water samples are illustrated in Figure 2. Among them, HAAs (33.8-856.1 µg/L) were of the highest level in lakes,

79 followed by THMs (0.99-14.3 µg/L), HNMs (ND-4.62 µg/L), HANs (ND-1.09 µg/L) and
80 NAs (0.0414-0.0861 µg/L). Only DCAA was detected among three HAAs and the
81 concentration exceeded the MCL of the Standards for Drinking Water Quality of China
82 (GB5749-2006) except for samples from MSH. The highest mean concentration of HAAs
83 was observed in JYH (856.1 µg/L), followed by NH (330.1 µg/L) and HH (123.7 µg/L).
84 Other DBPs were all below the MCL. For river samples, only waters from Yangtze River
85 (YR) had a wide variety of DBPs, and HAAs had the highest concentration (mean 487.1
86 µg/L), approximately 8.7 times higher than the MCL. In contrast, only limited DBPs were
87 detected in HR, and none of them exceeded the MCL.

88 *2.2 DBPs composition*

89 The composition of individual THMs, HANs, HNMs and NAs in water samples are
90 illustrated in Figure 3. It is clear that waters from five lakes of HJH, NH, LZH, MSH and
91 HGH had high levels of TCM (1.14-6.91 µg/L, Figure 3A), DBCM (1.36-7.35 µg/L, Figure
92 3A), TCAN (0-0.45 µg/L, Figure 3B) and TCNM (0.48-4.62 µg/L, Figure 3C), designated
93 as the type-I lakes. For the other four lakes of DH, HH, JYH and TXH (the type-II lakes),
94 they only had a low level of TCM (0.13-0.16 µg/L) and DBCM (0.84-1.33 µg/L), whereas
95 TCAN and TCNM were non-detectable. Waters from both YR and HR were similar as
96 those from the type-II lakes, containing mainly DBCM (2.24 and 1.52 µg/L, respectively).
97 It is worth mentioning that the concentrations and compositions of NAs, which were not
98 DBPs from chlorine-based disinfectants, were similar across all water samples (Figure
99 3D). More precisely, NDMA (0.00836-0.0566 µg/L) and NDEA (0.00408-0.0379 µg/L)
100 were the predominant NAs, followed by NPYR (0.00192-0.00998 µg/L) and NPIP (N.D.-
101 0.0181 µg/L).

102 *2.3 Toxicity and ecological risks of DBPs in lakes and rivers*

103 The cytotoxicity indices and fractions of each DBPs in lakes and rivers of Wuhan are
104 illustrated in Figure 4A and 4B, respectively. Total DBPs cytotoxicity ranged from 3.80×10^4

(HGH) to 3.62×10^5 (JYH) in lakes. HAAs and HANs accounted for 74.2-95.1% of total DBPs cytotoxicity, which was approximately 1-3 orders of magnitude higher than THMs, HNMs or NAs. Both HAAs and HANs exhibited the major cytotoxicity in the type-I lakes, whereas HAAs dominated the cytotoxicity in the type-II lakes. Only DBPs in the type-I lakes exhibited significant genotoxicities ranging from 5.24×10^3 to 5.17×10^4 (Figure 4C). HNMs contributed to the majority of genotoxicities (94.0-98.7%), approximately 16.0-49.4 times higher than HANs.

RQs represent the potential ecological risks of DBPs in aquatic ecosystem, and our data suggested neglectable risks of DBPs to fish and daphnid in lakes and rivers of Wuhan, as their RQs were less than 0.1 (Figure 5A and 5B). Nevertheless, DCAA exhibited significant ecological risks to green algae, which were adverse in HJH, JYH and YR (RQs>10) and moderate in other seven lakes ($1.0 < \text{RQs} < 10$) (Figure 5C). All the other DBPs showed insignificant risks (RQs<0.01) to green algae.

2.4 Water physiochemical variables and DOMs

The physiochemical variables of 110 surface waters in Wuhan are listed in Table 1. The mean values of pH, conductivity, turbidity, $\text{NH}_3\text{-N}$, TN and TP were 6.64-8.63, 114.0-358.64 $\mu\text{S/cm}$, 1.85-38.81 NTU, 0.08-0.52 mg/L, 0.52-2.94 mg/L and 0.02-0.21 mg/L, respectively. TOC ranged from 2.72 ± 0.18 to 16.0 ± 9.03 mg/L and HJH were the highest. COD ranged from 2.97 ± 1.80 mg/L to 61.5 ± 21.7 mg/L and was highest in HH.

Typical EEM spectra of natural DOMs in lakes and rivers are shown in Figure 6A. The spectra exhibited some similarities in peak locations among DOMs from the nine lakes, such as region IV (microbial by-products) and region V (humic acids). The peaks of tryptophan-like proteins in region II were prominent, whereas the peaks of tyrosine-like proteins in region I were less noticeable. Figure 6B illustrated a similar distribution of FRI among all lakes and rivers. Region II (22.7-29.2%) was the highest for all samples, followed by region III (19.3-27.1%), region IV (12.9-23.1%), region V (11.7-22.3%) and

region I (10.7-20.4%). These indicated the presence of many tryptophan-like proteins and fulvic acids in water resources of Wuhan. More precisely, HIX values (1.83-3.31) were less than 4.0 (Figure 6B), suggesting a weak humification degree of DOMs. BIX values ranging from 0.90 to 1.65 suggested the autochthonous production of most natural DOMs. In most lakes, FI values ranged from 1.92 to 2.15 and NOMs were freshly produced from biological activities, whereas the lower FI values in LZH (1.73), YR (1.83) and HR (1.72) documented their formation from both microbial and terrestrial activities. Peak T/C values in YR (1.21) and HR (1.41) were much lower than those in lakes (1.54-3.39), indicating poorer biodegradability of natural DOMs in rivers.

2.5 Links between DBPs, water physiochemical variables and DOMs

Correlation analysis (Figure 7A) showed the relationships between DBPs, water physiochemical variables and FI indices of natural DOMs. Among DBPs, there were significant and positive correlations between HANs and HNMs ($r^2=0.66$, $p<0.05$), THMs and HNMs ($r^2=0.51$, $p<0.05$), THMs and HANs ($r^2=0.41$, $p<0.05$). These hinted similar precursors for the formation of THMs, HNMs and HANs. Among water physiochemical variables, TOC exhibited significant and positive correlations with HNMs ($r^2=0.45$, $p<0.05$), THMs ($r^2=0.23$, $p<0.05$) and HANs ($r^2=0.21$, $p<0.05$), further confirming similar organic precursors for the formation of HNMs, THMs and HANs. The positive correlation between $\text{NH}_3\text{-N}$ and HANs ($r^2=0.21$, $p<0.05$) suggested the important roles of ammonia in HANs formation. Conductivity was positively correlated with HAAs ($r^2=0.22$, $p<0.05$), but exhibiting negative correlations with HNMs ($r^2=-0.30$, $p<0.05$), THMs ($r^2=-0.51$, $p<0.05$) and HANs ($r^2=-0.23$, $p<0.05$). Among FRI, region I exhibited significant and positive correlations with THMs ($r^2=0.23$, $p<0.05$) and HAAs ($r^2=0.34$, $p<0.05$), indicating that tyrosine-like proteins may be crucial precursors of both THMs and HAAs. Region III ($r^2=0.25$, $p<0.05$) and Region IV ($r^2=-0.29$, $p<0.05$) was positively and negatively correlated with THMs, respectively. Region V exhibited significant and negative

correlations with HAAs ($r^2=-0.31$, $p<0.05$). Among fluorescence indices, there were negative correlations between HIX and HAAs ($r^2=-0.23$, $p<0.05$), and BIX and HNMs ($r^2=-0.22$, $p<0.05$).

RDA score plot between water physiochemical variables and DPBs (Figure 7B) illustrates that the first (RDA1) and second (RDA2) components explained 19.76% and 2.58% of the total variance, respectively. Among all the variables, conductivity (10.4%, $p=0.002$), TOC (7.1%, $p=0.004$), turbidity (3.3%, $p=0.02$) and $\text{NH}_3\text{-N}$ (3.0%, $p=0.042$) contributed to the major variance of DBP composition. Water samples from the type-I lakes, type- I I lakes and rivers were clearly separated. DBPs in the type-I lakes were mainly linked with TOC, whereas conductivity had more critical roles in the type-II lakes and two rivers, segregated by $\text{NH}_3\text{-N}$. As for fluorescence indices of DOMs, they together explained 20.88% of the total variance of DBP composition (Figure 7C). Peak T/C (4.1%, $p=0.02$) and Region IV (3.9%, $p=0.01$) were significant variable.

3. Discussion

To fight against COVID-19, chlorine-based disinfectants are extensively or even over used for water, surface and personal care decontamination, raising the concerns about the formation, release and accumulation of DBPs in surrounding environment ⁷. Disinfection with chlorine-based disinfectants can directly generate high levels of DBPs in sewage and sludge, and the residual active chlorine might further react with NOMs in natural waters or sediments, causing secondary contamination in lakes, rivers and even surrounding soils ⁸. These DBPs hypothetically extravasate into the receiving rivers and lakes, posing a great risk to aquatic ecosystem and public health ⁷. Till now, there is still lack of direct evidence, and our study is the first report unraveling the secondary pollution and ecological risks of DBPs in lakes and drinking water sources of Wuhan, resulted from disinfectant overuse during the COVID-19 pandemic. Our findings comprehensively

visualized the occurrence and distribution of eighteen DBPs, assessed their toxicities and ecological risks, and discussed the formation mechanisms, showing great significance to evaluate the consequence of intensified disinfection on aquatic ecosystem.

DBP levels are reported to vary greatly across water samples, attributing to the difference in disinfectant type, dosage, water physiochemical variables, and the presence of organic and halogenated molecules in water ²⁷. Comparing to other previously reported DBPs levels in wastewater effluents (13.5 to 234.2 µg/L) ²⁸, drinking water (86.0-122.2 µg/L) ²⁹ and swimming pools (85.2-224.0 µg/L) ³⁰ (Table 2), our results suggested a significant increase and accumulation of DBPs in surface waters of Wuhan after using superfluous disinfectants to decontaminate wastewater, surface and personal care in the COVID-19 pandemic. Particularly, HAAs were 33.8-856.1 µg/L in almost all lakes and Yangtze River, much higher than the MCL of the Standards for Drinking Water Quality of China (GB5749-2006). Previous studies found different levels of HAAs in drinking water and wastewater effluents, highlighting the necessity to prevent HAAs formation in drinking water disinfection ^{23,28}. Nevertheless, HAAs accumulation in natural surface water has not been reported, and the significant increasing HAAs in lakes and rivers of Wuhan in this study prove that intensive disinfection in megacities during the COVID-19 pandemic can cause remarkable DBP contamination in surface water and raises special concerns of disinfection strategy.

DBP composition in surface waters of Wuhan was different from other studies. THMs were normally the most prevalent chlorinated DBPs ²³, whereas they were much lower in our study, only ranging from 0.99 to 14.26 µg/L and accounting for 0.52-21.11% of total DBPs. In contrast, the average levels of HAAs (33.8-856.1 µg/L) were much higher and dominated DBPs, consistent with Lee's work that HAAs (35.2-747.1 µg/L) was main DBPs in chlorinated-swimming pool water, accounting for 72.6% of total DBPs ³¹. It might be explained by higher volatility and stability of THMs than HAAs in natural environment.

209 Unlike previous laboratory studies collecting samples promptly after the addition of
210 chlorine-based disinfectants, it normally takes several hours to days for lakes and rivers
211 to receive sewage containing DBPs from pipeline networks, which have many lifting and
212 pumping operations from disinfection points to the final receiving water ¹⁶. Highly volatile
213 chemicals like THMs are therefore easier to evaporate into atmosphere and their
214 concentrations then decline in natural water. Another possible explanation is the
215 secondary formation of DBPs in the receiving water due to the excess active chlorine
216 residual in sewage after intensive disinfection activities. Higher temperature, microbial
217 activities and DOMs are reported to benefit HAA formation ^{23,32}. In this study, the lakes
218 and rivers of Wuhan had poor water quality with high levels of COD and TOC. More
219 precisely, COD in HH exceeded the standard of Class V according to the Environmental
220 Quality Standards for Surface Water (GB 3838-2002) regulated by Chinese Ministry of
221 Health ³³. Together with the relatively higher temperature ranging from 15 to 30 °C during
222 the sampling period, more HAAs might be formed than THMs in natural surface waters.
223 Last but not the least, higher dosage and residuals of chlorine-based disinfectants can
224 favor the formation of HAAs over THMs ³⁴. Accordingly, HAAs rather than other DBPs
225 exhibited a striking high level in lakes and rivers of Wuhan, and they should be specifically
226 monitored in other surface waters receiving disinfected sewage in cities with intensive
227 disinfection activities to fight against COVID-19.

228 Besides total amount, each DBP category exhibited unique compositions comparing to
229 previous studies. TCM and DBCM were the dominant THMs in lakes and rivers of Wuhan,
230 and BDCM and TBM were not detected (Figure 3A). It was different from another study
231 reporting high levels of TCM (20.9 µg/L, approximately 90% of THMs) but neglectable
232 DBCM and TBM (<0.2 µg/L) in chlorinated swimming pool waters ³¹. Among HAAs, only
233 DCAA was detected in lakes and YR, while TCAA and MCAA were non-detectable. Other
234 studies have inconsistent results that TCAA was the most common HAAs, followed by

DCAA, DBCAA, MCAA and MBAA, in drinking water ²³, chlorinated wastewater effluents ²⁸, and chlorinated swimming pools ³¹. The low levels of HANs in our study (ND-1.09 µg/L) were consistent with other studies on drinking water (0.6-24 µg/L) ²⁸ and wastewater effluents (2.9-48.6 µg/L) ³¹. However, TCAN was the major HANs (Figure 3B), different from a study on the chlorinated swimming pool waters that DCAN (74.7%), BCAN (15.8%) and DBCN (9.5%) were predominant HANs and TCAN was below the limit of detection ³¹. Only TCNM was detected among all HNMs in lakes (Figure 3C) and similar with the concentrations in the chlorinated drinking water (0.16-1.5 µg/L) ²⁸, meeting well with previous studies that the TCNM (>94%) were the major HNMs in chlorinated water ^{35,36}. The dominance of TCAN and TCNM might be explained by slow decomposition of TCAN through hydrolysis and natural transformation from HCNM to TCM ³¹. Our results suggested that DBPs in natural surface waters receiving sewage containing DBPs from intensive disinfection during the COVID-19 pandemic had unique signature and compositions.

We only found ng/L level of NAs in lakes and rivers of Wuhan (Figure 3D), consistent with previous studies on drinking waters and wastewater effluents ^{28,37}. Particularly, NDMA and NDEA were predominant NAs (Figure 3D), similar as a study on river waters that NDMA (0.0015-0.017 µg/L) and NDEA (0.0014-0.0095 µg/L) were major NAs ³⁸. Wang et al. reported that wastewater effluents from upstream cities might be a crucial source of NAs ³⁵, and the tap water in megacities like Shanghai are heavily polluted by NAs compared to other areas along Yangtze River. As NAs are formed by the reactions between monochloramine and organic amine precursors ³⁷ or nitrite chlorination in the presence of nitrosamine precursor ¹⁷, they are not correlated with the intensively used chlorine-based disinfectants during the COVID-19 pandemic in Wuhan. Accordingly, all NAs had similar concentrations and compositions across all lakes and rivers in this study, documenting a DBP background from other disinfection activities.

According to the occurrence and composition of DBPs, two types of lakes were classified in Wuhan (Figure 3). Type-I lakes (HJH, NH, LZH, MSH and HGH) had high levels of TCM, DBCM, TCAN and TCNM (Figure 3), and they are mainly located in urban areas with higher population and disinfection density. The intensive disinfection activities in these regions left excessive disinfectants on roads and surrounding soils, eventually extravasating into the receiving lakes through rain pipes after rain off ²⁵. These residual disinfectants might react with NOMs in lakes and formed secondary DBPs. It was supported by the higher explanation of TOC in DBPs composition in RDA score plots (Figure 7B) and positive correlations between three kinds of DBPs (HANs, HNMs, THMs) and TOC in PCA score plots (Figure 7A). Further evidence comes from the structural characteristics of DOMs across lakes and rivers using EEM spectra, which provide information of chromophores and fluorophores ³⁹. DOMs in type-I lakes exhibited low HIXs (<4.0), high BIXs (0.90 to 1.12), moderate FIs (1.72-2.06), and high Peak T/C (1.53-2.71), suggesting weak humification, autochthonous and microbial-driven features ⁴⁰. Previous studies revealed that THMs were strongly correlated with humic-like components (region V) ⁴¹. Despite the similar distribution of region V between type-I (11.8-20.9%) and type-II (11.7-22.3%) lakes, TOC in type-I lakes (5.10-16.0 mg/L) were much higher than type-II lakes (4.95-8.00 mg/L), indicating higher levels of precursors for THMs. Similarly, the precursors for HANs strongly correlated with protein-like component (region I and II) ⁴², which were also remarkably higher in type-I lakes than in type-II lakes.

In contrast, type-II lakes (DH, HH, JYH and TXH) only had a low level of TCM and DBCM (Figure 3), and they are in suburban regions with lower population density, large area and mainly rain-sewage mixed flow pipelines. There were relatively weaker disinfection activities, and the low levels of residual disinfectants in runoff might directly react with DOMs in sewage in the pipelines and only primary DBPs were formed. Therefore, type-II lakes exhibited different DBPs composition, predominantly explained by conductivity,

turbidity and $\text{NH}_3\text{-N}$ (main contaminants in sewage) in RDA results (Figure 7B). Similarly, there are many discharge points of rain-sewage mixed or diversion pipelines along Yangtze River (YR)⁴³, which received effluents from both type-I and type-II lakes and had high levels of HAAs and small amounts of THMs (Figure 2A). Instead, Han River (HR) is the main drinking water sources for Wuhan and nearly all the outfalls have been banned since 2002⁴⁴. Therefore, Han River did not receive sewage containing high levels of DBPs from intensive disinfection activities during the pandemic and there were only NAs irrelevant to the chlorine-based disinfectants in HR (Figure 2A). Our findings proved that the high levels of DBPs in Yangtze River were mainly derived from discharge from pipelines and surrounding lakes, and the residual DBPs might spread and threaten the downstream aquatic ecosystems of Yangtze River.

DBPs in lakes and rivers of Wuhan exhibited considerable cytotoxicities, significant genotoxicities and non-neglectable ecological risks (Figure 4). Cytotoxicities were mainly derived from HAAs and HANs, which were approximately 1-3 orders of magnitude higher than THMs, HNMs or NAs (Figure 4A). More precisely, HAAs exhibited the highest concentration (Figure 2A) and strong cytotoxicity⁴⁵, contributing to the majority of cytotoxicities (15.7-98.6%) in lakes. As for HR containing neglectable HAAs and HANs, NAs had the highest cytotoxicity (Figure 4B). Although THMs, HAAs and NAs were main DBPs, neither of them exhibited genotoxicity or lack information (Table S2)^{46,47}. Instead, HNMs contributed to the major genotoxicities (94.0-98.7%), approximately 16.0-49.4 times higher than HANs. All DBPs exhibited limited or nonnegligible ecological risks to fish, daphnid and green algae in lakes and rivers of Wuhan (Figure 5A and 5B), except for DCAA which imposed significant risks to green algae. All these results were consistent with some previous reports about the cytotoxicities of HANs in wastewater effluents^{28,45}, major cytotoxicities of HANs and NAs in chlorinated effluents²⁸, significant cytotoxicities of HANs, haloacetaldehydes and HAAs in chlorinated saline groundwater⁴⁸, stronger

genotoxicities of nitrogen-containing HANs and HNMs⁴⁹ in disinfected recreational pools⁵⁰, and remarkable ecological risks of HAAs for green algae in chlorinated wastewater effluents²⁸. Comparing to other studies on wastewater effluents (cytotoxicities of 10^5 - 10^7)²⁸ and drinking water (cytotoxicities of 10^2 - 10^5 and genotoxicities of 10^2 - 10^3)⁵¹, the risks of DBPs in lakes and rivers of Wuhan were nonnegligible. Regarding the facts that some lakes and HR are water sources of Wuhan and residual DBPs can transport to downstream of Yangtze River, DBPs derived from intensive disinfection activities in Wuhan had significant ecological risks and could threaten drinking water safety. It is therefore of urgency to reconsider the disinfection strategies for the prevention and control of emerging infectious diseases during the pandemics, and appropriate disinfection management including disinfectant type and dosage is critical to mitigate secondary environmental pollution in the post-COVID-19 era.

4. Conclusion

Our study first reported the increasing levels of DBPs, particularly HAAs, in lakes and rivers of Wuhan during the initial stage of the COVID-19 pandemic. They were mainly derived from the intensive disinfection activities in urban areas, and imposed nonnegligible toxicities and significant ecological risks to surrounding receptors or even downstream of Yangtze River. Additionally, the composition of DBPs varied across surface waters and were remarkably different from previous studies on chlorinated drinking water or wastewater, explained by the long-distance transport in sewage pipelines and secondary formation with NOMs in surface waters. Our findings provide the first evidence that the excessive disinfection activities in megacities during the pandemics do disperse DBPs in the surrounding receiving waters and threaten aquatic ecosystems, highlighting the importance for appropriate disinfection strategy and management for the prevention and control of emerging infectious diseases during the pandemics.

340 **5. Materials and Methods**

341 *5.1 Materials*

342 Eighteen DBPs, including four THMs (TCM, BDCM, DBCM and TBM), three HAAs
343 (monochloroacetic acid, MCAA; DCAA; TCAA), one HAN (trichloroacetonitrile, TCAN),
344 one HNM (trichloronitromethane, TCNM) and nine NAs (N-nitrosomethylethylamine,
345 NMEA; N-nitrosopyridine, NPYR; N-nitromorpholine, NMOR; N-nitrosodiethylamine,
346 NDMA; N-nitrosodiethylamine, NDEA; N-nitrosopiperidine, NPIP; N-nitrosodipropylamine,
347 NDPA; N-nitrosodibutylamine, NDBA; N-nitrosodiphenylamine, NDphA) were
348 investigated in this study. DBPs chemical standards were purchased from Sigma-Aldrich
349 (USA) and stored at 4 °C.

350 *5.2 Study area and sampling*

351 The study area is in Wuhan, Hubei Province (China), which is about 8569 km² with
352 estimated population of 11.2 million. As illustrated in Figure 1 and Table S1, two types of
353 surface water including nine lakes (Huangjiahu Lake, HJH; Nanhu Lake, NH; Liangzihu
354 Lake, LZH; Moshuihu Lake, MSH; Tangxun Lake, TXH; Houhu Lake, HH; Donghu Lake,
355 DH; Houguanhu Lake, HGH; Jinyinhu Lake, JYH) and two rivers (Yangtze River, YR;
356 Hanjiang River, HR) were collected in May and June 2020, which was the initial stage of
357 the COVID-2019 pandemic. The distances between sampling sites were about 0.3-4.0
358 km and 2.0-9.0 km in lakes and rivers, respectively. All sampling sites in two rivers are
359 drinking water sources of Wuhan. Water samples were transported to the laboratory
360 within 4 h, stored at 4 °C and filtered through a 0.2 µm membrane filter prior to
361 instrumental analysis.

362 *5.3 Chemical analysis of water physiochemical variables*

363 Total organic carbon (TOC) was analyzed on a Shimadzu TOC-Analyzer (TOC-L CPN,

Japan). Chemical oxygen demand (COD), total phosphorus (TP), total nitrogen (TN) and ammonia nitrogen ($\text{NH}_3\text{-N}$) were analyzed according to the standard methods ²⁶. The pH and conductivity were measured with a WTW Multi 3410 equipped with a SenTix 940 pH electrode (WTW, Germany) and an HI 9835 conductivity meter (Hanna, Italy), respectively.

5.4 Excitation-emission matrix fluorescence spectroscopic analysis

Besides anthropogenic sources, dissolved organic matters (DOMs) in natural waters are well-recognized as the crucial precursors of DBP. As a complex mixture of organic matters with diverse chemical structures and reactivities, DOMs were characterized by excitation-emission matrix (EEM) fluorescence spectroscopy with high sensitivity and selectivity ⁵², using a Hitachi spectrofluorometer (F-7000 FL, Japan) at room temperature (20-23 °C) with a 1-cm quartz cuvette. The test conditions were as follows: excitation wavelength range: 200-400 nm (step 5 nm), emission wavelength range: 250-500 nm (step 5 nm), scan speed: 2400 nm/min. The spectrofluorometer was auto-zeroed prior to analysis and the fluorescence intensities were normalized by the Raman peak of Milli-Q water at $\lambda_{\text{ex}}=348$ nm.

Fluorescence regional integration (FRI) and several fluorescence indices were used to delineate EEM data. EEM peaks were first divided into five regions including aromatic proteins such as tyrosine and tryptophan (regions I and II), fulvic acids (region III), soluble microbial by-products (region IV) and humic acids (region V). The distribution of five regions can be calculated according to a previous study ⁵³. The fluorescence indices of humification index (HIX), autochthonous biological index (BIX), fluorescence index (FI) and peak T/C (the intensity ratio of peak T to peak C) were then calculated. HIX and BIX reflects the humification degree and microbial aspect of DOM properties, respectively. FI indicates the possibility of microbial production, and peak T/C indicates the biodegradability of DOMs ⁴⁰. They were calculated by the following Equations (1) to (3).

$$HIX = \frac{\sum I_{Em} 435-480}{\sum I_{Em} 300-345}, \text{ at } E_x = 254 \text{ nm} \quad (1)$$

$$BIX = \frac{I_{Em} 380}{I_{Em} 430}, \text{ at } E_x = 310 \text{ nm} \quad (2)$$

$$FI = \frac{I_{Em} 450}{I_{Em} 500}, \text{ at } E_x = 370 \text{ nm} \quad (3)$$

389 5.5 DBPs analysis

390 Five categories of DBPs including four THMs, three HAAs, one HAN, one HNM and nine
 391 NAs were quantitatively analyzed. THMs measurement was conducted on a gas
 392 chromatograph-mass spectrometer (GC-MS) (Agilent 7890B-5977C, USA) equipped with
 393 a purge and trap autosampler and a DB-624 column (60 m×0.25 mm×1.4 μm). For HAAs,
 394 samples were filtered with a Ba/Ag/H column and 0.2 μm microporous membrane, and
 395 then analyzed on an ion chromatograph (IC) (Thermo Integrion HPIC, USA). HANs and
 396 HNMs analyses were performed on a GC equipped with an electron capture detector
 397 (GC-ECD) (Agilent 7890A) and a DB-17MS capillary column (30 m×0.25 mm×0.25 μm).
 398 Solid phase extraction (SPE) and liquid chromatograph-mass spectrometer (LC-MS)
 399 (Shim-pack LCMS-8) were used for the measurement of NAs.

400 5.6 Toxicity and ecological risk assessment of DBPs

401 Both cytotoxicity and genotoxicity are widely used to assess the overall toxicity of DBPs
 402 in drinking water ²⁸. The unit cytotoxicity and genotoxicity index values were defined as
 403 the reciprocal of the median LC₅₀ and median CHO SCGE genotoxic potency for each
 404 DBP, respectively ⁴⁶. Cytotoxicity and genotoxicity indices of each DBPs were calculated
 405 by multiplying unit toxicity indices and DBPs concentrations, as listed in Table S2 ^{46,47}.
 406 In addition, risk quotient (RQ) was used to assess the potential ecological risks of DBPs
 407 in aquatic ecosystems. Three different model organisms including fish, daphnid and green
 408 algae were evaluated at the acute level of DBPs using LC₅₀ or EC₅₀. RQs of each DBP

409 for a certain taxonomic group were calculated by the following equation (4)^{28,54}.

$$RQ = \frac{MEC}{LC_{50} \text{ or } EC_{50}} \quad (4)$$

410 Here, MEC is the concentration of DBPs in natural waters, and LC₅₀ and EC₅₀ of each
411 DBPs were derived from ECOSAR Program developed by USEPA (Table S3). RQ<0.1 is
412 regarded as no adverse effect; 0.1<RQ<1 represents low risk; 1<RQ<10 indicates
413 moderate risk; RQ>10 is considered adverse risk to aquatic organisms^{54,55}.

414 *5.7 Statistical data analysis*

415 Differences in DBP concentrations between sites, lakes and rivers were examined using
416 one-way analysis of variance (ANOVA) at $p=0.05$ level. Non-parametric Spearman's
417 correlation analysis between DBPs, water physiochemical variables, and fluorescence
418 indices were performed by SPSS 24.0. Redundancy analysis (RDA) was used to explore
419 the relationships between DBPs, water physiochemical variables, and fluorescence
420 indices using Canoco 5.0.

421

422 **Contributions**

423 Y.L., J.Q. and D.Z. designed the whole work. X.Z., L.W. H.L., W.L., C.Y. and X.W. collected
424 water samples. S.C. did the chemical analysis. X.Z. coordinated data analyses and wrote
425 the first draft. Y.X., X.H. and D.Z. edited the manuscript.

426

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432

433 **Ethics declarations**

434 Competing interests

435 The authors declare no competing interests.

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- 593

595 **Table 1.** Water physiochemical variables in lakes and rivers of Wuhan.

	<i>n</i>	pH	Conductivity (μS/cm)	Turbidity (NTU)	NH ₃ -N (mg/L)	TN (mg/L)	TP (mg/L)	COD (mg/L)	TOC (mg/L)
HJH	12	6.64±0.15	236.5±62.4	6.95±1.77	0.21±0.05	0.81±0.16	0.09±0.02	12.8±2.57	16.0±9.03
NH	10	7.41±0.27	281.2±28.9	9.62±3.46	0.52±0.15	1.73±0.51	0.17±0.08	28.7±4.36	9.89±6.02
LZH	9	8.00±0.04	114.0±4.55	1.85±0.84	0.10±0.03	1.00±0.21	0.02±0.01	6.19±1.75	5.10±1.75
MSH	8	8.22±0.26	251.7±40.8	13.00±6.12	0.45±0.10	2.94±0.99	0.19±0.05	37.9±5.80	7.46±1.44
HGH	11	8.63±0.10	240.1±19.7	5.85±2.77	0.20±0.05	0.77±0.14	0.04±0.01	14.6±2.46	5.02±0.95
DH	12	8.45±0.22	287.7±23.0	3.55±1.41	0.12±0.02	0.52±0.05	0.03±0.01	14.2±1.91	5.34±1.49
HH	8	6.88±0.54	206.4±41.2	38.81±32.80	0.41±0.15	2.01±1.07	0.20±0.14	61.5±21.7	8.00±0.75
JYH	12	7.77±0.09	321.2±53.4	6.37±3.77	0.20±0.10	1.13±0.23	0.08±0.07	18.10±2.91	4.95±1.91
TXH	11	8.11±0.17	358.6±59.9	11.39±4.05	0.36±0.05	1.58±0.36	0.21±0.06	35.0±5.93	6.96±1.54
YR	11	7.86±0.23	262.5±12.8	2.06±0.46	0.08±0.02	1.52±0.26	0.03±0.01	2.97±1.80	2.72±0.18
HR	6	7.57±0.04	285.3±18.4	2.44±0.59	0.08±0.01	1.07±0.13	0.03±0.01	8.33±0.96	2.99±0.16

597 **Table2.** Comparison of DBPs compositions between this study and other waters.

Water sample	THMs (µg/L)	HAAs (µg/L)	HANs (µg/L)	HNMs (µg/L)	NAs (µg/L)	Reference
Drinking water	0.7-32.8	0-17.2	N.D.	N.D.	N.D.	56
	27.6-50.9	35.1-94.6	N.D.	N.D.	N.D.	29
	0.13-458	0.16-136	0.12-30	N.D.	N.D.	57
Wastewater effluents	31.9-115.7	0-27.3	4.0-20.7	N.D.	N.D.	58
	2.3-60.7	7.3-120.1	2.9-48.6	1.0-7.4	0.35-1.19	28
	2.47-4.45	N.D.	0.14-0.66	N.D.	N.D.	45
Swimming pools	N.D.-49.0	35.2-747.1	0.8-14.3	N.D.	N.D.	31
	75-104.0	N.D.-119	N.D.	0.7-1.7	N.D.	30
	41.3-57.4	90.1-105.6	15.1-20.6	N.D.	N.D.	58
This study	0.99-14.3	33.8-856.1	0-1.09	0-4.62	0.0414-0.0861	

599 **Figure captions**

600 **Figure 1.** Geographic location of 110 sampling sites in nine lakes and two rivers of Wuhan,
601 China.

602 **Figure 2.** Total amount (A) and composition (B) of key DBPs in lakes and rivers of Wuhan
603 in the initial stage of COVID-19.

604 **Figure 3.** The composition of individual THMs (A), HANs (B), HNMs (C), and NAs (D) in
605 lakes and rivers of Wuhan in the initial stage of COVID-19.

606 **Figure 4.** Cytotoxicity indices (A) and fraction (B) of DPBs in in nine lakes and two rivers
607 of Wuhan. Genotoxicity indices (C) and fraction (D) of DPBs in in nine lakes and two rivers
608 of Wuhan.

609 **Figure 5.** Ecological risk assessment by RQ values of DBPs in rivers and lakes of Wuhan.
610 (A) Fish; (B) daphnid; (C) green algae.

611 **Figure 6.** (A) Typical excitation-emission matrix (EEM) of dissolved organic matters
612 (DOMs) in lakes and rivers of Wuhan. (B) Composition of fluorescence regional
613 integration (FRI) in different lakes and rivers. (C) Fluorescence indices (FI) of DOMs in
614 different lakes and rivers.

615 **Figure 7.** (A) Correlation analysis between BBPs, water physiochemical variables,
616 fluorescence regional integration (FRI) and fluorescence indices (FI) in lakes and rivers
617 of Wuhan. Blue and red ellipses represent significantly positive and negative correlations,
618 respectively. (B) Redundancy analysis (RDA) score plot of DBPs and water
619 physiochemical variables. Key variables (explanation) include conductivity (10.4%,
620 $p=0.002$), TOC (7.1%, $p=0.004$), turbidity (3.3%, $p=0.02$) and $\text{NH}_3\text{-N}$ (3.0%, $p=0.042$). (C)
621 RDA score plot of DBPs and fluorescence indices from excitation-emission matrix (EEM).
622 Key fluorescence indices (explanation) include Peak T/C (4.1%, $p=0.02$) and Region IV
623 (3.9%, $p=0.01$).

Figures

Figure 1

Geographic location of 110 sampling sites in nine lakes and two rivers of Wuhan, China. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Total amount (A) and composition (B) of key DBPs in lakes and rivers of Wuhan in the initial stage of COVID-19.

Figure 3

The composition of individual THMs (A), HANs (B), HNMs (C), and NAs (D) in lakes and rivers of Wuhan in the initial stage of COVID-19.

Figure 4

Cytotoxicity indices (A) and fraction (B) of DBPs in in nine lakes and two rivers of Wuhan. Genotoxicity indices (C) and fraction (D) of DBPs in in nine lakes and two rivers of Wuhan.

Figure 5

Ecological risk assessment by RQ values of DBPs in rivers and lakes of Wuhan. (A) Fish; (B) daphnid; (C) green algae.

Figure 6

(A) Typical excitation-emission matrix (EEM) of dissolved organic matters (DOMs) in lakes and rivers of Wuhan. (B) Composition of fluorescence regional integration (FRI) in different lakes and rivers. (C) Fluorescence indices (FI) of DOMs in different lakes and rivers.

Figure 7

(A) Correlation analysis between BBPs, water physiochemical variables, fluorescence regional integration (FRI) and fluorescence indices (FI) in lakes and rivers of Wuhan. Blue and red ellipses represent significantly positive and negative correlations, respectively. (B) Redundancy analysis (RDA) score plot of DBPs and water physiochemical variables. Key variables (explanation) include conductivity (10.4%, $p=0.002$), TOC (7.1%, $p=0.004$), turbidity (3.3%, $p=0.02$) and $\text{NH}_3\text{-N}$ (3.0%, $p=0.042$). (C) RDA score plot of DBPs and fluorescence indices from excitation-emission matrix (EEM). Key fluorescence indices (explanation) include Peak T/C (4.1%, $p=0.02$) and Region IV (3.9%, $p=0.01$).

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