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Contamination profiles of antibiotic resistance genes in the sediments at a catchment scale

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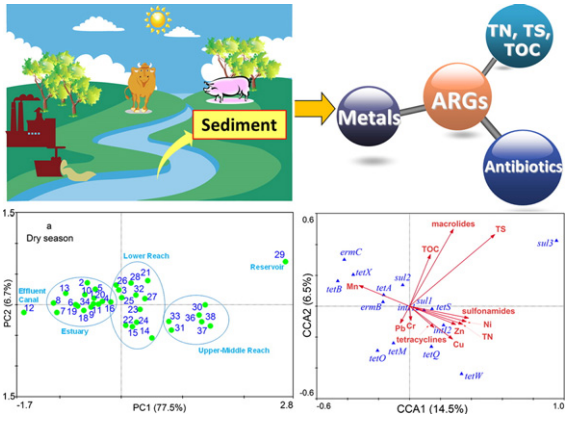
HIGHLIGHTS

- ARGs of tetracycline, sulfonamide, macrolide, and integrons detected in sediment
- *sul2* was the highest resistance gene and the *intI2* was found to be the lowest.
- The distribution of ARGs was correlated to the sediment properties.
- Sediment is a reservoir of ARGs and plays a key role in disseminating ARGs in basin.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this study was to investigate the contamination profiles of tetracycline, sulfonamide, and macrolide resistance genes, as well as integrons in sediments of Dongjiang River basin of South China by real time quantitative polymerase chain reaction. *sul2* was the most abundant resistance gene, with the average concentration of 6.97×10^8 copies/g and 1.00×10^8 copies/g in the dry and wet seasons, respectively, followed by *ermF*, *sul3*, *sul1*, *intI1*, *tetA*, *ermB*, *tetX*, *tetM*, *tetQ*, *tetO*, *tetW*, *tetS*, *ermC*, and *tetB*. The abundance of *intI2* gene was the lowest in the sediment samples. Significant correlations existed between the ARGs and sediment properties as well as metals (Cu and Zn) and corresponding antibiotic classes, suggesting that the contamination of ARGs is related to chemical pollution of the sediments in the river basin. Principal component analysis showed distinct groupings of the sampling sites, reflecting that human activities are the key player in the dissemination of ARGs in the catchment environment.

1. Introduction

The extensive application of antibiotics in human, livestock, and agriculture has led to environmental contamination of antibiotic residues,

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antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs), which has become a global public concern (Pruden et al., 2006). According to a World Health Organization (WHO) report, over two million Americans are infected with resistant pathogens each year, and 14 thousand people die consequently (WHO, 2000). As emerging environmental contaminants, ARGs have been broadly detected in diverse environmental compartments, such as hospital wastewater (Durham et al., 2010; Sidjabat et al., 2006; Vinue et al., 2010), waste water treatment plants (Caplin et al., 2008; LaPara et al., 2011; Zhang and Zhang, 2011), chicken farms (Xia et al., 2010), beef farms (Hoyle et al., 2006), pig farms (Xia et al., 2010), dairy farms (Srinivasan et al., 2005), aquaculture farms (Ishida et al., 2010; Tamminen et al., 2011), surface water and sediment (Pei et al., 2006; Storteboom et al., 2010a, 2010b; Yang and Carlson, 2003), drinking water treatment and distribution systems (Faria et al., 2009; Xi et al., 2009), and even food-producing animals (Deckert et al., 2010; Hughes et al., 2010; Schweitzer et al., 2011). ARGs may be transferred from human and animal sources to different environmental compartments at a regional scale, thus threatening human health; therefore, there is a need to understand their contamination profiles at the regional scale and their relationships to land uses and human activities.

ARGs could be disseminated via the water cycle in a catchment. The sediment phase of a river is potentially a reservoir of various contaminants including antibiotics and ARGs. Diverse ARGs have been reported in the sediment phase of rivers in several countries; for instance, tetracycline resistance genes and sulfonamide resistance genes in the USA (Pei et al., 2006; Pruden et al., 2006; Storteboom et al., 2010b) and in China (Luo et al., 2010), extended-spectrum beta-lactamase (ESBL) genes in China (Lu et al., 2010), and quinolone resistance genes in India and Sweden (Kristiansson et al., 2011). It is known that microorganisms can obtain the ARGs in the environment via horizontal gene transfer (HGT), such as plasmids, integrons, and transposons (Andersson and Levin, 1999; Kruse and Sorum, 1994; Pruden et al., 2006). Hence, the diversity and abundance of ARGs in sediments of a river basin are important information for us to understand the dissemination of antibiotic resistance at the catchment scale.

To understand the characteristics and contamination profiles of ARGs at the catchment scale, the Dongjiang River basin in South China was selected as the study area as it includes different land uses and economic development levels from the upper reach to estuary. The Dongjiang River provides the main drinking water source for a receiving population of over 40 million people in several metropolitan cities in the Pearl River Delta region. So far, little information has been known about the influence of human activities on contamination levels of ARGs in the Dongjiang River basin. The objective of this study was to investigate the contamination profiles of antibiotic resistance genes (ARGs) in the sediments of the Dongjiang River basin in both dry and wet seasons by real time quantitative polymerase chain reaction (qPCR), and evaluated the relationships between the spatial distribution of ARGs and the extent of chemical pollution at the catchment scale. Sediment samples were collected from 36 monitoring stations in the river basin from its upper reach to estuary. The ARGs selected in this investigation include tetracycline resistance genes, sulfonamide resistance genes, macrolide resistance genes, and integrons genes, which are related to some commonly used antibiotics. The results from this study can assist better understanding of the diversity, abundance, and dissemination of ARGs at the catchment scale, and their relationship with anthropogenic influence.

2. Materials and methods

2.1. Study sites and sample collection

Sediment samples were collected at 36 stations in the Dongjiang River basin in December 2008 (dry season) and July 2009 (wet season). The sampling sites include 4 sites in the upper reach, 5 sites in the middle reach, 10 sites in the lower reach, and 17 sites in the estuary of the Dongjiang River basin (Fig. 1). It should be noted that sites 17 and 35 out of all 38 monitoring stations in the river basin (Fig. 1) were not accessible during the sampling campaigns of this study. Basic information about water quality parameters, land use, population and livestock for each site, and different reaches are listed in Tables S1 and S2 (Supporting Information). Three sediment samples were aseptically collected from each site with sterile containers, immediately placed on ice, transported back to the laboratory, and stored in −20 °C until processing within a week.

2.2. Sediment characterization

Basic sediment quality parameters including total nitrogen (TN), total sulfur (TS), and total organic carbon (TOC) as well as particle size distribution were characterized and listed in Tables S3 and S4. Total organic carbon (TOC, %) of each sediment sample was determined by using an LECO C230 carbon analyzer (USA) after removal of carbonates with HCl, whereas its particle size distribution was analyzed by using the pipette method (Wang et al., 2011). The total nitrogen (TN) was determined by using the Kjeldahl method and the total sulfur (TS) was determined by using an elemental analyzer (Islam et al., 2004). Antibiotics in the sediment samples were quantified by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry with the previous method as described by Zhou et al. (2012). The selected antibiotics included five sulfonamides (sulfamethoxazole, sulfadiazine, sulfapyridine, sulfamethazine, trimethoprim), four tetracyclines (oxytetracycline, chlortetracycline, doxycycline, tetracycline), and three macrolides (erythromycin-H_2O, roxithromycin, oleandomycin). Six metal elements, i.e. chromium (Cr), manganese (Mn), nickel (Ni), copper (Cu), zinc (Zn), and lead (Pb), were determined by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500, Agilent, USA) (Yuan et al., 2004).

2.3. DNA extraction and purification

Sediment samples were freeze-dried, ground with a mortar and sieved through a 100-mesh screen. DNA was extracted from each sediment sample using the PowerSoil DNA Isolation Kit (Mobio, USA). Exactly 0.5 g of each sediment sample from every site was used for DNA extraction. The DNA extraction steps followed the protocol provided by the manufacturer. Then DNA was further purified using the DNA Spin Kit (Tiangen, China) to minimize PCR inhibition.

2.4. ARG quantification

Absolute quantification standard curve method was used to quantify the ARGs in the sediment samples. SYBR Green Real Time QPCR Kit (TOYOBO, Japan) was applied to quantitatively determine the abundance of resistance genes. The specific primers of 16 genes are listed in Table S5. Positive controls consisted of cloned and sequenced PCR amplicons obtained from the sludge of wastewater treatment plants and manures of livestock farms. Both positive and negative controls (Milli-Q water) were included in every run. A total of 40 cycles was applied to improve the chances of product formation from low initial template concentrations. A 20 μL PCR reaction solution was employed: 2 × THUNDERBIRD SYBR® qPCR Mix 10 μL, 0.05 mM each primer 0.08 μL, 50 × ROX reference dye 0.04 μL, template DNA 2 μL (DNA < 80 ng), and distilled water 7.8 μL (DNase I treated). The qPCR assays were run on an Applied Biosystems 7500 Fast Real-Time PCR System (ABI, USA). The temperature program for quantification of ARGs consisted of initial denaturing at 95 °C for 1 min, followed by 40 cycles for 15 s at 95 °C, 55 °C for 30 s, 72 °C for 30 s, and a final step for melting curve. The standard curve was used to calculate the copy number of ARGs, with the square of related coefficient (r^2) of the standard curve > 0.99, and the amplification efficiency ranging from 95% to 110%.

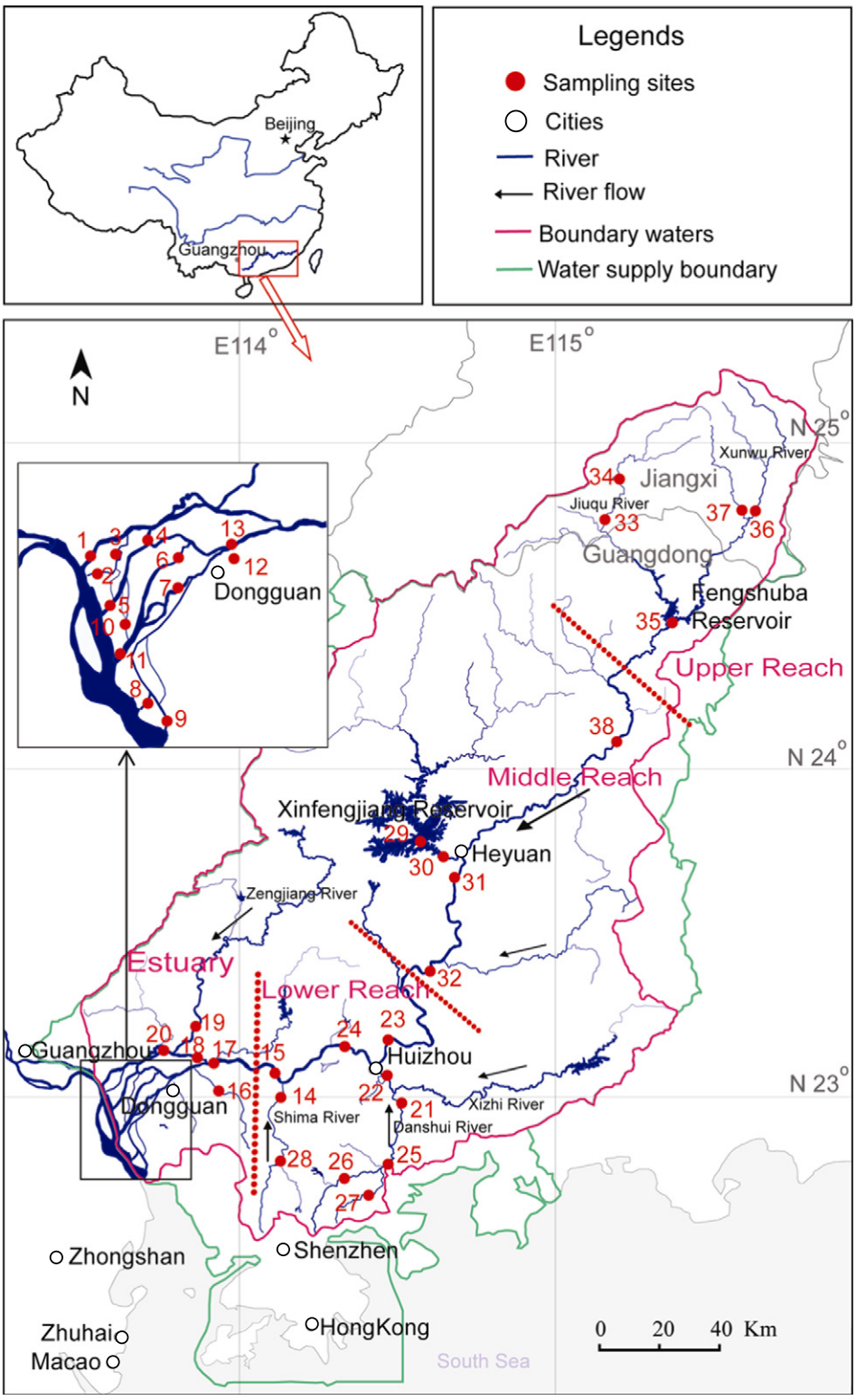

Fig. 1. Location map of the sampling sites in the Dongjiang River basin. The numbers in the map represent the sampling stations (1–38). It should be noted that sites S17 and S35 out of the 38 stations in the river basin were not accessible during the sampling campaigns of this study.

2.5. Statistics

Principal component analysis (PCA) and canonical correspondence analysis (CCA) were performed with Canoco for Windows (Version 4.5). Duncan's multiple range test was used to evaluate the statistical significance of difference with p-value <0.05. Averages and standard deviations were calculated with Microsoft Excel 2003.

3. Results

3.1. Chemical contaminants in the sediments

The sediments from different sites in the Dongjiang River basin exhibited large variations in their basic physiochemical properties. The TN, TS, TOC, and clay contents were 0.03–1.02%, 0.03–0.72%,

0.05–5.24%, and 2.91–40.84%, respectively (Tables S4 and S5). All the six heavy metals (Cr, Mn, Ni, Cu, Zn, and Pb) were detected in the sediments both in the dry and wet seasons (Tables S6 and S7). The concentrations of heavy metals in the sediments varied by three orders of magnitude, ranging from 1.78 μg/g (Cr, S29, in dry season) to 2.78×10^3 μg/g (Cr, S25, in dry season). The concentration ranges of these heavy metals were: Pb, 6.95–15.60×10^2 μg/g; Ni, 1.93–1.96×10^3 μg/g; Cr, 1.78–2.78×10^3 μg/g; Cu, 2.42–3.28×10^3 μg/g; Zn, 2.01–17.94×10^3 μg/g; and Mn, 1.18–43.47×10^3 μg/g.

The concentrations of antibiotics in the sediments were found ranging from ND to 3.46×10^2 ng/g (tetracycline, S26) in the wet season, and from non-detected (ND) to 1.64×10^3 ng/g (oxytetracycline, S19) in the dry season (Tables S8 and S9). Among the three classes of antibiotics, the sulfonamides were found with the lowest concentrations ranging from ND (S29, in both dry and wet seasons) to 43.9 ng/g (S19, in the dry season), followed by macrolides with the concentrations ranging from ND (S29, in the wet season) to 1.97×10^2 (S5, in the wet season). The concentrations of the tetracyclines were the highest, ranging from 2.02 ng/g (S36, in the wet season) to 2.28×10^3 ng/g (S19, in the dry season).

3.2. Contamination of antibiotic resistance genes in the sediments

All of 16 genes including two integron genes (*intI1* and *intI2*), three sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*), three macrolide resistance genes (*ermB*, *ermC*, and *ermF*), and eight tetracycline resistance genes (*tetA*, *tetB*, *tetM*, *tetO*, *tetQ*, *tetS*, *tetW*, and *tetX*) were detected in the sediments of the 36 sampling sites in both dry and wet seasons, even in the reservoir sediment (S29) (Fig. 2). Different from the work of Knapp et al. (2012), the ARGs in the sediments were found to be significantly more abundant in the dry season than in the wet season (ANOVA, $p < 0.05$). High flow events in the wet season might erode some of the top sediment layer.

The concentrations of 16 genes in the collected sediments of the river basin are listed in Tables S10 and S11. The concentrations of 16 genes varied greatly by eight orders of magnitude, ranging from 8.72×10^2 copies/g (*intI2*, S29, wet season) to 2.10×10^{10} copies/g (*sul2*, S12, dry season). Amongst the 16 genes determined, the highest ARG was *sul2*, with an average concentrations of 6.97×10^8 copies/g and 1.00×10^8 copies/g in the dry and wet seasons, respectively, followed by *ermF*, *sul3*, *sul1*, *intI1*, *tetA*, *ermB*, *tetX*, *tetM*, *tetQ*, *tetO*, *tetW*, *tetS*, *ermC*, and *tetB*. The *intI2* gene was found with the lowest average concentrations of 6.04×10^5 copies/g and 2.52×10^5 copies/g in the dry and wet seasons, respectively (Tables S10 and S11, Fig. 2).

A wide concentration range for each class of resistance genes was observed in both dry and wet seasons. The integron resistance genes were found the most prevalent, with 305-fold and 95-fold of differences in the dry and wet seasons, respectively, followed by tetracycline and macrolide resistance genes. Sulfonamide resistance genes were the least prevalent, with 1.9-fold and 2.8-fold of differences in the dry and wet seasons, respectively. Among the four classes of ARGs detected, the sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*) had the highest concentrations, with the total concentrations (*sul* genes quantified in 36 sampling sites) of 5.14×10^{10} copies/g and 7.36×10^9 copies/g in the dry and wet seasons, respectively, followed by the macrolide resistance genes (*ermB*, *ermC*, and *ermF*) and tetracycline resistance genes (*tetA*, *tetB*, *tetM*, *tetO*, *tetQ*, *tetS*, *tetW*, *and tetX*). The abundance of integron genes was the lowest, with the total concentrations of 6.64×10^9 copies/g and 8.68×10^8 copies/g in the dry and wet seasons, respectively. *Sul2* was the most abundant sulfonamide resistance gene, with the total concentrations of 2.51×10^{10} copies/g and 3.60×10^9 copies/g in the dry and wet seasons, respectively. Among the eight tetracycline resistance genes, *tetA* was the most abundant, with the total concentrations of 5.71×10^9 copies/g and 3.33×10^8 copies/g in the dry and wet seasons, respectively. Amongst the three macrolide resistance genes, *ermF* was the most abundant, with the total concentrations of

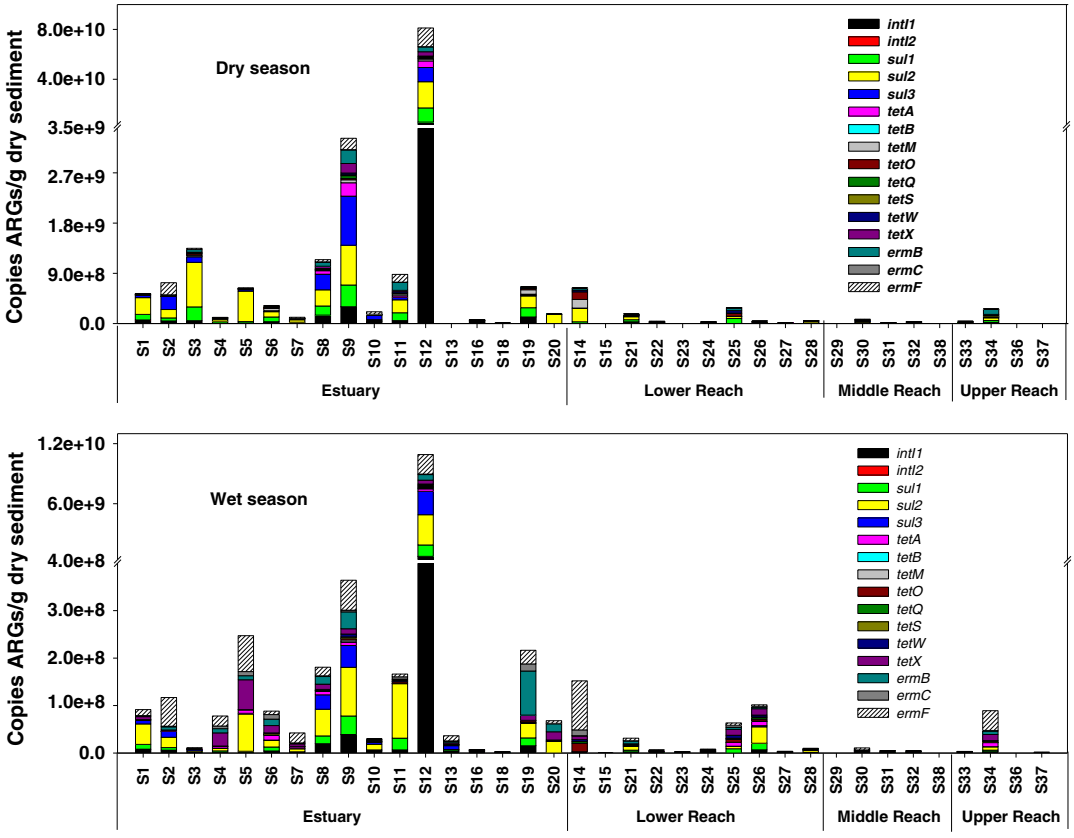

Fig. 2. Concentrations of 14 antibiotic resistance genes and two integron genes in the sediment of Dongjiang River basin in dry and wet seasons.

1.57×10^{10} copies/g and 2.46×10^{9} copies/g in the dry and wet seasons, respectively (Tables S10 and S11). Two integron genes (*intI1* and *intI2*) were determined with total concentrations of 6.62×10^{9} copies/g and 8.59×10^{8} copies/g in the dry and wet seasons, respectively. Clearly, *intI1* was much more abundant than *intI2*.

Significant differentiation in total concentrations of ARGs among the 36 sampling sites was observed at the catchment scale (Fig. 2). More abundant ARGs were found in the estuary and lower reach than in the upper and middle reaches (ANOVA, $p < 0.05$). Sampling site S12 (an effluent canal) in the estuary had the highest total concentrations of ARGs both in the dry and wet seasons, with 8.12×10^{10} copies/g and 1.09×10^{10} copies/g, respectively, followed by site S9. The lowest total concentrations of ARGs were detected at site S29 (a reservoir) in the middle reach, with 8.08×10^{4} copies/g and 4.91×10^{4} copies/g in the dry and wet seasons, respectively. Total concentrations of ARGs of the rest sampling sites ranged from 9.50×10^{5} copies/g (S38 in the middle reach) to 3.32×10^{9} copies/g (S9 in the estuary region) in the dry season and ranged from 1.12×10^{5} copies/g (S38 in the middle reach) to 3.64×10^{8} copies/g (S9 in the estuary region) in the wet season (Tables S10 and S11). Sampling site S12 was found with the highest concentration for each ARG, except for *intI2* in site 21. In contrast, site S29 was observed with the lowest abundances for *intI1*, *intI2*, *sul1*, *sul2*, *tetA*, *tetM*, *tetQ*, *tetS*, *tetW*, *tetX*, *ermB*, *ermC*, and *ermF*.

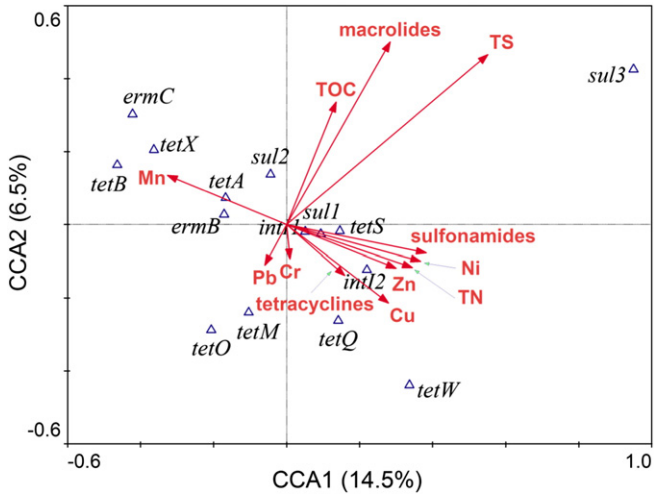

Fig. 3. Canonical correspondence analysis (CCA) of 14 antibiotic resistance genes and two integron genes (triangles) and the environmental variables (heavy metals, antibiotics, and properties parameters of sediment, shown in arrows). TN, total nitrogen; TS, total sulfur; and TOC, total organic carbon. CCA1 and CCA2 explained 14.5% and 6.5% of the total variance, respectively. CCA was performed with Canoco for Windows (Version 4.5).

4. Discussion

4.1. Relationships between the ARGs and environmental variables

Resistance genes of tetracycline, sulfonamide, macrolide, and integrons were measured quantitatively in the sediments both in dry and wet seasons at the catchment scale. Eight tetracycline resistance genes (*tetA*, *tetB*, *tetM*, *tetO*, *tetQ*, *tetS*, *tetW*, and *tetX*), three sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*), three macrolide resistance genes (*ermB*, *ermC*, and *ermF*), and two integron resistance genes (*intI1* and *intI2*) were detected in the sediments of the Dongjiang River basin in both seasons. This indicates that the sediment phase is an important reservoir of diverse antibiotic resistance genes, and plays a key role in storing and disseminating of various ARGs in the river basin.

Pruden et al. (2006) compared the occurrence of ARGs (*sul1*, *sul2*, *tetO*, and *tetW*) in various environmental compartments (dairy lagoons, ditches, effluents, and river sediments) in Northern Colorado, and found that dairy lagoons had the highest ARG concentrations. In the present study, the site (S12) from a canal receiving the discharge of effluent from an urban wastewater treatment plant was found to have higher ARG concentrations than those of dairy lagoons, suggesting a more abundant reservoir of ARGs in the Dongjiang River basin.

Among the detected ARGs in the sediments of the Dongjiang River basin, the abundance of *intI1* was found to have significantly strong correlations with the abundance of *sul1* (Fig. S1). This is consistent with the relationship of these two genes found in *Escherichia coli* isolates obtained from the Dongjiang River basin (Su et al., 2012). The finding from the present study suggests that the propagation of *sul1* is facilitated by class 1 integrons (Su et al., 2011; Vinue et al., 2010).

Canonical correspondence analysis (CCA) showed the relationships between the ARGs and environmental variables including heavy metals, antibiotics, and sediment properties (Fig. 3). The first two axes explained 21% of the cumulative percentage variance of the ARGs data with significance ($p = 0.01$). Based on the CCA, some ARGs such as *sul3* and *tetW* showed certain correlations with environmental variables such as TS, TOC, TN, Cu, Zn, and Ni. Pearson correlation analysis (Table 1) further demonstrated some significant correlations between the ARGs and environmental variables, especially sediment properties such as TN, TS, and TOC. Significant correlations existed between some ARGs (e.g. *tetM*, *sul1*, *intI1*, and *ermF*) and their corresponding antibiotics (Table 1). Significant correlations also existed between most detected ARGs and heavy metals like copper and zinc, which was in accordance with the study of Graham et al. (2011). The results suggest that the contamination of ARGs is related to anthropogenic activities in the river basin, for instance, agricultural activities, industrial activities, and sewage discharge. It is well established that wastewater treatment plants cannot completely eliminate ARGs (Caplin et al., 2008; LaPara et al., 2011; Zhang and Zhang, 2011). Wastewaters contain both chemical contaminants and ARGs. The results of this study may also reflect the same transport pathways for the ARGs and chemical contaminants via surface water in the river basin.

4.2. Spatial distribution of the ARGs in the river basin

Significant distinction in the abundance of ARGs in the river sediments at the catchment scale was observed (Fig. 2). The abundances of the detected ARGs in the lower reach and estuary of the Dongjiang River basin were much higher than those in the middle and upper reaches (ANOVA, $p < 0.05$). The sampling site S12 in a canal receiving the treated wastewater from a wastewater treatment plant was observed to have the highest total concentration of the ARGs in the river basin in both seasons. Furthermore, the abundances of most of the individual ARGs determined in this study were found the highest at site S12. It has been known that ARGs cannot be completely eliminated by existing wastewater treatment processes (Caplin et al., 2008; LaPara et al., 2011; Zhang and Zhang, 2011); thus, a more effective treatment technology is needed to remove the ARGs from the wastewater. The Xinfengjiang Reservoir (S29, less impacted by human activities) used as a drinking water source of the Pearl River Delta region was found to have the lowest total concentrations of ARGs in both seasons, and also observed to have the lowest abundance for most of the individual ARGs determined in the present study. Previous studies also showed detection of ARB and ARGs in drinking water (Cernat et al., 2007; Faria et al., 2009; Koksal et al., 2007; Ram et al., 2008), suggesting that some ARB and ARGs can survive drinking water treatment processes. It is documented that microorganisms can acquire ARGs in the environment with the mechanisms of plasmids, integrons, and transposons (Andersson and Levin, 1999; Kruse and Sorum, 1994; Pruden et al., 2006). Although the concentrations of ARGs in the reservoir of the present study were the lowest compared with the sites in the estuary and lower reach of the river basin, the Xinfengjiang Reservoir as the drinking water source is still a reservoir of various ARGs, which may pose potential threats to public health.

Table 1
Correlations between the antibiotic resistance genes and environmental variables[#].

Environmental variables[†]	*intI1*	*intI2*	*sul1*	*sul2*	*sul3*	*tetA*	*tetB*	*tetM*	*tetO*	*tetQ*	*tetS*	*tetW*	*tetX*	*ermB*	*ermC*	*ermF*
TN	0.62**		0.61**	0.48**	0.61**	0.63**	0.27*	0.53**	0.42**	0.62**	0.62**	0.62**	0.61**	0.62**		0.51**
TS	0.53**		0.46**	0.34**	0.5**	0.48**				0.46**	0.48**	0.41**	0.51**	0.48**	0.27**	0.5**
TOC	0.33**		0.32**					0.33**		0.33**	0.43**	0.33**	0.38**	0.33**	0.25*	0.35**
Sulfonamides	0.3*		0.32*					0.37**								
Tetracyclines	0.27*		0.29*					0.42**								
Macrolides																0.25*
Cr									0.26			0.65**		0.25*		
Mn																
Ni												0.36**				0.51**
Cu	0.29*		0.38**			0.27*			0.32**		0.37**	0.78**	0.25*	0.48**		0.31**
Zn	0.33**		0.41**		0.28*	0.31*			0.32**	0.28*	0.39**	0.78**	0.29*	0.49**		0.33**
Pb												0.32**				

[#] Values indicated the Pearson correlation coefficient (r).
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).
[†] Concentrations of the environmental variables in the sediment samples. TN, total nitrogen; TS, total sulfur; TOC, total organic carbon; sulfonamides, sum of sulfamethoxazole, sulfadiazine, sulfapyridine, sulfamethazine, and trimethoprim; tetracyclines, sum of oxytetracycline, chlortetracycline, doxycycline, and tetracycline; macrolides, sum of erythromycin-H_2O, roxithromycin, and oleandomycin; Cr, chromium; Mn, manganese; Ni, nickel; Cu, copper; Zn, zinc; Pb, lead.

Principal component analysis (PCA) (Fig. 4) showed distinct groupings of the 36 sampling sites in the river basin in both seasons. The reservoir (S29) and the canal receiving effluents of a wastewater treatment plant (S12) are located at the two ends of the PCA graphs. Site S34 is located in a tributary of the upper reach, but influenced by nearby livestock operations, hence resulting in a relatively high ARG abundance. The remaining sites in the Dongjiang River basin were generally grouped into three distinct groups: upper and middle reach, lower reach, and estuary. The sampling sites in the river basin were distributed from less impacted areas (i.e. reservoir, upper, and middle reaches) to highly impacted areas (lower reach, estuary, and effluent canal). This is consistent with the human activities in the river basin, with a much higher population density in the areas of the lower reach and estuary as shown in Tables S1 and S2. The results demonstrated that human activities in the river basin are the key player in the dissemination of ARGs in the catchment environment.

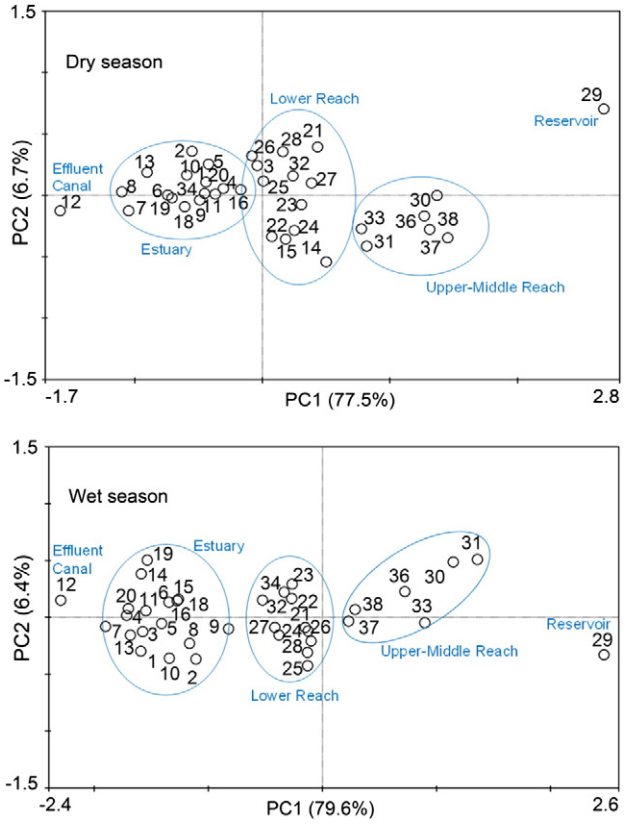

Fig. 4. Principle component analysis (PCA) of absolute concentrations of 14 antibiotic resistance genes and two integron genes in the Dongjiang River basin in the dry and wet seasons. The numbers in the graph represent the sampling stations (1–38). The first two principal components are shown in the graphs. The PC1 and PC2 explained 77.5%, 6.7% and 79.6%, 6.4% of the total variance in dry and wet seasons, respectively. PCA was performed with Canoco for Windows (Version 4.5).

The present study clearly demonstrated that sediment phase is a reservoir of various ARGs in the catchment environment, and their abundance is related to human activities at the catchment scale. In fact, diverse abundant ARGs were also detected in the river water in our previous study (Su et al., 2012). ARGs could be transferred between different microorganisms in riverine environments by HGT mechanisms (Andersson and Levin, 1999; Kruse and Sorum, 1994; Pruden et al., 2006). This could result in the development of multiple antibiotic resistance in microorganisms (El-Mansi et al., 2000; Ochman et al., 2000; Smillie et al., 2010). Since the Dongjiang River is an important drinking water source for metropolitan cities in the Pearl River Delta region including Guangzhou, Shenzhen, and Hong Kong, measures should be taken to remove ARGs in the final drinking water as a precaution where possible. Further study is needed to know which ARGs are of greatest health concern and what levels are safe.

5. Conclusions

Our study provided the first-hand data on prevalence of diverse antibiotic resistance genes in the sediments of Dongjiang River, South China at the catchment scale. The presence of 14 ARGs of tetracycline, sulfonamide, macrolide and two integron genes was ubiquitous throughout 36 sampling sites both in dry and wet seasons. The abundance of sulfonamide resistance genes was the highest. Among 16 genes tested, *sul2* was the most abundant on average, followed by *ermF*, *sul3*, *sul1*, *intI1*, *tetA*, *ermB*, *tetX*, *tetM*, *tetQ*, *tetO*, *tetW*, *tetS*, *ermC*, and *tetB*. The lowest gene was *intI2*. The distribution of ARGs was well related to the sediment properties, heavy metals, and corresponding antibiotic classes. Intensive human activities are one of the most important factors affecting the dissemination of ARGs in the catchment environment.

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Appendix A. Supplementary data

Basic information about the river basin and sampling sites (Tables S1–S4); primers used for qPCR (Table S5); concentrations of metals and antibiotics in the sediments (Tables S6–S9); concentrations of antibiotics resistance genes in the sediments (Tables S10–S11); and correlation analysis between the abundance of *intI1* and *sul1* genes (Fig. S1). Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.05.060.

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