Insight Into Impacts of Sewage Discharge and River Self-Purification on Microbial Dynamics and Pathogenicity in River Ecosystem

Yuyang Xie  
Shandong University

Xiaolin Liu  
Shandong University

Shou-Qing Ni  
Shandong University  
Email: sqni@sdu.edu.cn

Haiwei Wei  
Shandong University

Xue Chen  
Shandong University

Sherif Ismail  
Shandong University

Shakeel Ahmad  
Muhammad Nawaz Shareef University of Agriculture

Taeho Lee  
Pusan National University

Research Article

Keywords: Environmental parameters, microbial dynamics, raw sewage, river ecosystem, self-purification

DOI: https://doi.org/10.21203/rs.3.rs-608014/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Sewage directly discharge causes serious environmental problems. Here, the effects of treated and untreated sewage on the river ecosystem were investigated. The variations of microbial community structure, including infectious pathogenic bacteria and functional bacteria related to nitrogen, phosphorus, and COD metabolism were studied in detail. Bacterial diversity and richness were significantly decreased, while, *Proteobacteria* containing various infectious pathogens, such as *Vibrio* and *Helicobacter*, significantly increased after the discharge of raw sewage. Although the microbial structure was slightly restored and the abundance of most pathogenic bacteria was also slightly reduced through river self-purification, direct discharge of raw sewage caused severe and short-term irreversible damage to the river environment. Direct discharge also introduced various pollutants such as nitrogen, phosphorus, and COD, increasing the corresponding functional bacteria and their related genes. Furthermore, the high abundance of pathogenic bacteria of the drain outlet was mainly from raw sewage rather than bacteria reproduction caused by water deterioration according to the RDA analysis. With these results, direct discharge disturbed the ecological balance of the river. Therefore, more attention is needed to provide a hygienic situation for people and all sewage should be treated properly. In conclusion, all sewage should be treated properly before discharge into ecosystems to mitigate its negative impacts on receiving water bodies.

1. Introduction

With the massive worldwide population increase, water has been predicted to become one of the scarcest resources in the 21st century. Moreover, UN's World Water Development Report, 2017, said globally 80% of sewage (> 95% in developing countries) is directly released to the environment. Numerous questions have been raised about the ability of wastewater treatment programmes to remove pathogens from wastewater in which many waterborne diseases are associated supposedly treated water supplies. The untreated wastewater can threaten human and ecological system by enriching the pathogenic bacteria (Zhang et al., 2018). Moreover, a body of literature has confirmed the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in wastewater (Ahmed et al., 2020; Haramoto et al., n.d.; Zhang et al., 2020). The SARS-CoV-2 possibly spreads through wastewater treatment network (Naddeo and Liu, 2020). When raw sewage contaminates the aquatic environment, these pathogens can be transmitted through water to those who use the water for swimming, boating and fishing, causing a range of potential risks (Cornelisen et al., 2011). However, there exist many ways for untreated wastewater to enter rivers. When sewage treatment can not treat too much wastewater or when it was rains heavily, raw sewage will be discharged directly into river channels, polluting the environment and making the water quality worse. Natural disasters, such as earthquakes and floods, also make raw sewage flow into rivers, causing pathogens in rivers (Devane et al., 2014). Once sewage delivers to receiving waters, a series of physical and environmental factors changes will occur. Over time, river dilution, storage in sediments, and the intrinsic characteristics of the microorganisms and other related process may alter the destiny of the bacteria the pathogens of concern.
The direct discharge of sanitary wastewater may cause environmental diversity changes. Sewage discharge without proper treatment significantly altered the concentrations of different organic and inorganic contaminants in the receiving water bodies (Duttagupta et al., 2020), especially \( \text{NH}_4^+ \), \( \text{NO}_3^- \), total phosphorus (TP) and chemical oxygen demand (COD). The increase of these contaminants could trigger the aquatic organism by causing eutrophication of the catchment (Huelsen et al., 2016). David et al. (2014) found that the high \( \text{NH}_4^+ \) concentration negatively influenced the functional performance and taxonomic richness of the microbial community of 10 different WWTPs located across Switzerland (David et al., 2014). Moreover, phosphorus content was positively correlated with the microbial biomass (Lei, 2012) and occupied an important position among environmental factors that affect the microbial community of sediments (Jian, 2015; Yu et al., 2017).

Several common infectious pathogens, such as *Vibrio cholera*, *Staphylococcus aureus*, *Mycobacterium tuberculosis*, and *Helicobacter pylori*, exist in aquatic environments. *Vibrio cholerae*, a well-known internationally quarantinable infectious pathogenic bacterium could cause human enteric infection (Reidl and Klose, 2002). *Vibrio cholera* O1 is widely distributed in aquatic environments, namely rivers, ponds, sewage, and estuaries, in many developing countries such as Haiti (Chin et al., 2010). Reidl et al. (2002) isolated *Vibrio cholerae* from estuarine and aquatic environments (Reidl and Klose, 2002).

*Staphylococcus aureus* may cause local purulent infection, pneumonia, and colitis after enrichment in water (Minoru et al., 2017). Zieliński et al. (2020) found Sewage treatment plans are a sizeable source of drug-resistant staphylococci harboring virulence genes (Zieliński et al., 2020). Moreover, *Mycobacterium tuberculosis* normally enters the host via mucosal surfaces, usually through the lung after inhaling infectious droplets from an infected individual and occasionally via the intestine after ingesting infected material (DIETRICH and DOHERTY, 2009). Velayati et al. isolated *Mycobacterium tuberculosis* from water samples collected in Tehran, Iran metropolitan area (Velayati et al., 2015). Many diseases such as gastritis, peptic ulcer, and lymphoid proliferative gastric lymphoma were caused by *Helicobacter pylori* infection (Pereira and Medeiros, 2014). West et al. (1992) reported the capabilities of *Helicobacter pylori* to survive in various buffers at room temperature over a range of physical variables, which means that *Helicobacter pylori* may survive in a natural aquatic environment (West et al., 1992). Therefore, pathogenic bacteria in water environment will have direct or indirect effects on human beings or animals. The study research and analysis their microbial community composition and influencing factors is urgent for future treatment measure.

The lack of a thorough understanding of the survival and persistence of different microbial types in different conditions and environments is one of the major gaps in the knowledge of pathogenic microorganisms in wastewater. Previous studies focused on the abundance of pathogenic bacteria and the relationship between the contaminants and pathogenic bacteria in rivers contaminated by raw sewage discharge (McCarthy, 1996; Miller et al., 2006). However, it is unclear whether the source of pathogenic bacteria in the river comes from sewage itself or is caused by the environmental change after sewage discharge. Also, a deep insight into microbial and genetic responses to river self-purification needs further investigation. Thus, the main target of the present study is to fill in this knowledge gap by
investigating the environmental parameters and the microbial community structures of different sites along the Daxin River, representing treated and raw sewage discharge points. The specific objectives of this study are to (1) provide more information about the water quality and microbial community structure before and after domestic wastewater pollution, (2) explore the composition of pathogenic bacteria community in Daxin River based on PICRUSt and KEGG database, and (3) evaluate the relationship of infectious pathogens and functional bacteria to different environmental factors.

2. Materials And Methods

2.1. Description of Daxin River and sample collection

Jinan is located in the central and western part of Shandong Province, China. There are two major water systems, the Yellow River and the Xiaoqing River. Daxin River is a tributary of Xiaoqing River. Sediment and water samples were harvested from four different sites along the Daxin River. The four sites, distributed from upstream towards downstream of the river, were expressed as Site-1 (117°6′26″N, 36°42′35″E), Site-2 (117°6′24″N, 36°42′44″E), Site-3 (117°6′26″N, 36°44′1″E), and Site-4 (117°6′22″N, 36°44′17″E). Figure 1 shows the distribution of sampling sites along the Daxin River regarding the discharge points of treated and raw sewage. The raw sewage was discharged between Site-1 and Site-2; therefore, Site-2 was directly polluted by raw sewage. The source of the sewage discharged from the drain outlet was domestic wastewater from the nearby neighborhood. Site-3 was located about 3 kilometers downstream of Site-2. A sewage treatment plant discharge port is located between Site-3 and Site-4. The overlying water samples at each site were congregated with Niskin bottles and filtered through 0.45-µm syringe filters for measuring the concentrations of NO$_3^-$-N, NO$_2^-$-N, and NH$_4^+$-N. A Peterson mud sampler was used to collect the surface sediment samples by taking 0–10 cm depth. Sediment samples were frozen at -80 °C for subsequent analysis. Water quality parameters were measured immediately at sample sites.

2.2 Analytical methods

The concentration of NO$_3^-$-N, NO$_2^-$-N, and NH$_4^+$-N were measured according to the standard method (TU1810-PC, Purkinje General, China)(Natalie et al., 2018). The water temperature was measured by centigrade thermometer at each site. The pH of water was done using pH meter (PhS-3C, Rex Electric Chemical, China) during water sampling. The content of total phosphorus (TP) was determined by the colorimetric method of vanadium-molybdenum phosphoric acid. The measurement of chemical oxygen demand (COD) was carried out by COD rapid measuring instrument (MI-80K, China).

2.3 DNA extraction and quantitative real-time PCR (q-PCR) analysis

The Power Soil™ DNA Isolation Kit (Takara Company) was used to extract the total DNA of the freeze-dried sediment samples (Wang et al., 2016). The qPCR (Roche Light Cycler 480, Switzerland) was used to detect the gene concentration of infectious diseases. The qPCR reaction mixture (20 µL) included 2 ×
SYBR Green PCR master mix (10 µL), forward primer (0.4 µL), reverse primer (0.4 µL), template DNA (1 µL), and dd H$_2$O (8.2 µL). The primers used to identify the infectious pathogens were designed according to the conservative sequence of characteristic genes. The annealing temperature of qPCR was predicted using the software primer5.0 based on its "GC" ratio in the whole sequence (Wang et al., 2017).

2.4. High-throughput 16S rDNA sequencing

The next-generation sequencing was applied to determine the microbial community structure based on the universal primer pair of 16S rDNA (Liu et al., 2018). The V4 amplicons were sequenced using primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT) and the pair-ended method by Illumina Miseq sequencing platform (Lv et al., 2016). PICRUSt was used to predict the potential of a sample using 16S rRNA amplicon sequencing. KEGG Orthology (KO) was used to classify all genes that are homologous to a specific gene whose function is known to the same category (Minoru et al., 2017).

2.5. Data analysis

The community richness was estimated by the Chao1 and community diversity was estimated by Simpson and Shannon indices using the MOTHUR software (Yang et al., 2014). The SPSS 15.0 (IBM) software package was used for the general statistical analysis and a chi-square test. The chi-square values were converted into $p$-values by excel (Microsoft Office) to indicate whether the result of a data set is statistically significant (Marr-Lyon et al., 2012). Finally, CANOCO version 4.5 software was used for redundancy analysis (RDA) to evaluate the relationship between the environmental parameters and microbes.

3. Results

3.1 Water quality and environmental parameters of the sampling sites

The water quality parameters of the four sites were summarized in Table 1. COD and NH$_4^+$-N concentrations significantly increased from 47.3 and 25.6 mg/L to 74.3 and 73.3 mg/L, respectively, after the raw sewage was directly discharged into the river. Likewise, NO$_3^-$-N and TP concentration slightly increased from 0.2 to 1.2 mg/L and 1.6 to 5.7 mg/L at Site-2, respectively. However, due to river self-purification that occurred between Site-2 and Site-3, all water quality parameters returned to their average level before the discharge of raw sewage, except for COD which witnessed a slight decrease from 74.2 to 62.8 mg/L at Site-3. In contrast, there were relatively small changes after the discharge of STP effluent to the river. COD slightly decreased from 62.8 to 52.3 mg/L at Site-4, while NO$_3^-$-N increased from 0.23 to 12.2 mg/L. Also, STP effluent carried a lot of heat, resulting in an increase in temperature from 29.7 to 32.7 °C.
Table 1
Water quality and environmental parameters of the four sites

|            | Site-1          | Site-2          | Site-3          | Site-4          |
|------------|-----------------|-----------------|-----------------|-----------------|
| pH         | 7.8 ± 0.5       | 7.5 ± 0.7       | 8.3 ± 0.9       | 7.8 ± 0.5       |
| COD(mg/L)  | 47.3 ± 3.2      | 74.3 ± 4.2      | 62.8 ± 5.1      | 53.2 ± 3.2      |
| NH$_4^+$-N(mg/L) | 25.6 ± 1.5   | 73.8 ± 5.2      | 25.6 ± 2.1      | 8.7 ± 1.1       |
| NO$_3^-$-N(mg/L)| 0.2 ± 0.06    | 1.2 ± 0.1       | 0.2 ± 0.05      | 12.2 ± 1.5      |
| NO$_2^-$-N(mg/L)| 0.2 ± 0.04    | 0.1 ± 0.01      | 0.7 ± 0.06      | 0.1 ± 0.03      |
| T(° C)     | 28.1 ± 4.0      | 28.7 ± 2.1      | 29.7 ± 1.5      | 32.7 ± 2.3      |
| TP(mg/L)   | 1.6 ± 0.1       | 5.7 ± 0.8       | 1.2 ± 0.3       | 0.3 ± 0.1       |

3.2. Analysis of the community composition

Fifty-nine phyla were detected from the four sites, and ten predominant phyla were shown in Fig. 2 (A). Relative abundance of *Proteobacteria* increased from 11.8% at Site-1 to 88.2% at Site-2 and then gradually decreased to 62.5% at Site-3 and then 38.2% at Site-4. *Proteobacteria* include various pathogens, such as *Escherichia, Salmonella, Vibrio*, and *Helicobacter*, and many bacterial species responsible for nitrogen transformation (Elreedy et al., 2021). The high relative abundance of *Proteobacteria* at Site-2 could be due to the increase in NH$_4^+$-N concentration from the direct discharge of sewage to the river; the high ammonia provided an appropriate living environment for *Proteobacteria* growth (Elreedy et al., 2021). In a suitable environment, *Proteobacteria* competes with other native microorganisms and reduces microbial diversity (Shen et al., 2020). On the other hand, with the gradual decrease of NH$_4^+$-N concentration at Site-3 and Site-4, the abundance of *Proteobacteria* gradually declined to 62.5 and 38.2%.

As described in Fig. 2 (B), the dominant genera mainly related to nitrogen metabolism, COD degradation, phosphorous removal, and sulfur removal. *Treponema*, which can convert NO$_2^-$ to NO$_3^-$, had the highest abundance percentage (10.42%) at Site-1. Two denitrifying bacteria were enriched at Site-2, especially *Hydrogenophaga* (3.33%) (Kampfer and P., 2005). *Thauera*, a denitrifying related bacteria producing N$_2$ using NO$_2^-$ or NO$_3^-$ as the electron donor (Bu et al., 2017), was also enriched at Site-4. *Azoarcus* that performs nitrogen fixation (Zhou et al., 1995) appeared at Site-2 then kept growing in Site-3 and Site-4. The *Zoogloea* and *Pseudomonas* genera whose main function is to degrade organic matters increased sharply at Site-2. The *Pseudomonas* genus, responsible for the removal of phosphorus, was also dominant in Site-2 coupled with *Acinetobacter*. Their enrichment may be due to a large amount of phosphorus in the raw sewage, as evidenced by the highest TP content in Table 1 at Site-2. *Thiobacillus* and *Desulfovibrio* are related to sulfur removal and *Thiobacillus* was enriched at Site-3 and Site-4.
The total obtained operational taxonomy units (OTUs) were 28427, 28354, 28103, and 39105 for the four sites. Furthermore, four important functional microbes were extracted and classified as denitrifying bacteria (DNB), anammox bacteria, organic degrading bacteria (ODB), and phosphorus-accumulating bacteria (PAOs) as shown in Table 2. Three genera of DNB were detected in almost all samples. In particular, the relative abundance of *Hydrogenophaga* at Site-2 (3.333%) was more than 13-fold of that at Site-1 (0.253%); however, it suddenly decreased at Site-3 and Site-4. *Azoarcus* was absent at Site-1 and appeared at Site-2 (0.005%) then continued to grow in the latter two sites. *Thauera* also showed an increasing trend from Site-1 to Site-4. Table 2 shows that the discharge of raw sewage between Site-1 and Site-2 increased COD and nitrate contents, which provided sustainable and effective electron donors for denitrification, promoting the growth of denitrifying bacteria. Anammox bacteria were also detected at the genus level, and its highest abundance was 0.59% at Site-3. *Zoogloea* has been reported with high versatile metabolism capabilities such as nitrogen fixation, secretion of extracellular polymeric substance (EPS), and COD removal (Xia et al., 2018). The increase of ammonia nitrogen content at Site-2 may be related to the high *Zoogloea* abundance at this site. The contents of *Zoogloea* increased at Site-2 after the raw wastewater surged in and then decreased in the following two downstream sites. PAOs bacteria include *Pseudomonas, Arthobacter, Nocardia, Beyerinkia, Ozotobacter, Aeromonas, Microlunatus*, and *Rhodocyclus* (Wang et al., 2016). Only one PAOs genus was detected in all sites. The abundance of PAOs was much higher at Site-2 than that at Site-1, with a relative abundance of 2.69% and 0.08%, respectively. The relative abundance of all the above-mentioned functional bacteria at Site-2 is higher than those at Site-1, while only four bacteria related to COD and nitrate removal showed a continuous increase between Site-3 and Site-4. In other words, the discharge of both raw and treated wastewater caused changes in river water quality, which further altered the microbial community structure. The changes in water quality and microbial community at Site-2 might be mainly due to the high nutrients in the discharged wastewater. Site-4 subjected to the discharge of treated wastewater with high temperature, which further affected the microbial species and abundance.

### Table 2

| Name of bacteria | Site-1 | Site-2 | Site-3 | Site-4 |
|------------------|--------|--------|--------|--------|
| DNB, *Hydrogenophaga* | 0.253  | 3.333  | 0.315  | 0.438  |
| Thauera          | 0.012  | 0.856  | 0.888  | 3.429  |
| Azoarcus         | 0      | 0.005  | 0.194  | 0.244  |
| Anammox, *Planctomyces* | 0      | 0.016  | 0.592  | 0.097  |
| ODB, *Zoogloea*  | 0.047  | 6.28   | 0.093  | 0.497  |
| PAOs, *Pseudomonas* | 0.077  | 2.685  | 0.167  | 0.166  |

DNB, denitrifying bacteria; ODB, organic degrading bacteria; PAOs, phosphorus-accumulating bacteria

### 3.3. Analysis of the alpha diversity
The Chao1, Simpson, and Shannon indexes were calculated to determine the richness and diversity of the microbial community (Xu et al., 2018). The Chao1 index of sediment samples at Site-1 (874) was higher than that of Site-2 (869), as shown in Table 4; the same results were registered for Simpson and Shannon indexes with insignificant fluctuations. This may be because the direct discharge of sewage altered the environmental conditions of the river, caused changes in the microbial community, and eventually led to a decrease in species richness and diversity. The results indicated that the richness and diversity of the bacterial community were higher in Site-1 compared to Site-2. The values of Chao1 kept increasing from Site-2 to Site-4 indicating the increase of the richness of the microbial community. Discharging the treated sewage provided a relatively suitable environment for microorganisms in the river. Therefore, the species richness increased again during the process from Site-3 to Site-4.

### 3.4. Pathogenic gene abundance based on KEGG database and qPCR results

Pathogenic bacteria in water affect human and animal health. Pathogens could live in untreated wastewater for longer periods and spread further through water bodies (Casanova et al., 2009). Figure 3 shows the gene abundance of infectious diseases at four sites according to the forecast of the KEGG database (Fig. 3 (A)) and the relationship among four sites based on qPCR results (Fig. 3 (B)). There were significant differences in infectious diseases between Site-1 and Site-2, while no obvious variation was observed between Site-3 and Site-4. After polluted by raw sewage, the gene abundance of the infectious diseases increased sharply. The increased gene was related to *Vibrio cholera* pathogenic cycle process, whose gene amount increased from 4055 to 23556 reads. The gene abundance of *Helicobacter pylori* infection increased from 3797 to 13163 reads. The gene abundance of *Tuberculosis* also increased from 13545 to 19887 reads. The four kinds of pathogenic genes had the most remarkable variation among all the genes related to infectious diseases between Site-1 and Site-2. On the other hand, there is no remarkable big gap between the gene abundance of samples at Site-3 and Site-4. The values of these four infectious disease abundance were verified by qPCR, as shown in Fig. 3(B). Compared to Site-1, the abundance of these four infectious diseases in Site-2, Site-3, and Site-4 increased. The abundance of *Staphylococcus aureus* at Site-2 far exceeded that of Site-1. The abundance of these four infectious diseases decreased at Site-4, compared to Site-3.

### 3.5. The relationship of the infectious pathogens and functional bacteria to water quality parameters

The relationship between microbes and environmental variables was evaluated by redundancy analysis (RDA) (Fig. 4). Based on Fig. 4, there are significant differences between Site-1 and the other three sites. The seven water quality parameters have diverse behaviors in affecting the distribution of the infectious pathogens in the four sites. Site-2 has higher COD, TP, and NH\textsubscript{4}\textsuperscript{+} concentrations than other locations. Meanwhile, TP and NH\textsubscript{4}\textsuperscript{+} got a strong positive correlation with each other among all the environmental factors and they contributed to a large proportion in affecting the abundance of *Mycobacterium tuberculosis*. COD had less correlation to other parameters but strongly influenced the abundance of
Mycobacterium tuberculosis. Site-4, having the highest temperature and NO$_3^-$ concentration, had a low similarity with the other sites. Few functional bacteria were located in Site-1, while the discharge of raw sewage led to the most abundant and multiple distributions of bacteria at Site-2 (Fig. 4 (B)). Many bacterial species at Site-2 were affected by COD, NH$_4^+$, and TP. Moreover, NO$_3^-$ and temperature contributed to the microbial community diversity at Site-3 and Site-4. The functional microbes at Site-3 were influenced by pH and NO$_2^-$.

4. Discussion

4.1 The directly discharged sewage altered the microbial community structure

According to our analysis, the abundance of pathogens, which belong to *Proteobacteria*, witnessed a noticeable growth after raw wastewater flowed into the river, as shown in Fig. 2. The taxonomic analysis found that *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* were the most abundant phyla in all samples. The abundance of *Proteobacteria* decreased at Site-3 by the dilution or degradation of pollutants. The *Proteobacteria* also contains some bacteria whose function is to fix NH$_4^+$, and they had a positive correlation with NH$_4^+$ concentration (Naddeo and Liu, 2020). As shown in Table 3, all indexes were higher at Site-3 and Site-4, because the flowing water carried a large amount of sediment and bacteria to the river downstream. The most significant change between Site-1 and Site-2 among the functional bacteria was *Pseudomonas*, which can remove low concentrations of TP from wastewater (Li et al., 2012). RDA results suggested that functional bacteria, such as *Zoolea*, *Pseudomonas*, and *Hydrogenophaga*, were closely related to COD, NH$_4^+$, and TP. The DNB, which can restore NO$_2^-$, was enriched sharply at Site-2. The possible reason may be that NO$_2^-$, and NH$_4^+$ provide nutrition for DNB (Akizuki et al., 2015).

| Group | Reads | Chao1 | Simpson | Shannon |
|-------|-------|-------|---------|---------|
| Site-1 | 28427 | 874  | 0.95497 | 6.7710 |
| Site-2 | 28354 | 869  | 0.95311 | 6.4841 |
| Site-3 | 32810 | 1535 | 0.99000 | 9.2100 |
| Site-4 | 39105 | 2577 | 0.99000 | 9.1100 |

Notes: Chao1 indicates richness, Shannon and Simpson indicate diversity.

4.2 Raw sewage increased the pathogens content and the risk potential of human health
This paper revealed that raw sewage remarkably contributed to the enrichment of pathogens in sediment samples of the river. From the results of KEGG, the gene contents of four infectious diseases indeed increased after raw wastewater was discharged into the river, while most gene contents decreased after the discharge of treated wastewater. Further analysis proved that the KEGG prediction was in accord with the qPCR results, similar to a study conducted by Langille et al. (2013) (Langille et al., 2013). In general, the discharge of raw sewage into rivers changed the water quality of rivers and caused the growth of pathogenic bacteria in these rivers. The number of pathogenic genes of Site-2 showed a significant increase, which was higher than the other three sites. With the purification of the river, the water quality gradually changed and the abundance of pathogenic genes decreased in Site-3. When the treated wastewater was discharged into the river, however, the pathogenic genes of site-4 did not change dramatically. In the case of sediments being disturbed, it is likely that microorganisms, including pathogens, would remigrate into the water column. Medema et al. reported that protozoa can settle from the water column into the sediment and, due to their size, remain undisturbed for long periods (Medema et al., 1998). Thus, re-suspension events increase the potential risk potential to human health for those who are involved in activities in the aquatic environment. A suitable temperature is also an essential factor for the growth of pathogenic bacteria. When the pathogen is sown in pasteurized sewage, it can be infectious for up to 7 days, in deionized water at 25°C for 22 days, and in these media for up to 4 weeks or more at lower temperatures (< 4°C) (Casanova et al., 2009). *Vibrio* pathogens could be easy to remain active during treatment in rural sewage treatment plants and appear to be more abundant over a wide temperature range of 17.1 to 27.2°C (Igbinosa et al., 2009). Furthermore, it was reported that the relatively high temperature in the range of 5–30 °C was beneficial to the growth of *Vibrio cholera* (Goh et al., 2016).

4.3 Pathogenic bacteria content increased mainly due to raw sewage discharge rather than water quality change

The discharge of pollutants from industrial processes causes diverse adverse environmental impacts on aquatic ecosystems, such as eutrophication of water bodies, the introduction of pathogens, re-assembling of the microbial community structure in rivers, and so on (Liao et al., 2019; Marassi et al., 2020). With the change of water quality, mostly COD, in the river from Site-2 to Site-3, it was more suitable for *Mycobacterium tuberculosis* to be enriched than other bacteria. However, except for the *Mycobacterium tuberculosis* that related to COD, the correlation between other pathogenic bacteria and environmental factors was not noticeable. Through RDA analysis, functional bacteria are closely related to water quality changes, but most pathogenic bacteria most are inconsistent. This indicates that the sudden increase of pathogenic bacteria at Site-2 was probably since these pathogenic bacteria were contained in the sewage itself. In other words, the pathogenic bacteria in the Daxin river probably came from untreated wastewater directly rather than environmental changes.

4.4 Decreased pathogenic bacteria abundance may due to self-purification
After the raw sewage enters the river, the self-purification influenced the river's environmental conditions and then affected the further migration and deposition of pathogenic bacteria. The content of COD, $\text{NH}_4^+$, $\text{NO}_3^-$, and TP gradually decreased from Site-2 to Site-3, as well as the abundance of genes related to *Vibrio cholerae*, *Staphylococcus aureus*, and *Helicobacter pylori*, as shown in Fig. 3. The response of the ecosystem to chemically-driven environmental changes is modulated by physical and biological phenomena. Most pathogens, which came from raw sewage, could also be absorbed by animals, plants, and sediments in the river. Self-purification could the maintenance or restoration of river ecology and influencing factors may include seasonal fluctuations, the microbiological community present (Liao et al., 2019), and temperature (Wagner and Zalewski, 2016). Moreover, Silva et al. revealed that the self-purification potential increased in the dry season, influenced by the wastewater load (Silva et al., 2020). So the temperature in the Daxin river also contributed to self-purification. These biological processes are mainly carried out by endogenous and exogenous microorganisms that can remove organic and inorganic pollutants in contaminated water bodies, essential for maintaining a stable ecosystem dynamics state, in terms of function and structure (Clements and Rohr, 2009; Wagner and Zalewski, 2016). Another concern is that microbes could be shielded by physical embedding in organic matter, suspended particles, and occlusion of a biofilm, making them less susceptible to the inactivation action of disinfectants (Silva et al., 2020). So it is urgent to further focus on the migration and transformation mechanism of pathogenic bacteria in sediments.

### 4.5 Significance of this research

This study contributes to an improved understanding of the effects of sewage discharge on the changes of microbial communities and the abundance of pathogenic bacteria in river sediments. Despite water and sewage treatment technologies continue to achieve rapid progress in recent years, waterborne pollution remains a major threat to public health worldwide. This paper brings to the fore the need for comprehensive research into the movement and behavior of these microorganisms in river sediment after raw sewage is contaminated. And besides, with the evidence of increased pathogen abundance, we are here to demonstrate and highlight the risks of direct sewage discharge. Wastewater as a secondary source of transmission is not given full attention, especially in third-world countries. If wastewaters act as the source of transmission, it will be hard to break the chain of pathogen transmission in the third world countries and the consequence will be faced by developed nations as well. There is a common practice to discharge wastewater directly into rivers, canals, and lakes without any treatment in third-world countries. The wastewater carried pathogens can go to a big population of the world and create population crises in the world. So, measures should be taken as soon as possible in third-world countries to break the chain of pathogen transmission. Strict surveillance of wastewater treatment is suggested to stop the spread of pathogens in the human community.

### 5. Conclusion

This study shows that raw sewage discharge into rivers indeed changed the microbial community structure and the polluted water is a potential source of the spread of infectious diseases. Raw sewage
discharged directly into the river decreased the richness and diversity of the microbial community and introduced pathogenic bacteria. Although the self-purification of water slightly reduced the pollution, sewage directly discharge still caused irreversible effects. In conclusion, direct discharge of wastewater should be forbidden with regards to the negative impact on entering the ecosystem, especially on the lives that depend on the ecosystem for sustenance.

Declarations

Acknowledgment

The authors gratefully acknowledge the support from the National Natural Science Foundation of China (21777086, 22076100), Taishan Scholar Youth Expert Program of Shandong Province (tsqn201909005), Key Research & Developmental Program of Shandong Province (2019JZZY020308), Young Scholars Program of Shandong University (2016WLJH16, 2020QNQT012), and Qingdao Science and Technology Huimin Demonstration Guide Project (20-3-4-4-nsh).

Authorship contribution statement

S. N. contributed to the study conception and design. Y. X. and L. L. performed material preparation, data collection, and analysis. Y. X. and L. L. wrote the first draft of the manuscript. S. N., W. W., X. C., S. I., A. S. and L. T. revised the paper.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Consent for publication

Not applicable.

Conflict of interest/Competing interests

Authors declare no conflict of interest/competing interests.

Ethics approval and consent to participate

Not applicable.

References

1. Ahmed W, Angel N, Edson J, Bibby K, Bivins A, O'Brien JW, Choi PM, Kitajima M, Simpson SL, Li J, Tscharke B, Verhagen R, Smith WJM, Zaugg J, Dierens L, Hugenholtz P, Thomas KV, Mueller JF
(2020) First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. Sci Total Env 728:138764
2. Akizuki S, Wong TK, Aoki Y, Rodríguez GC, Niwa C, Toda T (2015) Effects of substrate COD/NO2-N ratio on simultaneous methanogenesis and short-cut denitrification in the treatment of blue mussel using acclimated sludge. Biochem Eng J 99:16–23
3. Bu C, Wang Y, Ge C, Ahmad HA, Gao B, Ni SQ (2017) Dissimilatory Nitrate Reduction to Ammonium in the Yellow River Estuary: Rates, Abundance, and Community Diversity. enitic Reports 7:6830
4. Casanova L, Rutala WA, Weber DJ, Sobsey MD (2009) Survival of surrogate coronaviruses in water. Water Res 43:1893–1898
5. Chin C-S, Sorenson J, Harris JB, Robins WP, Charles RC, Jean-Charles RR, Bullard J, Webster DR, Kasarskis A, Peluso P, Paxinos EE, Yamaichi Y, Calderwood SB, Mekalanos JJ, Schadt EE, Waldor MK (2010) The Origin of the Haitian Cholera Outbreak Strain. N Engl J Med 364:33–42
6. Clements WH, Rohr JR (2009) Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. Environ Toxicol Chem 28:1789–1800
7. Cornelisen CD, Gillespie PA, Kirs M, Young RG, Harwood VJ (2011) Motueka River plume facilitates transport of ruminant faecal contaminants into shellfish growing waters, Tasman Bay, New Zealand. New Zeal. J Mar Freshw Res 45:477–495
8. David R, Johnson T, Kwon, Lee J, Park, Kathrin F (2014) The functional and taxonomic richness of wastewater treatment plant microbial communities are associated with each other and with ambient nitrogen and carbon availability. Environ. Microbiol
9. Devane ML, Moriarty EM, Wood D, Webster-Brown J, Gilpin BJ (2014) The impact of major earthquakes and subsequent sewage discharges on the microbial quality of water and sediments in an urban river. ence Total Environ 485–486:666–680
10. DIETRICH JES, DOHERTY TM (2009) Interaction of Mycobacterium tuberculosis with the host: consequences for vaccine development. APMIS 117:440–457
11. Duttagupta S, Mukherjee A, Bhanja SN, Chattopadhyay S, Sarkar S, Das K, Chakraborty S, Mondal D (2020) Achieving Sustainable Development Goal for Clean Water in India: Influence of Natural and Anthropogenic Factors on Groundwater Microbial Pollution. Environ. Manage
12. Elreedy A, Ismail S, Ali M, Ni S-Q, Fujii M, Elsamadony M (2021) Unraveling the capability of graphene nanosheets and γ-Fe2O3 nanoparticles to stimulate anammox granular sludge. J Environ Manage 277:111495
13. Goh SG, Bayen S, Burger D, Kelly BC, Gin YH (2016) Occurrence and distribution of bacteria indicators, chemical tracers and pathogenic vibrios in Singapore coastal waters. Mar. Pollut. Bull. 114
14. Haramoto E, Malla B, Thakali O, Kitajima M, n.d. First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. ence Total Environ. 737
15. Huelsen T, Barry EM, Lu Y, Puyol D, Keller J, Batstone DJ (2016) Domestic wastewater treatment with purple phototrophic bacteria using a novel continuous photo anaerobic membrane bioreactor. Water
16. Igbinosa EO, Obi LC, Okoh AI (2009) Occurrence of potentially pathogenic vibrios in final effluents of a wastewater treatment facility in a rural community of the Eastern Cape Province of South Africa. Res Microbiol 160:531–537
17. Jian L (2015) Effects of nitrogen and phosphorus addition on soil microbial community in a secondary tropical forest of China. Biol Fertil Soils 51:207–215
18. Kampfer P (2005) Hydrogenophaga defluvii sp. nov. and Hydrogenophaga atypica sp. nov., isolated from activated sludge. Int J Syst Evol Microbiol 55:341
19. Langille MGI, Zaneveld J, Caporaso JG, Mcdonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 31:814–821
20. Lei ZJM (2012) Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. Soil Biol. Biochem
21. Li HF, Li BZ, Wang ET, Yang JS, Yuan HL (2012) Removal of low concentration of phosphorus from solution by free and immobilized cells of Pseudomonas stutzeri YG-24. Desalination 286:242–247
22. Liao K, Bai Y, Huo Y, Jian Z, Hu W, Zhao C, Qu J (2019) Use of convertible flow cells to simulate the impacts of anthropogenic activities on river biofilm bacterial communities. Sci Total Environ 653:148–156
23. Liu X, Ni SQ, Guo W, Wang Z, Ahmad HA, Gao B, Fang X (2018) N2O emission and bacterial community dynamics during realization of the partial nitrification process. RSC Adv. 8
24. Lv L, Ren LF, Ni SQ, Gao BY, Wang YN (2016) The effect of magnetite on the start-up and N2O emission reduction of the anammox process. RSC Adv. 6
25. Marassi RJ, Queiroz LG, Silva DCVR, Silva FTD, Silva GC, Paiva TCBD (2020) Performance and toxicity assessment of an up-flow tubular microbial fuel cell during long-term operation with high-strength dairy wastewater. J. Clean. Prod. 259
26. Marr-Lyon LR, Gupchup GV, Anderson JR (2012) An evaluation of the psychometric properties of the Purdue Pharmacist Directive Guidance Scale using SPSS and R software packages. Res Soc Adm Pharm 8:166–171
27. McCarthy SA (1996) Effects of temperature and salinity on survival of toxigenic Vibrio cholerae O1 in seawater. Microb Ecol 31:167–175
28. Medema G, Heijnen L, Elsinga G, Italiaander R, Brouwer A (2020) Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands. Environ Sci Technol Lett 7:511–516
29. Medema GJ, Schets FM, Teunis PFM, Havelaar AH (1998) Sedimentation of Free and Attached Cryptosporidium Oocysts and Giardia Cysts in Water. Appl Environ Microbiol 64:4460–4466
30. Miller WA, Miller MA, Gardner IA, Atwill ER, Byrne BA, Jang S, Harris M, Ames J, Jessup D, Paradies D, Worcester K, Melli A, Conrad PA (2006) Salmonella spp., Vibrio spp., Clostridium perfringens, and
Plesiomonas shigelloides in marine and freshwater invertebrates from coastal California ecosystems. Microb Ecol 52:198–206

31. Minoru K, Miho F, Mao T, Yoko S, Kanae M (2017) KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucl Acids Res. D353–D361

32. Naddeo V, Liu H (2020) Editorial Perspectives: 2019 novel coronavirus (SARS-CoV-2): what is its fate in urban water cycle and how can the water research community respond? Environ. Sci. Water Res. Technol. 6

33. Natalie H, Mufidat, Mamman, Hajara T, Ahmed A-G, Chuxia L (2018) Effects of softwood biochar on the status of nitrogen species and elements of potential toxicity in soils. Ecotoxicol. Environ. Saf

34. Pereira MI, Medeiros JA, 2014. Role of Helicobacter pylori in gastric mucosa-associated lymphoid tissue lymphomas. World J Gastroenterol 20, 684–698. https://doi.org/10.3748/wjg.v20.i3.684Reidl J, Klose KE, 2002. Vibrio cholerae and cholera: out of the water and into the host. FEMS Microbiol. Rev

35. Shen X, Xu M, Li M, Zhao Y, Shao X (2020) Response of sediment bacterial communities to the drainage of wastewater from aquaculture ponds in different seasons. Sci Total Environ 717:137180

36. Silva DCVR, Queiroz LG, Marassi RJ, Araújo CVM, Bazzan T, Cardoso-Silva S, Silva GC, Müller M, Silva FT, Montagner CC, Paiva TCB, Pompêo MLM (2020) Predicting zebrafish spatial avoidance triggered by discharges of dairy wastewater: An experimental approach based on self-purification in a model river. Environ Pollut 266:115325

37. Velayati AA, Farmia P, Mozafari M, Malekshahian D, Farahbod AM, Seif S, Rahideh S, Mirsaedi M (2015) Identification and Genotyping of Mycobacterium Tuberculosis Isolated From Water and Soil Samples of a Metropolitan City. Chest 147:1094–1102

38. Wagner I, Zalewski M (2016) Temporal changes in the abiotic/biotic drivers of selfpurification in a temperate river. Ecol Eng 94:275–285

39. Wang Z, Bin, Miao MS, Kong Q, Ni SQ (2016) Evaluation of microbial diversity of activated sludge in a municipal wastewater treatment plant of northern China by high-throughput sequencing technology. Desalin Water Treat 57:1–6

40. Wang Z, Liu X, Ni SQ, Zhang J, Zhang X, Ahmad HA, Gao B (2017) Weak magnetic field: A powerful strategy to enhance partial nitrification. Water Res 120:190–198

41. West AP, Millar MR, Tompkins DS (1992) Effect of physical environment on survival of Helicobacter pylori. J Clin Pathol 45:228–231

42. Xia J, Ye L, Ren H, Zhang XX (2018) Microbial community structure and function in aerobic granular sludge. Appl. Microbiol. Biotechnol

43. Xu F, Cao F, Kong Q, Zhou L, Yuan Q, Zhu Y, Wang Q, Du Y, Wang Z (2018) Electricity production and evolution of microbial community in the constructed wetland-microbial fuel cell. Chem Eng J 339:479–486

44. Yang S, Liebner S, Alawi M, Ebenh?H O, Wagner D (2014) Taxonomic database and cut-off value for processing mcrA gene 454 pyrosequencing data by MOTHUR. J Microbiol Methods 103:3–5
45. Yu SX, Pang YL, Wang YC, Li JL, Qin S (2017) Spatial variation of microbial communities in sediments along the environmental gradients from Xiaoqing River to Laizhou Bay. Mar Pollut Bull 76:1048–1056

46. Zhang D, Ling H, Huang X, Li J, Li W, Yi C, Zhang T, Jiang Y, He Y, Deng S, Zhang X, Wang X, Liu Y, Li G, Qu J (2020) Potential spreading risks and disinfection challenges of medical wastewater by the presence of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) viral RNA in septic tanks of Fangcang Hospital. Sci Total Environ 741:140445

47. Zhang Y, Wu J, Xu B (2018) Human health risk assessment of groundwater nitrogen pollution in Jinghui canal irrigation area of the loess region, northwest China. Environ Earth Sciences 77:273

48. Zhou J, Fries MR, Cheesanford JC, Tiedje JM (1995) Phylogenetic analyses of a new group of denitrifiers capable of anaerobic growth of toluene and description of Azoarcus tolulyticus sp. nov. Int J Syst Bacteriol 194:500–506

49. Zieliński W, Korzeniewska E, Harnisz M, Hubeny J, Buta M, Rolbiecki D (2020) The prevalence of drug-resistant and virulent Staphylococcus spp. in a municipal wastewater treatment plant and their spread in the environment. Environ Int 143:105914

Figures

Figure 1
Daxin River sampling points locations

**Figure 2**

Microbial composition in four sites at the phylum (A) and genus (B) levels.

**Figure 3**

Prediction of abundance of genes related to infectious diseases (A) and the multiple relationship among them by qPCR (B) at four sites.
Figure 4

Redundancy analysis (RDA) of water quality parameters at the four sampling sites and their relationship to the microbial community structure of infectious pathogens (A) and functional bacteria (B).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.jpg