



Antibiotic resistance genes in lakes from middle and lower reaches of the Yangtze River, China: Effect of land use and sediment characteristics



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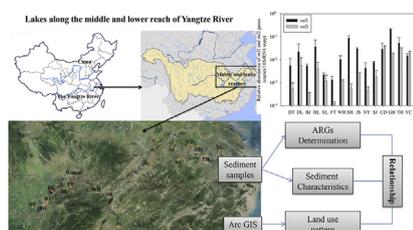
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HIGHLIGHTS

- Diversity and abundance of ARGs in 15 lakes along the Yangtze River were shown.
- Redundancy analysis was used to analyze the ARG abundance determined by qPCR.
- The lakes with high proportion of built-up land use had high ARG abundance.
- Sediment characteristics had no significant effect on ARG distribution.

GRAPHICAL ABSTRACT



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ABSTRACT

Freshwater lakes provided an ideal media for the accumulation and propagation of antibiotic resistance genes (ARGs), because they were susceptible to anthropogenic impacts. Land reclamation and urbanization exerted severe anthropogenic impacts on lakes from middle and lower reaches of the Yangtze River, China over the past decades. In this study, 15 lakes in the region were selected to understand the level and variability of ARGs. Proportion of different land use types was applied to display the land reclamation and urbanization around each lake. For sulfonamide resistance (*sul*) genes, *sul1* had the highest relative abundance in sediments, with maximum 2.11×10^{-1} copies/16SrRNA copy in Gehu Lake. For tetracycline resistance (*tet*) genes, *tetG* had the highest average value of relative abundance (4.74×10^{-3} copies/16SrRNA copy), followed by *tetB*, *tetA*, *tetQ* and *tetM*. Class I integron (*int1*) played an important role in acquisition and dissemination of *sul1* and *tetG*. Sediment characteristics (moisture, density, total nitrogen, total carbon, ammonium, and nitrate) were found to have no significant effect on ARG distribution. Taihu Lake and Yangcheng Lake which exhibited high *sul* and *tet* genes had the high proportion of built-up land use.

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1. Introduction

Antibiotics have saved millions of lives since their application in 1930s, but the increasing antibiotic resistance genes (ARGs) in bacteria was a growing cause of concern. Pathogens carrying ARGs

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have been becoming resistant to the most commonly prescribed antibiotic treatments, resulting in prolonged illness and greater risk of death (Cosgrove, 2006). The aquatic environment was an important pool for ARGs, because many pollutants from wastewater treatment plants, industrials, hospitals and swine farms finally circulated in water environments and drove the propagation of ARGs (Zhang et al., 2009; Liu et al., 2012; Zhu et al., 2013; Lavilla Lerma et al., 2014). Evidence showed that even subinhibitory concentrations of antibiotics might promote antibiotic resistance (Kümmerer, 2009). Among the aquatic environment, rivers have received the most attention during to its rapid transport of ARGs and obvious identification of pollution source and landscape in different reaches (Storteboom et al., 2010; Pruden et al., 2012; Chen et al., 2013; Rodriguez-Mozaz et al., 2015). However, lakes with long retention time of pollutants having the potential to store and accumulate more ARGs were paid little attention relatively (Czekalski et al., 2015).

China is a country with high human and veterinary antibiotic consumption, and antibiotic resistance has been a serious public health threat in China (Hvistendahl, 2012). Lakes along the middle and lower reaches of Yangtze River were in the rapid economic development region of China. Serious destruction (land reclamation and urbanization) in this region led to changes in lake morphology and pollution in lakes (Yang and Lu, 2014). The constructed hospitals and wastewater treatment plants during the urbanization might be a very important source of ARGs in lakes via receiving discharged effluents. Hence, we assumed that the land use might have significant effect on ARG distribution in lakes. The relative abundance of ARGs in sediment of 15 lakes along the Yangtze River was explored in this study. Eighteen different ARGs (*sul1*, *sul2*, *sul3*, *tetA*, *tetB*, *tetC*, *tetM*, *tetW*, *tetQ*, *tetO*, *tetG*, *qnrA*, *qnrB*, *qnrD*, *qnrS*, *ermA*, *ermB* and *ermC*) were scanned by conventional PCR analysis to give us a profile of ARG pollution in 15 lakes along the Yangtze River. Sediment was selected as the targeted media, because it reflected a long-term pollution status and stored easily for ARGs analysis. Different land use pattern was applied to display the land reclamation and urbanization around each lake. The effect of lake morphology and sediment characteristics on ARG abundance was also investigated.

2. Materials and methods

2.1. Sampling sites and sample collection

Many lakes of China are in the middle and lower reaches of the Yangtze River form a shallow lake group unique in the world. In this study, 15 lakes were selected non-randomly on the basis of ease of access in this region for ARGs analysis including Datong Lake, Bajiao Lake, Dongting Lake, Xiliang Lake, Honghu Lake, Wanghu Lake, Futou Lake, Saihu Lake, Junshan Lake, Nanyi Lake, Shijiu Lake, Gehu Lake, Changdang Lake, Taihu Lake, and Yangcheng Lake. The 15 lakes varying in size and geographic location (Fig. 1 and Table 1) were sampled during the summer from July 30 to August 7, 2012. Two sampling sites were set at each lake. At each site, three replicate surface sediments were randomly collected within an approximately 50 m² area from a boat using a Peterson dredge and then mixed and homogenized to form a composite sample. Then, 0.5 kg of sediment was collected, placed in a sealed plastic bag and stored at approximately 4 °C in a refrigerator until returned to the laboratory. After that, the samples were transported to the laboratory and stored at –80 °C prior to analysis.

2.2. DNA extraction and assays for detection of ARGs

2.2.1. DNA extraction and conventional PCR assay for ARGs

The sediment samples were lyophilized and ground. Then, exactly 0.5 g of sample was used to extract DNA by FastDNA Spin Kit for Soil (QBiogene, Carlsbad, CA) according to the protocol provided by the manufacturer. After that, GeneClean Spin Kit (QBiogene, Carlsbad, CA) was applied to purify the DNA to minimize PCR inhibition. Finally, spectrophotometer analysis (NanoDrop ND-2000c, Thermo) and 1.5% agarose gel electrophoresis were used to assess the quality and the concentration of DNA.

All conventional PCR assays were performed in a 25 µl volume reaction by 2 × Utaq PCR MasterMix (Beijing Zoman Biotechnology Co., Ltd.) according to the protocol provided by the manufacturer. Qualitative analysis of 18 different ARGs (*sul1*, *sul2*, *sul3*, *tetA*, *tetB*, *tetC*, *tetM*, *tetW*, *tetQ*, *tetO*, *tetG*, *qnrA*, *qnrB*, *qnrD*, *qnrS*, *ermA*, *ermB* and *ermC*), class I integron (*int1*) and bacterial 16S rRNA gene fragments were analyzed by agarose gel electrophoresis using the published primers (Aminov et al., 2001; Ng et al., 2001; Cummings et al., 2010; Luo et al., 2010; Gaze et al., 2011; Ji et al., 2012; Chen and Zhang, 2013; Mao et al., 2015). The PCR products were randomly selected for sequencing and blast in Antibiotic Resistance Genes Database (ARDB, <http://arbd.cbcb.umd.edu/>) to avoid bias in the analysis.

2.2.2. qPCR assays for ARGs

The qPCR reactions were performed in a 20 µL reaction mixture according to its protocol provided by the manufacturer. The experiment was carried out in 96 well plated in a 7500 Fast Real-Time PCR system (Applied Biosystems, USA). Standard curves were prepared from plasmids with targeted genes and constructed from serial 10-fold dilutions of plasmids containing the respective gene in a range of 10⁸ to 10² gene copies. The R² values for all standard curves were all higher than 0.99. The efficiency of our reactions ranged from 96% to 115%.

2.3. Sediment characteristics

Six sediment characteristics (moisture, density, total nitrogen, total carbon, ammonium and nitrate) in Table S1 were detected according to the procedures described in our previous study (Liu et al., 2015). Briefly, sediment moisture was measured gravimetrically (24 h at 105 °C) from 30 g sediment samples. Total nitrogen (TN) was measured using the Kjeldahl method after digesting samples in a digester using a sulfuric acid/mercuric oxide catalyst. Total carbon (TC) content of air-dried samples was analyzed by a TOC analyzer (Vario TOC cube, Elementar, Germany). Nitrate and ammonium were extracted from sediments with 2 M KCl and determined using a continuous flow analyzer (Skalar, the Netherlands).

2.4. Watershed land use pattern

The watershed land use was calculated using ArcGIS 10 software (ESRI, Redlands, California, USA) according to the methods described in our previous study (Liu et al., 2015). The data were interpreted from recent Landsat TM images and obtained from the Data Sharing Infrastructure of Earth System Science in China (<http://www.geodata.cn/>). Four main types of land use were calculated: (1) vegetation, including forest and grassland; (2) agriculture, including dry land and paddy fields; (3) built-up land, including urban areas, rural settlements and others such as industrial areas, roads, and airports; and (4) water bodies, including lakes, rivers, streams, reservoirs, ponds, and wetlands.

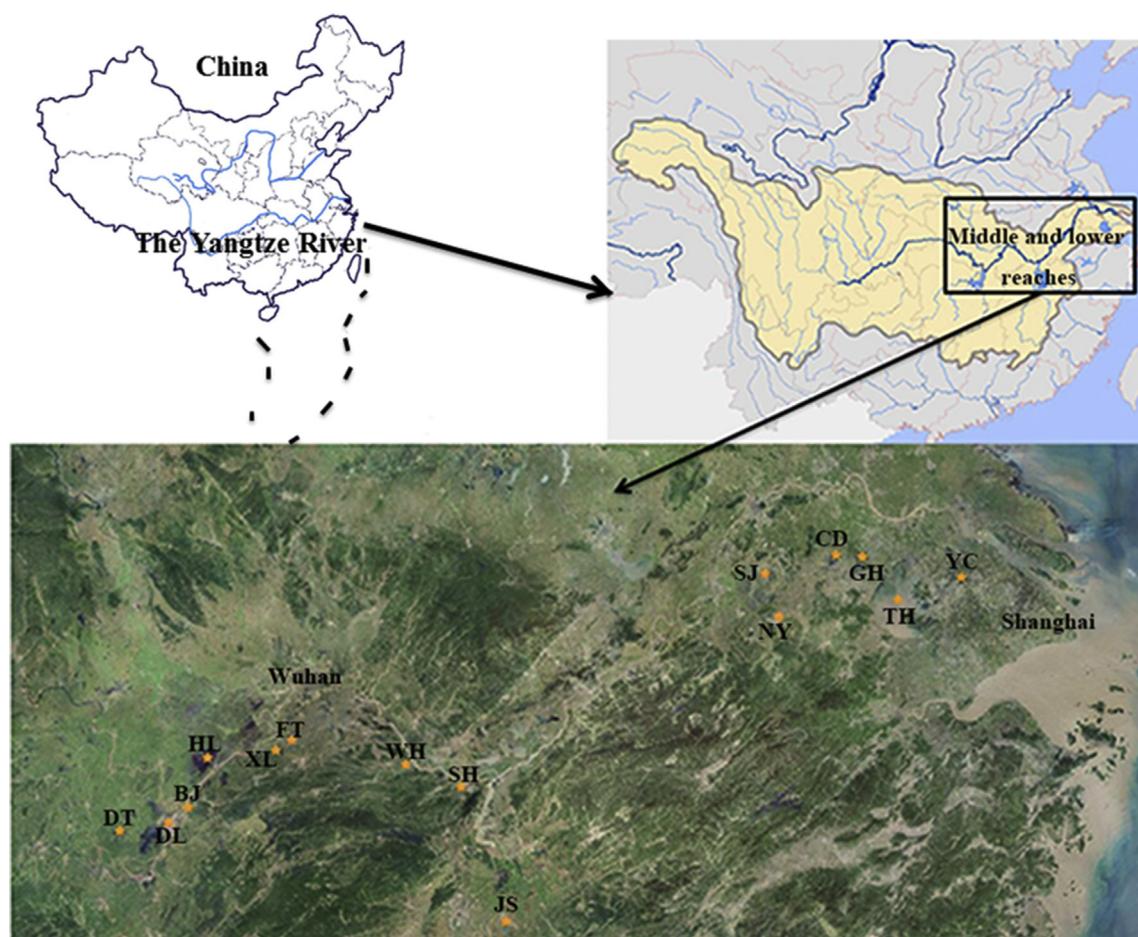


Fig. 1. The sketch map of 15 lakes from middle and lower reaches of the Yangtze River, China (Abbreviations of lake names are explained in Table 1).

Table 1

The information of lake morphology and land use type in 15 lakes from the middle and lower reach of the Yangtze River.

Lakes	Abbreviation	Location		Lake morphology		Land use type (%)			
		latitude (°N)	longitude (°E)	area (km ²)	depth (m)	vegetation	agriculture	built-up land	water
Datong Lake	DT	29.23	112.53	83.1	2.9	29.88	51.66	3.17	15.29
Dongting Lake	DL	29.13	112.79	2614.4	6.5	48.32	35.84	3.21	12.63
Bajiao Lake	BJ	29.45	113.21	10.2	3	28.33	40.12	6.11	25.44
Honghu Lake	HL	29.86	113.39	344.4	1.9	17.35	54.87	4.33	23.45
Xiliang Lake	XL	29.98	114.07	72.1	1.9	37.17	37.33	2.43	23.07
Futou Lake	FT	30.04	114.23	141.2	1.6	30.59	41.98	4.22	23.21
Wanghu Lake	WH	29.87	115.36	42.3	3.7	42.32	47.15	5.33	5.2
Saihu Lake	SH	29.69	115.9	53.3	2.2	38.93	40.58	4.71	15.78
Junshan Lake	JS	28.53	116.34	177.3	4	35.67	38.92	2.93	22.48
Nanyi Lake	NY	31.05	118.85	197.8	2.3	41.43	43.77	1.35	13.45
Shijiu Lake	SJ	31.46	118.97	222.1	5.3	42.03	41.27	1.28	15.42
Changdang Lake	CD	31.61	119.59	85	1.2	23.51	52.23	6.54	17.72
Gehu Lake	GH	31.51	119.81	139.6	1.2	23.17	50.51	7.33	18.99
Taihu Lake	TH	31.17	120.42	2537.2	2.1	33.05	42.33	13.05	11.57
Yangcheng Lake	YC	31.4	120.72	118.1	1.4	23.33	40.81	15.37	20.49

2.5. Statistical analysis

Pearson correlation analysis between ARGs and class I integron and *t*-test among tetracycline resistance (*tet*) genes were performed by SPSS software (Version 16.0, IBM, USA). Redundancy analysis between factors and ARGs were carried out by R software (Version 3.3.0) with “Vegan” package.

3. Results

3.1. Qualitative analysis of ARGs in 15 lakes

Eighteen different ARGs were analyzed in sediments of 15 lakes from middle and lower reaches of the Yangtze River (Table 2). For sulfonamide resistance (*sul*) genes, *sul1* and *sul2* were widely distributed in all the 15 lakes, while *sul3* could be observed in 10

Table 2
Qualitative analysis of ARGs in sediments of lakes from middle and lower reaches of the Yangtze River.

Lakes	<i>sul1</i>	<i>sul2</i>	<i>sul3</i>	<i>tetA</i>	<i>tetB</i>	<i>tetC</i>	<i>tetM</i>	<i>tetW</i>	<i>tetQ</i>	<i>tetO</i>	<i>tetG</i>	<i>qnrA</i>	<i>qnrB</i>	<i>qnrD</i>	<i>qnrS</i>	<i>ermA</i>	<i>ermB</i>	<i>ermC</i>
Datong Lake	+	+	+	+	+	+	+	-	+	+	+	-	+	-	+	+	+	+
Dongting Lake	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+
Bajiao Lake	+	+	-	+	+	+	+	-	+	+	+	-	+	+	+	-	+	-
Honghu Lake	+	+	+	+	+	+	+	-	+	+	+	-	+	-	+	+	+	+
Xiliang Lake	+	+	-	+	+	-	+	-	+	+	+	-	-	-	-	+	+	-
Futou Lake	+	+	-	+	+	-	+	-	+	+	+	-	-	-	-	-	+	+
Wanghu Lake	+	+	-	+	+	-	+	-	+	-	+	-	-	-	-	+	+	+
Saihu Lake	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	-
Junshan Lake	+	+	+	+	+	-	+	-	+	-	+	-	-	-	+	+	-	-
Nanyi Lake	+	+	-	+	+	-	+	-	+	-	+	-	-	-	+	+	-	-
Shijiu Lake	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+
Changdang Lake	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	+	+
Gehu Lake	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	+	+
Taihu Lake	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-
Yangcheng Lake	+	+	+	+	+	-	+	-	+	+	+	-	+	-	+	+	+	+

+: positive, -: negative.

lakes with exception of Xiliang Lake, Futou Lake, Bajiao Lake, Nanyi Lake and Wanghu Lake. For the two groups of tetracycline resistance (*tet*) genes including antibiotic efflux pumps (*tetA*, *tetB*, *tetC* and *tetG*) and ribosomal protection proteins (*tetM*, *tetO*, *tetQ* and *tetW*), *tetA*, *tetB*, *tetG*, *tetM* and *tetQ* occurred in all the 15 lakes. *tetO* was observed in 12 lakes except for Nanyi Lake, Wanghu Lake and Junshan Lake. *tetC* was found in 9 lakes and *tetW* was not observed in all the 15 lakes. All the four plasmid mediated quinolone resistance genes (*qnrA*, *qnrB*, *qnrD* and *qnrS*) were observed with low detection frequency (20%–53.33%) in the 15 lakes. For 3 erythromycin (*erm*) genes, *ermB* was observed in 13 lakes with exception of Nanyi Lake and Junshan Lake. *ermA* was found in 11 lakes except for Futou Lake, Bajiao Lake, Changdang Lake and Saihu Lake. *ermC* occurred in 9 lakes among the 15 lakes. The detection frequency of 18 ARGs in the 15 lakes were in the following order: *sul1*, *sul2*, *tetA*, *tetB*, *tetG*, *tetM*, and *tetQ* (100%) > *ermB* (86.67%) > *tetO* (80%) > *ermA* (73.33%) > *sul3*, *tetC* (66.67%) > *ermC* (60%) > *qnrB* (53.33%) > *qnrS* (40%) > *qnrD* (26.67%) > *qnrA* (20%) > *tetW* (0). It could be concluded that *qnr* genes were less detected compared with *sul*, *tet* and *erm* genes except for *tetW*. Hence, *sul1*, *sul2*, *tetA*, *tetB*, *tetG*, *tetM*, and *tetQ* with 100% detection frequency were selected for quantitative analysis of ARGs.

3.2. Quantitative analysis of ARGs in 15 lakes using qPCR

The relative abundance of sulfonamide resistance genes *sul1* and *sul2* in 15 lakes were showed in Fig. 2. The highest abundance

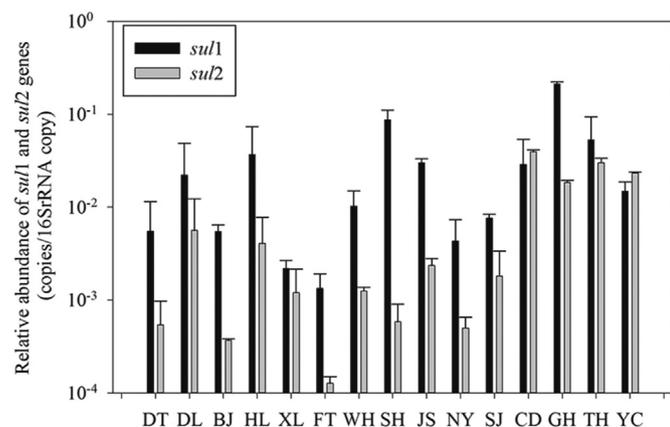


Fig. 2. The relative abundance of *sul* genes in sediments of 15 lakes (Abbreviations of lakes are the same as in Table 1).

of *sul1* was found in Gehu Lake (2.11×10^{-1} copies/16SrRNA copy), which was significantly different from levels determined in the remaining lakes. Dongting Lake, Honghu Lake, Wanghu Lake, Saihu Lake, Junshan Lake, Changdang Lake, Taihu Lake and Yangcheng Lake exhibited high relative abundance of *sul1*, ranging from 1.03×10^{-2} (Wanghu Lake) to 8.69×10^{-2} copies/16SrRNA copy (Saihu Lake). Datong Lake, Bajiao Lake, Xiliang Lake, Futou Lake, Nanyi Lake and Shijiu Lake presented low relative abundance of *sul1* gene, ranging from 1.33×10^{-3} (Futou Lake) to 7.64×10^{-3} copies/16SrRNA copy (Shijiu Lake). To give a more clear profile of *sul1* gene in 15 lakes, lower, median and upper quartiles of *sul1* gene were 5.48×10^{-3} , 1.85×10^{-2} and 4.49×10^{-2} copies/16SrRNA copy, respectively. With exception of Changdang Lake and Yangcheng Lake, *sul2* was less prevalent than *sul1* in sediments of sampled lakes (1.75–148.77 times). The highest relative abundance of *sul2* was found in Changdang Lake (3.95×10^{-2} copies/16SrRNA copy), followed by Taihu Lake (3.01×10^{-2} copies/16SrRNA copy), and Yangcheng Lake (2.34×10^{-2} copies/16SrRNA copy). The lower, median and upper quartiles of *sul2* gene in 15 lakes were 5.42×10^{-4} , 2.09×10^{-3} and 2.10×10^{-2} copies/16SrRNA copy, respectively. The lowest relative abundance of *sul2* gene was determined in Futou Lake (1.27×10^{-4} copies/16SrRNA copy). Futou Lake had the lowest relative abundance of *sul1* and *sul2* genes among the 15 lakes. Interestingly, Changdang Lake, Gehu Lake, Taihu Lake and Yangcheng Lake had high relative abundance of *sul1* and *sul2* genes. Pearson correlation analysis indicated abundance of *sul1* and *sul2* in the 15 lakes was not correlated ($r = 0.301$, $p = 0.276$).

The relative abundance of five *tet* genes determined by qPCR in 15 lakes was shown in Fig. 3. For *tetA* gene, Bajiao Lake, Changdang Lake and Yangcheng Lake exhibited high (upper quartile) relative abundance, ranging from Changdang Lake (7.85×10^{-3} copies/16SrRNA copy) to Bajiao Lake (9.54×10^{-3} copies/16SrRNA copy). The lower, median and upper quartiles of *tetA* gene were 7.95×10^{-4} , 2.96×10^{-3} and 5.59×10^{-3} copies/16SrRNA copy, respectively. For *tetB* gene, Yangcheng Lake had the highest relative abundance (3.32×10^{-2} copies/16SrRNA copy), followed by Changdang Lake (1.01×10^{-2} copies/16SrRNA copy), and Dongting Lake (9.25×10^{-3} copies/16SrRNA copy). The lowest relative abundance of *tetB* gene was observed in Nanyi Lake (3.37×10^{-4} copies/16SrRNA copy). The lower, median and upper quartiles of *tetB* gene were 7.61×10^{-4} , 1.24×10^{-3} and 3.73×10^{-3} copies/16SrRNA copy, respectively. For *tetG* gene, the relative abundance ranged from 3.33×10^{-4} copies/16SrRNA copy (Futou Lake) to 2.79×10^{-2} copies/16SrRNA copy (Dongting Lake). The lower, median and upper quartiles of *tetG* gene were 7.90×10^{-4} ,

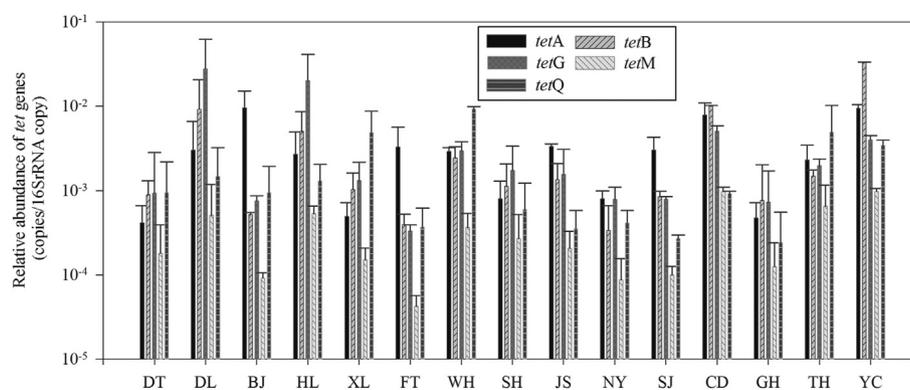


Fig. 3. The relative abundance of *tet* genes in sediments of 15 lakes (Abbreviations of lakes are the same as in Table 1).

1.65×10^{-3} and 3.49×10^{-3} copies/16SrRNA copy, respectively. The relative abundance of *tetM* in the 15 lakes was found in the range 4.25×10^{-5} copies/16SrRNA copy (Futou Lake)– 9.77×10^{-4} copies/16SrRNA copy (Yangcheng Lake). The lower, median and upper quartiles of *tetM* gene were 9.99×10^{-5} , 2.38×10^{-4} and 5.95×10^{-4} copies/16SrRNA copy, respectively. For *tetQ* gene, Xiliang Lake, Wanghu Lake and Taihu Lake exhibited high relative abundance (upper quartile), ranging from Xiliang Lake (4.89×10^{-3} copies/16SrRNA copy) to Wanghu Lake (9.29×10^{-3} copies/16SrRNA copy). The lower, median and upper quartiles of *tetG* gene were 3.71×10^{-4} , 9.32×10^{-4} and 4.17×10^{-3} copies/16SrRNA copy, respectively. Generally, *tetG* had the highest average value of relative abundance (4.74×10^{-3} copies/16SrRNA copy), followed by *tetB* (4.58×10^{-3} copies/16SrRNA copy), *tetA* (3.36×10^{-3} copies/16SrRNA copy), *tetQ* (2.03×10^{-3} copies/16SrRNA copy) and *tetM* (3.51×10^{-4} copies/16SrRNA copy). The relative abundance of *tetM* was significant lower than the other *tet* genes (*tetA*, *tetB*, *tetM*, and *tetG*) at $p < 0.05$ level. But, there was no significant difference between *tetA*, *tetB*, *tetM*, and *tetG*.

For the regional analysis, nine lakes (Datong Lake, Bajiao Lake, Dongting Lake, Xiliang Lake, Honghu Lake, Wanghu Lake, Futou Lake, Saihu Lake and Junshan Lake) located in the middle reach of Yangtze River, whereas the other six lakes were in the lower reach. The average relative abundances of *sul1*, *sul2*, *tetA*, *tetB*, *tetG*, *tetM*, and *tetQ* in lakes from the middle reach were 2.23×10^{-2} , 1.79×10^{-3} , 2.94×10^{-3} , 2.45×10^{-3} , 6.42×10^{-3} , 2.61×10^{-4} and 2.24×10^{-3} copies/16SrRNA copy, respectively. The average relative abundances of *sul1*, *sul2*, *tetA*, *tetB*, *tetG*, *tetM*, and *tetQ* in lakes from the lower reach were 5.33×10^{-2} , 1.90×10^{-2} , 3.98×10^{-3} , 7.77×10^{-3} , 2.23×10^{-3} , 4.86×10^{-4} and 1.70×10^{-3} copies/16SrRNA copy, respectively. *sul* genes in lakes from lower reach of the Yangtze River was significantly higher than the lakes from middle reach of the Yangtze River. But the *tet* genes showed varied trend depended on the *tet* gene types.

4. Discussion

4.1. Diversity and abundance of ARGs

The 15 lakes along the middle and lower reach of the Yangtze River suffered ARG pollution. For 18 tested ARGs during the period of qualitative analysis, most detected ARG types were found in Dongting Lake. Fifteen types of ARGs were observed in Datong Lake, Shijiu Lake, Taihu Lake and Honghu Lake. To get a clear view of detected ARG types in 15 lakes, negative and positive PCR reactions were renamed 0 and 1 for cluster analysis. Three groups of ARGs

detected by conventional PCR analysis were classified (Fig. 4a). The first group was Bajiao Lake, in which *sul3*, *tetW*, *qnrA*, *ermA* and *ermC* were not detected. The second group included Xiliang Lake, Futou Lake, Gehu Lake, Changdang Lake, Nanyi Lake, and Junshan Lake, in which 8 to 12 types of ARGs were found. Honghu Lake, Datong Lake, Yangcheng Lake, Dongting Lake, Shijiu Lake, Saihu Lake and Taihu Lake constituted the third group, in which 14 to 16 types of ARGs were observed. This result indicated that the lakes in the third group had more abundant ARG diversity.

The relative abundance of 7 ARGs in 15 lakes was also classified into two groups (Fig. 4b). The first group included Dongting Lake, Honghu Lake, Changdang Lake, Taihu Lake and Yangchang Lake. In this group, the average values of *sul1*, *sul2*, *tetA*, *tetB*, *tetG*, *tetM*, and *tetQ* were 3.11×10^{-2} , 2.05×10^{-2} , 5.06×10^{-3} , 1.18×10^{-2} , 1.18×10^{-2} , 7.30×10^{-4} and 2.41×10^{-3} copies/16SrRNA copy, respectively. Datong Lake, Bajiao Lake, Xiliang Lake, Wanghu Lake, Saihu Lake, Junshan Lake, Nanyi Lake, Shijiu Lake, and Gehu Lake comprised the second group. The average values of *sul1*, *sul2*, *tetA*, *tetB*, *tetG*, *tetM*, and *tetQ* in the second group were 3.32×10^{-2} , 2.48×10^{-3} , 2.28×10^{-3} , 8.83×10^{-4} , 1.09×10^{-3} , 1.47×10^{-4} and 1.67×10^{-3} copies/16SrRNA copy, respectively. This result indicated that the first group had both high relative abundance of *sul* and *tet* genes. The second group had high value of *sul1* and low values of *sul2*, *tetA*, *tetB*, *tetG*, *tetM* and *tetQ*. That is also to say, only 5 (Dongting Lake, Honghu Lake, Changdang Lake, Taihu Lake and Yangcheng Lake) of 15 lakes suffered heavy *sul* and *tet* pollution. Anthropogenic impacts and environmental pollution might be the main reason accounting for the high relative abundance of ARGs in these five lakes. For Dongting Lake, serious heavy metal pollution was observed in sediment (Makokha et al., 2016) and human disturbance was an important factor influencing the ecological function in this lake (Yuan et al., 2014). For Honghu Lake, it was a very important aquaculture lake and enclosure aquaculture had a great influence on the pollution status (Ban et al., 2010). For Changdang Lake, serious ecological risk level of heavy metals was observed in the sediment (Jiang et al., 2015). In Taihu Lake, it suffered high antibiotic and heavy metals pollution in sediment (Xu et al., 2014; Niu et al., 2015) and swine, chicken, and/or human sources had been identified as the reason for ARGs in bacteria (Zhang et al., 2015). Yangcheng Lake was very famous for the culture of Chinese mitten crab and enclosure culture also had a great influence on the pollution status (Ling et al., 2010). *sul* genes usually dominated ARG types in water samples, such as surface water in 21 Swiss lakes (Czekalski et al., 2015) and aquaculture environment (Gao et al., 2012). In this study, *sul* genes accounted for 25–99% of total ARG abundance in the sediment of 15 lakes.

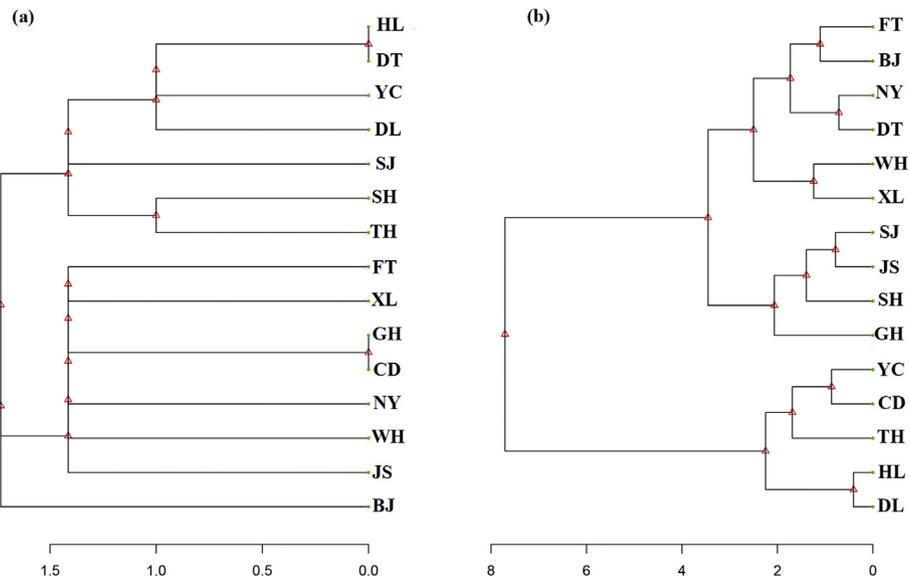


Fig. 4. The classified groups for qualitative analysis (a) and quantitative analysis (b) of ARGs in sediments of 15 lakes via cluster analysis (Abbreviations of lakes are the same as in Table 1).

4.2. Correlation with the class I integron

Mobile genetic elements mediated horizontal gene transfer was an important way for widespread dissemination of ARGs in environment, such as integrons and plasmids (Stalder et al., 2012; Marti et al., 2014). In this study, the relative abundance of *int11* in 15 lakes ranged from 1.91×10^{-3} (Dongting Lake) to 4.40×10^{-2} (Gehu Lake). Pearson analysis showed that *sul1* and *tetG* had positive correlation with *int11* at $p < 0.05$ ($r = 0.434$) and $p < 0.1$ ($r = 0.320$) level, respectively. While, the other five ARGs tested in this study had no significant correlation with *int11*. In Haihe River, extracellular DNA in river sediment facilitated antibiotic resistance gene propagation (Mao et al., 2013). The sediment characteristics were different from water samples, so the ARG propagation may be more

complex.

4.3. Factors influencing the ARG distribution

In this study, 13 factors were selected to investigate their effect on ARG distribution including lake morphology (area and depth), land use pattern (vegetation, agriculture, built-up land and water), sediment characteristics (moisture, density, total nitrogen, total carbon, ammonium, and nitrate) and *int11*. *int11* was also regarded as an environmental marker of anthropogenic pollution (Gillings et al., 2015). Fig. 5 showed the result of redundancy analysis between 13 factors and 7 ARGs. It indicated that *int11* had high correlation with *sul1*. Built-up land use and area had correlation with *tetM* gene. Most sediment characteristics had no correlation with

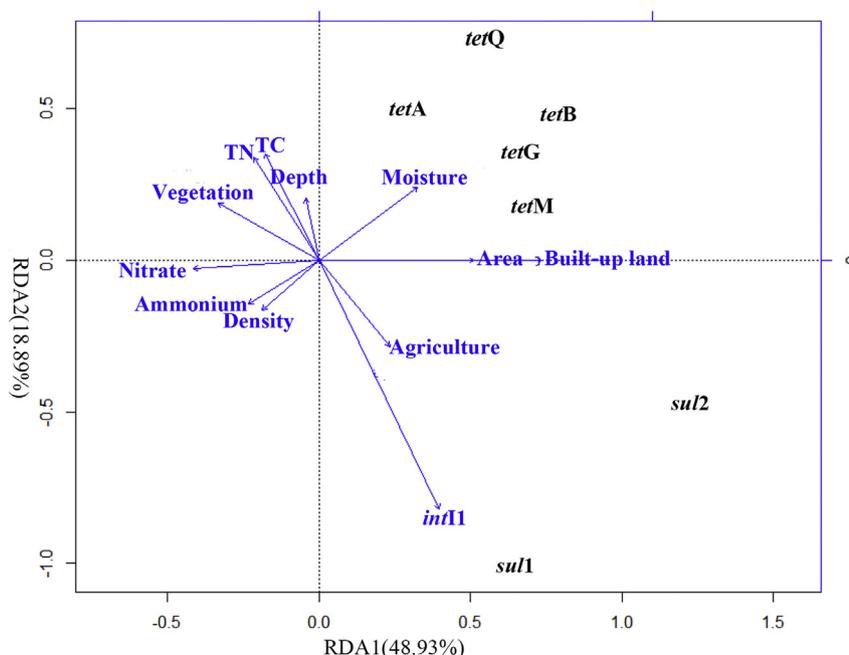


Fig. 5. Redundancy analysis (RDA) for the quantitative correlation between ARGs and lake morphology, land use pattern, sediment characteristics and *int11*.

ARGs, indicating that sediment characteristics had no significant effect on ARG distribution. In 21 Swiss lakes, agricultural rather than urban impacts were proposed as the main drivers of elevated ARG abundance in water of lakes (Czekalski et al., 2015). In this study, *int11* and built-up land use were the main drivers of ARG distribution in sediment of lakes. Taihu Lake and Yangcheng Lake which exhibited high *sul* and *tet* genes had the high proportion of built-up land use. Gehu Lake which had the highest relative abundance of *int11* and the third high proportion of built-up land use had high relative abundance of *sul* genes.

5. Conclusion

All the sediments of 15 lakes from middle and lower reaches of the Yangtze River, China experienced ARG pollution. Among the 15 lakes, Dongting Lake, Honghu Lake, Changdang Lake, Taihu Lake and Yangcheng Lake suffered heavy *sul* and *tet* pollution. With exception of Changdang Lake and Yangcheng Lake, *sul2* was less prevalent than *sul1* in the sediments of sampled lakes (1.75–148.77 times). No correlation between *sul1* and *sul2* was found in the lakes. Built-up land use was found to be an important factor driving the distribution of ARGs in lakes. The acquisition and dissemination of ARGs in sediments still need further search to understand the gene transfer mechanism and evolution of ARGs.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.chemosphere.2017.03.041>.

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