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A biological safety evaluation on reclaimed water reused as scenic water using a bioassay battery

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Abstract

An assessment method based on three toxicity tests (algae growth inhibition, daphnia immobilization and larval fish toxicity) was used to screen the biological safety of reclaimed water which was reused as sole replenishment for scenic water system in a park (SOF Park) in northern China. A total of 24 water samples were collected from six sites of water system in the SOF Park in four different seasons. The results indicated that: (1) the reclaimed water directly discharged from a reclamation treatment plant near the SOF Park as influent of park had relatively low biological safety (all samples were ranked as C or D); (2) the biological safety of reclaimed water was improved greatly with the ecological reclamation treatment processes composing of artificial wetland system and followed oxidation pond system; (3) the biological safety of reclaimed water in the main lake of SOF Park kept at a health status during different seasons (all samples were ranked as A); (4) there was some certain correlation (R^2 = 0.5737) between the sum of toxicity scores and dissolved organic carbon for the studied water samples. It was concluded that the assessment method was reliable to screen the safety of reclaimed water reused as scenic water, and the reclaimed water with further ecological purification processes such as artificial wetland and oxidation pond system can be safely reused as scenic water in park.

Key words: biological safety; water quality index; bioassay; reclaimed water

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Introduction

Water resource scarcity and water quality deterioration represent serious contemporary concerns for municipalities, industries, agriculture, and the environment in many parts of the world. Factors contributing to these problems include increasing population in urban areas, serious contamination of water environment, uneven distribution of water resources, and frequent droughts (Asano et al., 2007). Wastewater reclamation and reuse has drawn more and more attention as an integral part of water resources management, which has been proved to be an effective pathway to relieve the crisis on water resource (Chu et al., 2004). The situation of water shortage in north China, especially in Beijing is a typical case in point, of which has 230 m^3 freshwater per capita (World Bank, 2009). With the three decades developing, the reused volume of reclaimed wastewater reached 0.65 billion cubic meters in 2009, about 18% of total water consumption volume in Beijing (Lai, 2010). However, the safety of reclaimed water quality become bone of contention from the just beginning of wastewater reuse because there are many kinds of toxic chemicals and nutrient elements remained in the wastewater while the traditional reclamation treatment processes can not thoroughly remove these chemicals which would accumulate and cause potential risk on human health and ecological system during reuse of reclaimed water (Asano et al., 2007; Hu et al., 2002).

Traditionally, hazard or risk assessments of polluted environmental elements are conducted with physicochemical measurements, such as chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total organic carbon (TOC), total nitrogen (TN), total phosphate (TP). However, the physicochemical analysis alone can not sufficiently and effectively evaluate the safety of environmental elements, because it is impossible to cover all of the chemicals, and usually the synergistic or antagonistic interactive effects cannot be ignored for those chemicals co-existed in environmental elements (Fernández et al., 2005; Juvonen et al., 2000). Fortunately, biological toxicity tests are developed as useful tools for integrating the effects of all the bioavailable contaminants and of their interaction (Hernando et al., 2005).

To evaluate the environmental risks, toxicity test set composing of several species at different trophic levels

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of biological system and of different biological effects to complement physicochemical analyses has been recommended (Bispo et al., 1999; Békaert et al., 1999; Rila and Eisentraeger, 2003; Fernández et al., 2005). For example, a toxicity test set with three commonly recommended aquatic toxicity tests: acute fish lethal test, acute daphnia immobilization test and chronic algae growth inhibition test has been recommended to evaluate the toxicity of the water sample (Ferard and Ferrari, 2005). However, the common problem is that, the endpoints for different toxicity tests are different, and it is hard to establish a reasonable and simple assessment index to characterize the hazards of pollutants on aquatic organisms. In some of previous studies, researchers tried to develop a variation of assessment indices such as Pesticide Toxic Index (PTI), risk-based scoring system were developed (Kimerle et al., 1997; Persoone et al., 2003) to protect freshwater aquatic organisms, however, the PTI ignored the interaction (synergism, antagonism) among the coexisted chemicals in the environment (Munn and Gilliom, 2001), and the risk-based scoring system did not extrapolate the long-term hazard of pollutants to aquatic organisms. Recently, Wei et al. (2006) proposed a biological safety evaluation method basing on the risk extrapolation of chemical pollutants to overcome those limitations of previous toxicity classification systems. Simply, the proposed assessment method aimed on protecting living organisms, e.g., producer, primary consumer, secondary consumer in aquatic ecosystem, therefore, three of sensitive species algae (*Selenustrum capricornutum*, producer), crustacean (*Daphnia magna*, primary consumer) and fish (*Oryzias latipes* larvae, secondary consumer) locating on three typical trophic levels of food chain were selected as toxicity test species, to measure the real effects of mixed chemicals on aquatic living organisms, and the test results would be more reliable to evaluate and protect the safety for more living organisms in aquatic ecosystem.

SOF Park, an artificial park in northern China with 704 ha of total area and 70 ha of water area in the main lake, is an ideal site for citizen recreation and sightseeing. The water body of main lake in the park is supplemented by reclaimed water uniquely ditching from a wastewater reclamation treatment plant near the park. The inlet of reclaimed water locates at the north of park, and the influent of reclaimed water is divided into two parts, one part enters artificial wetland system for removing excessive nutrient elements and some toxic chemicals remained in reclaimed water, and the other part flows through a flower terrace with water falling for oxygen recharging. Then, the reclaimed water from both parts enter into an oxidation pond system for further treatment, pass through mixed function zone and finally enter into the main lake. With the run of water system circulating, reclaimed water in main lake will flow through a water gate and into another lake, and then piped to the artificial wetland system again as circulating water for further treatment. The flow direction and circulation of reclaimed water in the SOF Park is shown in Fig. 1, in which the arrow symbols represent the

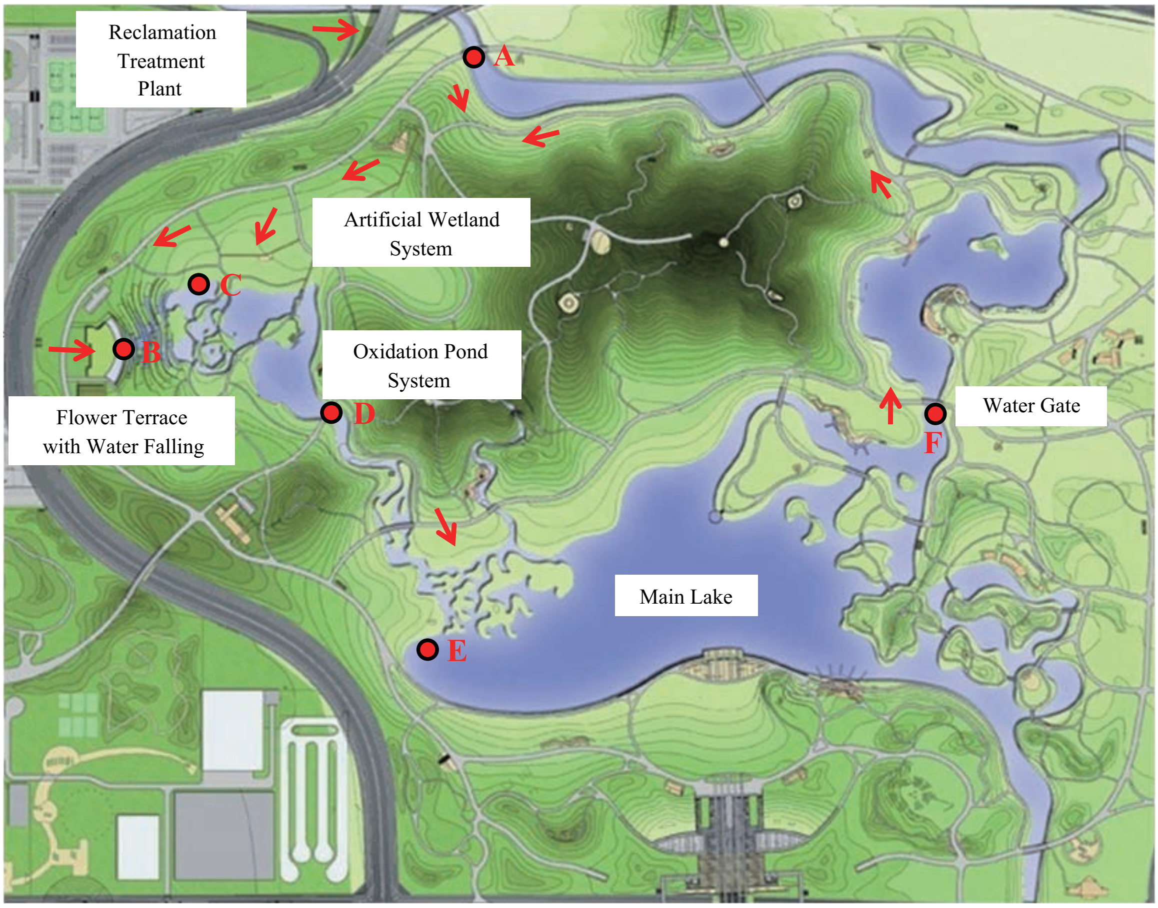


Fig. 1 Flow direction and distribution of sampling sites in the SOF Park.

direction of water flow in the SOF Park.

As mentioned above, the biological safety of the reclaimed water, especially for those in reuse, is worthwhile to be screened for protecting ecological system and human health. In this study, the biological safety of the reclaimed water reused as a sole replenishment source for the scenic lake in the SOF Park was measured by using the developed toxicity-based evaluation method, the toxicity removal efficiencies of the different ecological reclamation treatment processes and their variations during different seasons were evaluated as well. Moreover, the corresponding physicochemical parameters of water samples were determined, and the correlation between biological safety indices and physicochemical parameters were statistically tested as well.

1 Materials and methods

1.1 Water sampling

The reclaimed water reused in SOF Park is generated by a wastewater reclamation plant locating at north of the park, and the reclaimed water is partially ditched to the park. Six of sampling sites were designed at the inlet of reclaimed water for the SOF Park (site A, 116°22′55″E, 40°01′13″N), downstream of water falling through flower terrace (site B, 116°22′33″E, 40°00′59″N), outlet of artificial wetland system (site C, 116°22′40″E, 40°01′02″N), middle of oxidation pond system (site D, 116°22′41″E, 40°00′59″N), inlet of main lake (site E, 116°22′52″E, 40°00′45″N), and outlet (water gate) of the main lake for water system circulating (site F, 116°23′22″E, 40°00′53″N). The distribution of six sampling sites in the SOF Park is shown in Fig. 1. Four times of sampling were organized at spring (March 27), summer (June 5), autumn (August 26), and winter (November 10) in 2010, and a four-liter of water sample was collected at each sampling site. The artificial wetland was designed as vertical flow type, of which the total surface area was 41500 m^2, the treatment capacity was 2600 m^3/day for effluent from wastewater reclamation plant and 20000 m^3/day for circulated water from the main lake. The hydraulic loading was 0.41 $m^3/(m^2 \cdot day)$ and retention time was 23.2 hr. A run for more than one year showed that the water quality of effluent from wetland system kept relatively stable as: COD 22 mg/L, BOD_5 1.96 mg/L, TN 2.90 mg/L, and TP 0.04 mg/L.

1.2 Concentration and elution

Each sample (4 L) was filtrated through a glass fiber filter of 0.7 μm (Whatman GF/F), followed by flowing through two pre-conditioned Oasis HLB cartridges in tandem (6 cc/500 mg, Waters., USA) using a solid phase extraction apparatus (Visiprep DL SPE, Supelco, USA) and a vacuum pump (DOA-P504-BN, Gast, USA) at 10 mL/min. Oasis HLB cartridges have been reported and proved to adsorb many kinds of organic micropollutants in water or some other environmental media and have been widely applied to recover agricultural chemicals (Economou et al., 2009), pharmaceuticals (Vazquez-Roig et al., 2010; Huerta-Fontela et al., 2010), endocrine-disruptors (Pedrouzo et al., 2009), brominated flame retardants (Ramos et al., 2007), and microcystins (Dai et al., 2008) from complex environmental water samples. During each elution, air was first injected manually into the cartridge with a syringe to drive out the space water. A 10-mL volume of acetone was then pumped into each cartridge at 2.0 mL/min for elution, and two acetone solutions respectively eluated from the two cartridges were collected and mixed in a scaled centrifuge tube. Then, the acetone eluent was concentrated down to 500 μL by evaporation under nitrogen gas purge at 300 mL/min, and 2.0 mL distilled water was added to the residual solution and concentrated down to 2.0 mL in water bath of 35°C. The obtained samples which were concentrated 2000 times were diluted into different concentrations and prepared as exposure solutions for toxicity tests: maximum exposure concentrations as 10, 50 and 50-fold of reclaimed water for algae, daphnia and larval medaka test, respectively.

1.3 Toxicity tests and evaluation method

The stock solution of 50-fold of reclaimed water was prepared with dilution water (treated with activated carbon and ion-exchange resin), and was diluted into a series of duplicate exposure solutions. As for algae growth inhibition test, the serial exposure solutions were prepared as 2-, 4- and 10-fold of reclaimed water. The experimental conditions were: volume of exposure solution: 20 mL; initial cell density: 10^4/mL; shaking speed: 100 r/min; exposure duration: 72 hr; light intensity: 4000 lx; and light/dark: 24 hr/0 hr. A series of exposure solutions were prepared as 10-, 20- and 50-fold of reclaimed water for daphnia immobilization test. The operational parameters were: volume of exposure solution: 20 mL; neonate age: <24 hr old; neonate density: 10/concentration; exposure duration: 48 hr, and light/dark: 16 hr/8 hr. The solution preparation of larval medaka toxicity test was the same as daphnia test. The operational conditions were: volume of exposure solution: 20 mL; larvae age: 48–72 hr old; larvae density: 10/concentration; exposure duration: 48 hr; and light/dark: 16 hr/8 hr. The highest concentration at which adverse effect cannot be observed was recorded, and their corresponding safety score along with biological safety rank (BSR) based on the worst toxicity score among three toxicity tests according to the approach proposed in our previous research were determined (Wei et al., 2006).

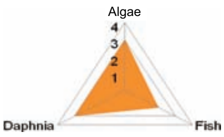
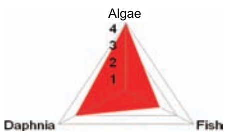
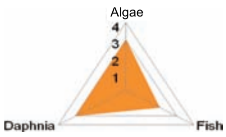
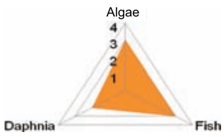
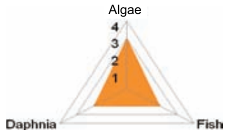
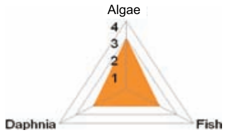
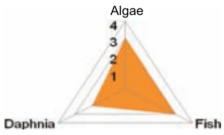
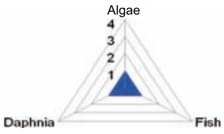
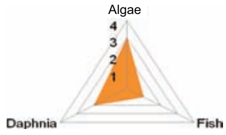
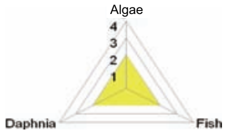
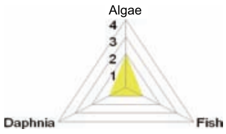
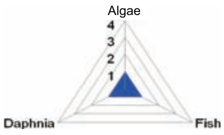
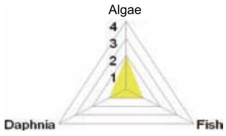
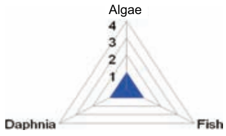
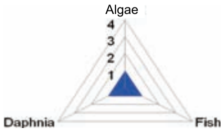
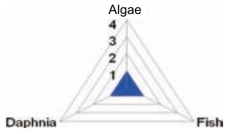
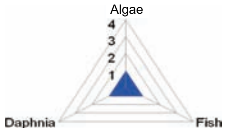
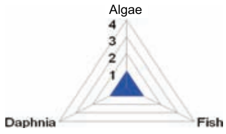
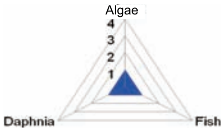
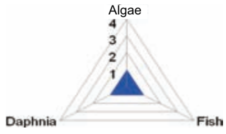
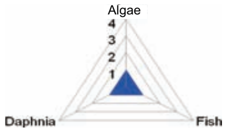
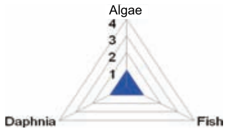
The advantages of the assessment method are the follows. (1) Direct toxicity tests were used to measure the objective biological effects of mixed compounds, which appear to be very promising in solving some of the issues facing water managers by providing chronic predictable biological safety of water quality using short-term toxicity tests. (2) Three of widely used species algae (*Selenustrum capricornutum*), daphnia (*Daphnia magna*) and fish (*Oryzias latipes* larvae) at three trophic levels in aquatic ecosystem were selected as a test set to measure the bio-toxicity of water sample. (3) Exposure concentrations of water sample for each toxicity test were respectively designed according to the toxicity extrapolation method of EU directives. (4) A novel assessment index "toxicity score" was proposed and determined according to the highest exposure concentration where adverse toxic effects could not be observed, of which designed into rank 1, 2, 3, and 4, and rank 1 is the safest. (5) A triangle figure was designed to visually describe the toxicity scores of three toxicity tests, three axes respectively shows the toxicity scores of three tests, and the different colors (blue, yellow, orange, red) were filled in the area surrounded by three toxicity scores. (6) An integrated assessment index "biological safety rank" (BSR) was proposed to wholly evaluate the biological safety of environmental water with three toxicity tests, of which designed as A, B, C and D four ranks, and rank A is the safest. The rank of BSR was determined according to the worst toxicity score among the three toxicity tests.

2 Results and discussion

2.1 BSRs changes of water samples during the reclamation processes in the SOF Park

The toxicity scores of three tests plotted as triangle shapes and their corresponding BSRs for twenty-four water samples collected in SOF Park are shown in Table 1. It can be seen that along the ecological reclamation treatment processes in the SOF Park, the biological safety

Table 1 Toxicity scores and biological safety rank (BSRs) for the water samples collected in SOF Park

Sampling sites	Mar 27, 2010	Jun 5, 2010	Aug 26, 2010	Nov 10, 2010
Site A	 BSR= C	 BSR=D	 BSR=C	 BSR=C
Site B	 BSR=B	 BSR=C	 BSR=C	 BSR=C
Site C	 BSR=A	 BSR=C	 BSR=B	 BSR=B
Site D	 BSR=A	 BSR=B	 BSR=B	 BSR=A
Site E	 BSR=A	 BSR=A	 BSR=A	 BSR=A
Site F	 BSR=A	 BSR=A	 BSR=A	 BSR=A

Among ranks A, B, C, D for BSRs and ranks 1, 2, 3, 4 for toxicity scores, rank A and rank 1 are the safest, respectively.

of reclaimed water improved significantly. As mentioned above, the water sample from site A represented the influent of reclaimed water in the park, which was the effluent of a wastewater reclamation treatment plant near the park. However, the BSRs of four samples collected at site A in different seasons were ranked as C, C, D and C, respectively, which implied that the water quality had some certain risk to aquatic organisms if directly reuse as scenic and recreational water in the park. As for water samples collected at site B, downstream of water falling through the flower terrace, equaling to an aerating treatment, some of volatile compounds might be removed and their BSRs had some certain of improvement after this process. The most interesting was that the artificial wetland system and oxidation pond system, which appeared high treatment capability on removing toxic compounds remaining in reclaimed water. After the treatment with artificial wetland system, the BSRs of four water samples decreased from rank C, D, C, and C to rank A, C, B and B, respectively, and they further reduced to rank A, B, B and A rank with the treatment in oxidation pond system. Subsequently, the BSRs of water samples collected at the inlet and outlet of main lake (water gate for water system circulating) kept excellent character that all of them ranked as A, which indicated that the reclaimed water in the main lake was relatively safe for aquatic organisms.

As for different seasons, the influents of the reclaimed water in the SOF Park, effluent of the wastewater reclamation treatment plant, had not appeared obvious differences on BSRs (from rank C to rank D) in different seasons, which indicated that the effluent of reclamation treatment plant had relatively stable removal capability for toxic substances although it appeared some potential risk to aquatic organisms. Moreover, considering the evaporation loss and the pollutants removal capability of artificial wetland system and oxidation pond system in different seasons, the amount of reclaimed water supplemented into the park was less in winter and spring than that in summer and autumn, and the circulating rate of water system in the park was slowed down in spring and winter as well. In fact, from the test results listed in Table 1, the removal capability of pollutants with water falling process was higher in spring than that in winter, while the artificial wetland system and oxidation pond system kept higher removal potential in spring and winter than that in summer and autumn, maybe attributed to the relatively less hydraulic loading in spring and winter seasons.

2.2 Variations of physicochemical parameters of water samples along the reclamation processes in SOF Park

The dissolved organic carbon (DOC) is commonly used to reflect pollution level of organics in water phase. DOC values of the 24 water samples collected in SOF Park were measured using TOC-VCPH Analyzer (Shimadzu Co., Japan), and their changes along the reclamation treatment processes are plotted in Fig. 2. Another surrogate parameter UV_{254}, ultraviolet light absorbance at 254 nm, describes the concentration of organic molecules with aromatic groups or extended conjugation (APHA, 2005). The UV_{254} of 24 water samples were measured with UV-2800 Spectrophotometer (Unico Co., China) and their changes along the ecological reclamation treatment processes are shown in Fig. 2. Basing on the above two parameters, a concept of specific ultraviolet absorption (SUVA), defined as UV_{254} (measured as m^{-1}) divided by the DOC concentration (in mg/L), was developed as an operational indicator for simply characterizing the nature of natural organic matter in water (Edzwald and van Benschoten, 1990). The SUVA values of 24 water samples were calculated and their changes along reclamation processes are listed in Table 2.

Totally, the variation trends of DOC and UV_{254} along the ecological reclamation treatment processes in different seasons were similar to those of BSRs. As for the changes of water quality parameters DOC and UV_{254}, the water samples collected from site A and site B had higher values than those from some other sampling sites, which implied that the influents of the park contained relatively high organic substances. However, both of the two parameters DOC and UV_{254} of water samples decreased significantly

Table 2 SUVA changes of water samples during the reclamation treatment processes in SOF Park (unit: L/(mg·m))

Sampling date	Site A	Site B	Site C	Site D	Site E	Site F
Mar 27, 2010	1.13	1.08	0.94	0.90	0.82	0.79
Jun 05, 2010	1.34	1.39	0.92	0.61	0.62	0.66
Aug 26, 2010	0.81	0.86	0.78	0.77	0.72	0.74
Nov 10, 2010	0.93	1.05	0.92	0.77	0.77	0.83

SUVA: specific ultraviolet absorption.

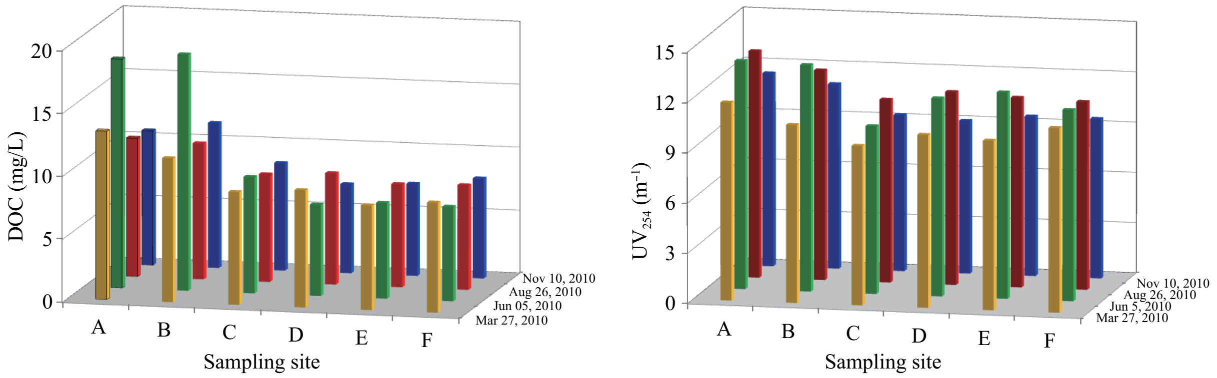


Fig. 2 Changes of DOCs and UV_{254} along the reclamation treatment processes in SOF Park.

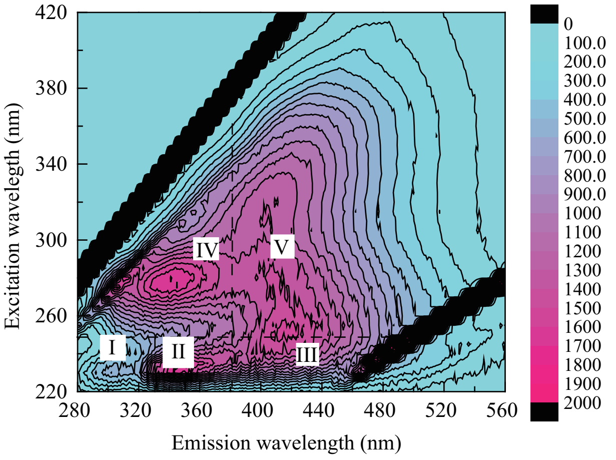


Fig. 3 3D-EEM spectra of water sample collected from SOF Park.

after flowing through the artificial wetland and oxidation pond systems, especially, the reduction percentage of DOC in June was much higher than that in some other seasons due to the higher DOC in the influent of park in June. Subsequently, DOC of water samples still kept at low level in the main lake, while the UV_{254} values appeared a slight increase at the inlet and outlet of main lake, which might be attributed to the generation of aromatic substances by aquatic organisms in the main lake (Sartoris et al., 2000). In addition, according to the method proposed by Edzwald and van Benschoten (1990) SUVA values of less than about 2 L/(mg·m) signify a water containing mostly non-humic material, low hydrophobicity and low molecular weight. And all SUVA values for reclaimed water collected in the SOF Park were less than 2 L/(mg·m), which implied that the main organic substances in the reclaimed water would be non-humic materials with low hydrophobicity and low molecular weight, which would be proved by the 3D-EEM (excitation emission matrix) spectrum of a water sample collected in SOF Park (Fig. 3). It can be seen from Fig. 3 that there is relatively low peak in region V, which implied that the content of humic acid in reclaimed water sample was low.

2.3 Comparison of BSRs and physicochemical parameters for reclaimed water samples

As mentioned above, physicochemical parameters such as DOC and UV_{254} have been commonly used as important indices for evaluating water pollution, which reflect the total dissolved (aromatic) organic pollutants in the water. However, since more and more toxic organic chemicals have been found in water, these indices cannot provide the real toxicity information of the organic pollutants and no longer sensitive enough for water quality protection although they are a little easier to be gotten than toxicity index BSR. However, toxicity index BSR has more advantages on describing the overall toxicity of the pollutants in water. As shown in Fig. 4, water samples with high DOC or UV_{254} tended to relate to high toxicity values, while low DOC or UV_{254} did not always equal to low toxicity effect. For example, BSRs could range from rank A to C and toxicity scores could range from 1 to 3 within corresponding DOC ranged from 6 to 10 mg/L (69% ranked as A, 25% ranked as B, and 6% ranked as C), and UV_{254}s ranged from 8 to 13 /m (67% ranked as A, 17% ranked as B, and 16% ranked as C). It was therefore concluded that BSR could be an effective bio-safety index for evaluating water quality.

To understand the correlation between toxicity index and physicochemical parameter DOC, UV_{254} for the studied water samples, the toxicity scores of three tests for each water sample was summed as dependant variable S_{TS}, and linear regression analysis was conducted. It could be found from Fig. 5 that the multiple correlation coefficient was 0.5737 for S_{TS} vs. DOC regression, while it was 0.3675 for S_{TS} vs. UV_{254} regression, which implied that there was slight correlation between toxicity index and DOC, but low correlation between toxicity index and UV_{254}. This result is different from the previous result which found there was low correlation between BSRs and DOCs. This phenomenon may be attributed to the variance of primary pollutants between reclaimed water and river water (Wei et al., 2008; Liu et al., 2007). Of course, the combination of chemical methods with biological/toxicological methods could be more useful in estimating the risk of contaminants and contaminated environmental elements (Fent, 2003; Tsui and Chu, 2003).

3 Conclusions

In this study, an assessment method based on three kinds of toxicity tests was used to evaluate the performance efficiencies of ecological reclamation treatment processes and the biological safety of reclaimed water which was reused

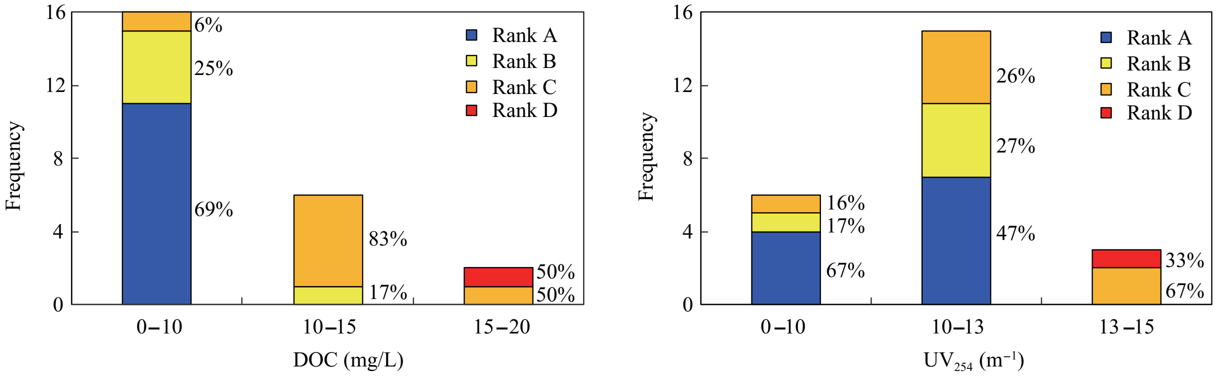


Fig. 4 Frequency distribution of BSRs depending on DOCs and UV_{254}.

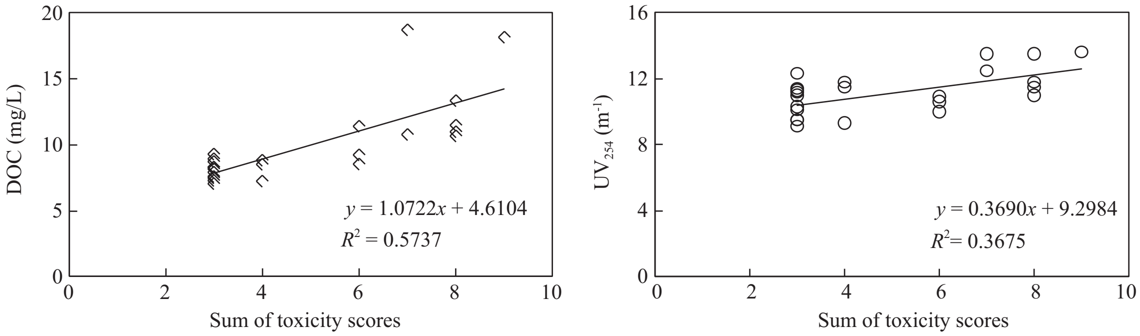


Fig. 5 Relationships between sum of toxicity scores and DOCs, and UV_{254}s.

as sole replenishment water source of a scenic lake in SOF Park, and the results indicated that the evaluation method based on the toxicity test set composing of algae growth inhibition test, daphnia magna immobilization test and larval medaka lethal test was very effective and sensitive, and it could successfully reveal the removal characteristics of toxic chemicals during reclamation processes. From the results, it was apparent that the reclaimed water generated from wastewater reclamation treatment plant still contained some toxic substances, the BSR was ranked as C or D, which was unsuitable to reuse directly. The ecological reclamation treatment processes designed in the SOF Park, including artificial wetland system and oxidation pond system, played an important role in improving the quality of reclaimed water, which effectively guaranteed the safety (A rank) for the aquatic organisms in the main lake. In addition, with conditioning amount of replenishment water and rate of water system circulating, the reclamation treatment efficiency was maintained at a relatively stable level (A rank) even in winter and spring seasons with low temperature. In addition, the evaluation indices, e.g., toxicity score and BSR, can reflect the properties of toxic pollutants and there was a slight relationship ($R^2 = 0.5737$) with physicochemical parameter DOC, which could be used as a useful and practical screening tool not only for evaluating and managing of reclaimed water, but also for surface water, lake water and reservoir water (Wei et al., 2008). As for the reuse in the SOF Park, the direct effluent of wastewater reclamation treatment plant should have a further reclamation treatment before its reuse, and the visitors in the park should be reminded that do not contact the water within the area of ecological reclamation treatment system.

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References

APHA, AWWA, WEF, 2005. Method 5910: UV-absorbing organic constituents/Ultraviolet absorption method. Standard Methods for the Examination of Water and Wastewater (21st ed.). American Public Health Association, the American Water Works Association, and the Water Environment Federation, Washington DC.

Asano T, Burton F L, Leverenz H L, Tsuchihashi R, Tchobanoglous G, 2007. Water Reuse – Issues, Technologies, and Applications. McGraw-Hill, New York. 1–1570.

Békaert C, Rast C, Ferrier V, Bispo A, Jourdain M J, Vasseur P, 1999. Use of *in vitro* (Ames and Mutatox tests) and *in vivo* (Amphibian Micronucleus test) assays to assess the genotoxicity of leachates from a contaminated soil. *Organic Geochemistry*, 30(8): 953–962.

Bispo A, Jourdain M J, Jauzein M, 1999. Toxicity and genotoxicity of industrial soils polluted by polycyclic aromatic hydrocarbons (PAHs). *Organic Geochemistry*, 30(8): 947–952.

Chu J Y, Chen J N, Wang C, Fu P, 2004. Wastewater reuse potential analysis: Implications for China's water resources management. *Water Research*, 38(11): 2746–2756.

Dai M, Xie P, Liang G D, Chen J, Lei H H, 2008. Simultaneous determination of microcystin-LR and its glutathione conjugate in fish tissues by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B*, 862(1-2): 43–50.

Economou A, Botitsi H, Antoniou S, Tsipi D, 2009. Determination of multi-class pesticides in wines by solid-phase extraction and liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, 1216(31): 5856–5867.

Edzwald J K, Van Benschoten J E, 1990. Aluminum coagulation of natural organic matter. In: Chemical Water and Wastewater Treatment (Hahn H H, Klute R, eds.). Springer-Verlag, New York. 341–359.

Fent K, 2003. Ecotoxicological problems associated with contaminated sites. *Toxicology Letters*, 140-141: 353–365.

Férard J F, Ferrari B, 2005. Wastoxhas: a bioanalytical strategy for solid wastes assessment: a review. In: Small-Scale Freshwater Toxicity Investigations: Volume 2-Hazard Assessment Schemes (Blaise C, Ferard J F, eds.). Springer, New Jersey. 331–375.

Fernández M D, Cagigal E, Vega M M, Urzelai A, Babín M, Pro J et al., 2005. Ecological risk assessment of contaminated soils through direct toxicity assessment. *Ecotoxicology and Environmental Safety*, 62(2): 174–184.

Hernándo M D, Fernández-Alba A R, Tauler R, Barceló D, 2005. Toxicity assays applied to wastewater treatment. *Talanta*, 65(2): 358–366.

Hu H Y, Wei D B, Dong C H, 2002. Assessment and management on water quality safety of sewage. *Environment Protection*, (11): 37–38.

Huerta-Fontela M, Galceran M T, Ventura F, 2010. Fast liquid chromatography-quadrupole-linear ion trap mass spectrometry for the analysis of pharmaceuticals and hormones in water resources. *Journal of Chromatography A*, 1217(25): 4212–4222.

Juvonen R, Martikainen E, Schultz E, Joutti A, Ahtiainen J, Lehtokari M, 2000. A battery of toxicity tests as indicators of decontamination in composting oily waste. *Ecotoxicology and Environmental Safety*, 47(2): 156–166.

Kimerle R, Barnthouse L, Brown R, de Beyssac B C, Gilbertson M, Monk K et al., 1997. Ecological effects (Chapter 4). In: Chemical Ranking and Scoring – Guidelines for Relative Assessments of Chemical: Proceedings of the Pellston Workshop on Chemical Ranking and Scoring (Swanson M B, Socha A C, eds.). SETAC Press, Florida. 154.

Lai Z, 2010. The utilization of reclaimed water was beyond surface water in Beijing City during the past two years. http://news.xinhuanet.com/environment/2010-03/25/content_13246457.htm. Beijing, 2010-03-25.

Liu R, Kameya T, Sawai A, Urano K, 2007. Application of a larval medaka assay to evaluate the fish safety level in Sagami River, Japan. *Environmental Monitoring and Assessment*, 130: 475–482.

Munn M D, Gilliom R J, 2001. Water-resources investigations report 01-4077: Pesticide toxicity index for freshwater aquatic organisms. United States Geological Survey (USGS), California. 1–61.

Pedrouzo M, Borrull F, Pocurull E, Marcé R M, 2009. Estrogens and their conjugates: Determination in water samples by solid-phase extraction and liquid chromatography–tandem mass spectrometry. *Talanta*, 78(4-5): 1327–1331.

Personne G, Marsalek B, Blinova I, Torokne A, Zarine D, Manusadzianas L et al., 2003. A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters. *Environmental Toxicology*, 18: 395–402.

Ramos J J, Gómara B, Fernández M A, Gonzalez M J, 2007. A simple and fast method for the simultaneous determination of polychlorinated biphenyls and polybrominated diphenyl ethers in small volumes of human serum. *Journal of Chromatography A*, 1152(1-2): 124–129.

Rila J P, Eisentraeger A, 2003. Application of bioassays for risk characterisation and remediation control of soils polluted with nitroaromatics and PAHs. *Water, Air, & Soil Pollution*, 148(1-4): 223–242.

Sartoris J J, Thullen J S, Barber L B, Salas D E, 2000. Investigation of nitrogen transformations in a southern California constructed wastewater treatment wetland. *Ecological Engineering*, 14(1-2): 49–65.

Tsui M T K, Chu L M, 2003. Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. *Chemosphere*, 52(7): 1189–1197.

Vazquez-Roig P, Andreu V, Blasco C, Picó Y, 2010. SPE and LC-MS/MS determination of 14 illicit drugs in surface waters from the Natural Park of L'Albufera (València, Spain). *Analytical and Bioanalytical Chemistry*, 397: 2851–2864.

Wei D B, Kisuno A, Kameya T, Urano K, 2006. A new method for evaluating biological safety of environmental water with algae, daphnia and fish toxicity ranks. *Science of the Total Environment*, 371(1-3): 383–390.

Wei D B, Lin Z F, Kameya T, Urano K, Du Y G, 2008. Application of biological safety index in two Japanese watersheds using a bioassay battery. *Chemosphere*, 72(9): 1303–1308.

World Bank, 2009. Addressing China's Water Scarcity: Recommendations for Selected Water Resource Management Issues. World Bank, Washington DC. 1–143.