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Process evaluation of urban river replenished with reclaimed water from a wastewater treatment plant based on the risk of algal bloom and comprehensive acute toxicity

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ABSTRACT

Municipal wastewater reuse has an important role to play with scarce water resources and serious water pollution. However, the impact of reclaimed water on the aquatic ecology and organisms of the receiving water needs to be assessed. This study investigated one ecological restoration project of an urban river replenished with reclaimed water, and evaluated the risk of algal bloom and acute biological toxicity in the river. Results showed that the concentrations of permanganate index and ammonia nitrogen in the river could stably remain below the standard values, the concentrations of total phosphorus were high and most of the monitoring values were between 0.42 and 0.86 mg/L. The content of chlorophyll a was relatively lower, ranging from 0.06 to 0.10 mg/m^3. The maximum value of F_v/F_m was 0.42, which was lower than the algal bloom prediction threshold of 0.63. Moreover, the results of luminescence inhibition rate on luminescent bacteria showed that the reclaimed water did not cause significant biological toxicity to the aquatic ecology. The study suggested that implementing urban reclaimed water reuse projects requires a series of ecological purification and restoration technologies in the receiving water, which can effectively guarantee the stability of water quality and the safety of water ecological environment.

Key words: algal blooms, ecological restoration, luminescent bacteria test, reclaimed water, water acute biological toxicity

HIGHLIGHTS

- Ecological restoration project of urban river replenished with reclaimed water was evaluated.
- Water quality of the receiving water was mainly affected by its water source.
- In the situation of relatively high nutrient concentration, the possibility of algal blooms was small.
- In the river replenished with reclaimed water, application of ecological restoration technologies could effectively maintain the water ecological safety.

INTRODUCTION

At present, many cities in the world, especially in south China, are cities with moderate water shortage, insufficient water in the inland rivers, and poor hydrodynamic conditions. The shortage of water resources has become the main factor restricting the economic and social development of these cities. In recent decades, relevant departments and institutions have explored a variety of engineering methods to achieve water environment improvement and river restoration (Lin *et al.* 2020). Municipal wastewater reuse has an important role in the cities with scarce water resources and serious water pollution. With the gradual grimness of the water crisis problem, the reuse of reclaimed water produced by wastewater treatment plants (WWTPs) has become one of the important approaches to resolving the shortage of water resources in many cities. As well, the reuse of reclaimed water is one of the effective ways to simultaneously solve the issues of sewage resources recycling, river water pollution control, and ecological environment improvement, which has obvious practical significances (Rose 2007). Projects using reclaimed water to recharge landscape rivers and lakes have been carried out in Japan, Australia, America, and other countries (Marks 2006; Mano *et al.* 2017). In contrast, Beijing, Urumqi, and many other cities in China have successively carried out the construction of reclaimed water reused in landscape waters, which has positively contributed to improve the urban water system and to realize the comprehensive utilization of water resources (Wang *et al.* 2015; Ye *et al.* 2019). Due to the differences in pollution background values and self-purification capacity of water environments, it is a matter of urgency to carry out targeted projects such as a river water replenishment project.

However, even if the reclaimed water from WWTPs has reached the water quality standard for landscape use, the concentration of nitrogen-phosphorus nutrients still cannot be ignored. Some studies have shown that the main cause of eutrophication and algal blooms was the response of nutrient enrichment and lower water velocity, which significantly affected public health and water ecosystem services (Schindler *et al.* 2016; Ho *et al.* 2019). Therefore, eutrophication control of surface landscape water is one of the most critical technical issues that need to be considered for the reuse of reclaimed water. Controlling the hydraulic characteristics and constructing a healthy ecosystem of water bodies to inhibit the growth of algae were conventional methods to avoid eutrophication and algal blooms in urban reclaimed water reuse projects.

Trace amounts of harmful and toxic pollutants were another limiting factor for reclaimed water reuse. A large number of emerging contaminants in treated wastewater can be identified and quantified with the progress of analytical chromatographic methods, such as pharmaceuticals and personal care products, disinfection by-products, and endocrine disrupting chemicals (Wang *et al.* 2017; Zhang *et al.* 2018; Liu *et al.* 2019b). However, the conventional treatment processes in WWTPs typically lacked the targeted removal of these hazardous contaminants. As a result, WWTPs have become one of the primary sources and sinks of emerging contaminants released into the global environment (Michael *et al.* 2013). Many experimental results have shown that these pollutants usually possess genotoxicity, developmental toxicity, acute toxicity, potential carcinogenicity, etc. (Petrie *et al.* 2015; Chai *et al.* 2018), which may exhibit various adverse biological effects in reclaimed water, thus posing potential threats to the ecological environment and even human health (Carlsson *et al.* 2006). Therefore, eutrophication and emerging contaminants have become prominent problems before reclaimed water reuse.

Apart from the detection and chemical analysis of conventional water quality indicators, water quality security should also focus more on integrated toxicities based on multiple pollutant effects. Thus, more attention regarding the toxicity of pollutants in reclaimed water should be considered, and the biological toxicity test can be an efficient way to vividly measure the safety level of contaminants in water (Xu *et al.* 2020). Moreover, the comprehensive biological effects of pollutants in the reclaimed water can be effectively estimated through the biological toxicity, and the potential hazards can also be evaluated.

Although there are various related studies and engineering applications around the world (Salgot & Folch 2018), the evaluation of reclaimed water based on actual projects still needs to be studied in China. Moreover, most of these researches mainly focused on analyzing eutrophication problems caused by the reclaimed water reuse and its treatment methods or biological toxicity of water bodies, respectively, rarely on comprehensively investigating reclaimed water reuse projects from multiple analysis perspectives. Hence, the objective of this research was to evaluate the urban river replenished with reclaimed water from the standpoint of ecological safety by combining specific aspects of conventional water quality indicators, eutrophication indicators, and biological toxicity indicators. The research was based on the ecological restoration project of the urban river replenished with reclaimed water from one wastewater treatment plant (WWTP) in Ningbo, Zhejiang Province. The reclaimed water treated by WWTP was reused in river L and then entered the surface water system to provide stable fresh water. By analyzing the water quality of reclaimed water effluent from the WWTP, as well as the water quality, the risk of algal bloom and the comprehensive acute toxicity in river L from May 2019 to September 2019 (the months with higher temperatures of one year), this study systematically evaluated the impact of the reclaimed water on river L, which may be helpful to develop future water reuse projects in Ningbo, China and other countries and regions.

MATERIALS AND METHODS

Description of the reclaimed water reuse project

The influent of this WWTP was domestic sewage, and the effluent, after secondary biochemical treatment, was further treated by denitrification filter, high-density sedimentation tank, D-type filter, chlorine dioxide disinfection, and ultraviolet disinfection, then the reclaimed water was supplied. The ecological restoration project of the urban river replenished with reclaimed water has undergone long-term monitoring since it was officially completed in December 2016. The reclaimed water discharged into river L through the drainage pipes, and the average daily emissions were basically stable at 15,000 m^3/d. In terms of the water quality, the reclaimed water treated by the WWTP met the GB/T 18921-2019 standard for scenic environment use (Standardization Administration China 2019). The concentrations of four main water quality indicators, chemical oxygen demand (COD_{Cr}), total phosphorus (TP), total nitrogen (TN), and ammonia nitrogen (NH_4^+-N) from May to September are shown in Table 1. Monitoring results indicated that the TP concentration cannot meet the standard requirements completely.

Table 1 | Concentration range and standard value of the reclaimed water

Indicators	COD_{Cr} (mg/L)	TP (mg/L)	TN (mg/L)	NH_4^+-N (mg/L)
Concentration range	15.0–26.0	0.10–0.84	5.44–10.90	0.03–0.62
Standard value	≤50*	≤0.5	≤15	≤5

*The reuse of urban recycling water – Water quality standard for scenic environment use (GB/T 18921-2019) does not specify the concentration of COD_{Cr}, thus the standard value referred to the concentration limit specified in China's Discharge standard of pollutants for municipal wastewater treatment plant (GB 18918-2002) (The State Environmental Protection Administration China 2002a).

Ecological restoration of river L

River L (29.90°N, 121.61°E), replenished with reclaimed water, is located in one residential area in the east of Ningbo, and the linear distance between river L and WWTP is about 1.1 km. River L is 800 m in length, 11–14 m in width, 1.2–1.5 m in depth, 9,000 m^2 in water area, and 13,000 m^3 in volume. To objectively study the impact of reclaimed water reuse and ecological restoration measures on the urban river, the project set up pollution interceptions on both banks of river L to prevent the input of surrounding point source and non-point source pollution, thus the reclaimed water from the WWTP became the primary replenishment water source of river L.

As shown in Figure 1, the project divided river L into two ecological zones, the oxygen control and denitrification enhancement zone, and the efficient reoxygenation and ecological purification zone. Different ecological restoration measures were taken in these two zones. The oxygen control and denitrification enhancement zone (N1–N3) was in the upper river L, with a water area of 3,000 m^2. In order to remove nitrogen and phosphorus and control eutrophication of the river, this zone adopted ecological restoration technologies with underwater biological filter bed and biological grid as the main part, and artificial aquatic plants (such as string-shaped artificial aquatic plants and multi-loop series artificial aquatic plants) as the supplement. The efficient reoxygenation and ecological purification zone (N3–N8) was in the middle and lower river L, with a water area of 6,000 m^2. In order to further achieve the purpose of water quality purification and algae bloom control in river L, this zone mainly used aeration and macrophyte remediation as the main ecological restoration technologies, supplemented by biomanipulation (such as adding fish). The main macrophytes used in the ecological restoration of river L were floating leaf plants (*Nymphaea tetragona*, etc.), emergent plants (*Thalia dealbata Fraser*, *Canna indica L.*, etc.), and submerged plants (*Vallisneria natans*, *Potamogeton wrightii Morong*, *Hydrilla verticillata*, etc.).

Water quality monitoring and analytical methods

Monitoring sections and sampling

The study set up eight monitoring sections (N1–N8) in river L (Figure 1), with each section having one sampling point in the center of the river, thus there were eight sampling points. For each sample, 100 mL of water was collected 0.5 m below the water surface at the sampling point. These samples were collected once a month and the sampling time was in the middle of each month from May to September. The water samples were stored in the refrigerator (4 °C) after being collected and measured within 2 days. The concentrations of permanganate index (COD_{Mn}), NH_4^+-N, and TP were measured based on the national standard method (The State Environmental Protection Administration China 2002c), and the concentration of chlorophyll-a (Chla) was measured based on the national standard method (HJ 897-2017) (Ministry of Ecology & Environment China 2017).

Early-warning indicator for algal bloom prediction

An early-warning indicator for algal bloom prediction in river L was investigated based on the detection of chlorophyll fluorescence parameter of the water body. In this project, F_v/F_m (the maximum quantum efficiency of photosystem II (PSII) photochemistry) was chosen to monitor and predict the algal blooms, reflecting the photosynthetic characteristics of algae (Yan *et al.* 2014; Sharma *et al.* 2015). F_v/F_m was commonly used to characterize the intrinsic PsII efficiency of chlorophyll and reflected the maximum quantum yield when all PsII reaction centers were in the open state at that time. F_v/F_m <0.60 indicated that algae were in the growth adaptation phase, and F_v/F_m >0.60 showed that algae were in the logarithmic growth phase. Chlorophyll fluorescence parameters of algae (such as F_v/F_m) could be more sensitive than the chlorophyll content, and the changing trend could also appear earlier (Zhu *et al.* 2011). By analyzing the correlation between F_v/F_m and the concentration of main pollutants, it could be seen that when the nutrient concentration level was slightly higher

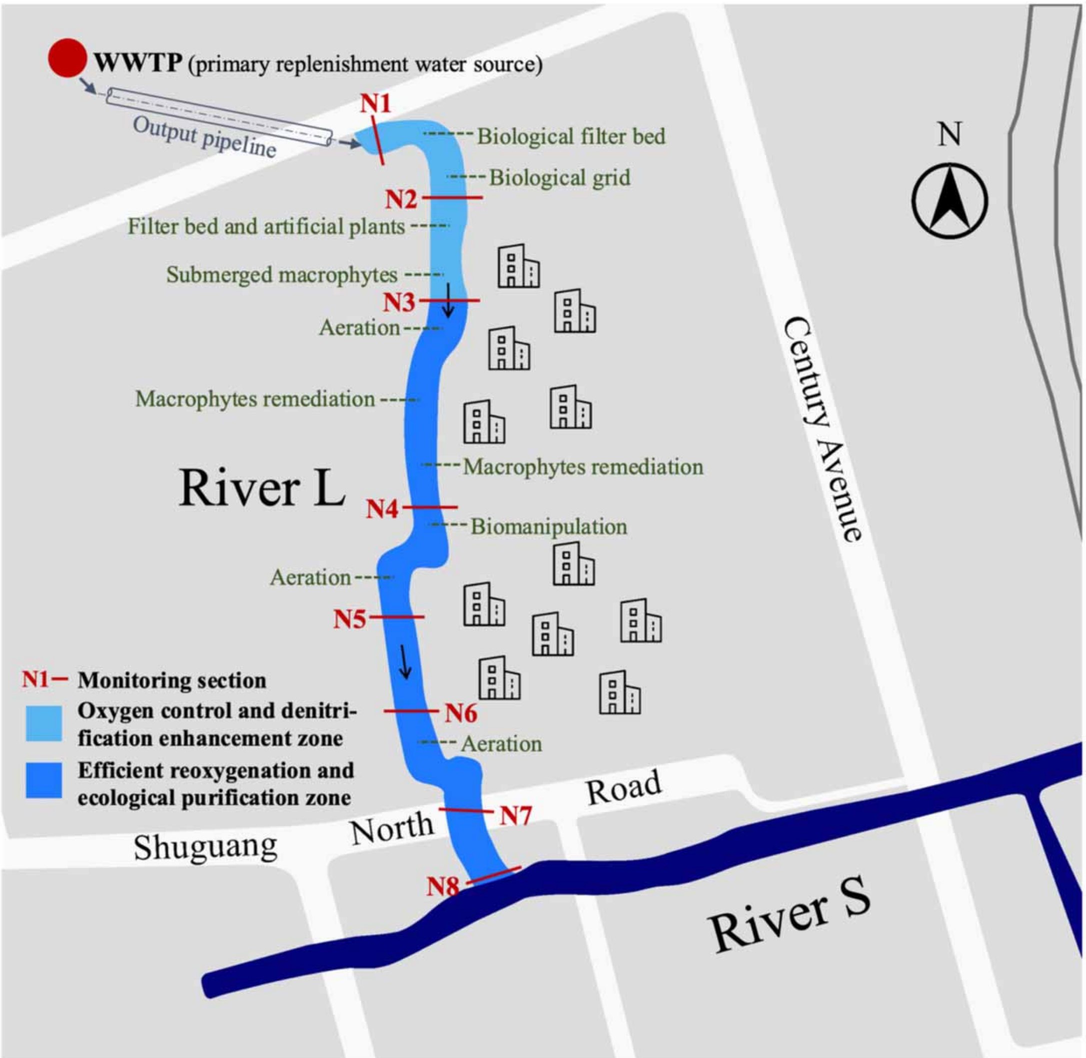

Figure 1 | Schematic diagram of ecological restoration project and monitoring section location of river L.

in the water body, the potential growth and reproduction ability of algae was strong, and compared with Chla, F_v/F_m could take into account the growth characteristics of algae and nutrient level of the water body better (He 2018). Therefore, F_v/F_m may be more suitable to be used as an early prediction indicator for the algal bloom. According to the study on the early warning and prediction of algal bloom in urban artificial water bodies in Shanghai, since river L has not been monitored to obtain the threshold of F_v/F_m, the algal bloom prediction threshold of $F_v/F_m = 0.63$ could be adopted as the reference threshold in this study (Yan *et al.* 2014). A handheld chlorophyll fluorescence measuring instrument (AquaPen-C AC100) was used on site to measure the value of F_v/F_m in each sampling point (N1–N8) of river L. The monitoring frequency was three times in June, four times in July and August, and twice in May and September.

Luminescent bacteria toxicity test

The luminescent bacteria method was a biological toxicity test of water quality that uses non-pathogenic *Photobacterium* as the test object to monitor the effects of toxic and harmful substances in water on its luminous intensity, and this method was currently used in comprehensive acute toxicity monitoring of water bodies (Girotti *et al.* 2008; Lopez-Roldan *et al.* 2012). The relative luminosity of luminescent bacteria showed a significantly negative correlation with the total concentration of toxic components in the water sample ($P \leq 0.05$), thus the acute toxicity level can be expressed by measuring the relative luminosity of water samples (Ye *et al.* 2011). The luminescent inhibition ratio (%) is equal to the difference value between 100% and relative luminosity (%), and it was significantly positively correlated with the total concentration of toxic components in the water sample (Liu *et al.* 2019a).

The test method of luminescent inhibition ratio in each of river L's sections referred to the Water quality-Determination of the acute toxicity-Luminescent bacteria test (GB/T 15441-1995) (The State Environmental Protection Administration China 1995). The freeze-dried powder of luminescent bacterium *Photobacterium phosphoreum* T_3 spp. was purchased from Nanjing Institute of Soil Science, Chinese Academy of Sciences, and the test instrument is the biological toxicity tester (DXY-3). The sampling method, number, location, and frequency were the same as those for monitoring COD_{Mn}, NH_4^+-N, TP, and Chla (the same source of water samples). The acute toxicity grade of water quality divided by the luminescent inhibition ratio referred to the percentage grade score standard which was recommended by Nanjing Institute of Soil Science, Chinese Academy of Sciences (Table 2).

RESULTS AND DISCUSSION

Water quality evaluation of river L replenished with reclaimed water

Four fundamental water quality indicators, COD_{Mn}, NH_4^+-N, TP, and Chla were chosen in the study to evaluate the overall water quality of river L. The concentration of COD_{Mn} and NH_4^+-N could stably meet the standard of Class III in Environmental quality standards for surface water (GB3838-2002) (The State Environmental Protection Administration China 2002b), but the concentration of TP was generally high and basically exceeded the standard of Class V. The variations of these main water quality indicators in sections N1 to N8 of river L from May to September are shown in Figure 2.

From the results, the COD_{Mn} (except in May) and TP concentrations were lower than the background value. On the time scale, the concentrations of COD_{Mn} and TP showed a slight trend of decreasing, which was mainly related to the water quality of the reclaimed water. With the continuous emission of COD_{Mn} in the previous period, organic matter pollutants did not accumulate and deteriorate.

COD_{Mn}

The concentrations of COD_{Mn} fluctuated slightly along the river except for the N6 section (5.95 mg/L) in May. On the time scale, the concentration of COD_{Mn} decreased generally, which was mainly related to the water quality of the reclaimed water. Except in May, the water quality of river L after being replenished with reclaimed water was better than before. The abnormal concentration of COD_{Mn} in river L in May may be attributed to the artificial maintenance of several ecological restoration measures in the river section, such as planting new submerged plants, which may cause sediment disturbance.

NH_4^+-N

The concentrations of NH_4^+-N fluctuated generally along river L, especially in June and July, and there was no obvious upward or downward trend in the concentrations. The data showed a low background value of ammonia nitrogen, thus the NH_4^+-N concentration in the river was mainly affected by the reclaimed water as the water supply source, but there were no significant effects for the oxygen control and denitrification enhancement zone from May to September. With continuous replenishment, the concentration kept decreasing during the experimental periods.

TP

The concentrations of TP in river L were about 1 mg/L before the reuse of the reclaimed water, which was at a relatively high concentration level. Since the TP concentration of the reclaimed water effluent was lower than the background value of river L, and the concentration of the water body decreased significantly, this showed that the implementation of the reuse project may be beneficial to improve the water quality. The overall water quality of river L was greatly affected by the quality of the reclaimed water, and the variations along the river were small. In addition, the ecological zones of the river had a limited

Table 2 | Classification standard for determining the acute toxicity level of water quality by luminescent bacteria

	I	II	III	IV	V
Luminescent inhibition ratio (R) (%)	$R < 30$	$30 \leq R < 50$	$50 \leq R < 70$	$70 \leq R < 100$	$R = 100$
Toxicity levels	Low toxicity	Intermediate toxicity	Heavy toxicity	High toxicity	Severely high toxicity

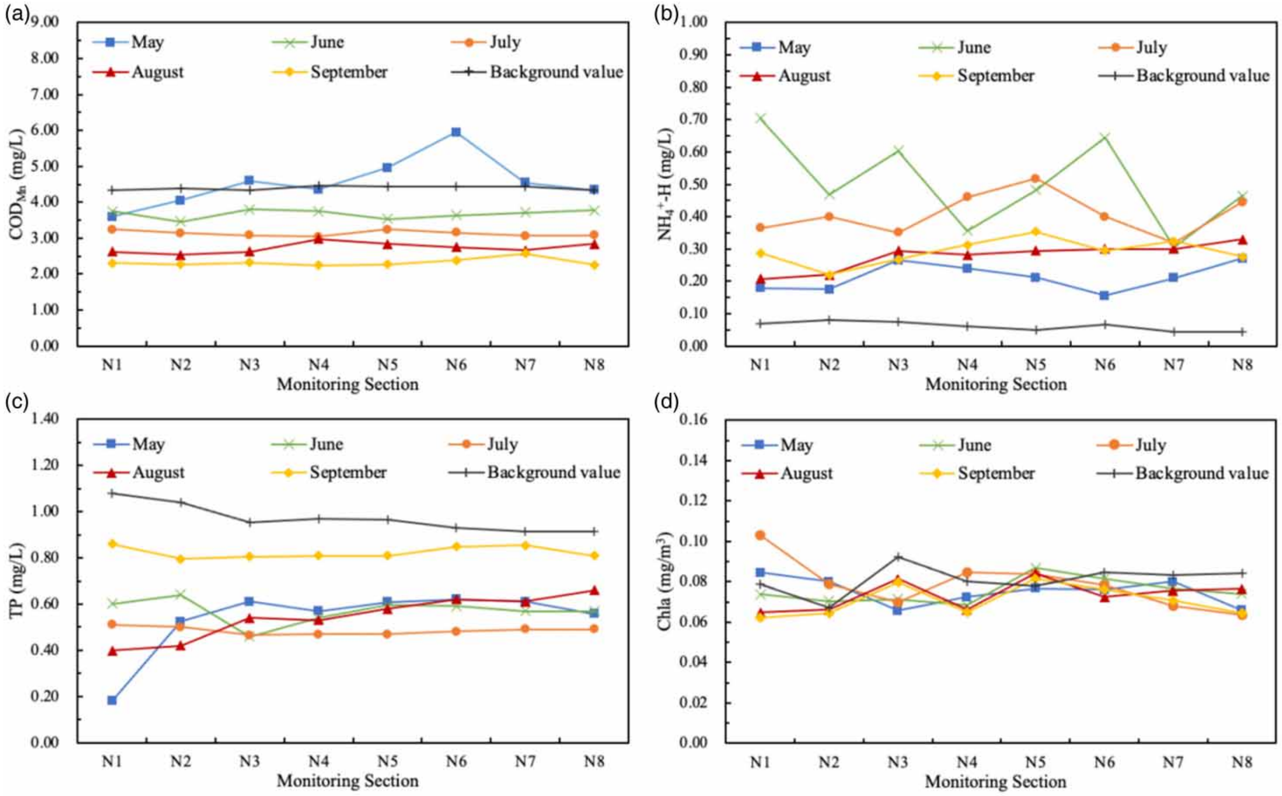

Figure 2 | Variation of four main water quality indicators in river L: (a) concentration of potassium permanganate index, (b) concentration of ammonia nitrogen, (c) concentration of total phosphorus, and (d) concentration of chlorophyll a.

ability to reduce the concentration of TP. Therefore, to improve the water quality qualified rate of the TP concentration in river L, the control of total phosphorus in WWTP was particularly important. Owing to the limited capacity of this WWTP in the project currently, the upgrading of treatment processes could be very useful to effectively improve the removal efficiency of TP and reduce its concentration in the river.

Chla

The content of Chla in the water body can indicate the content of phytoplankton and can also reflect the degree of eutrophication, to some extent. The Chla content in river L was basically maintained between 0.06 and 0.10 mg/m^3 from May to September, and there was a slight variation in the time series. The mean concentration among different sections had little differences, but larger variation deviation was presented. On the whole, compared with the background value of each section, the Chla content in river L generally decreased after the replenishment of the reclaimed water, and the water quality of the river showed a trend of improvement. Compared with the threshold for Chla of 10 mg/m^3 used in most studies (Huang *et al.* 2020), the possibility of eutrophication in river L was not high despite the relatively high concentration of phosphorus nutrients in the river. The ecological restoration measures in river L could maintain the stability of water quality to avoid initial deterioration. The roots of aquatic plants, especially the roots of emergent plants, were well developed, which was conducive to attachment and growth of microorganisms, thereby accelerating the decomposition of organic materials around the roots. Aquatic plants in the growing period can also absorb fixed nitrogen and phosphorus elements, consequently accelerating the remove of nitrogen and phosphorus from water (Zhou *et al.* 2016).

The risk assessment of algal bloom in river L

It is widely believed that nutrient levels, optimal light, and temperature are the three main factors that cause algal blooms in water bodies (Paerl *et al.* 2001). Under certain environmental conditions (rich nutrition, bright light, high temperature), the algae have extremely strong photosynthesis and thus grew rapidly, causing a short-term explosion of algae. In this study, the risk of algal bloom and safety assessments were evaluated by analyzing the variation of F_v/F_m monitoring values in river L. As shown in Figure 3(a), the value of F_v/F_m in river L fluctuated greatly, and basically it still presented an increasing trend along the river ($P < 0.5$). The monthly average values of F_v/F_m for the entire section remained below 0.40, which did not exceed the algal bloom prediction threshold of 0.63, thus, the application of ecological restoration measures in the river effectively suppressed the possibility of algal blooms. With the increase of temperature and light intensity, the photosynthesis of algae and the growth rate gradually increased to a better external environmental position. Therefore, the value of F_v/F_m in the river increased gradually from May to July and decreased successively in August and September.

As shown in Figure 3(b), the value of F_v/F_m in three typical sections of river L are analyzed in detail on a smaller time scale. N1 is the input point of the reclaimed water, N3 lies at the junction between the oxygen control and denitrification enhancement zone and the efficient reoxygenation and ecological purification zone, while N8 is located at the end of river L. There were almost no ecological restoration measures at N1 as it was the start of the reclaimed water reuse in the river. Therefore, the biological content here was low and the value of F_v/F_m was the lowest in the entire river. As the reclaimed water went through a series of ecological restoration measures in the river, the value of F_v/F_m increased to some extent, and these values between two ecological zones showed a slight difference. In the most suitable period for algae growth, the value of F_v/F_m in river L reached a record level of 0.42, along with a smaller risk of algal bloom and a higher level of river security. It can also be seen from the figure that there were certain fluctuations between the monitoring results in the same month, and this may be due to the water environmental conditions changing after the reclaimed water entered the ecological zones of the river, which also caused the change of F_v/F_m monitoring values along the river.

The nutrient concentration is a key limiting factor for algae growth, and the nitrogen and phosphorus nutrients' input control (especially phosphorus) was quite necessary to reduce the risk of eutrophication (Schindler *et al.* 2016). The concentration of TP in river L generally exceeded the internationally recognized critical value of 0.2 mg/L for eutrophication of water bodies from the above analysis (Wang *et al.* 2019). In this case, these ecological restoration technologies could be effective and necessary in the river. Although the F_v/F_m value was rising, the concentration was still well below the risk threshold. It can also be seen that the number of algae in river L was at a relatively low level from the value of Chla. Many studies have also shown that macrophytes (especially submerged plants) can effectively absorb and remove nitrogen and phosphorus in the water, and inhibit the growth of phytoplankton through the release of allelochemicals, so as to play a good role in the algal bloom control (Mohamed 2017).

Comprehensive biological toxicity assessment of river water quality

The variations of the luminescence inhibition rate on luminescent bacteria in river L from May to September are shown in Figure 4. The acute toxicity of water quality in all sections was at or below the toxicity level of II (intermediate toxicity), and the toxicity level of I (low toxicity) accounting for 57.5%. The acute toxicity of water quality in summer was significantly higher than that in May and June, which might be related to the incoming quality of the reclaimed water. The amount of chemical dephosphorization reagents, disinfectants, and other agents added may be higher than that in May and June in order to control the effluent quality of the reclaimed water in WWTP in summer; as a result, the luminescent inhibition ratio in the river increased correspondingly. However, the higher comprehensive biological toxicity of the water body in

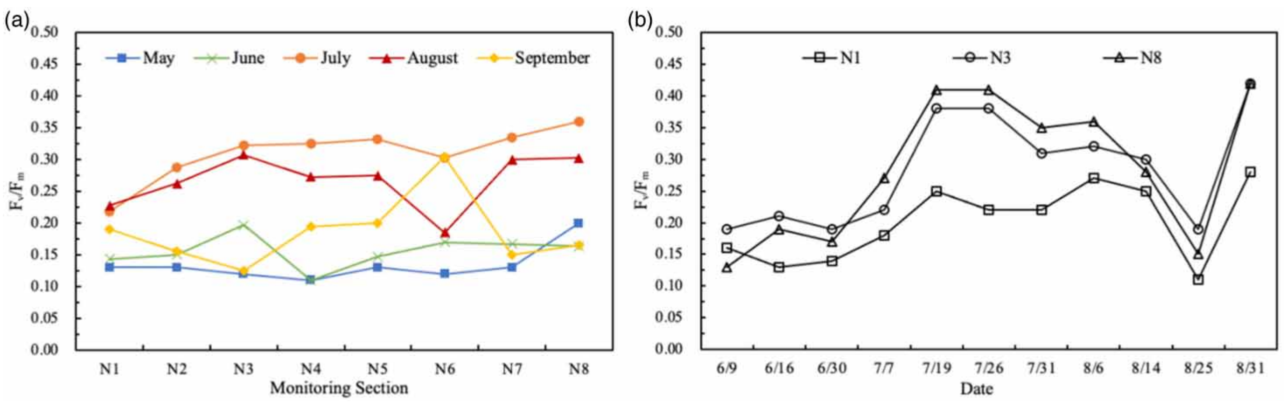

Figure 3 | Variation of optimal PSII efficiency in river L: (a) variation of F_v/F_m along river L (monthly average of the values) and (b) variation of F_v/F_m in N1, N3, and N8 (multiple measurements from June to August).

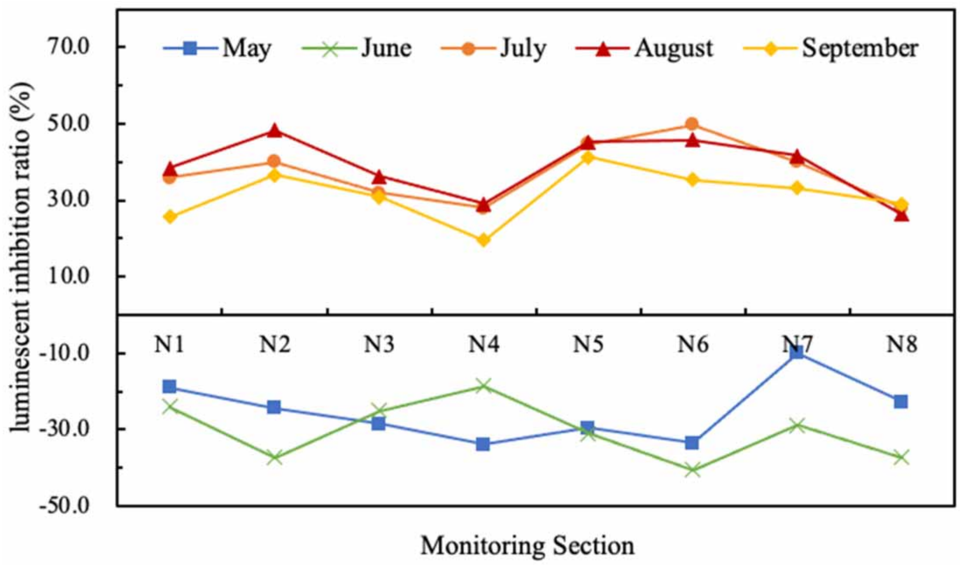

Figure 4 | Variation of luminescent inhibition ratio in river L.

summer did not inhibit the growth of various organisms in river L. Adversely, the growth of organisms was better from the observation on site. In addition, the luminescent inhibition ratio was negative in May and June, which was attributed to the promoted reproduction of luminescent bacteria by the water environment. The reason may be that when the water sample was less toxic or non-toxic, some nutrients in the water (such as NH_4Cl, $NaNO_3$, $NaNO_2$, NaH_2PO_4, etc.) could enhance the activity of luminescent bacteria, making the luminescent inhibition ratio negative (Zhu 2012).

The luminescent inhibition ratio along river L fluctuated but showed a downward trend in general of N1–N4. This result was attributed to the physical, chemical, physicochemical, and biological effects of the river ecological purification and restoration system, meaning the biological toxicity of the reclaimed water could be effectively controlled and alleviated. The semi-natural environment of the landscape water provided a favorable condition for the degradation of toxic organic chemicals (Ma *et al.* 2016). Chen *et al.* (2014) also showed that the macrophytes could remove disinfection by-products (DBPs) through plant uptake, phytovolatilization, and/or phytodegradation, thus reducing the water toxicity. However, there were several waterside terraces near N5–N7, and the residents of the community may wash their daily necessities (such as mops or rags) by the river in summer, causing some functional detergents to enter the river. Some of the chemicals may inhibit the luminescent bacteria, leading to an increment in the acute toxicity level of the water in these three sections.

CONCLUSION

In this study, the risk of algae blooms and the comprehensive biological toxicity of the urban river replenished with reclaimed water were investigated based on water quality monitoring. The dilution of the river L by reclaimed water decreased the concentration of the former COD_{Mn}, NH_4^+-N, and Chla. Subject to the limited effect of phosphorus treatment, the concentrations of TP were relatively high and basically exceeded 0.4 mg/L. Even so, the possibility of algal blooms in river L was still low according to the analysis of F_v/F_m value. Furthermore, the implementation of ecological restoration could reduce the acute toxicity of reclaimed water to water bodies.

Under the condition of high nutrient concentrations in the river, a series of ecological restoration technologies and measures, such as underwater biological filter bed, aeration, and macrophytes' remediation, could effectively prevent the occurrence of eutrophication and algal bloom. Therefore, when conducting reclaimed water reuse projects and maintaining the stability and safety of the water quality and aquatic ecology, it is necessary to suit the measures to the local conditions and carry out ecological purification and restoration of water bodies according to the water quality of the reclaimed water and the characteristics of the receiving water. Further monitoring and studies of other water quality indicators and biological toxicity can be conducted in the future, to explore the safety of urban reclaimed water reuse in multiple dimensions.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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