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Characterisation of acute toxicity, genotoxicity and oxidative stress posed by textile effluent on zebrafish

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Abstract

Textile industries are important sources of toxic discharges and contribute enormously to water deterioration, while little attention has been paid to the toxicity of textile effluents in discharge regulation. Bioassays with zebrafish were employed to evaluate the toxicity of wastewater samples collected from different stages at a textile factory and sewage treatment plants (STPs). Physico-chemical parameters, acute toxicity, genotoxicity and oxidative stress biomarkers were analyzed. The wastewater samples from bleaching, rinsing and soaping of the textile factory exhibited high acute toxicity and genotoxicity. The coexisting components of dye compounds, as assistants and oxidants, seemed to cause some effect on the toxic response. After treatment employing the anoxic-oxic (A/O) process in STPs, the color and the chemical oxygen demand (COD) were reduced by 40% and 84%, respectively, falling within the criteria of the Chinese Sewage Discharge Standard. In contrast, increases in acute toxicity and genotoxicity were observed in the anaerobic tank, indicating the formation of toxic intermediates. The genotoxicity of the effluent of the STP was not significantly different from that of the influent, suggesting the wastewater treatment processes were not effective in removing the genotoxicity of the dye wastewater. Results indicated that the effluent contains pro-oxidants since the activities of glutathione (GSH), malondialdehyde (MDA), and total anti-oxidation capacity (T-AOC) were all elevated. In addition, decreases in superoxide dismutase (SOD) and glutathione-S transferase (GST) activities observed can be interpreted as a cytotoxicity sign due to an over-production of reactive oxygen species (ROS). The results of the present study suggest that the STPs were not capable of reducing the toxicity of wastewater sufficiently. Further treatment is needed to remove the potential risks posed by textile effluent to ecosystems and human health, and employing a toxicity index is necessary for discharge regulation.

Key words: acute toxicity; genotoxicity; micronucleus; comet; textile effluent

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Introduction

The textile industry is one of China's traditional pillar industries and the Chinese textile industry is the largest in the world. But at the same time, it consumes a large quantity of water (up to 150 L of water to dye 1 kg of cotton) and generates a huge amount of wastewaters (Hai et al., 2007). Given the great variety of fibers, dyes, surfactants, process aids and finishing products in use, textile wastewaters contribute enormously to water deterioration (Prigione et al., 2008). Even at very low concentrations (10–50 mg/L), water-soluble azo dyes can cause streams, river or ponds to become highly colored (Anliker, 1977). The presence of dyes in effluent is highly visible and affects aesthetics, water transparency, and gas solubility in the receiving aquatic ecosystem. Moreover, wastewaters generated from textile plants usually have high levels of chemical oxygen demand (COD), biological oxygen demand (BOD), acidity, chlorides, sulphates, and various heavy metals (Lanciotti et al., 2004). The release of colored wastewater in the ecosystem is a significant source of eutrophication and perturbations in aquatic life (Prigione et al., 2008).

The potential hazards of textile effluent to ecosystems and human health have aroused great concern since textile effluents usually contain some toxic substances, such as additives, detergents, surfactants and dyes, which are carcinogenic, mutagenic or teratogenic to various organisms (Vanhulle et al., 2008). As a result, some primary cancers involving kidneys, urinary bladder, and liver were reported in workers of dye-related industries (Morikawa et al., 1997). Moreover, azo and nitro dye compounds have been reported to be reduced in sediments of aquatic bodies. Under reductive conditions, azo dyes may cleave into potentially carcinogenic aromatic amines that spread in the ecosystem (Chen, 2006). In addition, the presence of dyes or their degradation products in water can also cause human health disorders such as nausea, hemorrhage, and ulceration of skin and mucous membranes, and can cause severe damage to the kidney, reproductive system, liver,

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brain, and central nervous system (Puvaneswari et al., 2006).

These concerns have led to strict regulations on the discharge of textile wastewater, compelling the dye manufacturers and treatment plants to adopt more effective approaches. Chemical analysis is unable to predict the effect on the organisms in the aquatic ecosystem. Substances may be present in concentrations which are too low to be detected yet still have a negative impact, and toxic intermediates might be formed that are difficult or impossible to predict (Liu et al., 2008; Verma, 2008). Identification of toxic materials and evaluation of toxic risk by effect-directed analysis (EDA) are widely used in the countries of the European Union (Brack, 2003). The United States has begun to carry out the comprehensive toxic control of wastewater, and formulate the relevant toxic emissions standards (US EPA, 1991). Whole-effluent toxicity (WET) testing is an important component of the US EPA's integrated approach for controlling the discharge of toxic chemicals and other materials into surface waters (US EPA, 1993). Therefore, it is necessary to employ a toxicity index for discharge regulation of textile wastewater.

Acute toxicity tests are considered to be the first step in a tiered toxicity approach for setting maximum acceptable concentrations of pollutants as they are relatively rapid, simple and cost-effective (Chapman, 1995). Most of the regulatory agencies and research organizations use the conventional acute toxicity test with fish and *Daphnia* (ISO, 1996; OECD, 2004). Moreover, it has been proposed to assess the genotoxicity of environmental contaminants in environmental biomonitoring studies, which could provide a basis for an ecotoxicological risk assessment of genotoxic substances in discharged waters (Diekmann et al., 2004). Furthermore, the assessment of genotoxic potential in textile dyeing sewage is of particular importance considering the toxic characteristics of dye compounds and their intermediates. Both the micronucleus assay and the single cell gel electrophoresis (SCGE, also known as comet assay) are extensively applied genotoxicity bioassays for effluents, being capable of detecting DNA damage (Lemos et al., 2007; Rajaguru et al., 2003). In addition, oxidative stress has been proved to closely correlate with genotoxicity (Tsangaris et al., 2011).

The advantages of using fish as model organisms in water quality studies include the fact that fishes depend on the aquatic environment, where significant deposition of environmental pollutant discharges occurs, and they react sensitively to environmental changes. Special attention has been given in some studies to fish as possible biological monitors in genotoxicity assessment (Bolis et al., 2001; Rajaguru et al., 2003; Sumathi et al., 2001), because these organisms respond to toxic agents similarly to higher vertebrates, allowing the evaluation of substances that are potentially teratogenic, mutagenic, and carcinogenic to humans. And due to prolific reproduction and the external development of its transparent embryo, the zebrafish is used as prime model for genetic and developmental studies, as well as research in toxicology and genomics (Shin and Fishman, 2002).

In the present study, the toxicological characteristics of the effluents from the textile industry and wastewater treatment plant were evaluated using combined indicators, involving acute toxicity, genotoxicity assessed by micronucleus frequency and comet assay, and oxidative stress. Zebrafish was employed as the monitor organism. These assays allowed an evaluation of the potential risks of textile effluents to ecosystems.

1 Materials and methods

1.1 Dye wastewater sampling

The dye-contaminated effluents of the representative processes of bleaching, fiber scouring, rinsing and soaping were obtained from a typical dyeing factory in Haicheng, Liaoning Province of China. The factory is one of the largest textile factories in the Northeast of China. In addition, the wastewater of diverse treatment processes was taken from a dyeing wastewater treatment plant. The plant receives the printing and dyeing wastewater of 34 textile enterprises in nearby areas and treats about 40,000 tons of wastewater daily. The plant performs the anoxic-oxic (A/O) treatment process, in which the hydraulic retention time (HRTs) of the anoxic tank and oxic tank are about 2 and 4.5 hr, respectively. Nearby samples were collected over a 24-hr period according to composite sampling methods (US EPA, 2002). The samples included influent, wastewater from the anaerobic process, oxic tank, secondary sedimentation tank, flocculation and effluent process, respectively. They were delivered to the laboratory the same day. After filtering through a glass fiber filter (Whatman GF/D) and adjusting the value of pH to 7.7–8.0, the wastewater was stored at –20°C until the day of the test.

1.2 Measurement of physico-chemical parameters

Water quality parameters including pH, color, salinity and COD were measured according to the standard methods of the NEPA (2002). The physico-chemical characteristics of wastewater collected from representative processes in the textile factory and sewage treatment plant (STP) are given in Table 1. Wastewater from the textile factory had

Table 1 Physico-chemical characteristics of wastewater from textile factory and sewage treatment processes

Wastewater	pH	Color	Salinity (‰)	COD (mg/L)
Textile factory				
Bleaching	9.4	17	0	528 ± 7.9
Fiber scouring	7.3	21	2	–
Rinsing	8.6	7	2	311 ± 2.1
Soaping	12	38	5	578 ± 23.5
Sewage treatment process				
Influent	7.5	15	2	378 ± 14.2
Anaerobic tank	9.1	30	3	586 ± 12.9
Oxic tank	7.7	16	4	359 ± 10.4
Secondary sedimentation tank	7.6	17	4	316 ± 19.9
Flocculation	7.5	15	4	27.6 ± 7.8
Effluent	7.5	9	3	60.5 ± 6.4

–: less than detection limit (50 mg/L).

a relatively high COD, ranging from 311 to 578 mg/L (Table 1). Except for the wastewater from the rinsing process, wastewater from other stages showed a relatively high color, ranging from 17 to 38. After the treatment by the A/O in the STP, both the color and the COD were effectively removed. With a reduction of 40% and 84% in color and COD, respectively, the color of the effluent from the STP met the 1-Class B Criteria of the Chinese Sewage Discharge Standard. In this standard (30 for color and 60 mg/L for COD, GB18918-2002).

1.3 Zebrafish culturing

Zebrafish were purchased from the Xianglujiao Aquarium Market at Dalian, China, and kept in 200 L aquaria supplied with aerated water. The water temperature was (25 ± 1)°C. Before the experiments they were acclimated under laboratory conditions for 7 days in the aerated water, fed with commercial fish food pellets every day and not during the toxicity assay.

1.4 Exposure

Before the toxicity assay, the wastewater was diluted by the aerated water to different concentrations (*V*/*V*). Zebrafish were exposed to water samples for 4 days at 1 g fish/L water. The aerated water without toxicant served as negative control (NC) groups. The positive controls employed potassium dichromate and 4-nitroquinoline-1-oxide (4-NQO), diluted in aerated water, which are typical genotoxic compounds.

1.5 Acute toxicity test

Lethality is the most commonly used acute toxicity test endpoint, as it is an unambiguous measure of response. The mortality was counted at various concentrations of exposure, and to determine the maximal tolerance concentration to be used in the genotoxicity test. In the negative control, 100% survival was observed.

1.6 Comet assay

The comet assay requires only minute quantities of tissue, making this technique suitable for assays involving very small fish (Wilson et al., 1998). A zebrafish was placed on a filter, and the blood was removed by cutting the tail. Livers from three fishes per treatment were dissected into centrifuge tubes, rinsed in phosphate buffered solution (PBS) (Solarbio, China) three times, and then digested to single cells in 400 μL trypsine (Solarbio, China). After digestion, the cells were dispersed into 800 μL Dulbecco's Modified Eagle Medium (DMEM) (Solarbio, China) and filtered through a 74 μm nylon cloth into centrifuge tubes. Cells were collected by centrifugation for 5 min at 1000 r/min and incubated with DMEM. After adjusting the density of cells, the resulting cell suspensions were processed in the comet assay.

Thirty microliters of the cell suspension were mixed with 90 μL of 0.7% low melting agar (LMA) (Solarbio, China) at 37°C and pipetted onto a fully frosted slide precoated with a layer of 100 μL of 1% normal melting agar (NMA) (Solarbio, China). After solidification, slides were immersed into lysis solution (2.5 mol/L NaCl, 10 mmol/L Tris, 100 mmol/L EDTA, NaOH of pH 10.0, 1% Na-sarcosinate (Solarbio, China), 10% dimethylsulfoxide (DMSO) (Sigma, USA)) and 1% Triton X-100 (Sigma, USA) for 1.5 hr. Slides were then incubated in an electrophoresis tank containing 300 mmol/L NaOH with 1 mmol/L EDTA for 20 min prior to electrophoresis for 20 min at 25 V (300 mA). Then, slides were neutralized (0.4 mol/L Tris, pH 7.5) (Solarbio, China) and stained with 40 μL Gelred (0.2 μL/mL) (Biotium, USA) for fluorescence microscopy (IX71, Olympus, Japan) analysis using a digital imaging system (DP71, Olympus, Japan).

1.7 Micronucleus assay

The zebrafish peripheral erythrocytes which were obtained from a caudal section were added to slides containing fetal bovine serum and dried. Then the erythrocytes were fixed in absolute methanol for 20 min, air dried, stained with 10% Giemsa (SCRC, China) for 10 min, washed with PBS, and dried at room temperature just before the evaluation with an optical microscope (MODEL YS100, Nikon, Japan) using a 1000× oil-immersion lens. The micronuclei are interpreted as particles with color and structure similar to those of the nucleus, without shining or refraction, of size between 1/5 and 1/100 of the main nucleus, and next to but not touching the main nucleus (Minissi et al., 1996).

1.8 Quality control/quality assuarance

The results of the toxicity test were validated using both the negative control and the positive control. The control tests were run parallel with each series of real samples. Aerated water was used as the negative control, while the reference compounds potassium dichromate ($K_2Cr_2O_7$) and 4-nitroquinoline 1-oxide (4-NQO) were employed as positive control in the toxicity tests. The results of the positive control indicated that the comet assay was more sensitive than the micronucleus assay (Fig. 1). DNA damage induced for fish exposed to Cr^{6+} at concentrations of 5 mg/L and 20 μg/L of 4-NQO was significantly different compared with NC values, while significant effect was caused in micronucleus frequency compared with the negative control at concentrations of 20 mg/L and 30 μg/L respectively.

1.9 Oxidative stress biomarkers

The livers were homogenized in ice-cold physiological saline (0.9%). 5% of the homogenate was centrifuged (2000 r/min for 10 min at 4°C) and the supernatant was used for assays of activities of superoxide dismutase (SOD), glulathiones-S transferase (GST), glulathione (GSH), malondildehyde (MDA) and and total anti-oxidation capacity (T-AOC) with a commercially-available kit (Nanjing Jiancheng Bioengineering Institute, China).

1.10 Statistical analysis

The parameter used to quantify the extent of DNA damage was the tail moment, which was the integrated value of relative fluorescence intensity in the tail multiplied by the

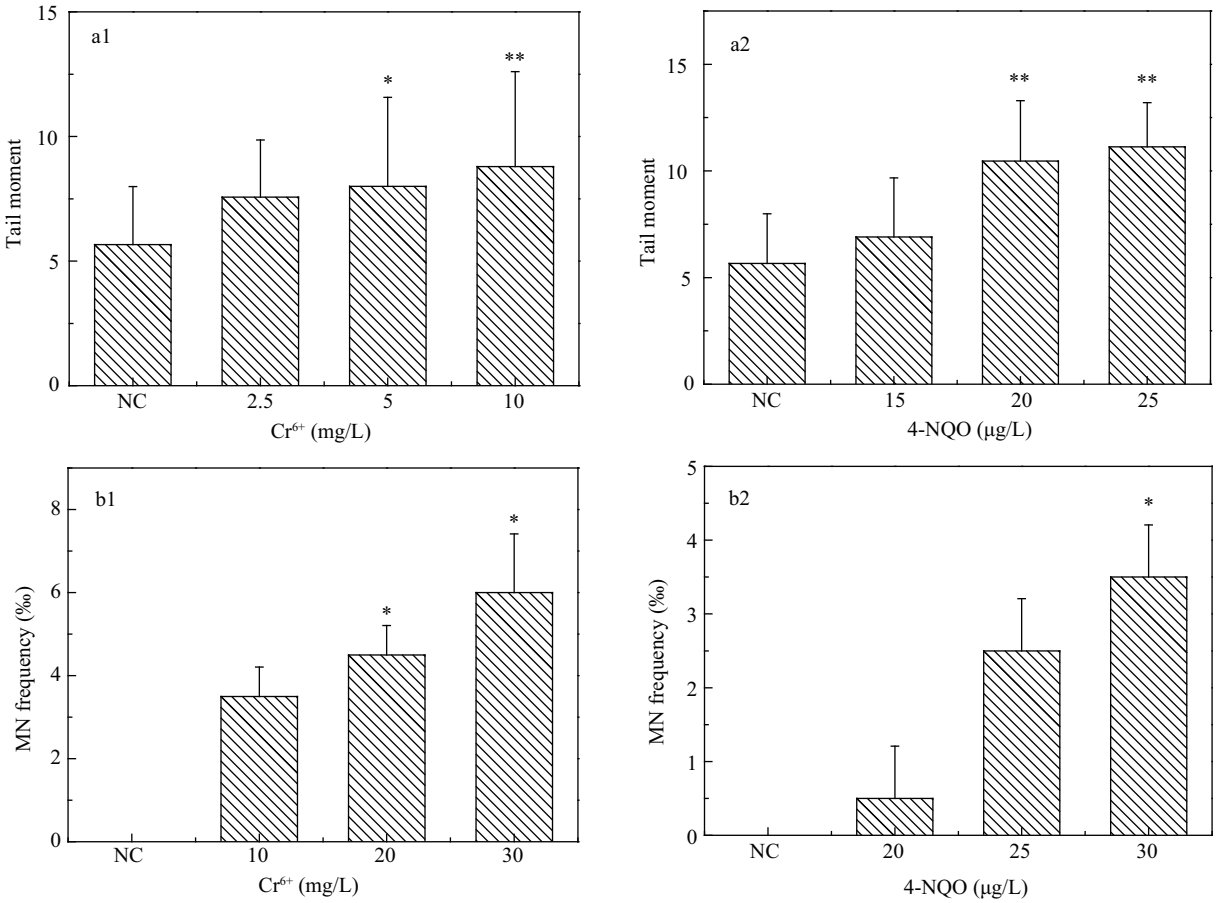

Fig. 1 DNA damage (a) and micronucleus frequency (b) in the hepatocytes of zebrafish after exposure to positive controls at different concentrations. * and ** represent significant differences at $p < 0.05$ and $p < 0.01$, respectively compared with negative control (NC) values.

migration distance of the DNA fragments. For statistical analysis, the tail moment from 100 cells were measured and the results are presented as mean ± SD. Comparisons between the parallel negative control and the treatments, and between different treatment groups were analyzed by one-way ANOVA for group mean comparison. Mean micronucleus frequencies, expressed as number of micronucleus per 1000 erythrocytes, were calculated for each group. Micronucleus frequencies were compared between wastewater samples also by one-way ANOVA.

2 Results and discussion

2.1 Acute toxicity of the effluent

The acute toxicity of water samples collected during the textile industry processing on zebrafish is presented in Table 2. Except for the water samples from the fiber scouring process, water samples from bleaching, rinsing and soaping all caused the lethality of zebrafish to different extents. At a concentration of 20%, wastewater from the bleaching process showed 100% lethality, while the maximum non-lethal dose was 5%. Compared with the water from other processes, the acute toxicity of wastewater from the bleaching process was the highest. Considering that the color of the wastewater from the bleaching process was comparable to that from other processes (Table 1), suggesting similar concentrations of dye compounds, the assistant employed in the bleaching process possibly resulted in the high acute toxicity. Assistants were most commonly used in the textile industry as emulsifiers and solubilizing, wetting, and dispersing agents. In addition, the bleaching process was also preceded by hydrogen peroxide. All residual reagents from the previous stages were eliminated in the fiber scouring process. The rinsing dye effluent was found to be comparatively less toxic, causing 100% lethality at 50% of effluent. Additional assistants were employed to optimize the color of the textile fabric in the process of soaping, which were toxic and possibly caused the acute toxicity of water from the soaping process.

Table 2 Lethality of zebrafish caused by wastewater from textile factory and sewage treatment processes at different concentrations

Dilution concentration	5%	10%	20%	30%	50%
Textile factory					
Bleaching	0	60	100	100	100
Fiber scouring	–	0	0	0	0
Rinsing	–	0	0	0	100
Soaping	–	0	0	100	100
Sewage treatment process					
Influent	–	–	0	0	20
Anaerobic tank	–	–	0	0	100
Oxic tank	–	–	0	0	0
Secondary sedimentation tank	–	–	0	0	0
Flocculation	–	–	0	0	0
Effluent	–	–	0	0	20

–: no acute toxicity test arranged.

The acute toxicity of wastewater collected from the representative processes of STPs on zebrafish at various concentrations is presented in Table 2. The influent caused 20% lethality at the concentration of 50%. However, an increase in acute toxicity was observed in the anaerobic tank. Some toxic intermediates might be generated after the anaerobic reaction of the dye wastewater. It should be noted that the wastewater still exhibited acute toxicity on zebrafish after exposure to the effluent wastewater, where a 20% lethality was observed at 50% concentration. It seemed that the current technology employed in the STPs was incapable of removing the acute toxicity of the dye wastewater and further treatment is warranted. No significant acute toxicity was observed for textile wastewater from the oxic tank, secondary sedimentation tank and flocculation wastewater at and below 50% of concentration, suggesting lower acute toxicity.

2.2 Genotoxicity of the textile wastewater

According to Fig. 2, a gradual increase in tail moment was observed as the concentration of the exposure increased in all the samples. The erythrocytes from fish exposed to samples of bleaching, fiber scouring, rinsing and soaping had mean tail moments that were significantly higher than the negative control values at 2.5%, 10%, 5%, 10% of effluent, respectively, and above. While the effluent from fiber scouring process causing lower acute toxicity, the effluent induced genotoxicity at the 10% concentration. The lowest concentration of the wastewater from the textile factory which did not cause significant effect on tail moment compared with the NC was used to determine the dilution multiple, with the aim of comparing the genotoxicity among the water samples (Fig. 3a). Wastewater from the bleaching process exhibited the highest potential of DNA damage, where a dilution of 80 fold was required to eliminate its genotoxicity. Moreover, the results of the micronucleus assay showed a similar trend as the comet assay, with decreasing genotoxicity of wastewater in the sequence of bleaching > soaping = rinsing > fiber scouring (Fig. 3b).

Similar to the results of the acute toxicity test, the assistant, oxidant and salt existing in the textile wastewater seemed to cause an effect on the genotoxicity of the effluents, although the dye compounds and their intermediates were the major concern. Due to the characteristics of the textile effluents, many pollutants enter aquatic ecosystems as mixtures of chemicals. The combined effect of the dye compounds and the coexisting components in the textile effluents need to be further studied.

A gradual increase in mean tail moment with increasing concentration of exposure was also observed in the

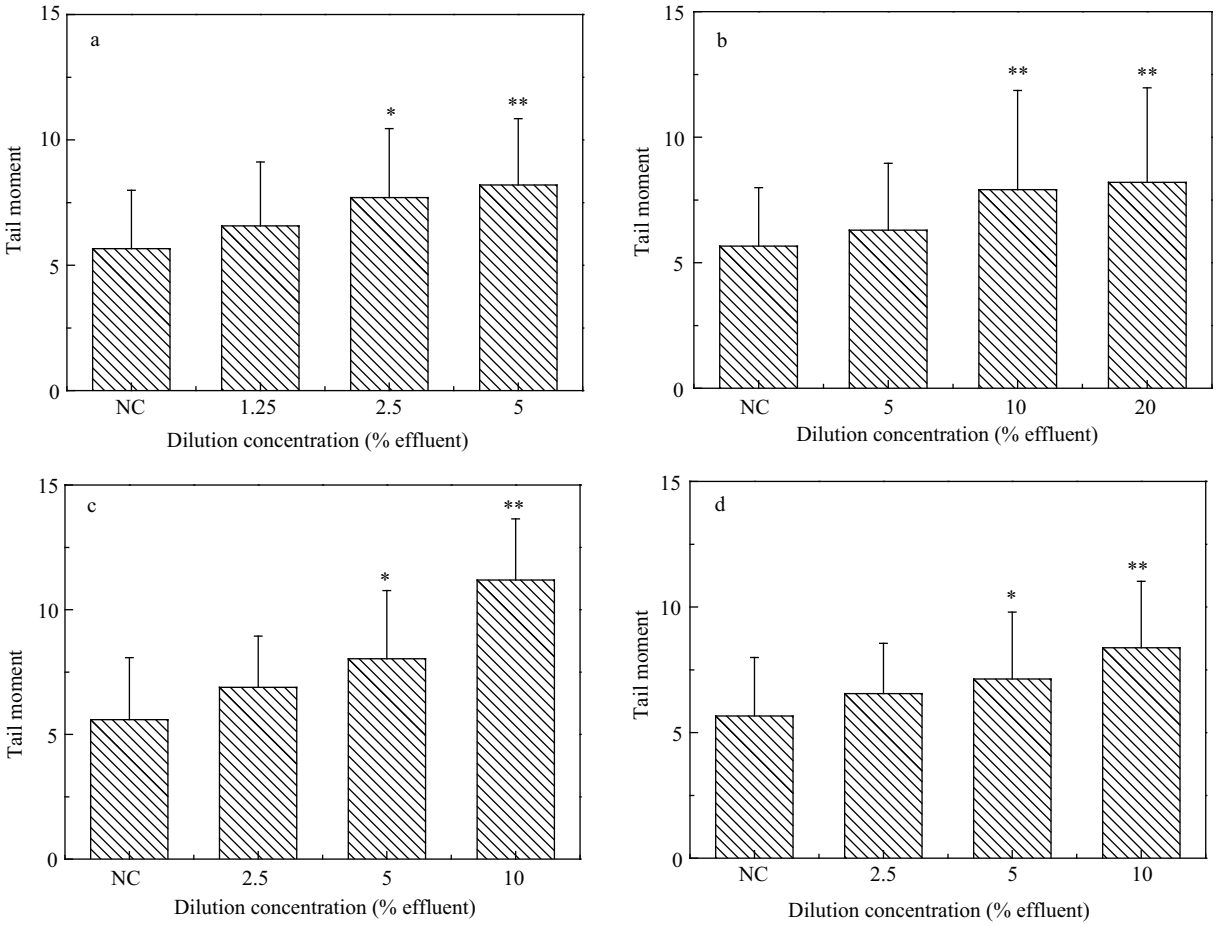

Fig. 2 DNA damage in the hepatocytes of zebrafish after exposure to effluents from the typical process stages of textile industry at different concentrations. (a) bleaching; (b) fiber scouring; (c) rinsing; (d) soaping. * and ** represent significant differences at $p < 0.05$ and $p < 0.01$, respectively compared with NC values.

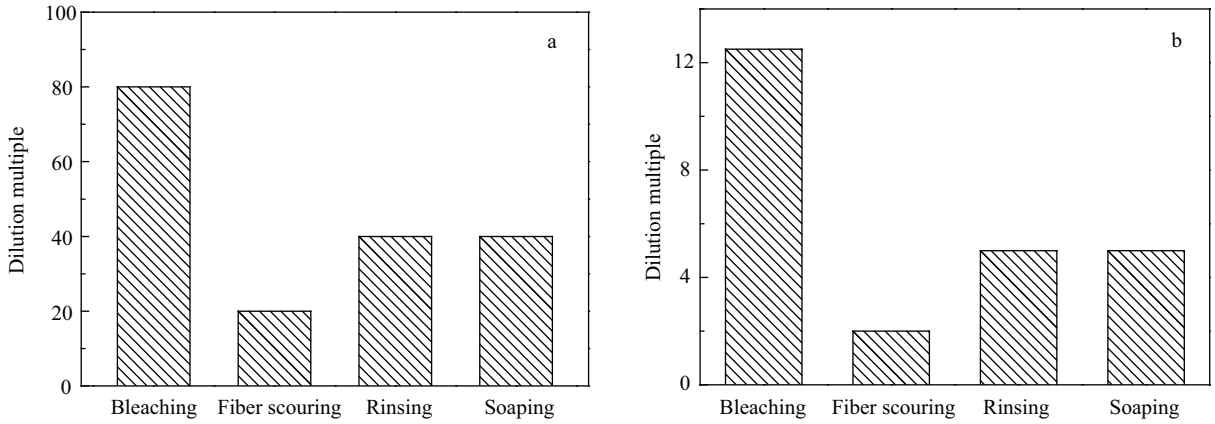

Fig. 3 Dilution multiple of wastewater collected from the textile factory, where no significant DNA damage (a) and different micronucleus frequency in comparison to NC (b) was observed.

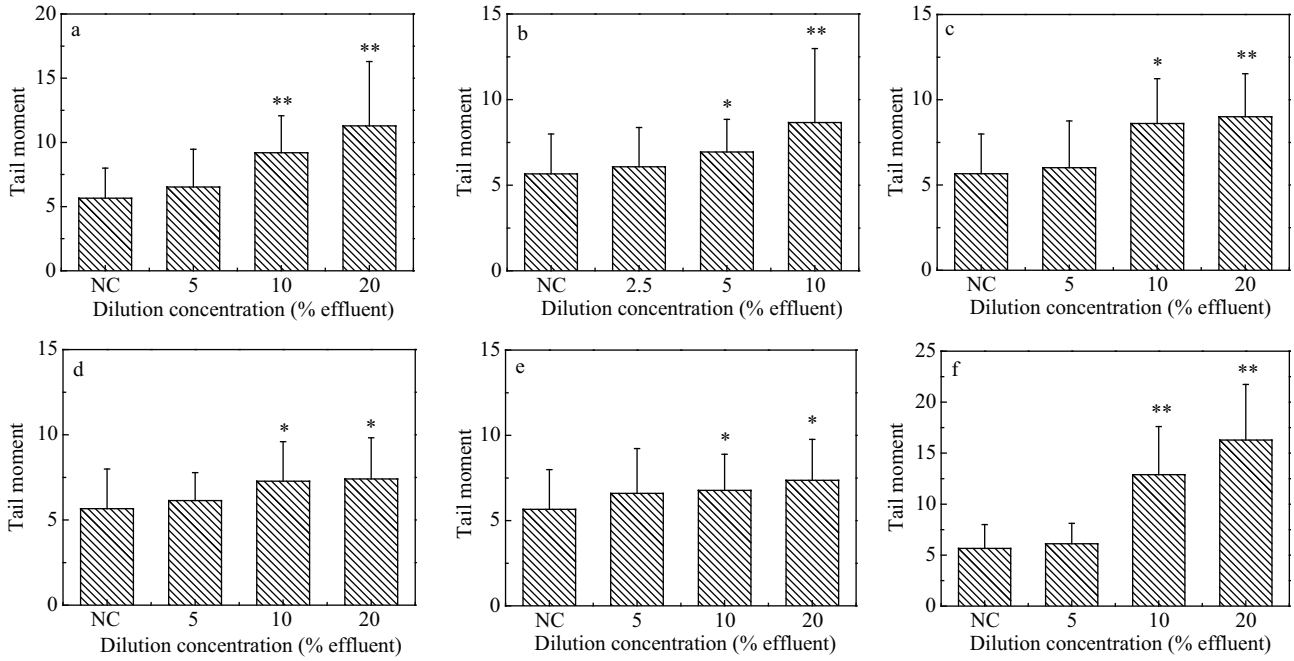

Fig. 4 DNA damage determined by tail moment of the hepatocytes caused by exposure of zebrafish to various concentrations of wastewater samples collected in the representative stages from the textile dyeing sewage treatment plant. (a) influent, (b) anaerobic tank, (c) oxic tank, (d) secondary sedimentation tank, (e) flocculation, and (f) effluent. * and ** represent significant differences at $p < 0.05$ and $p < 0.01$, respectively, compared with NC values.

wastewater samples collected from the dyeing sewage treatment plant (Fig. 4). The influent involving dye-contaminated wastewater exhibited significant toxicity, to which exposure at 10% concentration caused a significantly higher tail moment of the hepatocytes relative to the negative control ($p < 0.01$). DNA damage caused by wastewater samples collected from the STPs at the same concentration of 30% were compared (Fig. 5). An increase in the genotoxicity during the STPs was observed in the anaerobic tank. At 5% concentration, wastewater from the anaerobic tank resulted in significant DNA damage, while wastewater samples caused a significant genotoxicity at 10% concentration (Fig. 4). Although reactive textile dyes can be decolorized due to reduction of the azo bond under anaerobic conditions, azo dyes cleave into one of the 22 aromatic amines which are potentially carcinogenic and resist further degradation (Sweeney et al., 1994). Consequently, the wastewater from the anaerobic tank caused the highest genotoxicity during the STP. Because the toxicity of the dye contaminants could be eliminated through bacterial fission of the aromatic ring, where oxygen was required (Seshadri et al., 1994), a decrease of genotoxicity was observed following aerobic treatment. As the treatment stages processed, a gradual decrease in the genotoxicity was achieved in the secondary sedimentation tank, and flocculation wastewater. However, an increase in the DNA damage was observed in the ultimate effluent. It was not clear why such phenomena occurred. Similarly, the micronucleus assay exhibited the same trend in the genotoxicity changes during the sewage treatment process (Fig. 6). The results from the micronucleus assay further supported the results of the comet assay. It should be noted that the genotoxicity of the effluent was not significantly different with that of the influent, suggesting the incapability of the STPs in removing the genotoxicity of the dye wastewater.

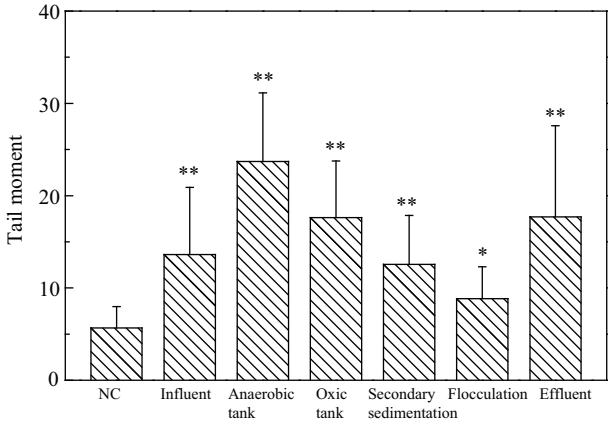

Fig. 5 DNA damage of zebrafish exposed to NC and 30% of textile wastewater in the representative process stages from the sewage treatment plant. * and ** represent significantly different at $p < 0.05$ and $p < 0.01$, respectively in comparison to NC.

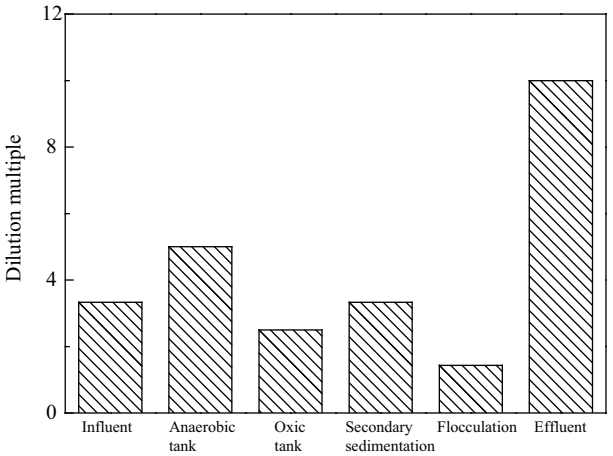

Fig. 6 Dilution multiple of wastewater from different stages of the textile dyeing sewage treatment plant, which caused identical micronucleus frequency.

2.3 Oxidative stress biomarkers

The oxidative stress biomarkers values measured after zebrafish exposure in different dilutions of wastewater from the typical process stages are shown in Fig. 7. Antioxidant enzymes can be induced by increased generation of reactive oxygen species (ROS) as a protection mechanism to inhibit oxidative stress, suggesting toxicity (Sturve et al., 2008). The enzyme response to toxic chemicals shows a bell-shaped trend with an initial increase in activity due to enzyme induction, and then followed by a decrease in activity due to enhanced catabolic rate and/or direct inhibition by toxic chemicals (Viarengo et al., 2007). The two analyzed antioxidant enzymes, SOD and GST, exhibited significantly lower activities in the influent and anaerobic tank exposure group at the concentration of 20%, and decreased in the oxic tank and effluent group during the course of the study, although not significantly. The decreases in SOD and GST activities observed after exposure can be interpreted as a cytotoxicity sign to compensate an over-production of ROS, due to high levels of pollutant exposure (Dazy et al., 2008). Scandalios (1997) have demonstrated that excess superoxide radicals ($O_2^{\bullet -}$), which are a precursor to other ROS, disturbed the signal transduction triggering the genes responsible for antioxidant enzymes like SOD. Since then many researchers have reported that ROS could damage DNA and lead to genotoxicity. Studies have shown that a higher formation of MDA was believed to be related to the induced cytotoxicity and genotoxicity effects on A-549 cells by oil fumes (Dung et al., 2006). After exposure, even if no clear dose-effect relationship could be highlighted, it was possible to state that zebrafish showed higher concentrations of GSH, MDA, and T-AOC compared with NC in the high exposure group. The MDA increase suggested that exposure to effluents can lead to oxidative damage such as lipid peroxidation in fish, and the elevated level of GSH activity after 4 days exposure

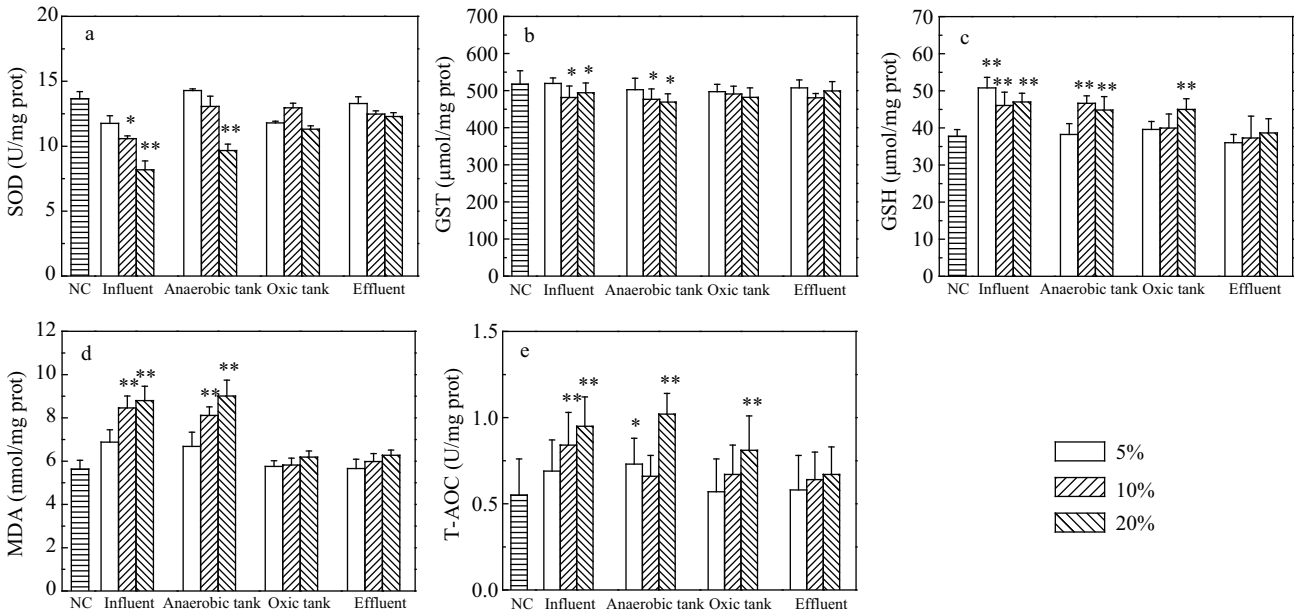

Fig. 7 Oxidative stress biomarkers of liver in zebrafish exposed to different dilutions of wastewater from a typical textile factory. (a) SOD, (b) GST, (c) GSH, (d) MDA and (e) T-AOC. Results are presented as mean ± standard. * and ** represent significant differences at $p < 0.05$ and $p < 0.01$, respectively, compared with NC values.

to the effluents, also suggests the presence of pro-oxidant compounds in the effluent. Wu (2005) has reported a GSH response caused by azo dyes. Induction of antioxidants, especially GSH, represents a first front of cellular defense to compensate for the toxicity of ROS (Filho et al., 2001). T-AOC levels increased in tissue homogenates of zebrafish exposed to effluent, meanwhile, suggesting an oxidative stress condition. The results suggested that the oxidative stress caused by the textile effluents possibly resulted in the genotoxicity.

3 Conclusions

In the present study, the authors showed that wastewater from typical stages of a textile factory and sewage treatment plant exhibited varying degrees of toxicity using a zebrafish bioassay. The coexisting components of dye compounds, as assistants and oxidants in textile effluents, caused some effects on the toxic response. After treatment employing the A/O process in the STPs, the physicochemical indexes met the criteria of the Chinese Sewage Discharge Standard. In contrast, an increase in acute toxicity and genotoxicity were observed in the effluent, indicating that the wastewater treatment processes were not effective in removing the toxicity of the dye wastewater, emphasizing the need for further wastewater remedial treatments. The response of the antioxidant suggests the presence of pro-oxidants in the effluents from the textile factory which could cause oxidative stress. Additionally, results suggest that effluents also contain compounds that may inhibit components in the antioxidant enzymes, e.g., SOD and GST. The correlations between biomarkers of oxidative stress and genotoxicity suggest that the observed effects were due to contaminants exhibiting oxidative stress potential that can also induce genotoxicity.

The toxicity to zebrafish is an efficient method to suggest potential damage to various receptor ecosystems and increases concern about the need to reassess methods of treatment in STPs. The results of the present study demonstrated that the toxicity test has a viable role to play in water quality monitoring, and provided a basis for further environmental risk assessment of textile effluent.

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