Journal of Hazardous Materials 324 (2017) 573–582

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Insight into the risk of replenishing urban landscape ponds with reclaimed wastewater

Rong Chen[a,b,*], Dong Ao[a], Jiayuan Ji[b], Xiaochang C. Wang[a,**], Yu-You Li[b], Yue Huang[a], Tao Xue[a], Hongbing Guo[a], Nan Wang[a], Lu Zhang[a]

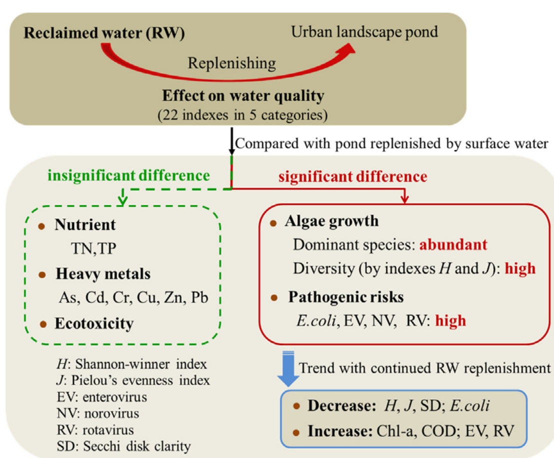
[a] Key Lab of Northwest Water Resources, Environment and Ecology, Ministry of Education, Xi'an University of Architecture and Technology, Xi'an 710055, PR China

[b] Department of Civil and Environmental Engineering, Graduate School of Engineering, Tohoku University, 6-6-06 Aza-Aoba, Aramaki, Aoba-ku, Sendai, Miyagi 980-8579, Japan

HIGHLIGHTS

- Significant risk of replenishing landscape ponds by reclaimed water were identified.
- Great differences were observed in algae and pathogens between RW- and SW-ponds.
- RW-ponds showed Cyanophyta-Chlorophyta-Bacillariophyta type with high algal diversity.
- Health risk is relatively higher in RW-ponds and viral pathogens are the main driver.
- Duration of RW replenishment remarkably affects algal growth and pathogen risk.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 August 2016
Received in revised form 15 October 2016
Accepted 9 November 2016
Available online 10 November 2016

Keywords:
Reclaimed water
Replenishment
Landscape pond
Algal growth
Pathogen risk

ABSTRACT

Increasing use of reclaimed wastewater (RW) for replenishing urban landscape ponds has aroused public concern about the water quality. Three ponds replenished with RW in three cities in China were chosen to investigate 22 indexes of water quality in five categories. This was achieved by comparing three pairs of ponds in the three different cities, where one pond in each pair was replenished with RW and the other with surface water (SW). The nutrients condition, heavy metal concentration and ecotoxicity did not differ significantly between RW- and SW-replenished ponds. By contrast, significant differences were observed in algal growth and pathogen risk. RW ponds presented a Cyanophyta-Chlorophyta-Bacillariophyta type with high algal diversity while SW ponds presented a Cyanophyta type with low diversity. Regrowth of bacterial pathogens and especially survival of viral pathogens in RW, was the main driver behind the higher risk for RW ponds compared with SW ones. The duration of RW replenishment was proved to

* Corresponding author at: Key Lab of Northwest Water Resources, Environment and Ecology, Ministry of Education, School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, Xi'an 710055, PR China.
** Corresponding author.
E-mail addresses: chenrong@xauat.edu.cn (R. Chen), xcwang@xauat.edu.cn (X.C. Wang).

http://dx.doi.org/10.1016/j.jhazmat.2016.11.028
0304-3894/

have a marked impact on the algal growth and pathogen risk. With continued RW replenishment, non-dominant algal species subjected to decrease while dominant species were enhanced resulting in the biomass increasing but diversity declining, and the risk posed by viral pathogens might become greater.

1. Introduction

Water shortages are a dire concern in many countries and regions of the world because of population growth and accelerated industrialization and urbanization. In this context of scarcity, alternative water resources (AWRs) are becoming increasingly prominent as a way to meet different water needs. Such AWRs mainly include rainwater, industrial recycled water and reclaimed wastewater (RW). Due to its stable quality and continuity of supply, RW has been widely used [1,2].

Although RW can ease the problem of water shortages, the safety of wastewater reuse is occasionally questioned, as some compounds commonly found in RW, such as nutrients and micro-pollutants, have negative consequences for reuse. For agricultural use, salts and heavy metals can accumulate in soils following extended RW irrigation and can have consequent deleterious effects on yield [3–5]. For municipal use, health risks can be generated by pathogens from the spray irrigation of a green area with RW [6,7]. For industrial use, pipes can become corroded through microbial action [8]. Finally, for environmental use, the high concentration of nutrients in RW may have negative impacts on ecosystems in the receiving water bodies in the environment [2,9].

With increasing water shortages comes the need in the urban waterscape for new water resources, and RW is gradually being used more often for replenishing urban landscape ponds. In California, USA, about 25% of the effluent from wastewater treatment plants (WWTPs) is used for lake replenishment [10]. About 50% of the effluent from WWTPs is used to improve water features in nearby recreational leisure venues in Osaka, Japan [10]. In recent years, China has experienced rapid development and there is an increased demand in urban life for attractive water scenes [11]. China has taken great effort to conserve and augment the limited water resources available to meet the growing water demands. Also, water reclamation, has been established as a key components of the national water strategy [12]. In most cities, RW has become the first option of water resource management for replenishing landscape ponds, such as at Olympic Park in Beijing [13], Eco-wetland Park in Tianjin (the first theme park focused on environmental protection in China [14]) and Kunming Pool in Xi'an (the largest artificial lake in northwestern China [15]).

Although RW can satisfy the Chinese water quality standards for environmental reuse [16], use of RW instead of the traditional surface water (SW) for landscape needs will certainly affect the water quality of the receiving ponds. In urban landscape scenes, the primary objective of RW use is to help create an aesthetically pleasing water landscape, and until now, most studies on RW use in this context focused on aesthetic aspects, such as controlling algal growth and eutrophication [13,14,17]. Few studies have measured non-sensory indexes of water quality for RW-replenished water features, and research attention has mainly focused on the concentration of heavy metals in the water column and sediment, as well as their accumulation characteristics and corresponding technologies for controlling them [18,19]. Furthermore, there are even fewer studies on the impact of toxic organic micro-pollutants and pathogens from RW replenishment. Toxic organic chemicals are one of the dominant hazardous components of wastewater, and conventional treatment processes cannot remove them thoroughly [1]. Of the various pollutants, persistent organic pollutants, disinfection by-products and microbial metabolic products have received much attention because they are slow to degrade, can bio-accumulate and can be ecologically toxic and carcinogenic [20,21]. In addition, although most pathogens are killed during wastewater treatment processes by disinfection, the bacterial pathogens can regrow because of the availability of nutrients in the water bodies replenished with RW, and the viral pathogens may survive and remain infective in the water environment due to their high resistance to the inactivation of disinfection of wastewater treatment, especially for chlorination [22]. In recent years, health problems caused by pathogens following wastewater reuse are being reported more and more frequently. This includes acute gastroenteritis caused by rotavirus, vomiting and abdominal pain caused by norovirus, and enteric fever caused by *Salmonella* [6,23,24]. In short, it is necessary to analyse the impact of replenishing urban landscape waters with RW in a more comprehensive manner. This should mainly focus on the impacts on the trophic condition, algal growth, accumulation of heavy metals, toxicity of organic micro-pollutants and health risks posed by pathogens.

The water quality in such replenished water bodies will also be influenced by external environmental factors such as the local climate and atmospheric deposition, and intrinsic factors such as depth, coverage and hydraulic retention time. Özkan et al. [25] assessed the relative roles of different environmental factors in phytoplankton growth by investigating 195 Danish lakes and ponds in a spatially explicit framework, and found that the main influencing factors were lake chemistry (especially total nitrogen [TN] and total phosphorus [TP]) and lake morphology (especially lake depth), but that climate conditions (including air temperature and solar radiation) had little effect. Phillips et al. [26], through analysing a large dataset of 1138 European lakes and ponds, also concluded that the water quality was affected by lake morphology and local climate. Wang et al. [27] concluded that the water cycle plays an even more important role in stabilizing water quality than does the control of nutrient levels. Therefore, the present approach to studying the effect of replenishing urban ponds with RW was to compare these RW ponds with ponds replenished by SW located in the same region and with similar characteristics, including hydraulic retention time, coverage and depth.

The objective of this research was to gain insight into the effect of RW replenishment on urban landscape ponds by comparing such RW ponds with counterparts replenished by SW, and to reveal the differences related to visual effects, ecology and health risk. In total, three RW-replenished landscape ponds in three different cities were chosen to investigate 22 indexes of water quality in five categories: trophic condition, algal growth, heavy metals, ecotoxicity and pathogenic risk. This was done by comparing the RW ponds with nearby ponds replenished by SW in each of the three cities. The paper identified the key effects on the water quality from RW replenishment, which will be useful for landscape planners and managers to determine what should be monitored and what proactive steps should be taken to minimize any negative effects of RW use. Such insights will also help to promote further use of RW as a water source for replenishing urban landscape ponds and to relieve the urban water shortage problem.

2. Materials and methods

2.1. Selected ponds and water sources for pond replenishment

The RW-replenished landscape ponds of Cuihu in Kunming city (designated R1), Fengqing in Xi'an city (R2) and Lingang in Tianjin city (R3) in China, which are solely replenished by RW, were chosen for water sampling and analysis (Fig. 1). Corresponding SW-replenished ponds were chosen to match the key characteristics of the three RW ponds: Yueya in Kunming city (S1), Lianhua in Xi'an city (S2) and Changhong in Tianjin city (S3), respectively. Table S1 lists the basic information for all of the ponds. All six ponds have similar hydraulic and morphologic features, with hydraulic retention time (HRT) ranging from 28 to 40 days, average depth from 1.5 to 2.2 m and surface area from 5×10^4 to 12×10^4 m^2.With respect to the RW ponds, by 2015 Cuihu, Fengqing and Lingang had been receiving RW for around 9, 5 and 3 years, respectively. The basic properties of RW and SW as source water were showed in Table 1. All the RW are transferred from related WWTPs to the RW ponds by pipelines. The SW for S1 and S3 ponds come from two urban natural rivers while that for S2 is transferred by an artificial underground tunnel.

2.2. Water sampling

Field investigations for most indexes were conducted from January to December 2015, with the exception of samples for the algal identification, which were collected in July, August and September when the growth rate of algae was expected to be the highest. Samples were collected once a month and all samples were collected at approximately 11:00–11:30 A.M. every day. Five sampling sites were chosen for each pond, including one site at each inflow (Fig. S1). The samples were collected using a water sampler (2000 mL) at a depth of 0.5 m below the surface (except the inflow water sample of RW ponds, which was cascading water, collected at pipe ends). Eight litres of water samples were collected from each sampling site, and kept in glass bottles. Two-litre samples for phytoplankton detection were immediately preserved in Lugol's iodine solution and then concentrated to approximately 100 mL in the laboratory. The pH value, total dissolved solids (TDS), residual chlorine (with standard methods [16]) and transparency (Secchi disk clarity, SD) were measured in situ.

2.3. Water sample analysis

As Table 2 shows, 22 indexes in five categories of water quality were selected and measured as follows: (1) parameters related to the trophic condition, including nitrogen, phosphorus, chemical oxygen demand (COD), chlorophyll-a (Chl-a), and SD; (2) parameters related to algal growth, including algae species, dominant algae and their richness and evenness; (3) parameters related to heavy metals, including the concentrations of As, Cr, Cu, Cd, Zn and Pb; (4) ecotoxicity indexes; and (5) concentrations of three typical bacterial pathogens, namely *Escherichia coli (E. coli)*, *Salmonella* and *Shigella*, and three typical viral pathogens, namely enterovirus (EV), norovirus (NV) and rotavirus (RV).

In addition, heavy metal pollution index (HPI) and hazard index (HI) were used to evaluate the ecological and health risk due to heavy metals respectively. Comprehensive ecotoxicity was measured by detecting effective phenol concentration ($EC_{50\text{-phenol}}$). Infection rate by pathogens were quantified to assess their health risks. The details of all the selected indexes described above were referred to Supplementary materials (detailed water sample analysis).

2.4. Statistical analysis

Statistical analyses were conducted using SPSS software (v. 19.0, Statistical Product and Service Solutions, IBM, USA) to identify the effects of RW replenishment on all the measured water quality indexes and to reveal their linear correlations with the duration of RW replenishment. In order to increase the normality of the data and the suitability of the linear regression model, and to stabilise the variances, all variables were logarithmically transformed. The significance and strength of the relationship were shown by the *P*-value and the multivariate Pearson's correlation coefficients ρ, respectively.

3. Results and discussion

3.1. Impacts on the trophic condition

As Fig. 2a shows, the TP level in each of the RW-replenished ponds was remarkably lower than the TP level in the corresponding source water: 25%, 22% and 43% lower for R1, R2 and R3, respectively; however, there was significantly less difference in TP between the source and pond water for the SW-replenished ponds. Based on related studies, PO_4^{3-}-P is the dominant form of phosphorus absorbed by planktonic microbes in water [9,14]. Accordingly, the dominant component of TP in RW from a sewage treatment process wasPO_4^{3-}-P, whereas in SW, the proportion of PO_4^{3-}-P in TP was not as high. Thus, the significant decrease in TP in the RW ponds can be attributed to the high percentage of PO_4^{3-}-P in the RW source water. In addition, relative high concentration of PO_4^{3-}-P was possible to reduce the toxic effect of acid (H^+) concentration on freshwater biota, and further to stimulate primary productivity and nutrient assimilation [29]. In addition, TP in all the SW ponds was a little higher than in the source SW water, but PO_4^{3-}-P was converse. As a reason, on one hand, phosphorus was probably released from sediments which tended to be more abundant in the SW ponds than in the RW ponds due to the relative long history of SW ponds; on the other hand, rapid algae growth was certain to consume most of PO_4^{3-}-P in the ponds.

Regarding nitrogen shown in Fig. 2b, the source water TN concentration declined in all ponds except S2, but the decline was particularly notable in the RW ponds, the average decreases were 46% and 67% for the SW and RW ponds, respectively. Due to the aerobic treatment unit in WWTPs that provided the RW, most of the nitrogen in the original untreated wastewater had already been converted to NO_3^--N, which accounted for the majority of TN in the RW. By contrast, organic nitrogen is abundant in SW, and it is not readily absorbed by aquatic plants and phytoplankton [9,14]. Due to large absolute algae quantities which can be learned from Chl-a concentrations shown in Fig. 2d, TN showed much more declination in the RW ponds than in the parallel SW ponds. In particular, abundant Bacillariophyta in the RW ponds (see Fig. 3a) would consume plenty of nitrate accounting for a high proportion of TN in RW, because it is an algae division significantly associated with the uptake of oxidized nitrogen which will be further discussed in the following impact on algal growth.

Besides, COD and Chl-a are also main indicators of trophic condition and their performances were shown in Fig. 2c and d. The COD concentrations in all the ponds were higher than in the corresponding source water, which probably attributed to the contribution of algae cells to COD because it was measured without any filtration, and COD concentrations in RW ponds were higher than in the corresponding SW ponds, conforming to the results of Chl-a very well. As an indicator for algal quantity and growth, Chl-a tended to be much higher in all the RW ponds than in the compared SW ponds, which

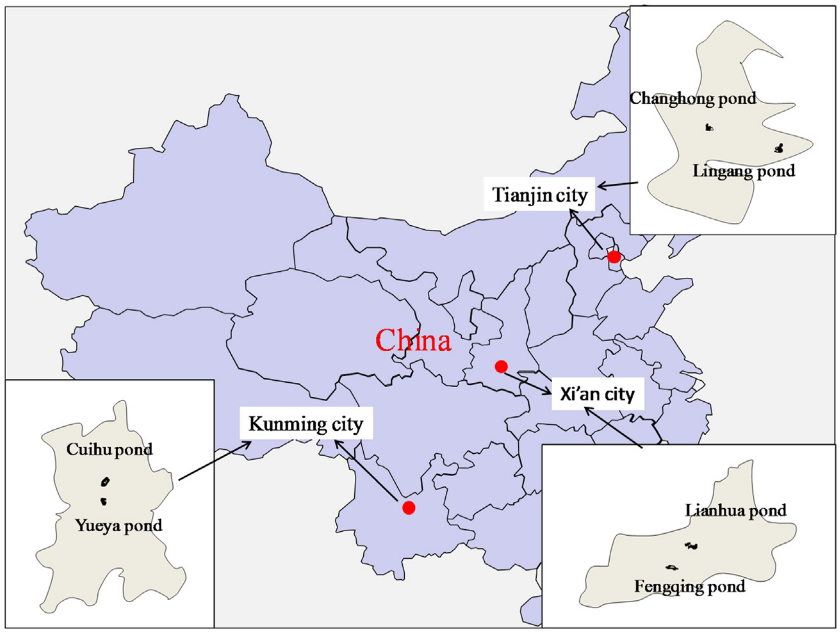


Fig 1. Location of the three selected ponds replenished by RW and the three corresponding ponds replenished by SW.

Table 1
Basic properties of reclaimed (R) and surface (S) source water for pond replenishment.

Items	pH	TDS (mg/L)	COD (mg/L)	TN (mg/L)	NO_3^--N (mg/L)	NH_4^+-N (mg/L)	TP (mg/L)	PO_4^{3-}-P (mg/L)
R1	7.95 ± 0.02	500 ± 20	19.24 ± 3.32	6.39 ± 0.52	4.63 ± 0.40	1.10 ± 0.10	0.31 ± 0.15	0.25 ± 0.14
R2	7.73 ± 0.04	401 ± 25	23.12 ± 2.23	9.51 ± 0.40	8.89 ± 0.42	0.35 ± 0.08	0.58 ± 0.20	0.42 ± 0.21
R3	8.01 ± 0.03	–	20.93 ± 3.21	9.78 ± 0.34	7.01 ± 0.27	1.95 ± 0.52	0.36 ± 0.14	0.34 ± 0.12
S1	8.40 ± 0.03	372 ± 24	14.42 ± 5.08	4.57 ± 0.42	1.35 ± 0.21	3.02 ± 0.51	0.20 ± 0.07	0.13 ± 0.04
S2	7.40 ± 0.04	277 ± 30	17.63 ± 4.23	6.14 ± 0.33	4.53 ± 0.37	0.36 ± 0.05	0.22 ± 0.13	0.15 ± 0.12
S3	8.37 ± 0.03	–	15.08 ± 4.01	5.03 ± 0.23	1.30 ± 0.13	1.28 ± 0.12	0.15 ± 0.06	0.06 ± 0.05

Table 2
Water quality indexes selected for analysis and those detection methods.

Five categories	22 indexes	Detection method
Trophic condition	Nitrogen (TN, NO_3^--N and NH_4^+-N)	EPAC Standard Methods[a]
	Phosphorus (TP and PO_4^{3-}-P)	EPAC Standard Methods
	COD	$K_2Cr_2O_7$ titrimetry
	Chl-a	EPAC Standard Methods
	SD	Secchi disc
Algal growth	Algae identify	Counting method
	Dominant species	Cells count more than 10% [9,11]
	Species richness	Shannon–Wiener index (*H*)[b] [11,14]
	Species evenness	Pielou's evenness index (*J*)[c] [11,14]
Heavy metals [d] [28]	As	ICP-AES (λ = 189.0 nm)[e]
	Cr	ICP-AES (λ = 267.7 nm)
	Cu	ICP-AES (λ = 324.7 nm)
	Cd	ICP-AES (λ = 228.8 nm)
	Zn	ICP-AES (λ = 213.8 nm)
	Pb	ICP-AES (λ = 220.3 nm)
Ecotoxicity	$EC_{50\text{-phenol}}$	Acute toxicity to Q67[f] [21]
Pathogens	*E. coli*	RT PCR[g]
	Salmonella	RT PCR
	Shigella	RT PCR
	EV	RT PCR
	NV	RT PCR
	RV	RT PCR

[a] EPAC (Environmental Protection Agency of China), Standard Methods for the Examination of Water and Wastewater.
[b] Shannon–Wiener index (*H*) represents the abundance and the evenness of a species present, but it is not significantly affected by rare species.
[c] Pielou's evenness index (J) is used as the measure of species evenness.
[d] Selected heavy metals are the major metal contaminants listed by EPAC.
[e] ICP-AES means inductively coupled plasma atomic emission spectrometry and λ is the analysing spectrum lines for detection.
[f] Q67 is a luminescent bacterium.
[g] RT PCR means real-time quantitative PCR.

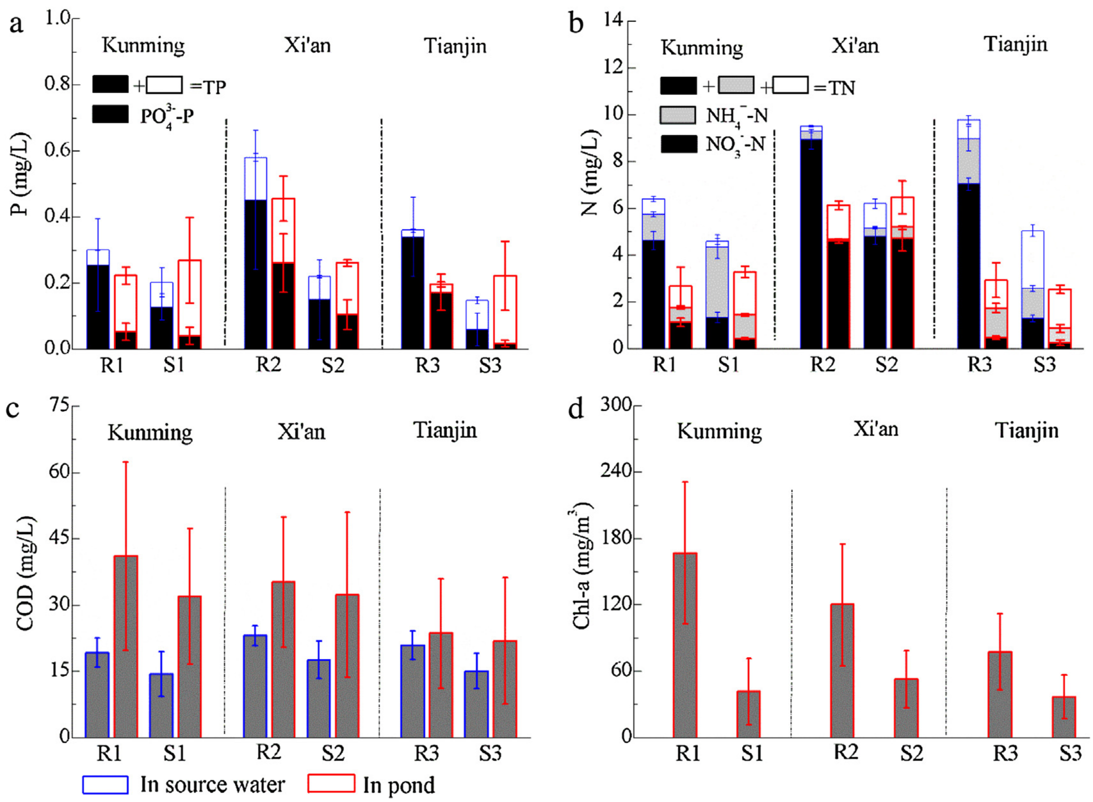


Fig. 2. Changes in the levels of trophic condition between source and pond water.

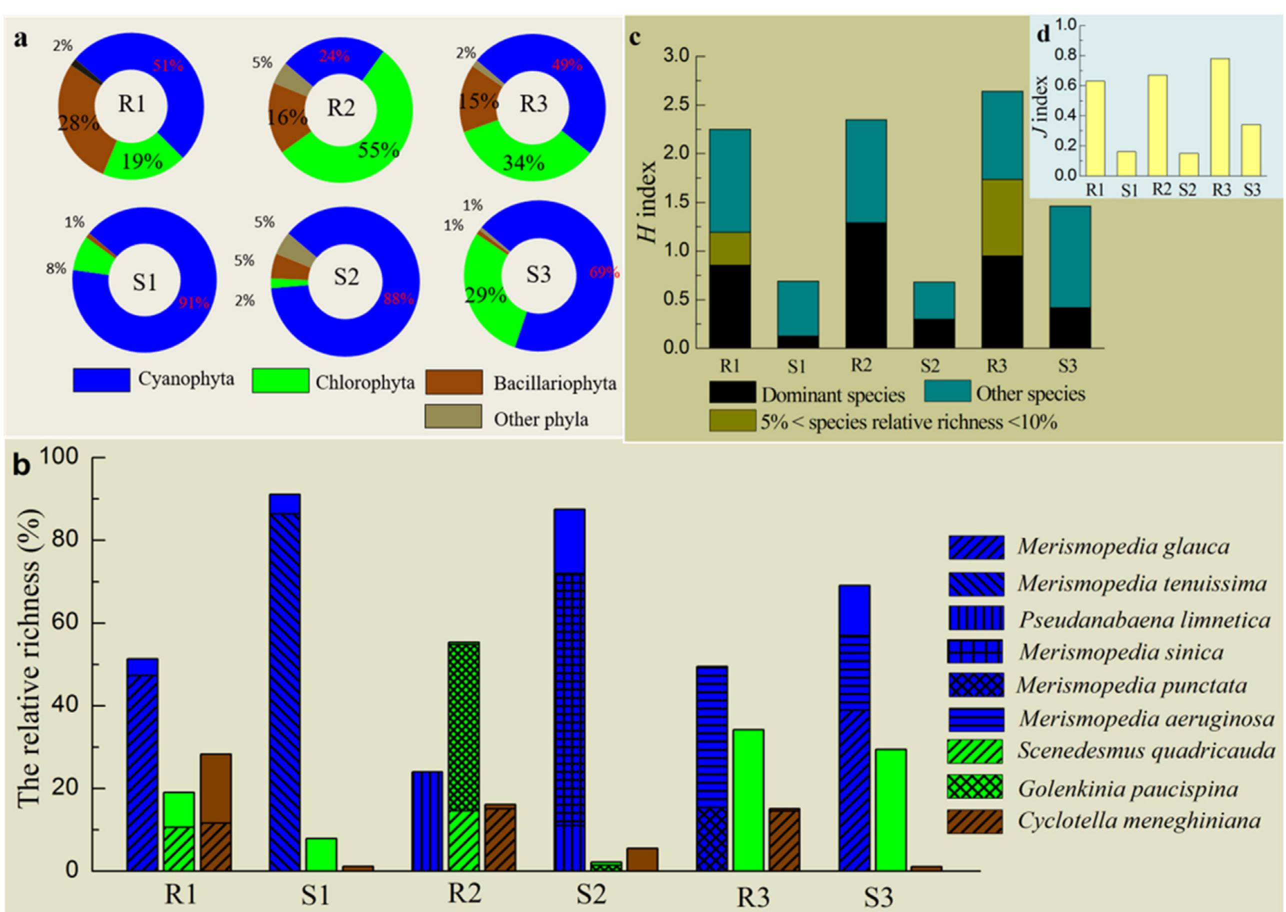


Fig. 3. Difference in the algal diversity between RW and SW ponds.

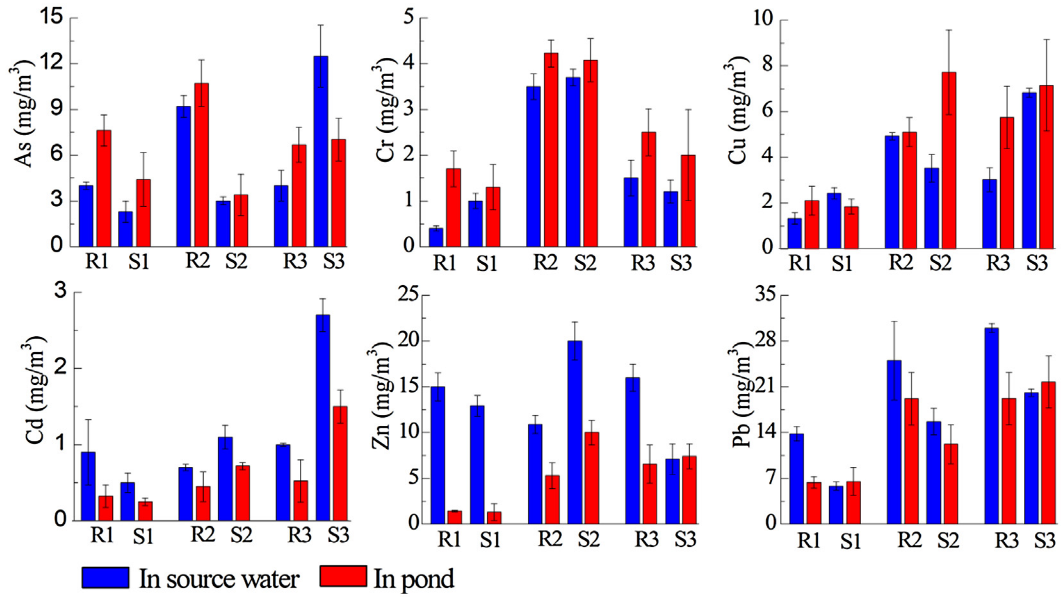


Fig. 4. Changes in As, Cr, Cu, Cd, Zn and Pb levels between source and pond water.

could also explain rapid consumption of nitrogen and phosphorus in the RW ponds.

3.2. Impacts on algal growth

All species of algae in the ponds were identified and their populations were counted. As shown in Fig. 3a, the proportions of Cyanophyta were 51% and 91% in R1 and S1 in Kunming, 24% and 88% in R2 and S2 in Xi'an, and 49% and 69% in R3 and S3 in Tianjin, respectively. It can be concluded that the richness of Cyanophyta, which is an abundant phylum of algae in eutrophic natural lakes, was much lower in RW ponds than in SW ponds. This can be attributed to the fact that RW contains more NO_3^--N, and Cyanophyta are less competitive in utilizing that than other divisions of phytoplankton [30,31]. In contrast Bacillariophyta potentially take up nitrate to serve as a sink for electrons during periods of imbalance between light energy harvesting and utilization, and this mechanism is apparently not present in non-diatom species, thus Bacillariophyta growth well in RW ponds are associated with high nitrate supply [30]. Besides, Chlorophytes have higher ability of nutrients predation and may outcompete Cyanophyta at sufficient PO_4^{3-}-P supply in RW ponds [32]. In addition, it should be noted that the RW was disinfected in the WWTPs, while the SW was not. For this reason, the concentrations of residual chlorine in the three RW ponds were measured irregularly but they were usually below a detection limitation of 0.05 mg/L, possibly due to chlorine volatilization and water dilution, and they were far less than the concentration for algae growth inhibition which was reported to be around 1 mg/L [33]. Therefore, the impact of residual chlorine on the algae growth was negligible. As a result, ponds supplemented with RW tend to be of the Cyanophyta–Chlorophyta-Bacillariophyta type, but those supplemented with SW tend to be of Cyanophyta type.

Moreover, from the analysis of dominant species (Fig. 3b) which defined as any species whose cells count accounts for more than 10% of the total cells, three species (*Merismopedia glauca*, *Scenedesmus quadricauda* and *Cyclotella meneghiniana*) were identified in R1, while only one species (*Merismopedia tenuissima*) was identified in S1. Four species (*Scenedesmus quadricauda*, *Golenkinia paucispina*, *Cyclotella meneghiniana* and *Pseudanabaena limnetica*) were identified in R2, while only two species (*Merismopedia sinica* and *Pseudanabaena limnetica*) were identified in S2. Three species (*Microcystis aeruginosa*, *Merismopedia punctata* and *Cyclotella meneghiniana*) were identified in R3, while only two species (*Merismopedia glauca* and *Microcystis aeruginosa*) were identified in S3. In summary, each RW pond had no less than three dominant species, which belonged to at least two kinds of algal division, but for SW ponds there were no more than two dominant species, and these belonged only to the Cyanophyta phylum. Principally dominant species distribution was matched to the opportunities provided by environment, and all the identified dominant algae in RW ponds appeared great growth potential in the high nutrients condition [34].

In addition, as shown in Fig. 3c and d, RW resulted in higher values for the Shannon–Wiener index (H) and Pielou's evenness index (J). The H values were 2.25, 2.35 and 2.68 in R1, R2 and R3, respectively, while they were 0.63, 0.67 and 1.33 in S1, S2 and S3, respectively. The J index also revealed a substantial gap between the RW and SW ponds. It can therefore be concluded that the algal diversity in the RW ponds is much greater and more stable (as shown by H and J, respectively) than in the SW ponds. Note worthily not only rich dominant species but also non-dominant species whose richness were between 5% and 10% in RW ponds contributed largely the diversity expressed as H and J. Reportedly high NO_3^--N and PO_4^{3-}-P in RW could enable many new species to become established in the system and further increase the lake's algae diversity [35].

3.3. Impacts on heavy metals

Six heavy metals were measured, namely As, Cr, Cu, Cd, Zn and Pb, and the changes in their levels between source and pond water for RW and SW ponds are shown in Fig. 4. Water sources had no significant effect on the concentrations of heavy metals in ponds. Moreover, the final concentrations of all metals in ponds changed from the levels in the source water for both RW and SW ponds. For example, in most of ponds, the concentrations of As, Cr and Cu were lower in the source water than in the ponds, while Cd, Zn and Pb largely followed the opposite trend.

The results showed that the concentration of heavy metals in source water might not be the only one that have a significant impact on the subsequent heavy metal levels in ponds, and some

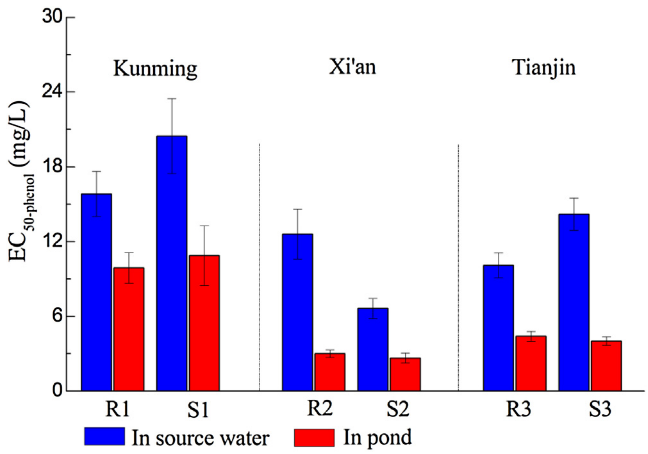


Fig. 5. Changes in $EC_{50\text{-phenol}}$ between source and pond water.

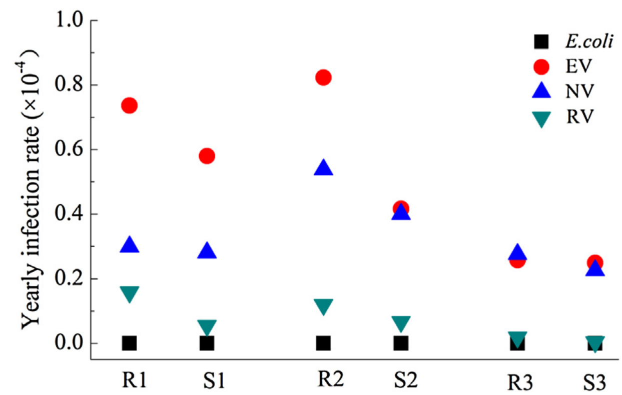


Fig. 6. Yearly infection risk from human exposure to the RW and SW ponds.

other factors, such as sediment adsorption, atmospheric deposition and algae absorption, may play more important roles than the replenishment water source. As related studies have mentioned, sediment has a great ability to accumulate Cd, Zn and Pb [36], and the heavy metal content in atmospheric deposition was found to be 5–9-fold higher than in ponds [37,38]. Furthermore, the adsorption capabilities of different algae species differ depending on the metal [18,39].

The risks of heavy metals on ecosystem and human health were also evaluated by means of HPI and HI method, respectively (see Supplementary materials). The possible maximum HPI values were determined as 10.3, 7.5, 17.6, 15.2, 21.5 and 33.6 in R1, S1, R2, S2, R3 and S3, respectively, which also presented no obvious difference between RW-ponds and SW-ponds. Furthermore all the HPIs were much lower than a critical value of 100 [40], which indicated the selected ponds were not critically polluted by heavy metals. In addition, the HI values due to heavy metals in ponds were calculated, where the exposure frequency and ingestion rate were assumed as 40 d/y and 100 mL/d (all of that through ingestion exposure in this text for conservative assumption) respectively, according to a related research [41]. The results showed that the possible maximum HI values due to heavy metals were 1.3×10^{-2}, 1.1×10^{-2}, 3.2×10^{-2}, 1.8×10^{-2}, 2.9×10^{-2} and 3.2×10^{-2} in R1, S1, R2, S2, R3 and S3, respectively, which were smaller than the safe risk threshold of unity [40].

3.4. Impacts on ecotoxicity

Fig. 5 illustrates the value of $EC_{50\text{-phenol}}$ in source water and in the receiving ponds. The concentrations of $EC_{50\text{-phenol}}$ in SW and RW source water were very different, but after entering the ponds, the gap decreased in all cases. In this analysis, the ecotoxicity in ponds had little relationship with the source water, which was probably because the influent ecotoxic compounds were within the scope of the ponds' self-purification capacity. For instance, some disinfection by-products (DBPs) in the RW such as trihalomethanes (THMs), haloacetic acids (HAAs), halogen acetonitriles (HANs), etc. tend to be ecologically toxic, but most of them have low vapour pressure and weak resistance to sunlight irradiation thus would readily evaporate or degraded when they entered a pond with a large surface area [20,42,43]. And also the sediments can act as a sink for ecotoxic compounds through direct sinking of the algae cells capable of removing ecotoxic compounds or through incorporation in zooplankton fecal pellets and subsequent sinking [44,45]. In addition, some organochlorine pesticides and organic biocides which was available in the SW [44,46], and pharmaceuticals in the RW [21], are always less water soluble and nonpolar, and are easily adsorbed to sediment particles and accumulated in the fatty tissues of organisms [47,48].

3.5. Impacts on pathogen risk

As Table 3 showed, among the six selected bacterial and viral pathogens, only four were identified, comprising *E. coli*, EV, NV and RV, and all samples had a detection rate of 100%. The activity of pathogens might be inhibited by the residual chlorine in RW ponds, but the concentrations of all pathogens were still much higher in RW ponds than in SW ponds, except the concentration of EV in R1, which was lower than in S1. The high rate of detection of pathogens in RW ponds was mainly attributable to their high resistance to the external environment and other influences in the water environment. Reportedly, the fate of pathogens is crucial determined by inactivation by ultraviolet (UV) radiation [23,49]. Commonly low transparency caused by high algae biomass in RW ponds made pathogens affected by less UV radiation [23,49].

The high rate of detection of bacterial pathogens in RW ponds was also attributable to the contribution of the relatively high nutrient content of RW, e.g., assimilable organic carbon, nitrogen, phosphorus, and necessary microbial trace elements, which are conducive to regrowth of bacterial pathogens in the ponds [23,50,51]. Regarding viruses, they are not directly involved in nutrient metabolism as they are obligate intracellular parasites and are not living entities, however, it is likely that the high biomass in RW ponds enhanced virion attachment and promoted viral persistence by microbial biofilm formation and extracellular polymeric substance flocculation in situ [52,53], which meant the majority of virion in RW ponds are more stable or remain infectious. In addition, pathogens adsorbed onto suspended particles are readily settled down and accumulated in the bottom sediments, and are therefore protected from inactivation by natural or artificial disturbance, under such condition, pathogens would survive longer in the RW ponds sediments which contained available nutrients contents [54].

Through field investigations, we identified that the main means of public exposure to pond water were fishing and boating. To calculate the daily risk of infection, the mean volume of water ingested was assumed to be 5 mL (the possibility of swallowing those volume water was about 1%) per day [24], and the exposure frequency was assumed to be 40 events per year. The yearly infection rate for pathogens in all the ponds was shown in Fig. 6. In each city, the total risk for the RW pond was higher than that for the SW pond. For *E. coli*, the difference was quite small, while for EV, NV and RV, the differences observed were notable; and for EV, the difference was statistically significant. Notably, in Kunming and Xi'an, the yearly infection risk for viral pathogens was 1.2×10^{-4} and 1.4×10^{-4}, respectively, in ponds replenished with RW, which exceeds the

Table 3
Concentration of bacterial and viral pathogens in RW and SW ponds.

	Unit	R1	S1	R2	S2	R3	S3
E. coli	Copies/L	801 ± 256	656 ± 240	3632 ± 620	3028 ± 740	4560 ± 1120	2735 ± 750
EV	Copies/L	760 ± 354	796 ± 468	1130 ± 338	762 ± 372	354 ± 131	342 ± 85
NV	Copies/L	55 ± 11	50 ± 24	90 ± 21	57 ± 17	51 ± 20	39 ± 14
RV	Copies/L	340 ± 80	142 ± 25	280 ± 36	204 ± 49	64 ± 23	36 ± 18

*The *Salmonella* and *Shigella* pathogens were not identified because their concentrations were below the detection limits.

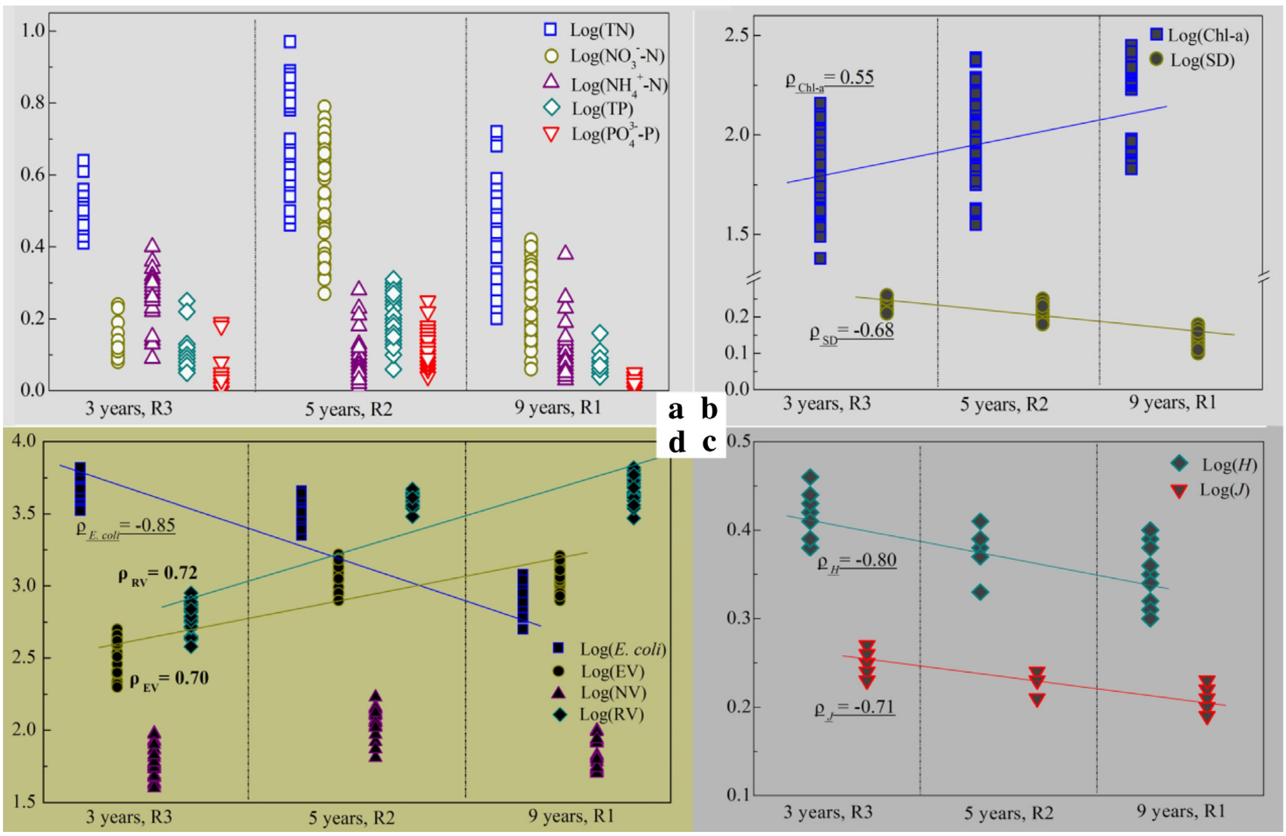


Fig. 7. Scatterplots and linear regression lines for the relationships between the number of years of RW replenishment and indexes of water quality in RW ponds.

World Health Organization's recommended safe threshold of 10^{-4}. The health risk of landscape ponds replenished with RW thus warrants attention.

3.6. Impacts of the duration of RW replenishment

In addition to the above, the duration of RW replenishment was found to have an important effect on the water quality in the RW ponds as indicated by the various indexes. From the analyses in the previous Sections 3.3 and 3.4, it was already seen that the heavy metal concentrations and $EC_{50\text{-phenol}}$ in ponds had little relationship to the source water type. The correlations of other indexes with the duration of RW use are shown in Fig. 7. As can be seen from Fig. 7a, nitrogen and phosphorus concentrations in ponds were less affected by duration of RW replenishment. Commonly an urban pond has a much shorter hydraulic retention time than a natural large lake, for this reason, most of the physicochemical parameters of water quality including nitrogen and phosphorus in the ponds may tend to appear close correlation to the input quality instead of time.

However, the Chl-a level, which is closely related to SD, had a significant ($P < 0.05$) and positive ($\rho = 0.55$) relationship with the RW replenishment duration (Fig. 7b). Furthermore, the *H* and *J* indexes showed a significant ($P < 0.05$) and strongly negative ($\rho < -0.70$) linear relationship with the RW replenishment duration (Fig. 7c). As mentioned the Chl-a level was closely related to the amount of dominant algae species, while the diversity expressed as *H* and *J* was contributed not only by dominant species but also mainly by abundant non-dominant species. With continued RW replenishment, non-dominant species subjected to decrease significantly while dominant species were enhanced due to long-term competitions [55,56], which resulted in the biomass increasing but diversity declining. Researchers made similar findings for many large SW-replenished lakes monitored continuously for many years, such as Chaohu Lake (8 years), Taihu Lake (11 years) and Vortsjarv Lake (40 years), which showed with the time going, the biomass of algae increases while the algal diversity declines [57–59].

As for pathogens, a chlorination process is the ultimate treatment for the three WWTPs producing RW and the concentrations of various pathogens presented no significant difference in the three RW source water based on irregular sample detections. However, it was noticeable that most of the detected pathogens in the RW ponds displayed linear relationships with the replenishment duration. A significant ($P < 0.05$) and strongly negative ($\rho = -0.85$) correlation was observed for *E. coli*, but trends were different for viral pathogens, especially for EV and RV (Fig. 7d). The concentrations of EV and RV were highly significantly ($P < 0.01$) and strongly positively ($\rho > 0.70$) related to the replenishment duration, and increased 2- and 3.86-fold, respectively, from 3 to 9 years of RW replenishment. These results indicated that as the duration of RW replenishment extends, the health risk posed by viral pathogens might become greater. The difference in the trends for *E. coli* and viral pathogens can mostly be attributed to their different survival abilities in ponds [23,51], as well as the different main factors affected their survival abilities [23]. Viruses generally are more environmentally resistant than enteric bacteria in pond's water body and sediment [50,60], so that can survive long enough for the virion levels to accumulate and which would promoted by the high content of viruses in sediment, and eventually to infect human hosts.

4. Conclusions

The effects of replenishing urban landscape ponds with reclaimed water (RW) were studied by comparing three ponds replenished RW with three ponds replenished with surface water (SW) in the same cities and with similar hydrologic and hydraulic characteristics. Algal growth was significantly affected by RW replenishment, showing that RW ponds presented a Cyanophyta-Chlorophyta-Bacillariophyta type with high algal diversity while SW ponds presented a Cyanophyta type with low diversity. The health risk from pathogens was the other indicator significantly affected by RW replenishment, due to regrowth of bacterial pathogens and especially survival of viral pathogens in RW, which proved to be the main driver behind the higher risk level for RW ponds compared with SW ones. The duration of RW replenishment was also found to have a great impact on the transformations of algal growth and pathogen risk. With continued RW replenishment, non-dominant algal species subjected to decrease while dominant species were enhanced resulting in the biomass increasing but diversity declining, and the risk posed by viral pathogens might become greater. The research results help to enable a better understanding of RW as a source for replenishing urban landscape ponds, and thus to improve the management of RW reuse and promote the appropriate utilization of RW.

Acknowledgements

This work was supported by the China National Major Project of Water Pollution Control (Grant No. 2012ZX07313001-002), the National Natural Science Foundation of China (No. 51308439), JSPS Postdoctoral Fellow Program (NO. 15F15353), Shaanxi Provincial Program for Science and Technology Development (No. 2013KJXX-55) and Program for Innovative Research Team (No. 2013KCT-13).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2016.11.028.

References

[1] I. Michael-Kordatou, C. Michael, X. Duan, X. He, D.D. Dionysiou, M.A. Mills, D. Fatta-Kassinos, Dissolved effluent organic matter: characteristics and potential implications in wastewater treatment and reuse applications, Water Res. 77 (2015) 213–248.
[2] G.Z. Teklehaimanot, B. Genthe, I. Kamika, M.N. Momba, Prevalence of enteropathogenic bacteria in treated effluents and receiving water bodies and their potential health risks, Sci. Total Environ. 518–519 (2015) 441–449.
[3] F. Lopez-Galvez, A. Allende, F. Pedrero-Salcedo, J.J. Alarcon, M.I. Gil, Safety assessment of greenhouse hydroponic tomatoes irrigated with reclaimed and surface water, Int. J. Food Microbiol. 191 (2014) 97–102.
[4] Q. Li, Y. Chen, H. Fu, Z. Cui, L. Shi, L. Wang, Z. Liu, Health risk of heavy metals in food crops grown on reclaimed tidal flat soil in the Pearl River Estuary China, J. Hazard. Mater. 227–228 (2012) 148–154.
[5] J. Xu, L. Wu, A.C. Chang, Y. Zhang, Impact of long-term reclaimed wastewater irrigation on agricultural soils: a preliminary assessment, J. Hazard. Mater. 183 (2010) 780–786.
[6] T.T. Gao, X.C. Wang, R. Chen, H.H. Ngo, W.S. Guo, Disability adjusted life year (DALY): a useful tool for quantitative assessment of environmental pollution, Sci. Total Environ. 511 (2015) 268–287.
[7] C.C. Wang, Z.G. Niu, Y. Zhang, Health risk assessment of inhalation exposure of irrigation workers and the public to trihalomethanes from reclaimed water in landscape irrigation in Tianjin, North China, J. Hazard. Mater. 262 (2013) 179–188.
[8] Y. Cui, S. Liu, K. Smith, K. Yu, H. Hu, W. Jiang, Y. Li, Characterization of corrosion scale formed on stainless steel delivery pipe for reclaimed water treatment, Water Res. 88 (2016) 816–825.
[9] P. Muhid, T.W. Davis, S.E. Bunn, M.A. Burford, Effects of inorganic nutrients in recycled water on freshwater phytoplankton biomass and composition, Water Res. 47 (2013) 384–394.
[10] J.S. Marks, Taking the public seriously: the case of potable and non potable reuse, Desalination 187 (2006) 137–147.
[11] H.J. Zhao, Y. Wang, L.L. Yang, L.W. Yuan, D.C. Peng, Relationship between phytoplankton and environmental factors in landscape water supplemented with reclaimed water, Ecol. Indic. 58 (2015) 113–121.
[12] T. Fu, X.C. Zheng, J.N. Chen, Strategy and orientation concerning wastewater reclamation, China Water Wastewater 23 (2007) 5–9.
[13] D. Li, D. Huang, C. Guo, X. Guo, Multivariate statistical analysis of temporal-spatial variations in water quality of a constructed wetland purification system in a typical park in Beijing China, Environ. Monit. Assess. 187 (2015) 4219.
[14] Y.L. Jiang, J.Q. Xiong, X.C. Wang, Y.T. Zhang, N. Wang, Q.Q. Zhang, Water fields cluster division and water quality indexes spatial and temporal distribution characteristics of an artificial lake complmented by reclaimed water, Chin. J. Environ. Eng. 9 (2015) 4746–4752.
[15] Xi'an Water Authority, Water Management for the Eight-Rivers Project, 2010 http://www.xawater.gov.cn/ptl/trs_ci_id_1803517.html.
[16] General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (AQSIQ), The Reuse of Urban Recycling Water—Water Quality Standard for Scenic Environment Use(GB/T 18921–2002), Standards Press of China, Beijing, 2002.
[17] S. Gao, M. Du, J. Tian, J. Yang, J. Yang, F. Ma, J. Nan, Effects of chloride ions on electro-coagulation-flotation process with aluminum electrodes for algae removal, J. Hazard. Mater. 182 (2010) 827–834.
[18] O. Rouane-Hacene, Z. Boutiba, B. Belhaouari, M.E. Guibbolini-Sabatier, P. Francour, C. Risso-de Faverney, Seasonal assessment of biological indices bioaccumulation and bioavailability of heavy metals in mussels Mytilus galloprovincialis from Algerian west coast, applied to environmental monitoring, Oceanologia 57 (2015) 362–374.
[19] K.C. Cheung, B.H.T. Poon, C.Y. Lan, M.H. Wong, Assessment of metal and nutrient concentrations in river water and sediment collected from the cities in the Pearl River Delta, South China, Chemosphere 52 (2003) 1431–1440.
[20] M. Ezechias, S. Covino, T. Cajthaml, Ecotoxicity and biodegradability of new brominated flame retardants: a review, Ecotoxicol. Environ. Saf. 110 (2014) 153–167.
[21] X.Y. Ma, X.C. Wang, Y.J. Liu, Study of the variation of ecotoxicity at different stages of domestic wastewater treatment using *Vibrio-qinghaiensis* sp.-Q67, J. Hazard. Mater. 190 (2011) 100–105.
[22] O.M. Lee, H.Y. Kim, W. Park, T.H. Kim, S. Yu, A comparative study of disinfection efficiency and regrowth control of microorganism in secondary wastewater effluent using UV, ozone, and ionizing irradiation process, J. Hazard. Mater. 295 (2015) 201–208.
[23] J.D. Brookes, J. Antenucci, M. Hipsey, M.D. Burch, N.J. Ashbolt, C. Ferguson, Fate and transport of pathogens in lakes and reservoirs, Environ. Int. 30 (2004) 741–759.
[24] S. Dorevitch, S. Panthi, Y. Huang, H. Li, A.M. Michalek, P. Pratap, M. Wroblewski, L. Liu, P.A. Scheff, A. Li, Water ingestion during water recreation, Water Res. 45 (2011) 2020–2028.
[25] K. Özkan, E. Jeppesen, M. Søndergaard, T.L. Lauridsen, L. Liboriussen, J.C. Svenning, Contrasting roles of water chemistry lake morphology, land-use, climate and spatial processes in driving phytoplankton richness in the Danish landscape, Hydrobiologia 710 (2012) 173–187.
[26] G. Phillips, O.P. Pietiläinen, L. Carvalho, A. Solimini, A. Lyche Solheim, A.C. Cardoso, Chlorophyll–nutrient relationships of different lake types using a large European dataset, Aquat. Ecol. 42 (2008) 213–226.
[27] W. Liping, Z. Binghui, Prediction of chlorophyll-a in the Daning River of Three Gorges Reservoir by principal component scores in multiple linear regression models, Water Sci. Technol. 67 (2013) 1150–1158.
[28] Environmental Protection Agency of China (EPAC), The Twelfth Five-Year Plan for Comprehensive Prevention and Control of Heavy Metal Pollution, 2011 http://www.zhb.gov.cn/gzfw13107/ghjh/zxgh/201605/P020160522268332644308.pdf.
[29] Ø. Kaste, A. Lyche-Solheim, Influence of moderate phosphate addition on nitrogen retention in an acidic boreal lake, Can. J. Fish. Aquat. Ssi. 62 (2005) 312–321.

[30] G.M. Berg, M. Balode, I. Purina, S. Bekere, C. Béchemin, S.Y. Maestrini, Plankton community composition in relation to availability and uptake of oxidized and reduced nitrogen, Aquat. Microb. Ecol. 30 (2003) 263–274.

[31] M.B. Rothenberger, J.M. Burkholder, T.R. Wentworth, Use of long-term data and multivariate ordination techniques to identify environmental factors governing estuarine phytoplankton species dynamics, Limnol. Oceanogr. 54 (2009) 2107–2127.

[32] L.P. Iensen, E. Jeppesen, K. Olrik, P. Kristensen, Impact of nutrients and physical factors on the shift from Cyanobacterial to Chlorophyte dominance in shallow lakes, Can. J. Fish. Aquat. Ssi. 51 (1994) 1692–1699.

[33] E. Emmanuel, G. Keck, J.M. Blanchard, P. Vermande, Y. Perrodin, Toxicological effects of disinfections using sodium hypochlorite on aquatic organisms and its contribution to AOX formation in hospital wastewater, Environ. Int. 30 (2004) 891–900.

[34] C.S. Reynolds, What factors influence the species composition of phytoplankton in lakes of different trophic status, Hydrobiologia 369 (1998) 11–26.

[35] R. Ptacnik, A.G. Solimini, T. Andersen, T. Tamminen, P. Brettum, L. Lepisto, E. Willen, S. Rekolainen, Diversity predicts stability and resource use efficiency in natural phytoplankton communities, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 5134–5138.

[36] W. Guo, S. Huo, B. Xi, J. Zhang, F. Wu, Heavy metal contamination in sediments from typical lakes in the five geographic regions of China: distribution, bioavailability, and risk, Ecol. Eng. 81 (2015) 243–255.

[37] J.Y. Hou, J.C. Liu, S.P. Cao, X.J. Cheng, Y.N. Zhang, W.X. Wang, Study on the dry and wet atmospheric deposition in the urban area of Tianjin, Geol. Surv. Res. 36 (2013) 131–135.

[38] F.M. Mei, C.Y. Xu, L. Zhou, Chenmical species and bioavailbility of Cu, Pb Zn, Ni and Cd of dustfall from Xi'an parks in China, Environ. Chem. 30 (2011) 1284–1290.

[39] S. Dwivedi, S. Srivastava, S. Mishra, B. Dixit, A. Kumar, R.D. Tripathi, Screening of native plants and algae growing on fly-ash affected areas near National Thermal Power Corporation, Tanda Uttar Pradesh, India for accumulation of toxic heavy metals, J. Hazard. Mater. 158 (2008) 359–365.

[40] X.F. Yang, J.M. Duan, L. Wang, W. Li, J.L. Guan, S. Beecham, D. Mulcahy, Heavy metal pollution and health risk assessment in the Wei River in China, Environ. Monit. Assess. 187 (2015) 110–111.

[41] T. Asano, L.Y.C. Leong, M.G. Rigby, Evaluation of the California wastewater reclamation criteria using enteric virus monitoring data, Water Sci. Technol. 26 (1992) 1513–1524.

[42] H. Zhi, Z. Zhao, L. Zhang, The fate of polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) in water from Poyang Lake, the largest freshwater lake in China, Chemosphere 119 (2015) 1134–1140.

[43] I. Abusallout, G.H. Hua, Photolytic dehalogenation of disinfectio n byproducts in water by natural sunlight irradiation, Chemosphere 159 (2016) 184–192.

[44] H.H. Hong, W.Q. Chen, L. Xu, X.H. Wang, L.P. Zhang, Distribution and fate of organochlorine pollutants in the pearl river estuary, Mar. Pollut. Bull. 39 (1999) 376–382.

[45] J. Dachs, S.J. Eisenreich, J.E. Baker, F.C. Ko, J.D. Jeremiason, Coupling of phytoplankton uptake and air-water exchange of persistent organic pollutants, Environ. Sci. Technol. 33 (1999) 3653–3660.

[46] I.K. Wittmer, H.P. Bader, R. Scheidegger, H. Singer, A. Lück, I. Hanke, C. Carlsson, C. Stamm, Significance of urban and agricultural land use for biocide and pesticide dynamics in surface waters, Water Res. 44 (2010) 2850–2862.

[47] A. Marrucci, B. Marras, S.S. Campisi, M. Schintu, Using SPMDs to monitor the seawater concentrations of PAHs and PCBs in marine protected areas (Western Mediterranean), Mar. Pollut. Bull. 75 (2013) 69–75.

[48] F. Polesel, B.G. Plosz, S. Trapp, From consumption to harvest: environmental fate prediction of excreted ionizable trace organic chemicals, Water Res. 84 (2015) 85–98.

[49] A. Rzezutka, N. Cook, Survival of human enteric viruses in the environment and food, FEMS Microbiol. Rev. 28 (2004) 441–453.

[50] D.G. Bambic, B.J. Kildare-Hann, V.B. Rajal, B.S.M. Sturm, C.B. Minton, A. Schriewer, S. Wuertz, Spatial and hydrologic variation of Bacteroidales adenovirus and enterovirus in a semi-arid, wastewater effluent-impacted watershed, Water Res. 75 (2015) 83–94.

[51] J.H. Zhou, X.C. Wang, Z. Ji, L. Xu, Z. Yu, Source identification of bacterial and viral pathogens and their survival/fading in the process of wastewater treatment reclamation, and environmental reuse, World J. Microbiol. Biotechnol. 31 (2015) 109–120.

[52] K. Helmi, S. Skraber, C. Gantzer, R. Willame, L. Hoffmann, H.M. Cauchie, Interactions of Cryptosporidium parvum Giardia lamblia, vaccinal poliovirus type 1, and bacteriophages phiX174 and MS2 with a drinking water biofilm and a wastewater biofilm, Appl. Environ. Microbiol. 74 (2008) 2079–2088.

[53] P. Vasickova, I. Pavlik, M. Verani, A. Carducci, Issues concerning survival of viruses on surfaces, Food Environ. Virol. 2 (2010) 24–34.

[54] A. Bosch, R.M. Pintó, F.X. Abad, Survival and Transport of Enteric Viruses in the Environment, Viruses in Foods, Springer, 2006.

[55] V. Dakos, E. Beninca, E.H. van Nes, C.J. Philippart, M. Scheffer, J. Huisman, Interannual variability in species composition explained as seasonally entrained chaos, Proc. Biol. Sci. R. Soc. 276 (2009) 2871–2880.

[56] F. Pomati, J. Jokela, M. Simona, M. Veronesi, B.W. Ibelings, An automated platform for phytoplankton ecology and aquatic ecosystem monitoring, Environ. Sci. Technol. 45 (2011) 9658–9665.

[57] D.E. Akyuz, L. Luo, D.P. Hamilton, Temporal and spatial trends in water quality of Lake Taihu, China: analysis from a north to mid-lake transect, 1991–2011, Environ. Monit. Assess. 186 (2014) 3891–3904.

[58] P. Nõges, U. Mischke, R. Laugaste, A.G. Solimini, Analysis of changes over 44 years in the phytoplankton of Lake Võrtsjärv (Estonia): the effect of nutrients, climate and the investigator on phytoplankton-based water quality indices, Hydrobiologia 646 (2010) 33–48.

[59] L. Yang, K. Lei, W. Meng, G. Fu, W. Yan, Temporal and spatial changes in nutrients and chlorophyll-α in a shallow lake, Lake Chaohu, China: an 11-year investigation, J. Environ. Sci. 25 (2013) 1117–1123.

[60] F.J. Simmons, I. Xagoraraki, Release of infectious human enteric viruses by full-scale wastewater utilities, Water Res. 45 (2011) 3590–3598.