Ecotoxicology and Environmental Safety 76 (2012) 143–152

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

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

Use of TIE techniques to characterize industrial effluents in the Pearl River Delta region

Yi-Xiang Fang, Guang-Guo Ying*, Li-Juan Zhang, Jian-Liang Zhao, Hao-Chang Su, Bin Yang, Shan Liu

State Key Laboratory of Organic Geochemistry, CAS Centre for Pearl River Delta Environmental Pollution and Control Research, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China

ARTICLE INFO

Article history:
Received 7 July 2011
Received in revised form
3 October 2011
Accepted 6 October 2011
Available online 22 October 2011

Keywords:
TIE
Industrial effluents
Whole effluent toxicity
Lux bacteria

ABSTRACT

We investigated the acute toxicity of various industrial effluents in the Pearl River Delta region using lux bacteria, duckweed, green algae, crustaceans and zebrafish. The potential toxicants in the industrial effluents were identified and evaluated by lux bacteria bioassay and chemical analysis. The results show that green algae (*Pseudokirchneriella subcapitata*) and crustacean (*Ceriodaphnia dubia*) were more sensitive to the effluents from electronic and electroplate factories than other test species, while lux bacteria were more sensitive to all the other effluents. The toxicities of effluents from electronic and electroplate factories to the six test organisms were significantly higher than those of the other industrial effluents, and mainly caused by metals. Noticeably, organic pollutants were the main contributing factor to the toxicity of effluents from textile and dyeing plants, pulp and paper mills, fine chemical factories and municipal wastewater treatment plants.

1. Introduction

Industrial effluents have been regarded as the main input source of various pollutants to the aquatic ecosystem (Chan et al., 2003; Lah et al., 2004; Smolders et al., 2004), and contain various organic and inorganic substances potentially toxic to aquatic biota (Gomez et al., 2001). Discharging effluents without proper treatments may have an adverse effect on the receiving water bodies (Kim et al., 2008). This brings a necessity to identify, characterize and evaluate the toxicants in various industrial effluents for the purpose of setting acceptable discharge levels.

Both the chemical-based approach and whole effluent toxicity (WET) assay can be used to assess and quantify the toxicity of industrial effluents (Sarakinos et al., 2000; Teodorovic et al., 2009). Unfortunately, by the chemical-based approach alone, we can only know the concentrations of individual compounds in the effluents (Sarakinos et al., 2000), which is not sufficient enough to assess ecological effects of toxic chemicals in industrial effluents (Burgess et al., 1995; Rosa et al., 2001). By WET testing alone, we can only measure the toxic effect of an effluent as a whole and account for uncharacterized sources of toxicity (Smolders et al., 2003), but it is difficult to identify the toxicants without chemical analysis of the pollutants (Fjällborg et al., 2006). Therefore there is a great need for methods to combine the chemical analysis and bioassay to evaluate the toxicity of effluents from different industries.

Toxicity Identification and Evaluation (TIE) methods developed by USEPA (1992, 1993a, 1993b), which are an integrated tool to determine what fraction of chemicals has caused the observed impacts on the bioassay, exactly meet the need. The methods have been widely used in determination of toxic constituents in environmental samples, and the utility of conducting TIE for characterizing, identifying and confirming key toxicants in a variety of industrial effluents has been realized (Jo et al., 2008; Mount and Hockett, 2000). TIE consisits of three phases: Phase I (Characterization) characterizes the physical and chemical natures of the constituents causing toxicity; Phase II (Identification) isolates and identifies the toxicants characterized in Phase I with the aid of chemical analytical techniques and toxicity evaluation (Hogan et al., 2005) and Phase III (Confirmation) confirms the true toxicant responsible for the toxicity (USEPA, 1993b). For the examination of WET and TIE, various aquatic species can be selected as the test species, such as *Daphnia magna*, *Pseudokirchneriella subcapitata* (Deanovic et al., 1999), *Danio rerio*, *Ceriodaphnia dubia* and *Pimephales promelas* (Fjällborg et al., 2006; Hogan et al., 2005; Isidori et al., 2003; Ra et al., 2007; Strom et al., 2009; USEPA, 1992).

The Pearl River Delta (PRD) region is one of the fastest ecomonic growth regions in China with various manufacturing industries including electronic, textile, paper making and fine chemical industries. With rapid economic development, large amounts of wastewaters are generated from these industries and discharged into the aquatic environments with an annual discharge of 417 million tonnes. This has resulted in serious water pollution problems since these effluents may contain toxic chemicals. It is essential to understand the toxicity and toxicants of these industrial effluents.

* Corresponding author. Fax: +86 20 85290200.
E-mail addresses: guangguo.ying@gmail.com, guang-guo.ying@gig.ac.cn (G.-G. Ying).

0147-6513/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2011.10.003

The aim of this study was to perform WET test using lux bacteria (*Escherichia coli* HB101 pUCD607), duckweed (*Lemna minor*), green algae (*P. subcapitata*), crustaceans (*D. magna* and *C. dubia*) and zebrafish (*D. rerio*) to evaluate the toxicity of representative effluents from textile and dyeing plants, electronic and electroplate factories, pulp and paper mills, fine chemical factories and municipal wastewater treatment plants in the PRD region. Considering that lux bacterial bioassay systems are particularly applicable to rapid toxicity testing on account of their ease of use, low cost and sensitivity to a wide range of pollutants (Tiensing et al., 2002), lux bacteria was selected to conduct the TIE toxicity test to identify the toxicity-causing substances. The results from the above studies could provide useful information for regulating and monitoring effluent discharge levels.

2. Materials and methods

2.1. Sample collection

Effluent samples were collected in the PRD region from fifteen wastewater treatment plants of five industries including municipal wastewater treatment plants, each industry category having three plants. Of the fifteen industrial effluent samples collected, samples S-1, S-2 and S-3 were from three textile and dyeing plants located in Guangzhou, samples S-4, S-5 and S-6 were from three pulp and paper mills in Dongguan, samples S-7, S-8 and S-9 were from three municipal wastewater treatment plants in Huizhou, samples S-10, S-11 and S-12 were from three electronic and electroplate factories in Huizhou and samples S-13, S-14 and S-15 were from three fine chemical factories in Heyuan. The location of these factories is shown in Fig. 1.

During the sampling campaign, 5 L of each effluent was collected from each plant every 15 min and they were mixed into a 24-h composite sample in a big container. We collected 50 L of each effluent from the mixing containers. It should be noted here that before sample collection, each bottle was pre-rinsed with effluents three times. These samples were transported in coolers to the laboratory and promptly stored at 4 °C. Of the 50 L collected effluents, 20 L were used for baseline toxicity tests, 20 L for TIE treatment and 10 L for *in vitro* assays and chemical analysis after solid-phase extraction (SPE).

2.2. Whole effluent toxicity bioassay

2.2.1. C. dubia acute lethality test

The 48-h acute lethality test for *C. dubia* was conducted following the standard methods outlined by Environment Canada (2007). Briefly, each sample was two-fold diluted using Diluted Mineral Water (DMW) in five series, and the test concentration series were 100, 50, 25, 12.5 and 6.25 percent. DMW was prepared

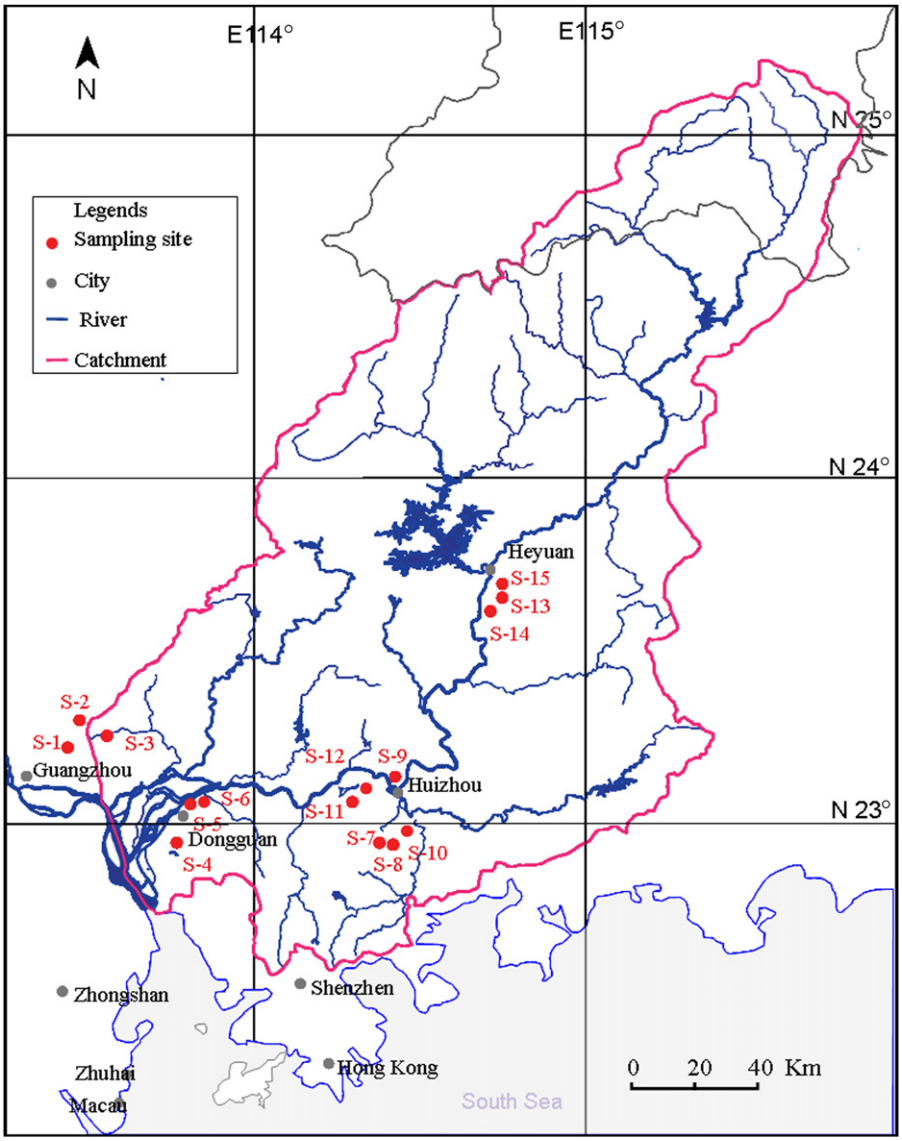

Fig. 1. Sketch map of sampling sites for various effluents in the Pearl River Delta region. S-1, S-2 and S-3 were textile and dyeing plants located in Guangzhou; S-4, S-5 and S-6 were pulp and paper mills in Dongguan; S-7, S-8 and S-9 were municipal wastewater treatment plants in Huizhou; S-10, S-11 and S-12 were electronic and electroplate factories in Huizhou and S-13, S-14 and S-15 were fine chemical factories in Heyuan.

according to the method described by Kszos and Stewart (2003). Four replicates of five neonates of 24-h-old per vessel were used for each concentration and for the control. Exposure experiments were conducted in 50-ml glass beakers containing 20 ml of test solution. Mortality, defined as lack of movement after gentle prodding, was recorded at 24-h and 48-h intervals.

2.2.2. D. magna acute lethality test

Based on the standard methods described in Environment Canada (1990), *D. magna* 48-h acute lethality test was performed with a similar procedure as mentioned in *C. dubia* acute lethality test. But it should be noted here that the dilution water was Moderately Hard Water (MHW) instead of DMW water. MHW was prepared in deionized water by adding the following salts on a per liter basis: $NaHCO_3$ (96 mg), $CaSO_4 \cdot 2H_2O$ (60 mg), $MgSO_4$ (60 mg) and KCl (4 mg), which was described by Yang et al. (2006).

2.2.3. L. minor growth inhibition test

The duckweed growth inhibition test was performed in accordance with the methods recommended by Organization for Economic Cooperation and Development (OECD, 2006a). The testing procedures were given as follows. Effluent, after being added ten-fold SIS medium (the Swedish standard *L. minor* growth medium) with the proportion of 1:9 (v/v, culturing medium:effluent), was diluted on a dilution factor two using SIS media in five series, and the final test concentration series were 90, 45, 22.5, 11.25 and 5.63 percent. Then 10 ml prepared test solution was added to six vials of each concentration, four for replicates and two for pH determination after the test was terminated. A three-frond plant was transferred into each vial, including pH vials. The vials were randomly placed into an incubation cabinet at 24 ± 2 °C. Also, the incubation was maintained on a continuous fluorescent light cycle (cool white light at 60–80 μmol photons/s/m^2). The number of fronds of each replicate vial was counted on day 2, day 5 and day 7.

2.2.4. D. rerio acute lethality test

The *D. rerio* 96-h acute lethality test was carried out according to the procedure described in ISO (1996) with a few modifications. Briefly, the sample was diluted to five concentrations (100, 80, 60, 40 and 20 percent) with standard dilution water, having triplicates for each concentration and blank control. Five healthy zebrafish with a length of 30 ± 5 mm and a weight of 0.3 ± 0.1 g were transferred to each glass 2-L beaker containing 1.5 L test solution. Mortality was recorded at 24 h, 48 h and 96 h intervals.

2.2.5. Green alga growth inhibition test

The green alga (*P. subcapitata*) 72-h growth inhibition test was conducted in terms of OECD (2006b) method with a few modifications. In brief, the sample, after being added ten-fold USEPA medium with the proportion of 1:9 (v/v, culturing medium:effluent), was two-fold diluted using USEPA medium (without EDTA) in five series, and the final test concentration series were 90, 45, 22.5, 11.25 and 5.63 percent. Then 50 ml of the prepared test solution was transferred into 3 flasks of each concentration; the same volume of prepared green algae cell suspension was added into each flask to an initial cell concentration of approximately 1×10^4 cells/ml. The flasks were placed randomly and incubated at 24 ± 1 °C under continuous illumination (4000 lux, cool white fluorescence) in an incubator for 72 h. The final cell yield after 72 h exposure was determined by measuring the optical density of the cell suspension at a wavelength of 430 nm using a multi-functional microplate reader (FLUOstar Omega, BMG LABTECH, Germany) and then the biomass was calculated using a linear relationship. Percentage inhibition of algal growth was calculated and compared with the control.

Toxicity of industrial effluents to green algae was measured based on cell yield. Percentage inhibition of algae growth was calculated using the following equation:

$$I = \frac{Rc - R}{Rc} \times 100$$

where I is the percentage inhibition of algae growth for each test concentration replicate, Rc is the mean cell yield for the control and R is the cell yield for each test concentration replicate.

2.2.6. Lux bacteria toxicity test

The lux bacteria toxicity test was performed in accordance with the methods described by Preston et al. (2000). The test organism used was *E. coli* HB101 pUCD607, which had been genetically modified to contain the plasmid pUCD607, which encodes the lux CDABE genes from *Vibrio fischeri* under the control of the tetracycline resistance promoter.

Strains for the test were prepared by growing cells in LB (Luria–Bertani) broth containing 30 mg/L kanamycin at 25 °C and shaking for about 18 h until late log phase. Late log phase was reached after inoculation and was determined by measurement of optical density at 550 nm (OD=1) and light output (1.4×10^6 relative light units [RLUs]). The cultures were stored at 4 °C for later use within 2–3 days. When required, 30 ml cultures were centrifuged at the speed of 2000*g* at 4 °C for 40 min, and the supernatant was discarded. Prior to the test, the strains were resuscitated for 10 min in 10 ml of 0.1 M KCl at 25 °C.

The effluent, after 1 M KCl with the proportion of 1:9 (v/v, KCl:effluent) being added, was two-fold diluted by 0.1 M KCl in six series and they were 90, 45, 22.5, 11.25, 5.63 and 2.82 percent. Then 200 μL of each test solution was pippetted into a white 96-well microplate. The bioassay was carried out in triplicate for each concentration, blank control and Zn reference test. Blank control and Zn standard curve were also included in each microplate. Then 50 μl resuscitated strains were transferred to a microplate filled with test solution. The bioluminescence, after being exposed for 5 min and 15 min, was measured using a BMG microplate reader (BMG Lab technologies, Offenburg, Germany) and the toxicity response was expressed as a reduction percentage of relative light units (RLUs) which was calculated as:

$$R = \frac{Lc - L}{Lc} \times 100$$

where R is a reduction percentage of the relative light units (RLUs) using *E. coli* HB101 pUCD607 for each test concentration, Lc is the mean relative light units (RLUs) for the control and L is the RLUs for each test concentration.

In TIE toxicity assay, the toxicity of baseline whole effluent and all treated effluents were also determined using the lux bacteria, three replicates for each treatment, blank control and treatment control (0.1 M KCl solution treated with the same method as the effluent samples). Baseline whole effluent toxicity test was repeated each time when additional manipulation tests were performed. The test procedure was exactly the same as described above.

2.3. TIE procedures

2.3.1. TIE manipulation on effluents

Upon the arrival of the samples at the laboratory, water quality parameters of industrial effluents, such as pH, conductivity, dissolved oxygen and whole effluent toxicity, were measured. The physiochemical characteristics of the collected effluents are shown in Table 1. Meanwhile, the effluents were treated with the procedure shown in Fig. 2. The methods for each treatment are described in detail as follows: (1) *Aeration*: 50 ml effluents were aerated at a flow rate of 200 ml/min for 12 h. The air passed through the filtrated membrane to remove bacteria; (2) *EDTA addition*: EDTA was added to the effluents at the concentration of 30 mg/L, then the solution was mixed and then kept in a stable state for 12 h; (3) *Sodium thiosulfate* (*STS*) *addition*: $Na_2S_2O_3$ was added to each effluent at the concentration of 30 mg/L, then the solution was mixed and kept in a stable state for 12 h; (4) *Filtration*: 2.5 L of each effluent was filtered through pre-baked glass fiber filters (GF/F, Whatman 0.45 μm); (5) *Solid phase extraction* (*SPE*) *over C18 column*: 1 L of each filtered effluent was passed through a C18 cartridge at a flow rate of 5 ml/min under vacuum (with two replicates), and 200 ml of the effluent was collected after the original effluent was passed through the C18 column for 5 min; (6) *Filtration and EDTA addition*: 25 ml of each filtered effluent was treated as in

Table 1
Water quality parameters of industrial effluents.

Effluent[a]	TD			P			MW			E			FC		
Sample	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10	S-11	S-12	S-13	S-14	S-15
pH	7.19	6.84	7.02	7.99	7.6	7.78	6.53	6.97	6.83	7.11	7.06	8.14	8.14	7.56	7.48
Cond.[b] (μs/cm)	2685	861	882	381	1245	273	361	408	470	1386	1410	4310	300	238	324
DO[c] (mg/L)	1.83	1.58	2.05	2.47	2.73	1.09	1.58	4.39	2.7	6.6	5.8	2.62	5.1	6	3
NH_4^+–N (mg/L)	1.06	3.34	6.25	14	2.57	6.9	1.87	1.6	0.71	15.35	2.42	79.9	2.89	LOD	9.39

[a] TD: textile and dyeing plants; P: pulp and paper mills; MW: municipal wastewater treatment plants; E: electronic and electroplate factories and FC: fine chemical factories.
[b] Cond.: conductivity.
[c] DO: dissolved oxygen.

treatment 2; (7) *Filtration and sodium thiosulfate* (*STS*) *addition*: 25 ml of each filtered effluent was treated as in treatment 3; (8) *Solid phase extraction* (*SPE*) *over C18 column and EDTA addition*: 25 ml extracted effluent was treated as in treatment 2; (9) *Solid phase extraction* (*SPE*) *over C18 column and sodium thiosulfate* (*STS*) *addition*: 25 ml extracted effluent was treated as in treatment 3.

2.4. Chemical analysis

The content of ammonia in industrial effluents was measured by Nessler's reagent colorimetric method (MEP China, 2009). The concentrations of metals (Fe, Al, Cd, Pb, Cr, Cu, Zn, As, Ni, Mn, As, Se, Sn, Sb, Ag and Hg) in the collected effluents were analyzed by inductively coupled plasma mass spectrometry (ICP-MS: ELAN 6000, PerkinElmer Co., Ltd., USA). The concentrations of endocrine disrupting chemicals (EDCs) were determined in accordance with the method reported by Zhao et al. (2009) using gas chromatography–mass spectrometry under negative chemical ionization mode (GC-NCI-MS: Agilent 6890 N gas chromatograph connected to an Agilent 5975B mass spectrometer with a chemical ionization source). Polycyclic aromatic hydrocarbons (PAHs) in the effluents were determined using the method recommended by Barco-Bonilla et al. (2009). Identification and quantification of the dioxins (PCDD/Fs), polychlorinated benzophenyls (PCBs) and polybrominated diphenylethers (PBDEs) were carried out using a GC/MS (Agilent 6890 N-5975B, Agilent Ltd., USA) according to the methods reported by Moon et al. (2008).

2.5. Data analysis

The median lethal concentration (LC50) value of effluents on *D. magna*, *C. dubia* and *D. rerio* was calculated by probit analysis with their 95 percent confidence intervals using the software SPSS 16.0. EC50 values (median effect concentration) of industrial effluents on lux bacteria, duckweed and green algae were calculated using EC50 calculator program developed by the Commonwealth Scientific and Industrial Research Organisation (CSIRO, Adelaide, Australia) (Fortunati et al., 2005). Means and standard deviations were calculated with Microsoft Excel 2003. One-way ANOVA was used to compare the significant difference ($p < 0.05$) between the toxicity data of the whole effluents without any treatment and the toxicity data of the effluents with various treatments by software SPSS 16.0. The toxic unit (TU) was calculated by the formula: TU=100/LC50 or TU=100/EC50. If there is no significant difference for the lethal rate or inhibitor rate between the sample with 100 percent concentration and the blank control, the TU of the sample can be regarded as 0 (Ra et al., 2008).

For TIE bioassay, the curve of the Zn standard control test on the lux bacteria can be fitted using a sigmoid equation (Hill equation), which can be fitted using Origin 7.5. The equation is

$$R = R_{max} - \frac{R_{max} - R_{min}}{[1 + (C/EC50)^p]}$$

where R_{max} is the maximum value that the curve infinitely closes to, R_{min} is the minimum value that the curve closes to, p is Hill slope, EC50 is 50 percent effective concentrations and C is the concentration of the sample.

The Zinc equivalent concentration (*ZnEQ*) of each treatment was calculated by the Hill equation of the curve of the Zn standard control test. The toxic units of each treatment can be calculated by the following equation:

$$TUs = \frac{ZnEQ}{EC50_{Zn}}$$

where *ZnEQ* refers to Zinc equivalent concentration of each treatment and $EC50_{Zn}$ is 50 percent effective of Zn concentrations.

2.6. Quality assurance and quality control

All data generated from both the bioassay and chemical analysis were subjected to strict quality control procedures. As for chemical analysis, with each set of samples analyzed, a solvent blank, a standard and a procedure blank were run in sequence to check for background contamination, peak identification and quantification. In addition, surrogate standards were added to all the samples to monitor matrix effects. As for bioassay, with each set of samples analyzed, blank control and reference reagent have to be included to test the stability of test species and the experimental environment. As for TIE procedures, baseline test, blank control and manipulation control have to be included to investigate the effect of TIE manipulations on samples.

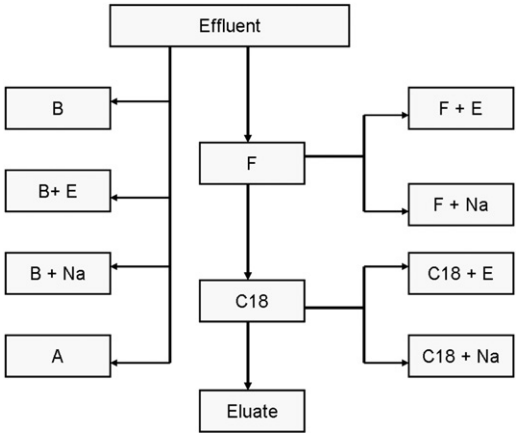

Fig. 2. Scheme of effluent manipulation for TIE Phase I. B: whole effluent without any treatment; A: aeration treatment; B+E: EDTA addition treatment; B+Na: sodium thiosulfate addition treatment; F: filtration treatment; F+E: filtration and EDTA addition treatment; F+Na: filtration and sodium thiosulfate addition treatment; EW: extracted water; C18: C18 solid phase extraction treatment; C18+E: C18 solid phase extraction and EDTA addition treatments; C18+Na: C18 solid phase extraction and sodium thiosulfate addition treatment.

3. Results

3.1. Whole effluent toxicity of industrial effluents

The toxicity of effluents from the electronic and electroplate factories was significantly higher than that of another four kinds of industrial effluents (Table 2). Different species have different sensitivities to the same kind of industrial effluent. Green alga (*P. subcapitata*) was the most sensitive to the effluents from the electronic and electroplate factories, and followed by *C. dubia*. Lux bacteria (*E. coli* HB101 pUCD607) showed a much higher sensitivity to the effluents from textile and dyeing factories, pulp and paper factories, fine chemical factories and municipal wastewater treatment plants than the other test species. As for the effluents from the textile and dyeing factories, *D. magna*, *C. dubia* and duckweed also showed a relatively high sensitivity. On the whole, lux bacteria (*E. coli* HB101 pUCD607) showed a high sensitivity to all kinds of effluents. Moreover, lux bacteria was also the test species, which showed little variability in sensitivity among all kinds of effluents. Therefore, it was selected as the test species for TIE manipulations.

Table 2
Acute toxicity units of industrial effluents to test species.

Effluent[a]	*D. rerio*	*C. dubia*	*D. magna*	*E. coli* HB101 pUCD607	*P. subcapitata*	*L. minor*
TD	0.00 ± 0.00[b]	1.09 ± 1.43	0.90 ± 1.01	1.64 ± 0.24	0.72 ± 0.92	1.35 ± 0.91
P	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.78 ± 0.13	0.00 ± 0.00	0.23 ± 0.40
MW	0.15 ± 0.26	0.29 ± 0.30	0.26 ± 0.26	1.99 ± 0.25	0.00 ± 0.00	0.00 ± 0.00
E	2.54 ± 3.87	3.92 ± 3.82	2.85 ± 2.59	3.02 ± 0.69	4.80 ± 2.20	3.70 ± 4.00
FC	0.00 ± 0.00	0.15 ± 0.25	0.11 ± 0.18	1.53 ± 0.19	0.46 ± 0.8	0.11 ± 0.20

[a] TD: textile and dyeing plants; P: pulp and paper mills; MW: municipal wastewater treatment plants; E: electronic and electroplate factories and FC: fine chemical factories.
[b] Mean ± STD (standard deviation) (n=3).

3.2. Toxicity identification evaluation for industrial effluents

3.2.1. Toxicity identification for effluents from textile and dyeing factories

Compared to the baseline whole effluent toxicity, the toxicity of effluents from textile and dyeing plants (S-1, S-2 and S-3), after the treatment of C18 SPE column, was decreased by 0.60, 0.97 and 0.80 TUs, respectively, which were all higher than 50 percent of whole effluent toxicity (Fig. 3). The toxicity of eluates was all nearly 0.4 TUs. For the samples S-1 and S-3, compared with the toxicity of whole effluent without any treatment, only the C18 SPE treatment group (C18, C18+E and C18+Na) elicited significant toxicity reduction, while the other treatments did not demonstrate significant toxicity change. As for the sample S-2, the following manipulations, aeration treatment, EDTA addition treatment, sodium thiosulfate addition treatment and filtration treatment, contributed nearly 20–40 percent of toxicity reduction. This indicated that non-polar organic pollutants contributed a large percentage to the toxicity of effluents from the textile and dyeing factories. It should be noted here that metals, oxidative and volatile substances contributed a small percentage to the effluent toxicity.

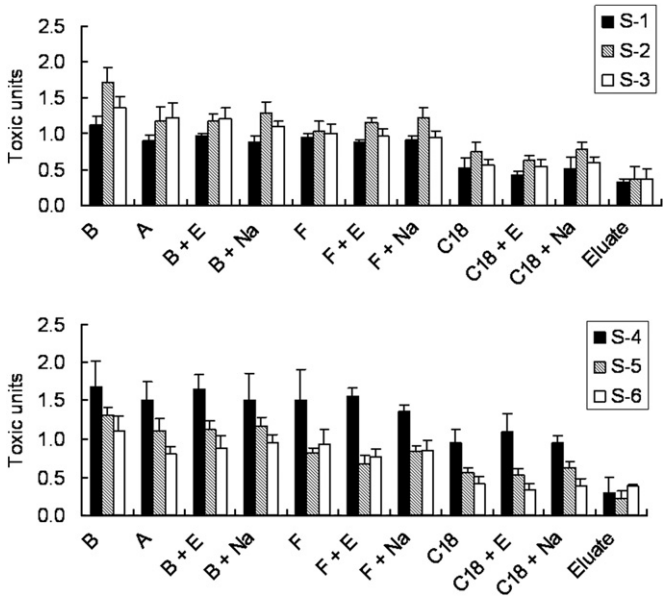

Fig. 3. Toxicity unit response of effluents from textile and dyeing factories (S-1, S-2 and S-3) and from pulp and paper mills (S-4, S-5 and S-6) with various treatments determined by lux bacteria *E. coli* HB101 pUCD607. Error bars represent standard deviations of the measurements ($n=3$). B: whole effluent without any treatment; A: aeration treatment; B+E: EDTA addition treatment; B+Na: sodium thiosulfate addition treatment; F: filtration treatment; F+E: filtration and EDTA addition treatment; F+Na: filtration and sodium thiosulfate addition treatment; C18: C18 solid phase extraction treatment; C18+E: C18 solid phase extraction and EDTA addition treatments; C18+Na: C18 solid phase extraction and sodium thiosulfate addition treatment.

The concentrations of some target compounds including EDCs, PCBs, PAHs, PCDD/Fs, PBDEs and metals in the industrial effluents are listed in Tables 3–5. As we can see in Table 5, only the concentrations of Cr and Se in the sample S-1 were slightly higher than the Chinese water quality standards for class I (WQS). The concentrations of Fe and Mn in the sample S-2 were approximately two and four times higher than the WQS, respectively. In the sample S-3, the concentrations of Mn and Ni were approximately twelve and six times higher than the WQS, respectively. However, the toxicity of Mn, Ni and Fe was very low as demonstrated by the lux bacteria toxicity tests. Thus the metals may, to some degree, cause some toxicity, but not much, which is in agreement with the toxicity change in TIE treatment. GC–MS scan of the eluates of all the samples showed the characteristic peaks of 4-nonylphenol (4-NP), 4-nonylphenol-ethoxylate (NPEOs), phthalates (PAEs) and aniline. The concentrations of 4-NP in the samples S-1, S-2 and S-3 reached 23.0, 129 and 86.6 μg/L, respectively (Table 3). The concentrations of both phthalates (PAEs) and aniline were estimated to be higher than 1.0×10^4 μg/L in the effluents. This indicated that the organic compounds including 4-NP, NPEOs, PAEs and aniline were mainly responsible for the toxicity of effluents from textile and dyeing industry.

Table 3
Concentrations of endocrine disrupting chemicals in industrial effluents.

Effluent[a]	Sample	Compounds[b] (μg/L)					
		4-t-OP ($\times10^{-2}$)	4-NP	BPA	E1 ($\times10^{-3}$)	E2 ($\times10^{-3}$)	TCS ($\times10^{-3}$)
TD	S-1	0.61 ± 0.22[c]	23.0 ± 1.05	0.52 ± 0.07	ND[d]	ND	2.20 ± 0.00
	S-2	17.0 ± 4.94	1.29 ± 17.5	1.33 ± 0.05	1.62 ± 0.04	ND	3.82 ± 0.23
	S-3	7.13 ± 0.90	86.6 ± 5.30	0.47 ± 0.00	5.57 ± 0.12	ND	2.74 ± 1.73
P	S-4	0.08 ± 0.21	1.09 ± 0.10	0.49 ± 0.06	1.02 ± 0.13	ND	5.57 ± 0.70
	S-5	0.72 ± 0.50	1.76 ± 0.27	1.66 ± 0.05	ND	< LOQ[e]	4.51 ± 1.59
	P-6	2.14 ± 1.79	6.63 ± 0.46	0.98 ± 0.01	1.4 ± 0.16	ND	1.46 ± 0.26
MW	S-7	0.03 ± 0.11	1.11 ± 0.31	0.37 ± 0.00	4.56 ± 0.21	< LOQ	117 ± 1.39
	S-8	0.02 ± 0.15	1.03 ± 0.15	0.66 ± 0.01	5.25 ± 0.35	< LOQ	132 ± 1.32
	S-9	0.04 ± 0.05	2.41 ± 0.26	0.75 ± 0.07	3.85 ± 0.48	ND	58.4 ± 1.87
E	S-10	3.21 ± 1.63	7.33 ± 0.53	1.04 ± 0.14	ND	ND	< LOQ
	S-11	< LOQ	8.06 ± 2.53	0.50 ± 0.00	ND	ND	< LOQ
	S-12	0.39 ± 1.20	16.5 ± 1.21	0.57 ± 0.03	ND	ND	9.17 ± 1.34
FC	S-13	0.02 ± 0.06	1.23 ± 0.14	0.71 ± 0.03	2.96 ± 3.17	11.6 ± 0.00	2.90 ± 0.00
	S-14	0.32 ± 0.90	9.43 ± 1.91	0.55 ± 0.08	7.81 ± 6.02	1.80 ± 0.00	8.76 ± 7.54
	S-15	6.29 ± 11.6	58.5 ± 37.3	0.57 ± 0.06	21.9 ± 3.37	4.23 ± 0.30	28.6 ± 2.86
	LOQ	0.1	0.007	0.002	0.5	1	0.5

[a] TD: textile and dyeing plants; P: pulp and paper mills; MW: municipal wastewater treatment plants; E: electronic and electroplate factories and FC: fine chemical factories.
[b] 4-t-OP: 4-tert-octylphenol; 4-NP: 4-nonylphenol; BPA: bisphenol-A; TCS: triclosan; E1: estrone and E2: 17β-estradiol.
[c] Mean ± STD (standard deviation) ($n=2$).
[d] Not detected.
[e] Method limit of quantitation.

Table 4
Concentrations and TEQs of PCBs, PAHs, PCDD/Fs and PBDEs in all industrial effluents.

Effluent[a]	Sample	PCBs[b] (μg/L)		PAHs[c] (μg/L)		PCDD/Fs[d] (μg/L)		∑PBDEs[e] ($\times 10^{-4}$) (μg/L)
		∑PCBs ($\times 10^{-3}$)	TEQ ($\times 10^{-8}$)[f]	∑PAHs	TEQ ($\times 10^{-3}$)	∑PCDD/Fs ($\times 10^{-5}$)	TEQ ($\times 10^{-6}$)	
TD	S-1	0.41	1.76	0.12	0.26	0.33	0.59	0.85
	S-2	1.67	6.31	0.08	0.28	0.65	0.76	5.86
	S-3	0.59	4.79	0.19	1.27	0.42	0.68	4.16
P	S-4	0.2	0.26	0.30	1.11	4.21	0.76	1.97
	S-5	1.17	3.00	0.23	5.14	5.81	0.99	8.74
	P-6	0.24	1.87	0.14	0.57	2.94	2.25	0.71
MW	S-7	0.16	0.09	0.09	0.34	2.07	0.14	4.12
	S-8	0.07	1.93	0.17	0.49	0.74	0.17	0.70
	S-9	0.08	0.06	0.19	0.49	4.86	0.39	0.96
E	S-10	1.54	0.06	0.18	0.33	0.43	0.56	1.52
	S-11	0.25	0.04	0.23	0.56	0.34	0.24	0.55
	S-12	0.11	0.06	0.20	0.55	0.21	0.19	1.08
FC	S-13	0.24	0.05	0.19	0.48	0.25	0.20	4.42
	S-14	0.07	0.06	0.13	0.17	0.14	0.12	0.04
	S-15	0.09	0.10	0.29	1.11	2.23	0.15	6.79

[a] TD: textile and dyeing plants; P: pulp and paper mills; MW: municipal wastewater treatment plants; E: electronic and electroplate factories and FC: fine chemical factories.
[b] Nine indicative polychlorinated biphenyls and twelve dioxin-like polychlorinated biphenyls.
[c] Sixteen US EPA priority polycyclic aromatic hydrocarbons.
[d] Seven 2,3,7,8-polychlorinated dibenzodioxin (4–8 chlorines substituents) and ten polychlorinated dibenzofuran (4–8 chlorines substituents).
[e] Polybrominated diphenylethers (1–10 bromine substituents).
[f] Toxic equivalent concentrations (TEQs).

Table 5
Concentrations of dissolved metals in all industrial effluents.

Effluent[a]	Sample	Metals (μg/L)												
		Al	Cr	Fe	Mn	Ni	Cu	Zn	As	Cd	Pb	Se	Sn	Sb
TD	S-1	47.6 ± 1.4[b]	14 ± 0.6	96.0 ± 3.7	16.3 ± 0.6	1.8 ± 0.1	5.31 ± 0.1	9.2 ± 0.2	2.7 ± 0.0	0.03 ± 0.0	1.0 ± 0.0	12.7 ± 0.6	0.3 ± 0.0	2.2 ± 0.1
	S-2	20.1 ± 1.5	6.6 ± 0.3	576 ± 22.5	432 ± 25.9	2.4 ± 0.0	8.5 ± 0.7	25.4 ± 1.1	2.3 ± 0.1	0.05 ± 0.0	1.7 ± 0.1	8.6 ± 0.5	11.9 ± 0.6	0.3 ± 0.0
	S-3	89.8 ± 7.8	4.9 ± 0.1	40.3 ± 2.0	1280 ± 47.2	130 ± 0.1	8.1 ± 0.4	16.5 ± 0.2	1.8 ± 0.2	0.07 ± 0.0	1.6 ± 0.1	5.8 ± 0.7	0.1 ± 0.0	5.8 ± 0.1
P	S-4	0.03 ± 0.0	0.6 ± 0.0	5.3 ± 0.2	0.07 ± 0.0	ND[c]	ND	0.02 ± 0.0	0.1 ± 0.0	ND	ND	0.3 ± 0.0	ND	ND
	S-5	109 ± 2.6	2.5 ± 0.1	54.8 ± 2.9	62.9 ± 2.3	5.3 ± 0.1	3.2 ± 0.2	9.9 ± 0.3	1.7 ± 0.0	0.09 ± 0.0	1.0 ± 0.0	2.4 ± 0.2	0.2 ± 0.0	1.6 ± 0.0
	P-6	122 ± 4.4	4.6 ± 0.1	101 ± 2.5	51.1 ± 1.9	11.9 ± 0.5	4.7 ± 0.2	17.5 ± 0.1	2.6 ± 0.2	0.05 ± 0.0	1.3 ± 0.0	4.8 ± 0.3	0.2 ± 0.0	0.9 ± 0.0
MW	S-7	21.5 ± 0.8	3.6 ± 0.1	120 ± 7.2	127 ± 3.7	11.6 ± 0.7	52.3 ± 1.1	52.2 ± 3.0	10.5 ± 0.4	0.16 ± 0.0	25.7 ± 2.0	4.3 ± 0.4	0.3 ± 0.0	0.5 ± 0.0
	S-8	15.9 ± 3.1	3.1 ± 0.2	61.6 ± 1.5	115 ± 4.2	7.7 ± 0.1	3.3 ± 0.1	35.5 ± 0.5	10.4 ± 0.7	0.07 ± 0.0	1.4 ± 0.0	3.7 ± 0.2	0.2 ± 0.0	0.4 ± 0.0
	S-9	11.3 ± 0.8	6.8 ± 0.2	30.1 ± 2.1	81.5 ± 1.2	21.6 ± 0.7	5.6 ± 0.2	20.5 ± 1.1	3.8 ± 0.1	0.26 ± 0.0	0.4 ± 0.0	5.2 ± 0.4	0.2 ± 0.0	0.6 ± 0.0
E	S-10	140 ± 7.9	2.4 ± 0.1	436 ± 30.9	13.7 ± 0.6	5.4 ± 0.1	455 ± 8.7	7.1 ± 0.2	1.1 ± 0.0	0.02 ± 0.0	0.6 ± 0.0	5.3 ± 0.1	8.0 ± 0.0	0.3 ± 0.0
	S-11	542 ± 20.0	16.7 ± 1.9	51.2 ± 2.1	2.5 ± 0.1	143 ± 3.9	9.8 ± 0.5	15.7 ± 0.6	2.5 ± 0.0	0.05 ± 0.0	1.4 ± 0.1	12.4 ± 1.3	0.4 ± 0.1	0.3 ± 0.0
	S-12	189 ± 12.3	50.4 ± 0.6	72.7 ± 2.5	241 ± 8.0	469 ± 28.6	87.9 ± 0.3	1150 ± 17.2	5.2 ± 0.1	0.04 ± 0.0	1.1 ± 0.0	19.2 ± 0.3	1.1 ± 0.0	2.1 ± 0.0
FC	S-13	93.1 ± 2.4	6.0 ± 0.1	99.6 ± 5.0	16.3 ± 0.2	0.9 ± 0.0	1.4 ± 0.1	96.3 ± 3.3	3.7 ± 0.2	0.05 ± 0.0	1.3 ± 0.0	3.6 ± 0.0	0.8 ± 0.1	0.3 ± 0.0
	S-14	90.1 ± 2.9	5.0 ± 0.1	39.7 ± 2.8	1.5 ± 0.0	1.5 ± 0.0	3.5 ± 0.1	5.0 ± 0.4	1.5 ± 0.0	0.04 ± 0.0	0.8 ± 0.0	2.7 ± 0.2	0.1 ± 0.0	0.2 ± 0.0
	S-15	27.9 ± 1.7	5.2 ± 0.23	459 ± 18.4	174 ± 3.8	1.9 ± 0.1	3.2 ± 0.1	20.3 ± 0.2	2.6 ± 0.1	0.05 ± 0.0	0.9 ± 0.0	2.8 ± 0.2	0.1 ± 0.0	0.7 ± 0.0
WQS[d]		≤ 200	≤ 10	≤ 300	≤ 100	≤ 20	≤ 10	≤ 50	≤ 50	≤ 1	≤ 10	≤ 10		≤ 5
EC50[e]		64 000	6500	2.6×10^5	$> 2 \times 10^6$	2750	490	620		509	389	$> 5 \times 10^6$		
LOD[f]		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

[a] TD: textile and dyeing plants; P: pulp and paper mills; MW: municipal wastewater treatment plants; E: electronic and electroplate factories and FC: fine chemical factories.
[b] Mean ± STD (standard deviation) ($n=2$).
[c] Not detected.
[d] China's water quality standard for class I (GHZB 1-1999).
[e] Toxicity of metals on *E. coli* HB101 pUCD607 determined in the present study.
[f] LOD: method limit of detection.

3.2.2. Toxicity identification for effluents from pulp and paper mills

Fig. 3 shows that the toxicity of effluents from pulp and paper mills (S-4, S-5 and S-6) after being extracted by C18 SPE column, was significantly reduced by 0.75, 0.75 and 0.69 TUs, which were 44, 58 and 62 percent of baseline whole effluent toxicity, respectively. Aeration treatment, EDTA addition treatment, sodium thiosulfate addition treatment and filtration treatment did not produce an obvious toxicity change on the samples S-4 and S-6, but filtration treatment led to 38 percent toxicity reduction on the sample S-5. The toxicities of eluates of the samples S-4, S-5 and S-6 were 0.29, 0.23 and 0.38 TUs, respectively. This indicates that non-polar organics played a major role in the toxicity of effluents from

pulp and paper mills, while other pollutants such as metals, ammonia and oxidative materials did not contribute much to the whole effluent toxicity. For the sample S-5, some toxicity could be removed by filtration treatment, suggesting some contribution of suspended solids.

As shown in Table 5, the concentrations of metals in the effluents from pulp and paper industry were lower than the Chinese WQS, implying that the effluent toxicity from metals would be very low. It is in line with the slight toxicity change of EDTA treatment to the sample effluents. When scanning the SPE extracts of the samples S-4, S-5 and S-6 with GC–MS, some characteristic peaks of 4-NP, BPA, PAEs and some sterol derivatives were identified. With 4-NP quantitatively analyzed, the concentrations of 4-NP in the samples S-4, S-5 and S-6 were found up to 1.09, 1.76 and 6.63 μg/L, respectively (Table 3). The concentrations of BPA in the samples S-4, S-5 and S-6 were found up to 0.49, 1.66 and 0.98 μg/L, respectively. And those of PAEs and sterol derivatives in the effluents were estimated both higher than 1000 μg/L. Therefore, organic compounds including 4-NP, BPA, PAEs and sterol derivatives were most likely responsible for the toxicity of effluents from paper and pulp industry.

3.2.3. *Toxicity identification for effluents from municipal wastewater treatment plants*

The toxicity of effluents from municipal wastewater treatment plants (S-7, S-8 and S-9), after being extracted by C18 SPE column, was decreased by 0.59, 0.74 and 0.50 TUs, respectively. The toxicity reduction rate, compared to the whole effluent toxicity (Fig. 4), was 42, 44 and 31 percent, respectively. Besides, after these samples were added with EDTA or sodium thiosulfate, their toxicity was obviously reduced, too. The toxicity of eluates for the samples S-7, S-8 and S-9 was 0.23, 0.30 and 0.41 TUs, respectively. Therefore, we can conclude that the toxicity of municipal effluents was mainly attributed to non-polar organics followed by metals and oxidative substances.

Table 5 illustrates that the concentrations of Cu, Pb and Zn in sample S-7 were approximately five, three and one times higher than the China WQS, respectively, while the concentrations of other metals were all lower than the WQS. In the sample S-9, only the concentration of Ni was slightly higher than the WQS. It should be noted here that the concentrations of all the metals in the sample S-8 were below the WQS. The characteristic peaks for the toxicants such as PAEs, sterol derivatives and triclosan were found when scanning the effluent extracts using GC–MS and their concentrations were all above 1000 μg/L. The sterol derivatives in the municipal wastewater may originate from the feces of human beings (Chou and Liu, 2004). Chemical data indicate that Cu and Pb were contributing some percentages to the toxicity of effluents from municipal wastewater treatment plants. The organic toxicants in the samples, such as PAEs, sterol derivatives and triclosan, contributed more to the toxicity (about 20 percent).

3.2.4. *Toxicity identification for effluents from electronic and electroplate factories*

As shown in Fig. 5, the toxicity of the whole effluents from electronic and electroplate industry (S-10, S-11 and S-12), after the EDTA addition treatment, was reduced by 1.07, 0.57 and 1.49 TUs, respectively. And the toxicity reduction rate, compared to the baseline whole effluent toxicity, was 44, 35 and 67 percent, respectively. For those filtrated effluents, after the EDTA addition treatment, their toxicity had a tendency of a significant reduction. Clearly, metals predominantly accounted for the toxicity of effluents from the electronic and electroplate industry.

As for the samples S-10, S-11 and S-12, after being extracted by C18 SPE column, an obvious toxicity reduction was observed (Fig. 5). But there was no significant change in the effluent toxicity after the following manipulations: aeration treatment, sodium thiosulfate addition treatment and filtration treatment. The toxicity of the eluates of the samples S-10, S-11 and S-12 was 0.60, 0.19 and 0.27 TUs, respectively. Thus non-polar organics were another important source for the toxicity of these effluents.

As shown in Table 5, the concentration of Cu in the sample S-10 was 44.5 times as high as the WQS. The concentration of Fe was slightly higher than the WQS, while the concentrations of the other metals were all below the WQS. In the sample S-11, only the concentrations of Cr, Ni and Se were found approximately two, seven and one times as high as the WQS, respectively. In the sample S-12, the concentrations of Cr, Ni, Cu and Zn were much

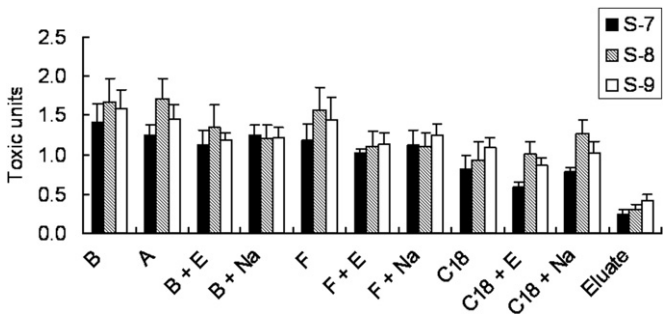

Fig. 4. Toxicity unit response of effluents from municipal wastewater treatment plants (S-7, S-8 and S-9) with various treatments determined by lux bacteria *E. coli* HB101 pUCD607. Error bars represent standard deviations of the measurements (n=3). B: whole effluent without any treatment; A: aeration treatment; B+E: EDTA addition treatment; B+Na: sodium thiosulfate addition treatment; F: filtration treatment; F+E: filtration and EDTA addition treatment; F+Na: filtration and sodium thiosulfate addition treatment; C18: C18 solid phase extraction treatment; C18+E: C18 solid phase extraction and EDTA addition treatments; C18+Na: C18 solid phase extraction and sodium thiosulfate addition treatment.

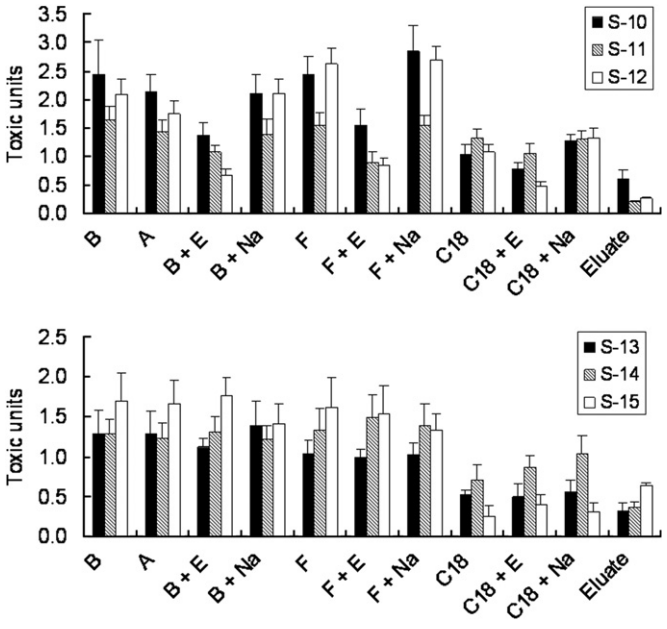

Fig. 5. Toxicity unit response of effluents from electronic and electroplate factories (S-10, S-11 and S-12) and from fine chemical factories (S-13, S-14 and S-15) with various treatments determined by lux bacteria *E. coli* HB101 pUCD607. Error bars represent standard deviations of the measurements (n=3). B: whole effluent without any treatment; A: aeration treatment; B+E: EDTA addition treatment; B+Na: sodium thiosulfate addition treatment; F: filtration treatment; F+E: filtration and EDTA addition treatment; F+Na: filtration and sodium thiosulfate addition treatment; C18: C18 solid phase extraction treatment; C18+E: C18 solid phase extraction and EDTA addition treatments; C18+Na: C18 solid phase extraction and sodium thiosulfate addition treatment.

higher than the WQS. The toxicity of Ni was comparatively lower than that of Cu, indicating that the toxicity of metals in the sample S-10 was higher than that in the samples S-11 and S-12, which is consistent with the toxicity change in TIE manipulations. It can be concluded that metals contributed most to the toxicity, with the contribution mainly from Cu, Ni and Zn. As for the samples S-10 and S-12, there was a significant toxicity reduction after being extracted by C18 SPE column. GC–MS scan of the extracts of S-10 and S-12 showed the characteristic peaks of NPEOs, 4-NP and 2-methylbenzene-1-sulfonamide and the concentrations of NPEOs were estimated higher than 1000 μg/L. The quantitative analysis found NP higher than 1 μg/L. The higher concentrations of NPEOs and 4-NP were related to their wide use in metal clean processing (Footitt et al., 1999). NPEOs were easily absorbed by C18 column, suggesting that the toxicity reduction of C18 column treatment was likely due to the fact that organic compounds NPEOs and NP were removed by C18 column.

3.2.5. Toxicity identification for effluents from fine chemical factories

The toxicity of effluents collected from fine chemical factories (S-13, S-14 and S-15), after being extracted by C18 SPE column, was significantly reduced and reached 0.75, 0.57 and 1.44 TUs, respectively (Fig. 5). The toxicity reduction rates for the three effluents was 59, 45 and 89 percent, respectively, when compared to the whole effluent toxicity. Compared with the baseline whole toxicity, no significant toxicity reduction was observed after the following treatments: aeration, filtration, EDTA addition and sodium thiosulfate addition. The toxicity of eluates for the samples S-13, S-14 and S-15 was 0.33, 0.37 and 0.64 TUs, respectively. Therefore, non-polar organics were mainly responsible for the toxicity of effluents from fine chemical industry.

Chemical analysis also showed that the concentrations of metals in the three effluents were lower than the WQS except for Zn in sample S-13 and Mn in sample S-15 (Table 5), which suggests that metals were not likely to contribute much to the effluent toxicity. It is consistent in line with the slight toxicity change of the effluent samples following EDTA treatment. GC–MS scan of the effluent extracts showed characteristic peaks of PAEs, BPA, NPEOs and 4-NP. The concentrations of 4-NP in the three effluents were found to reach 1.23, 9.43 and 58.5 μg/L, respectively (Table 3). And the concentrations of PAEs and NPEOs were all higher than 10,000 μg/L. Therefore, PAEs, NPEOs, BPA and 4-NP most likely contributed to the toxicity of effluents from the fine chemical industry. In the fine chemical factories, PAEs, NPEOs, BPA and 4-NP might be used as materials, products or by-products, and released into the effluents. Therefore, it is reasonable to assume that the concentrations of these chemicals were high in the effluents.

4. Discussion

The analytical results show that various contaminants including EDCs, PCBs, PAHs, PCDD/Fs, PBDEs and metals were present in the industrial effluents (Tables 3–5). Among the six representative EDCs analyzed, high concentrations were found at >1 μg/L for 4-nonylphenols (4-NP) and >0.36 μg/L for bisphenol-A (BPA) (Table 3). The highest concentration (129 μg/L) for 4-NP was detected in the effluent of textile and dyeing industry, while the highest concentration (1.66 μg/L) for BPA was detected in the effluent of paper and pulp mills.

Highest estrone (E1) and estradiol (E2) concentrations were detected in the effluents of fine chemical industry (having domestic effluent input), while that for triclosan (TCS) was found in the municipal effluents. These highest concentrations are above the predicated no-effect concentrations (PNEC) (4-t-OP: 0.12 μg/L; 4-NP: 1.12 μg/L; BPA: 1.5 μg/L; E1: 3×10^{-3} μg/L; E2: 1.5×10^{-3} μg/L and TCS: 5.8×10^{-2} μg/L) for these EDCs to aquatic organisms (Nwaogu et al., 2008; EC, 2002, 2010, 2007, 2009; Gross-Sorokin et al., 2007; Zhao et al., 2011, 2010; Maycock et al., 2007; EU, 2008) . Discharge of these effluents might affect the organisms in the receiving environments although the adverse effects will not be acute.

The concentrations of sixteen USEPA PAHs were converted into TEQ (toxic equivalent) of benzo[a]pyrene (B[a]P) according to the toxic equivalency factors (TEFs) of all PAHs (Petry et al., 1996). The TEQ of B[a]P in the industrial effluents were all far below 0.015 μg/L, which was the PNEC of B[a]P for aquatic organisms reported by von der Ohe et al. (2011). Therefore, we can assume that it is unlikely for PAHs to have an adverse effect on receiving water bodies as well as its ecosystem.

According to USEPA water quality criteria, 2,3,7,8-TCDD (USEPA, 1984) is no higher than 1.0×10^{-5} μg/L. The concentrations of PCDD/Fs were converted into TEQ of 2,3,7,8-TCDD, according to corresponding TEFs of 2,3,7,8-TCDD (Van den Berg et al., 1998); the TEQ-PCDD/Fs of industrial effluents were all far below 1.0×10^{-5} μg/L. Therefore, it is concluded that no observed acute toxicity was caused by PCDD/Fs in the industrial effluents.

The PNEC of PBDEs (TriBDE, HexaBDE and TriBDE) for water was 0.53 μg/L (European Chemicals Bureau, 2001), while the concentrations of PBDEs in all industrial effluents were far below the value, which is indicative of the fact that PBDEs in the effluents are unlikely to have significant adverse acute effects on the receiving environments.

It is regulated in USA Water Quality Criteria that the concentration of total PCBs in natural waters is no higher than 1.4×10^{-2} μg/L (USEPA, 2009). In the present study, the TEQ concentrations for PCBs were calculated using the TEFs provided by World Health Organization (WHO) in 1998 (Van den Berg et al., 2006). The TEQ concentrations of total PCBs in all industrial effluents were all far below 3×10^{-3} μg/L, indicating that it is unlikely for PCBs to have an adverse acute effect on receiving water bodies as well as its ecosystem.

Various metals were measured in the effluents of different industries (Table 5). Ag and Hg in all of those industrial effluents were below the limit of quantitation. However, the concentrations of the other metals higher than the Chinese water quality standards (class I, for drinking water source) were found in at least one effluent sample. Some effluent samples also had some metal concentrations more than the corresponding PNECs (Al: 55 μg/L; Fe: 300 μg/L; Mn: 100 μg/L; Ni: 17.2 μg/L; Cu: 7.8 μg/L; Zn: 6.1 μg/L and Cr: 3.4 μg/L) for these metals to aquatic organisms, suggesting high risks to aquatic organisms (CSIRO, 2008; EC, 2007, EU, 2008; Maycock et al., 2007).

In summary, due to the complexity of industrial effluents, the toxicity of effluents was not caused by a single toxicant, but by a mixture of various substances in the industrial effluents. This makes TIE confirmation phase very difficult to perform. The mixture effects could be synergistic, additive or antagonistic. There might be an interaction between different toxics. The existence of metals in industrial effluents may damage the cell membrane, which made it easier for non-polar organic contaminants to enter the cell and cause toxicity. Reinforcing the conclusion are the findings (Cabral, 1990; Lue-Kim et al., 1980), which showed some metals such as Cu and Cd may damage the cell membrane and result in an increase in the passage of Zn ions across the membrane. It was observed in the TIE experiment that the reduced toxic unit of an effluent sample after extraction by C18 column was far higher than the toxic unit of eluates, indicating that other substances such as metals were also a toxicity contributor in some effluents (*E. coli* HB101 pUCD607). The present study on TIE of five types of effluents demonstrates that organics are the predominant toxicants in the industrial effluents except for the effluents from the electronic and electroplate industry with metals as the dominant toxicants.

5. Conclusions

The six test species used in the present study had showed different toxicity responses to the industrial effluents. The effluents from electronic and electroplate industry had the highest toxicity to the test organisms among the five types of effluents. Lux bacteria were in general sensitive to all kinds of industrial effluents and also applied in TIE experiments of the fifteen industrial effluents. The toxicity of all industrial effluents was attributed to the mixture effect of various compounds, which was consistent with chemical analysis results. TIE manipulations demonstrated that the predominant toxicants in the effluents of five types of industries were various organic compounds except for the effluents from electronic and electroplate industry with metals as the dominant toxic substances. Metals and some oxidative substances also contributed to the toxicity of the effluents from textile and dyeing industry and municipal wastewater treatment plants.

Acknowledgments

We wish to acknowledge the financial support from National Water Research Project (2009ZX07528-001), the Chinese Academy of Sciences (KZCX2-EW-108), the National Natural Science Foundation of China (NSFC 40821003, 20977092 and 40688001), Guangdong Provincial Natural Science Foundation (8251064004000001) and the Earmarked Fund from the State Key Laboratory of Organic Geochemistry (SKLOG2009A02). This is a Contribution No. 1397 from GIG CAS.

References

Barco-Bonilla, N., Vidal, J.L.M., Frenich, A.G., Romero-Gonzalez, R., 2009. Comparison of ultrasonic and pressurized liquid extraction for the analysis of polycyclic aromatic compounds in soil samples by gas chromatography coupled to tandem mass spectrometry. Talanta 78, 156–164.

Burgess, R.M., Ho, K.T., Tagliabaue, M.D., Kuhn, A., Comeleo, R., Comeleo, P., Modica, G., Morrison, G.E., 1995. Toxicity characterization of an industrial and a municipal effluent discharging to the marine environment. Mar. Pollut. Bull. 30, 524–535.

Cabral, P.S.J., 1990. Cupric ions induce both an efflux of potassium and low-molecular mass metabolites in *Pseudomonas syringae*. FEMS Microbiol. Lett. 72, 109–112.

Chan, Y.K., Wong, C.K., Hsieh, D.P.H., Ng, S.P., Lau, T.K., Wong, P.K., 2003. Application of a toxicity identification evaluation for a sample of effluent discharged from a dyeing factory in Hong Kong. Environ. Toxicol. 18, 312–316.

Chou, C.C., Liu, Y.P., 2004. Determination of fecal sterols in the sediments of different wastewater outputs by GC–MS. Int. J. Environ. Anal. Chem. 84, 379–388.

CSIRO (Commonwealth Scientific and Industrial Research Organisation), 2008. Water Quality Screening Risk Assessment of Acid Sulfate Soil Impacts in the Lower Murray, SA. CSIRO Land and Water Science Report 45/08, Australia, pp. 1–156.

Deanovic, L., Connor, V.M., Knight, A.W., Maier, K.J., 1999. The use of bioassays and toxicity identification evaluation (TIE) procedures to assess recovery and effectiveness of remedial activities in a mine drainage-impacted stream system. Arch. Environ. Contam. Toxicol. 36, 21–27.

Environment Canada, 1990. Biological Test Method: Acute Lethality Test Using *Daphnia spp*. Ottawa, Canada.

Environment Canada, 2007. Biological Test Method: Test of Reproduction and Survival Using the *Cladoceran Ceriodaphnia dubia*. Ottawa, Canada.

European Chemicals Bureau, 2001. European Union Risk Assessment Report Diphenyl Ether, Pentabromo Derivative (Pentabromodiphenyl Ether), 1st Priority List, vol. 5.

EC (European Commission), 2002. European Union Risk Assessment Report: 4-nonylphenol (branched) and nonylphenol. European Union Risk Assessment Report. European Chemicals Bureau, pp. 1–224.

EC (European Commission), 2007. Risk Assessment Report on Zinc, Environmental Part. Scientific Committee on Health and Environmental Risks, Brussels, pp. 1–15.

EC (European Commission), 2009. Risk Assessment Report on Nickel and its Compounds, Environmental Part. Scientific Committee on Health and Environmental Risks. European Commission, Brussels, pp. 1–16.

EC (European Commission), 2010. European Union Risk Assessment Report: 4,4′-isopropylidenediphenol (bisphenol-A)—Complete risk assessment in one document. European Union Risk Assessment Report. European Chemicals Bureau, pp. 1–695.

EU (European Union), 2008. Voluntary Risk Assessment Of Copper, Copper II Sulphate Pentahydrate, Copper(I) Oxide, Copper(II)Oxide, Dicopper Chloride Trihydroxide. European Union Risk Assessment Report. European Copper Institute, Brussels, pp. 1–11.

Fjällborg, B., Li, B., Nilsson, E., Dave, G., 2006. Toxicity identification evaluation of five metals performed with two organisms (*Daphnia magna* and *Lactuca sativa*). Arch. Environ. Contam. Toxocol. 50, 196–204.

Footitt, A., Virani, S., Corden, C., Grahann, S., 1999. Nonylphenol Risk Reduction Strategy. Department of the Environment, Transport and the Regions, Risk & Policy Analyts Limited, Loddon, Norfolk, pp. 1–167.

Fortunati, P., Lombi, E., Hamon, R.E., Nolan, A.L., McLaughlin, M.J., 2005. Effect of toxic cations on copper rhizotoxicity in wheat seedlings. Environ. Toxicol. Chem. 24, 372–378.

Gomez, C.E., Contento, L., Carsen, A.E., 2001. Toxicity tests to assess pollutants removal during wastewater treatment and the quality of receiving waters in Argentina. Environ. Toxicol. 16, 217–224.

Gross-Sorokin, M.Y., Roast, S.D., Brighty, G.C., 2007. Assessment of feminization of male fish in English rivers by the environment agency of England and Wales. Environ. Health Perspect. 114, 147–151.

Hogan, A.C., Stauber, J.L., Pablo, F., Adams, M.S., Lim, R.P., 2005. The development of marine toxicity identification evaluation (TIE) procedures using the unicellular alga *Nitzschia closterium*. Arch. Environ. Contam. Toxicol. 48, 433–443.

Isidori, M., Lavorgna, M., Nardelli, A., Parrella, A., 2003. Toxicity identification evaluation of leachates from municipal solid waste landfills: a multispecies approach. Chemosphere 52, 85–94.

ISO (Organisation for Economic Co-operation and Development), 1996. Water Quality – Determination of the Acute Lethal Toxicity of Substances to a Freshwater Fish [*Brachydanio rerio* Hamilton–Buchanan (Teleostei, Cyprinidae)] – Part 1: Static method. ISO7346-1. ISO, Geneva, Switzerland.

Jo, H.J., Park, E.J., Cho, K., Kim, E.H., Jung, J., 2008. Toxicity identification and reduction of wastewaters from a pigment manufacturing factory. Chemosphere 70, 949–957.

Kim, E.H., Jun, Y.R., Jo, H.J., Shim, S.B., Jung, J.H., 2008. Toxicity identification in metal plating effluent: implications in establishing effluent discharge limits using bioassays in Korea. Mar. Pollut. Bull. 57, 637–644.

Kszos, L.A., Stewart, A.J., 2003. Review of lithium in the aquatic environment: distribution in the United States, toxicity and case example of groundwater contamination. Ecotoxicology 12, 439–447.

Lah, B., Gorjanc, G., Nekrep, F.V., Marinsek-Logar, R., 2004. Comet assay assessment of wastewater genotoxicity using yeast cells. Bull. Environ. Contam. Toxicol. 72, 607–616.

Lue-Kim, H., Wozniak, P.C., Fletcher, R.A., 1980. Cadmium toxicity on synchronous populations of *Chlorella ellipsoidae*. Can. J. Bot. 58, 1780–1788.

MEP China (Ministry of Environmental Protection of the People's Republic of China), 2009. Water Quality – Determination of Ammonia Nitrogen – Nessler's Reagent Spectrophotometry. HJ 535-2009. Beijing, China.

Moon, H.B., Yoon, S.P., Jung, R.H., Choi, M., 2008. Wastewater treatment plants (WWTPs) as a source of sediment contamination by toxic organic pollutants and fecal sterols in a semi-enclosed bay in Korea. Chemosphere 73, 880–889.

Mount, D.R., Hockett, J.R., 2000. Use of toxicity identification evaluation methods to characterize, identify, and confirm hexavalent chromium toxicity in an industrial effluent. Water Res. 34, 1379–1385.

Maycock, D., Sorokin, N., Atkinson, C., Rule, K., Crane, M., 2007. Proposed EQS for Water Framework Directive Annex VIII Substances: Chromium (VI) and Chromium (III) (dissolved). Environment Agency, Almondsbury, Bristol, 1–131.

Nwaogu, T.A., Manager, P., Zarogiannis, P., 2008. 4-tert-Octylphenol Risk Reduction Strategy and Analysis of Advantages and Drawbacks. Department for Environment, Food and Rural Affairs, London, pp. 1–120.

OECD (Organisation for Economic Cooperation and Development), 2006a. Guidelines for the Testing of Chemicals: *Lemna sp*. Growth Inhibition Test. OECD Guideline 221. Paris, France.

OECD (Organisation for Economic Cooperation and Development), 2006b. Guidelines for the Testing of Chemicals, Freshwater Alga and Cyanobacteria Growth Inhibition Test. OECD Guideline 201. Paris, France.

Petry, T., Schmid, P., Schlatter, C., 1996. The use of toxic equivalency factors in assessing occupational and environmental health risk associated with exposure to airborne mixtures of polycyclic aromatic hydrocarbons (PAHs). Chemosphere 32, 639–648.

Preston, S., Coad, N., Townend, J., Killham, K., Paton, G.I., 2000. Biosensing the acute toxicity of metal interactions: are they additive, synergistic, or antagonistic? Environ. Toxicol. Chem. 19, 775–780.

Ra, J.S., Kim, H.K., Chang, N.I., Kim, S.D., 2007. Whole effluent toxicity (WET) tests on wastewater treatment plants with *Daphnia magna* and *Selenastrum capricornutum*. Environ. Monit. Assess. 129, 107–113.

Ra, J.S., Lee, B.C., Chang, N.I., Kim, S.D., 2008. Comparative whole effluent toxicity assessment of wastewater treatment plant effluents using *Daphnia magna*. Bull. Environ. Contam. Toxicol. 80, 196–200.

Rosa, E.V.C., Simionatto, E.L., Sierra, M.M.D., Bertoli, S.L., Radetski, C.M., 2001. Toxicity-based criteria for the evaluation of textile wastewater treatment efficiency. Environ. Toxicol. Chem. 20, 839–845.

Sarakinos, H.C., Bermingham, N., White, P.A., Rasmussen, J.B., 2000. Correspondence between whole effluent toxicity and the presence of priority substances in complex industrial effluents. Environ. Toxicol. Chem. 19, 63–71.

Smolders, R., Bervoets, L., Blust, R., 2004. In situ and laboratory bioassays to evaluate the impact of effluent discharges on receiving aquatic ecosystems. Environ. Pollut. 132, 231–243.

Smolders, R., Bervoets, L., Wepener, V., Blust, R., 2003. A conceptual framework for using mussels as biomonitors in whole effluent toxicity. Hum. Ecol. Risk Assess. 9, 741–760.

Strom, D., Ralph, P.J., Stauber, J.L., 2009. Development of a toxicity identification evaluation protocol using chlorophyll-a fluorescence in a marine microalga. Arch. Environ. Contam. Toxicol. 56, 30–38.

Teodorovic, I., Becelic, M., Planojevic, I., Ivancev-Tumbas, I., Dalmacija, B., 2009. The relationship between whole effluent toxicity (WET) and chemical-based effluent quality assessment in Vojvodina (Serbia). Environ. Monit. Assess. 158, 381–392.

Tiensing, T., Strachan, N., Paton, G.I., 2002. Evaluation of interactive toxicity of chlorophenols in water and soil using lux-marked biosensors. J. Environ. Monit. 4, 482–489.

USEPA (United States Environmental Protection Agency), 1984. Ambient Water Quality Criteria for 2,3,7,8-Tetrachlorodibenzo-p-dioxin. EPA 440/5-84-007. USEPA, Washington, DC.

USEPA (United States Environmental Protection Agency), 1992. Methods for Aquatic Toxicity Identifications Evaluations. Phase 1, Toxicity Characterization Procedures, second edition. EPA/600/6-91/005F. USEPA, Washington, DC.

USEPA (United States Environmental Protection Agency), 1993a. Methods for Aquatic Toxicity Identifications Evaluations. Phase 2, Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic toxicity, second edition. EPA/600/R-92/080. USEPA, Washington, DC.

USEPA (United States Environmental Protection Agency), 1993b. Methods for Aquatic Toxicity Identifications Evaluations. Phase I, Toxicity Confirmation Procedures for Samples Exhibiting acute and Chronic Toxicity, second edition. EPA/600/R-92/081. USEPA, Washington, DC.

USEPA (United States Environmental Protection Agency), 2009. National Recommended Water Quality Criteria for Priority Pollutants. 〈http://www.epa.gov/ost/criteria/wqctable/〉. USEPA, Washington, DC.

Van den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775–792.

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93, 223–241.

von der Ohe, P.C., Dulio, V., Slobodnik, J., De Deckere, E., Kuhne, R., Ebert, R.U., Ginebreda, A., De Cooman, W., Schuurmann, G., Brack, W., 2011. A new risk assessment approach for the prioritization of 500 classical and emerging organic microcontaminants as potential river basin specific pollutants under the European water framework directive. Sci. Total Environ. 409, 2064–2077.

Yang, W.C., Spurlock, F., Liu, W.P., Gan, J.Y., 2006. Effects of dissolved organic matter on permethrin bioavailability to Daphnia species. J. Agric. Food. Chem. 54, 3967–3972.

Zhao, J.L., Ying, G.G., Wang., L., Yang, J.F., Yang, X.B., Yang, L.H., Li, X., 2009. Determination of phenolic endocrine disrupting chemicals and acidic pharmaceuticals in surface water of the Pearl Rivers in South China by gas chromatography–negative chemical ionization–mass spectrometry. Sci. Total. Environ. 407, 962–974.

Zhao, J.L., Ying, G.G., Liu, Y.S., Chen, F., Yang, J.F., Wang, L., 2010. Occurrence and risks of triclosan and triclocarban in the Pearl River system, South China: from source to the receiving environment. J. Hazard. Mater. 179, 215–222.

Zhao, J.L., Ying, G.G., Chen, F., Liu, Y.S., Wang, L., Yang, B., Liu, S., Tao, R., 2011. Estrogenic activity profiles and risks in surface waters and sediments of the Pearl River system in South China assessed by chemical analysis and in vitro bioassay. J. Environ. Monit. 13, 813–821.