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Contributions of flumequine and nitroarenes to the genotoxicity of river and ground waters

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ABSTRACT

The SOS/*umuC* assay was performed in conjunction with analytical measurements to identify potential genotoxins in river and adjacent ground waters in the Jialu River basin, China. The major genotoxic activities of the river and adjacent ground waters occurred in the same two fractions (F4 and F11) when assayed using the *Salmonella typhimurium* strain TA1535/pSK1002. This indicates that ground water near the Jialu River was influenced by the river water. LC–MS/MS analysis indicated that flumequine accounted for 86% and 76% of the genotoxicity in fraction F11 of the river and adjacent ground waters, respectively. When HPLC fractions were tested using the strain NM3009, three fractions showed genotoxic activities for river water sample, while no fractions from ground water samples elicited genotoxic activities. The specific response to the strain NM3009 in one fraction compared with the strain TA1535/pSK1002 suggested the presence of nitroarenes. However, we failed to identify the exact nitroarenes when GC–MS analysis was used to analyze nitroarenes which are well detected in air and soil samples in previous papers.

1. Introduction

Contamination of water from industrial, agricultural, and urban discharges has caused widespread concern over declining water quality, especially when the water body is used as a source of drinking water (Ohe et al., 2003, 2004; Wang et al., 2011). A number of studies have highlighted the genotoxicity or mutagenicity of chemicals in river and ground water samples using bioassays such as the Ames test and micronucleus test (Kummrow et al., 2003; Siddiqui and Ahmad, 2003; Umbuzeiro et al., 2004; Boettcher et al., 2010). This has led to a great concern over the safety of drinking water sources since chronic exposure to genotoxins or mutagens may increase the risk of cancer development (Wogan et al., 2004). While monitoring the genotoxicity or mutagenicity of chemicals in the environment is helpful for understanding water quality, identifying the causative chemicals is of greater importance for risk assessment and management.

Bioassay-directed chemical analyses, which consists of high-throughput bioassays that are performed in conjunction with instrumental analysis, have been reported to be powerful tools for identifying key compounds in various types of environmental matrices, such as soil, sediment, and wastewater (Zhen et al., 2009; Luebcke-von Varel et al., 2011; Reifferscheid et al., 2011). However, due to the presence of numerous chemical pollutants in environmental samples and the low concentrations of many genotoxic compounds, identifying the chemicals responsible for the genotoxicity is a difficult task. Bioassay tests which specifically respond to compounds with certain structures can provide some information about the identity of genotoxins in environmental samples (Brack, 2011). It has been reported that the genotoxic/mutagenic activities of nitroarenes require activation by nitroreductase (NR) and *O*-acetyltransferase (*O*-AT), and consequently the strains that overexpress NR and/or *O*-AT might be particularly sensitive to the genotoxic/mutagenic activities of these chemicals. In the Ames test, the NR and *O*-AT overproducing strains YG1041 and YG1042 have been successfully used to detect the mutagenicity of sediments, air, and river water samples caused by nitroarenes (Azuma et al., 1997; Ohe et al., 2003; Umbuzeiro et al., 2005; Reifferscheid et al., 2011). In the SOS/*umuC* test, the NR and *O*-AT overproducing strain NM3009 was used to identify nitroarenes in airborne particulate matter (Oda et al., 2004). Compared to the regular Ames test, the SOS/*umuC* test has some merits such as shorter test time, lower cost and less amount of sample although the sensitivity of Ames test has been improved by adding increased numbers of bacterial cells (Kado et al., 1983). Therefore, it can be expected that the NM3009 strain would be a potential sensitive method to identify nitroarenes in other environmental media, especially in water.

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Genotoxic compounds always present in low concentrations in environmental matrices. Therefore, methods for the extraction of these chemicals are required in order to conduct chemical analysis and bioassays. Blue rayon and XAD resins are widely used to concentrate genotoxins present in surface waters and positive results were observed when those extracts were analyzed in different short-term tests (Meier et al., 1987; Tolosa et al., 1996; Kataoka et al., 2000; Watanabe et al., 2002; Kummrow et al., 2003; Ohe et al., 2004; Valente-Campos et al., 2009). While blue rayon mainly absorbs substances with a planar polycyclic molecular structure and XAD resins require large volumes of water and have low extraction efficiency for polar chemicals (Fontanals et al., 2007), the HLB (Hydrophilic–Lipophilic Balance) solid phase extraction cartridge, formed by the macroporous copolymer poly(divinylbenzene-co-N-vinylpyrrolidone) with both hydrophilic and lipophilic retention characteristics, is more suitable for retaining a wide spectrum of both polar and non-polar compounds (Peruzzi et al., 2000).

Infiltration by contaminated surface water has been reported to be a main source of ground water pollution (Fenz et al., 2005; Nakada et al., 2008) although the impacts of leakage from decrepit sewer pipes, sewage recharge and soil contamination on the ground water quality could not be neglected. The Jialu River, which is an important tributary of the Huaihe River, is impacted by the direct discharge of industrial and domestic wastewater. Due to the lack of safe water supplies and water treatment systems in rural areas, many residents living along the river use shallow ground water as their main source of drinking water without water treatment. To assess whether water from the river is a source of genotoxic chemicals in ground water, a river water sample, an adjacent ground water sample, and a reference ground water sample collected at a site far from the river were extracted by HLB cartridge and subjected to both the parental strain TA1535/pSK1002 and NR and *O*-AT overproducing strain NM3009. Subsequently, bioassay-directed fractionation was employed to identify the compounds responsible for the observed genotoxic activity.

2. Materials and methods

2.1. Standards and reagents

The standards of 16 nitroarenes (5-nitroacenaphthene, 3-nitrobiphenyl, 4-nitrobiphenyl, 2-nitrofluorene, 9-nitrophenanthrene, 3-nitrophenanthrene, 9-nitroanthracene, 2-nitroanthracene, 1,3-dinitropyrene, 1,6-dinitropyrene, 1,8-dinitropyrene, 1-nitropyrene, 3-nitrofluoranthene, 6-nitrochrysene, 7-nitrobenz[a]anthracene, 6-nitrobenz[a]pyrene) were purchased from Accustandard (New Haven, CT). Twenty quinolone and fluoroquinolone (FQ) antibiotics were analyzed in this study. Cinoxacin, lomefloxacin hydrochloride, pipemidic acid, enoxacin, ofloxacin, danofloxacin, enrofloxacin, ciprofloxacin, sarafloxacin, difloxacin, sparfloxacin, and fleroxacin were purchased from Sigma (St. Louis, MO, USA); norfloxacin, oxolinic acid, pefloxacin, and flumequine were from Dr. Ehrenstorfen (GmbH, Germany); nalidixic acid was from Acros Organics (Morris Plains, NJ, USA); piromidic acid was from Wako (Osaka, Japan), and gatifloxacin was from LKT Laboratories Inc. (St. Paul, MN, USA). Formic acid, methanol, acetone, hexane, and methylene chloride and acetonitrile were HPLC grade purchased from Fisher Chemical (Fair Lawn, NJ, USA). Hydrochloric acid was analytical grade (Beijing Chemicals, Beijing, China). HPLC-grade water was prepared using a Milli-Q RC apparatus (Millipore, Bedford, MA, USA).

2.2. Sample collection and preparation

The study area, located in the southeast part of Henan Province, China, is underlain by an alluvial complex of fluviatile origin deposited by the ancestral Yellow River and Huaihe River. The soil is mainly composed of silt, fine and medium sand, and therefore having high soil permeability. Lateral seepage of the river water to ground water could not be neglected since the river water level is higher than ground water table in this area. Three water samples, each at a volume of 62 L, were collected from two sites along the Jialu River on 22 September, 2009 and 14 March, 2010. A river water sample (Sample 1, S1) and an adjacent ground water sample (Sample 2, S2; <1 km away from the Jialu River; well depth: 10 m) at site A as shown in Fig. 1 were collected. For comparison, a reference ground water sample (Sample 3, S3; well depth: 10 m) was collected at Site B, which is about 20 km from the Jialu River and is almost not affected by the river. There was no rainfall during the sampling periods. All samples were collected in amber glass bottles and kept on ice while being transported to the laboratory. All the samples were filtered with a glass microfiber filter (GF/C, 1.2 μm) (Whatman, Maidstone, UK) before being extracted on the day of collection. Polar chemicals usually have a high-leaching potential from surface water to ground water. To extract potential polar genotoxic chemicals, the samples were adjusted the pH to 3.0 by adding hydrochloric acid. Thirty-one Oasis HLB cartridges (6 mL, 500 mg, Waters, USA) which had been previously conditioned with 10 mL of hexane, 20 mL of acetone, 10 mL of methanol, and 10 mL of ultrapure water, were used to extract each water sample at the flow rate of 5–10 mL min^{-1}. After extraction, the cartridges were dried under a flow of nitrogen gas. To determine the genotoxic activities of the three crude concentrated water samples by the SOS/*umuC* test, 20 mL of acetone was used to elute each cartridge, and the elutions were dried under a gentle flow of nitrogen prior to dissolving in 100 μL DMSO. Experimental blanks were prepared by extracting 2 L of ultrapure water using the same method as described above. No genotoxic activity was observed for the blanks.

2.3. HPLC fractionation

To characterize the potential agents responsible for the genotoxic activities observed in the water samples, further fractionation was conducted by reversed-phase HPLC. As shown in Fig. 2, twenty milliliter of acetone was used to elute each cartridge, and the eluants of 60 L water samples were dried under a gentle flow of nitrogen and then dissolved in 2 mL methanol. The samples were then fractionated at room temperature using a 4.6 mm × 250 mm symmetry shield RP18 column (Waters, USA) at a flow rate of 1 mL min^{-1}. Solvent A was ultrapure water and solvent B was acetonitrile. The gradient was started at 20% B, and was held for 3 min (0–3 min). Then the gradient was brought to 100% B over the course of the next 27 min (3–30 min), and held for 10 min (30–40 min).

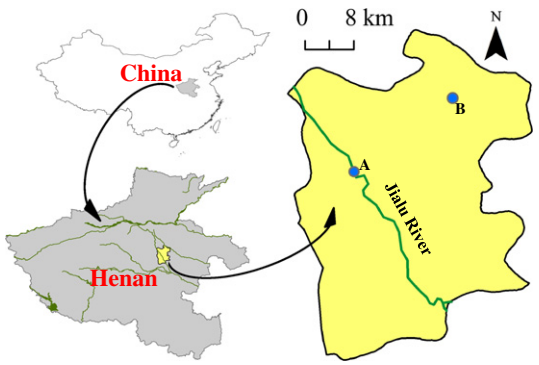

Fig. 1. Study area and sampling locations. A river water sample (S1) and an adjacent ground water sample (S2) were collected from site A. A reference ground water sample (S3) was collected from site B.

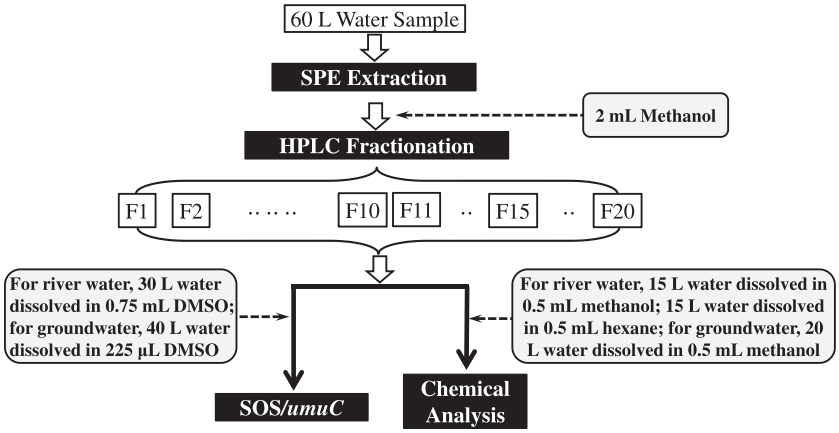

Fig. 2. Sample preparation procedure for the identification of genotoxic compounds in river and ground waters.

Finally, the gradient was brought down to 20% B over the course of 0.1 min, and maintained at this percentage for 10 min until the next injection. The UV detector was set at double wavelengths of 254 and 350 nm. Discrete fractions were collected at 2 min intervals from 0–40 min. The fractions were freeze dried and divided into two portions for subsequent bioassays and chemical analyses. For the SOS/*umuC* test, freeze dried fractions of 30 L of river water and 40 L of ground water were dissolved in 0.75 mL DMSO and 225 μL DMSO, respectively, and consequently, the river water and ground water were concentrated 4×10^4 and 2.67×10^5 fold, respectively; for instrumental analysis, the remaining fractions from 30 L of river water were separated into two equal parts and dissolved in 0.5 mL methanol and 0.5 mL hexane, respectively, while the remaining fractions from the 20 L ground water samples were dissolved in 0.5 mL methanol. The concentration factors were 3×10^4 and 4×10^4 for river water and ground water, respectively.

2.4. SOS/umuC bioassay

Two *Salmonella typhimurium* strains, the parental strain TA15 35/pSK1002 and the nitroreductase (NR)- and *O*-acetyltransferase (*O*-AT)-overproducing strain NM3009, were used to evaluate the genotoxic activities of the water samples without S9 activation. The test strain was grown in L-Broth medium overnight at 37 °C with continuous shaking. Growth of the strain was monitored by determining the absorbance at 595 nm of 150 μL of culture in a 96-well plate on a BioRad 550 microplate reader (BioRad, Hercules, CA, USA). The absorbance of the culture was adjusted to 0.03 by adding tryptone glucose ampicillin (TGA) medium and further cultured at 37 °C for 1.5 h with continuous shaking. Next, 300 μL of culture and 15 μL of DMSO solution diluted to the desired concentrations were cultured at 37 °C for 2 h with continuous shaking, and the concentration of 150 μL of the culture was determined at 595 nm. For the determination of β-galactosidase activity, 100 μL of the culture was mixed with 900 μL Z-buffer (0.1 M sodium phosphate (pH 7.4), 10 mmol L^{-1} KCl, 1 mmol L^{-1} $MgSO_4$ containing 1 mg mL^{-1} mercaptoethanol, 50 μl of 1 mg mL^{-1} SDS, and 50 μL chloroform and the enzymatic reaction was started by adding 100 μl of 4 mg mL^{-1} 2-nitrophenyl-β-D-galactopyranoside (ONPG, Tokyo Kasei, Tokyo, Japan). The reaction was allowed to proceed for 20 min at 30 °C and the reaction was stopped by adding 500 μl of 1 M Na_2CO_3. Finally, 150 μl aliquots of the reaction mixture were added to each well of a 96-well plate, the absorbance at 415 and 570 nm was determined. The β-galactosidase activity (Unit) was calculated according to following equation:

$$\text{Unit} = 1000 \times ([OD_{415}] - [1.75 \times OD_{570}])/([t] \times [v] \times [OD_{595}]) \quad (1)$$

where t represents the reaction time (min), v is the volume of the culture used in this assay (mL), OD_{595} is the cell density at the start of the assay, OD_{415} is the absorbance by *o*-nitrophenol at the end of the reaction, and OD_{570} is the light scattering at the end of the reaction. DMSO was used as a solvent control, and 4-nitroquinoline-1-oxide (4-NQO) and 1-nitropyrene (1-NP) were used as positive control for the strain TA1535/PSK1002 and the strain NM3009, respectively. A 2-fold increase in β-galactosidase activity above the control levels was considered to be positive. Eight doses of 2-fold dilutions were tested for each sample and the samples and solvent blank were tested in triplicate.

To evaluate the genotoxic potential of the concentrated water samples, the equivalent 4-NQO concentration ($TEQ_{4\text{-NQO}}$) or 1-NP concentration ($TEQ_{1\text{-NP}}$) of water samples was calculated based on the dose–response curves of 4-NQO or 1-NP and the water sample to quantitatively express the genotoxic effect, according to following equations:

$$TEQ_{4-NQO}\ (\mu g\ L^{-1}) = Slope_{sample}/Slope_{4-NQO} \quad (2)$$

$$TEQ_{1-NP}\ (ng\ L^{-1}) = Slope_{sample}/Slope_{1-NP} \quad (3)$$

where $Slope_{4\text{-NQO}}$, $Slope_{1\text{-NP}}$ and $Slope_{sample}$ represent the slope value of linear part of the dose–response curves of 4-NQO (Unit μg^{-1} 4-NQO) or 1-NP (Unit ng^{-1} 1-NP) and the concentrated sample (Unit L^{-1} water).

2.5. Chemical analysis

Four nitroarenes in extracts of water samples were analyzed by GC–MS which performed in a GC-2010 (Shimadzu, Japan) with a DB-17 MS column (30 m, 0.25 mm ID, 0.25 μm, J&W Scientific/Agilent Technologies, Santa Clara, CA, USA) using helium at 1.2 mL min^{-1} as a carrier gas and the following oven temperature program: 40 °C for 2 min, then temperature programmed to 200 °C at 20 °C min^{-1}, and then to 300 °C at 2 °C min^{-1}. 1 μL of sample was injected in splitless mode at 300 °C. MS analysis was performed using a negative chemical ionization (NCI) source at 200 °C using methane as ionization gas (40 mL min^{-1}). Data acquisition was conducted in selected ion monitoring mode. The instrumental detection limits for 3-nitrobiphenyl, 4-nitrobiphenyl, 5-nitroacenaphthene, and 2-nitrofluorene are 0.3 μg L^{-1}, 3 μg L^{-1}, 1 μg L^{-1}, and 0.2 μg L^{-1}, respectively.

The analysis for quinolone and FQ antibiotics were followed by our previous method (Xiao et al., 2008). The LC apparatus was an Acquity Ultra Performance LC (Waters, USA). Acquity UPLC® BEH C18 column (100 × 2.1 mm, 1.7 μm particle size) (Waters, USA) was used to separate antibiotics. The column was maintained at

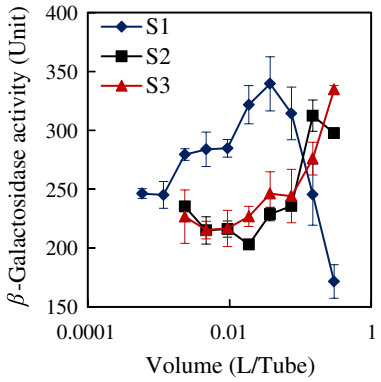

Fig. 3. Dose–response curves for the induction of SOS/*umu*C gene expression in the TA1535/pSK1002 strain exposed to the three crude water samples Each value represents the mean of three replicate measurements ± SD. S1: river water; S2: adjacent ground water collected from the same site as S1; S3: ground water collected from site far from the Jialu river (20 km).

40 °C and a flow rate of 0.2 mL min^{-1}, and the injection volume was 2 μL. Methanol (A) and purified water containing 0.1% formic acid (v/v) (B) were used as mobile phases. The gradient conditions were initiated with 10% A followed by a linear increase to 30% A in 6 min and then to 50% in 3 min. Then A was increased to 100% in 0.5 min and maintained for 3 min. In the next 0.1 min, A was sharply changed back to the initial percentage and held for 4 min until the next injection.

Mass spectrometry was performed using a Waters Premier XE detector, which was operated with electrospray ionization (ESI) in the positive ion (PI) mode. The MS parameters were as follows: capillary voltage, 3.6 kV; source temperature, 110 °C; desolvation temperature, 350 °C; source gas flow, 50 L h^{-1}; and desolvation gas flow, 800 L h^{-1}. Finally, the data acquisition was performed under time-segmented conditions based on the chromatographic separation of the target compounds to maximize the sensitivity of detection.

The instrumental detection limits ranges from 0.1 μg L^{-1} to 1.3 μg L^{-1}.

3. Results and discussion

3.1. Genotoxicity of water samples tested by SOS/umuC bioassay

A dose-dependent increase in β-galactosidase activity was observed when the strain TA1535/pSK1002 was exposed to crude extracts of the three samples collected in September 2009 (Fig. 3). The β-galactosidase activity of sample S1, a river water,

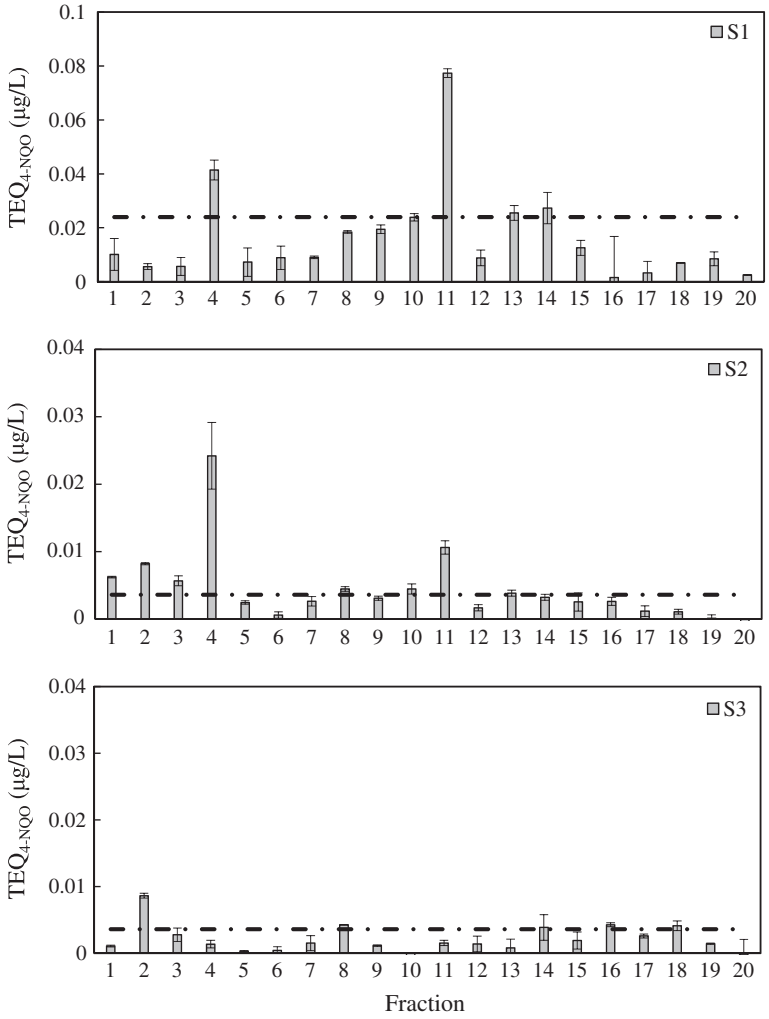

Fig. 4. Genotoxicity profiles of three water samples in the TA1535/pSK1002 strain. Discrete fractions were collected at 2 min intervals from 0 to 40 min. The concentration factors of river water and groundwater were 4 × 10^4 and 2.67 × 10^5, respectively. The β-galactosidase activity was expressed as $TEQ_{4\text{-NQO}}$ (the equivalent 4-nitroquinoline-1-oxide concentration). Dashed lines represent concentration of 4-NQO at 2-fold greater than the control response. Each value represents the mean of three replicate measurements ± SD. S1: river water; S2: adjacent ground water collected from the same site as S1; S3: ground water collected from site far from the Jialu River (20 km).

was found to be increased with increasing water volume in culture tube from 0.001 L to 0.0375 L. However, when the water volume in culture increased to 0.075 L, the β-galactosidase activity elicited obvious decrease, showing a potential cytotoxic effects, which would masked the genotoxic effects of the water. The β-galactosidase activity of sample S2, a ground water, was increased with increasing water volume in culture tube from 0.075 L, showing relatively low genotoxic activity compared with sample S1. It should be noted that the dose–response curve of sample S3, a ground water from the reference site, was similar to that of sample S2. Since sample S3 is far from the river, the contamination from soil would contribute the genotoxic activity as reported in previous paper (Valente-Campos et al., 2009).

Bioassay-guided fractionation of the three water samples was performed in order to characterize the agents responsible for the genotoxic effects. For the river water sample (S1), the major genotoxic activities were observed in Fraction 4 (F4: 0.041 μg $TEQ_{4\text{-}NQO}$ L^{-1}) at a retention time of 6–8 min and Fraction 11 (F11: 0.07 μg $TEQ_{4\text{-}NQO}$ L^{-1}) at 20–22 min when the fractions were tested by the strain TA1535/pSK1002 and the activities were expressed as $TEQ_{4\text{-}NQO}$ (Fig. 4). Similarly, the genotoxic activities of the adjacent ground water sample (S2) were 0.024 μg $TEQ_{4\text{-}NQO}$ L^{-1} for F4 and 0.011 μg $TEQ_{4\text{-}NQO}$ L^{-1} for F11, which were lower than those in the corresponding fractions of S1. It should be noted that Fractions 1, 2, and 3 of S2, which corresponded with retention times of 0–2 min, 2–4 min, and 4–6 min respectively, also elicited weak genotoxic activities with $TEQ_{4\text{-}NQO}$ values of 0.0062 μg L^{-1}, 0.0082 μg L^{-1}, and 0.0057 μg L^{-1}, respectively. However, no genotoxic effects of the corresponding fractions of S1 were detected. This result indicates that other genotoxic agents may present in the ground water. As for the ground water sample (S3) collected far away from the river, only Fraction 2 elicited weak genotoxicity with a $TEQ_{4\text{-}NQO}$ of 0.009 μg L^{-1}, largely different from sample S2. Similar genotoxic profiles based on HPLC fractionation were also observed for the samples collected in March 2010.

When HPLC fractions were tested using the strain NM3009, the activity was expressed as $TEQ_{1\text{-}NP}$. As shown in Fig. 5, the profile of genotoxic activity obtained using the strain NM3009 was different from the profile obtained using the strain TA1535/pSK1002. Specifically, Three fractions of S1, Fraction 11 (F11; 1.575 ng $TEQ_{1\text{-}NP}$ L^{-1}) at 20–22 min, Fraction 13 (F13; 1.526 ng $TEQ_{1\text{-}NP}$ L^{-1}) at 24–26 min, and Fraction 15 (F15; 2.711 ng $TEQ_{1\text{-}NP}$ L^{-1}) at 28–30 min, induced obvious genotoxic activities while no genotoxic fraction was observed in both of the ground water samples. Similar genotoxic

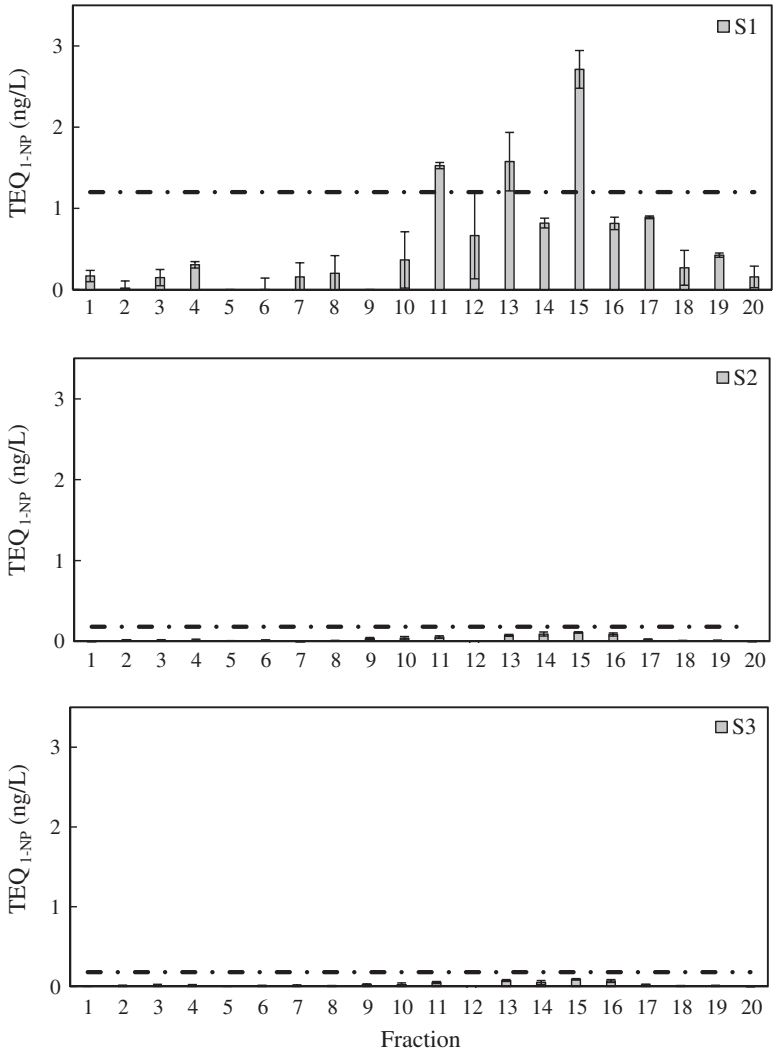

Fig. 5. Genotoxicity profiles of three water samples in the NM3009 strain. Discrete fractions were collected at 2 min intervals from 0 to 40 min. The concentration factors of river water and groundwater were 4×10^4 and 2.67×10^5, respectively. The β-galactosidase activity was expressed as $TEQ_{1\text{-}NP}$ (the equivalent 1-nitropyrene concentration). Dashed lines represent the concentration of 1-nitropyrene at 2-fold greater than the control response. Each value represents the mean of three replicate measurements ± SD. S1: river water; S2: adjacent ground water collected from the same site as S1; S3: ground water collected from site far from the Jialu river (20 km).

profile was also observed in the sample of S1 which was collected in March 2010 while the activity was relatively weak. As NM3009 is a NR- and *O*-AT overproducing strain, this strain shows high sensitivity for detecting nitroarenes, but the sensitivity for other chemicals is always lower than the parent strain. The minimum positive concentrations of 4-NQO and monomethyl sebacate were 0.15 μmol L^{-1} and 230 μmol L^{-1} when tested using the strain TA1535/pSK1002, but were 0.52 μmol L^{-1} and 600 μmol L^{-1} when tested using the NM3009 (Oda et al., 2004). This can explain why fractions elicited genotoxic effects in the strain TA1535/pSK1002 while not in the strain MN3009.

3.2. Identification of genotoxins

Because the river water (S1) had greater genotoxic effects than both of the ground water samples it was chosen for further cause-effect analysis. The major fractions showing genotoxic activities by the strain TA1535/pSk1002 and the strain NM3009 were F4, F11, F13 and F15 as shown in Figs. 4 and 5. Thus, we further compared their dose–response curves by the strain TA1535/pSK1002 with those by the strain NM3009 to explore potential classes of genotoxins (Fig. 6). While the genotoxic activities of F4, F11, and F13 determined using the strain NM3009 assay were comparable to or less than those determined using the strain TA1535/pSK1002 assay, the genotoxic activity of F15 was much greater in the strain NM3009 assay than in the strain TA1535/pSK1002 assay. Since the strain NM3009 overexpresses the NR and *O*-AT, which are needed when nitrosarenes are metabolized to display their genotoxic activities, it can conclude that the major causative agents of F15 should be nitroarenes.

Nitroarenes are a group of potent genotoxic and carcinogenic compounds that have raised considerable concern in recent years. Nitroarenes such as 2-nitrofluoranthene, 9-nitroanthracene, 1-nitropyrene, 1-nitronaphthalene were frequently detected in the air (Bamford and Baker, 2003; Reisen and Arey, 2005; Umbuzeiro et al., 2008), and nitroarenes have also been detected in the soil (Niederer, 1998; Watanabe et al., 1999). Nitroarenes enter aquatic environments through precipitation or discharged industrial wastewater. It has been reported that 1-nitropyrene has been detected at a concentration of 1 ng L^{-1} in water from the Yodo River (Ohe and Nukaya, 1996). To determine whether nitroarenes were responsible for the genotoxic effects of the river water sample, 16 commonly detected nitroarenes (5-nitroacenaphthene, 3-nitrobiphenyl, 4-nitrobiphenyl, 2-nitrofluorene, 9-nitrophenanthrene, 3-nitrophenanthrene, 9-nitroanthracene, 2-nitroanthracene, 1,3-dinitropyrene, 1,6-dinitropyrene, 1,8-dinitropyrene, 1-nitropyrene, 3-nitrofluoranthene, 6-nitrochrysene, 7-nitrobenz[a]anthracene, 6-nitrobenz[a]pyrene) at a concentration of 1 mg L^{-1} were injected into the HPLC system as described above. The peaks corresponding to 3-nitrobiphenyl, 4-nitrobiphenyl, 5-nitroacenaphthene, and 2-nitrofluorene, were observed within the retention times from 28–30 min, which overlapped with F15, indicating that these four nitroarenes might be the causal genotoxic agents in F15. To further confirm the identity of the causative agents, the concentrations of these four nitroarenes in F15 were analyzed by GC–MS, but all of them were below the limits of detection. The biological detection limits of 3-nitrobiphenyl, 4-nitrobiphenyl, 5-nitroacenaphthene, and 2-nitrofluorene are 1250 μg L^{-1}, 150 μg L^{-1}, 25 μg L^{-1}, and 0.8 μg L^{-1}, respectively. Because these concentrations are much greater than the corresponding instrumental detection limits of chemical analysis, it is unlikely that the four nitroarenes are responsible for eliciting the genotoxic effects of the river water.

Quinolone and FQ antibiotics elicit genotoxic effects based on the SOS/*umuC* test (Hartmann et al., 1998; Hu et al., 2007). This class of antibiotics is largely used in human medicine and veterinary therapy. Although they are partially metabolized in humans and livestock (Daughton and Ternes, 1999) and are removed during the wastewater treatment process (Gulkowska et al., 2008), a

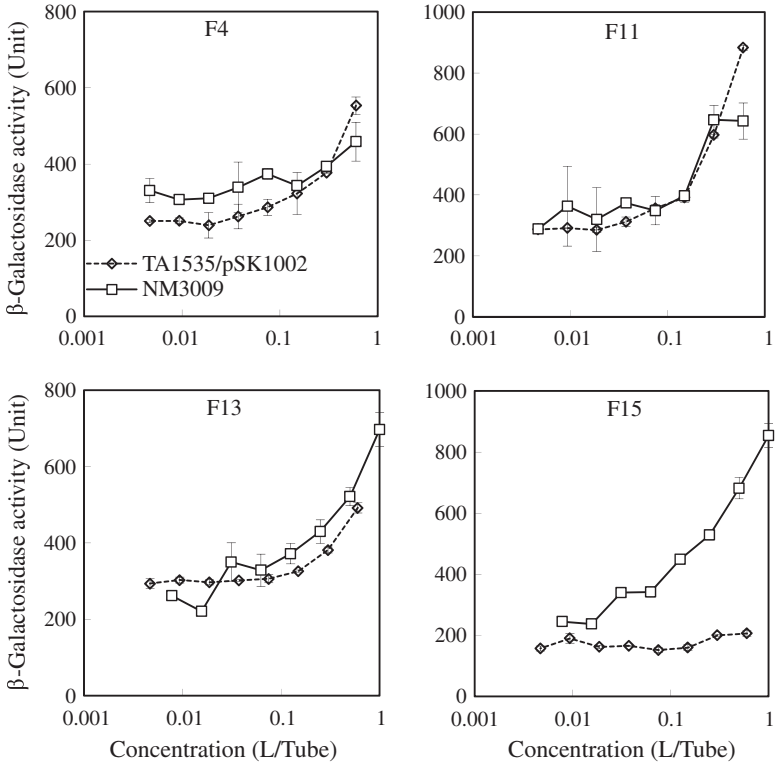

Fig. 6. Dose–response curves of the genotoxicty inducing HPLC fractions of S1 in the TA1535/pSK1002 and NM3009 strains. Each value represents the average of three replicate measurements ± SD.

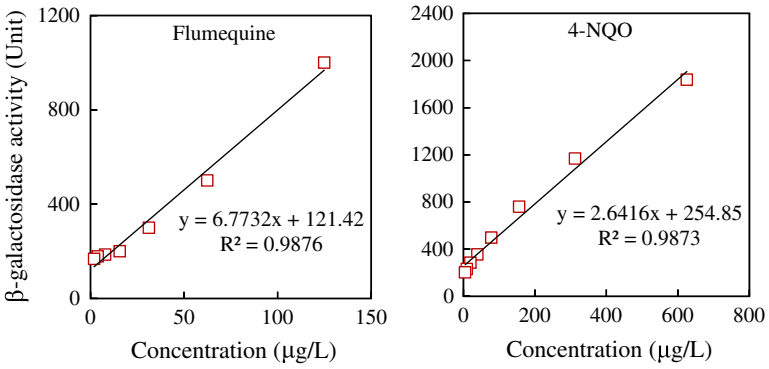

Fig. 7. Dose–response curves of flumequine and 4-NQO in the TA1535/pSK1002 strain.

considerable quantity can still be released into aquatic environments. In surface water samples, seven antibiotics (pipemidic acid, norfloxacin, ciprofloxacin, ofloxacin, lomefloxacin, gatifloxacin, and moxifloxacin) were detected with concentrations ranging from 1.3 ng L^{-1} (lomefloxacin) to 535 ng L^{-1} (ofloxacin) (Xiao et al., 2008). In order to determine whether the genotoxic effects of the S1 and S2 waters were due to antibiotics, F4 and F11 of S1 and S2 were analyzed by UPLC–MS/MS for twenty quinolone and FQ antibiotics. The concentrations of each of the antibiotics in F4 were below the limits of detection, whereas flumequine was detected in F11 at 25.9 ng L^{-1} for S1 and 12.3 ng L^{-1} for S2. Based on its dose–response curves (Fig. 7), the chemical-derived $TEQ_{4\text{-NQO}}$ was calculated to be of 0.066 μg L^{-1} for S1 and 0.031 μg L^{-1} for S2, which contributed to 86% and 76% of the corresponding biological-derived $TEQ_{4\text{-NQO}}$, suggesting that flumequine was the main genotoxic agent in F11.

As a broad-spectrum and high potent antibiotic, flumequine is widely used in aquaculture to cure and prevent skin infections in fish and in veterinarian medicine for the treatment of enteric infection (Albrecht, 1977). Moreover, its low bioavailability may result in high concentrations in the aquatic environment (Anadon et al., 2008). In the Seine River of France, flumequine was reported at concentration of up to 32 ng L^{-1} (Tamtam et al., 2008). Toxicity and carcinogenicity studies of flumequine have been performed using rats and mice (WHO, 1998; Yoshida et al., 1999). Oral administration of flumequine induced hepatic toxicity in CD-1 mice, and flumequine induced hepatocellular tumors in an 18-month carcinogenicity study in CD-1 mice (WHO, 1998). In another study it was determined that flumequine could induce hepatocellular foci in the livers of mice with or without any prior initiation treatment (Yoshida et al., 1999). Although the concentration of flumequine in the S1 and S2 samples was relatively low compared to the amount of acceptable daily intake (WHO, 2004), the presence of this genotoxic agent is of potential concern.

Overall, the present study found that the major genotoxic activities of the river water and adjacent ground water occurred in the same two fractions when assayed using the strain TA1535/pSK1002, although the genotoxicity in the river was stronger than the ground water. These results indicate that ground water near the Jialu River was influenced by the river water. LC-MS/MS analysis identified that flumequine was one of the causal agents causing the genotoxicity. The specific response to the strain NM3009 compared with the strain TA1535/pSK1002 demonstrated the presence of nitroarenes in the river sample, although the exact chemicals could not be identified by analyzing the nitroarenes commonly detected in the environment.

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