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Environmental factors influencing the distribution of total and fecal coliform bacteria in six water storage reservoirs in the Pearl River Delta Region, China

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Abstract

The Pearl River Delta (PRD) is one of the most developed and densely populated regions in China. Quantifying the amount of pathogens in the source of drinking water is important for improving water quality. We collected water samples from six major water storage reservoirs in the PRD region in both wet and dry seasons in 2006. Results showed that external environmental factors, such as precipitation, location, as well as the internal environmental factors, i.e., physicochemical properties of the water, were closely related with the distribution of coliforms. Seasonally, the coliform bacterial concentrations in wet season were one to two orders of magnitude greater than those in dry season. Spatially, coliform bacterial levels in reservoirs near urban and industrial areas were significantly higher ($p < 0.05$) than those in remote areas. Correlation analyses showed that the levels of coliforms had close relationships with pH, temperature, suspended solid, organic and inorganic nutrients in water. Principal components analysis further demonstrated that total coliforms in the reservoirs were closely related with water physicochemical properties, while fecal coliforms were more associated with external input brought in by seasonal runoff.

Key words: coliform bacteria; location; rainfall; physicochemical parameters; reservoir

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Introduction

Surface water supports microbiological communities, including disease-causing microorganisms. In order to protect public health, quantifying the changes and sources of these microbes in source water for drinking is important for an effective water treatment. Generally speaking, the more pathogens present in raw water, the higher degree of disinfection is needed, which involves the high doses of disinfectant applied and a long reaction time (WHO, 2006). However, due to the presence of natural organic matter in source water, high doses of disinfectant and long reaction time also lead to high levels of harmful disinfection byproducts (Hong et al., 2007, 2008). Therefore, controlling microbial levels at the source is the key to provide microbiologically and chemically safe drinking water.

Pathogens are primarily contained in fecal materials, derived from human, livestock and wild animals, and mostly get into surface water through sewage discharge, agriculture, urban and stormwater runoff (Medema et al., 2003). Many kinds of pathogens may present in surface water, and various detection methods were required for different pathogens. As a result, it is unfeasible to test all of them in water. Numbers of fecal coliform bacteria are widely used as microbiological parameters indicating fecal pollution, while those of total coliforms which are of fecal but also of environmental origin can serve as a parameter to provide basic information on surface water quality (WHO, 2006). Epidemiological studies have documented an increased risk of contracting gastrointestinal and respiratory illnesses after contact with waters with elevated concentrations of coliform bacteria (Dewailly et al., 1986; Haile et al., 1999).

The levels of coliform bacteria in surface water are influenced by several environmental factors. Externally, the surrounding land use pattern and the occurrence of storm water runoff are two major external factors causing microbiologically water quality degradation in surface water (Kistemann et al., 2002; Tong and Chen, 2002; George et al., 2004). In general, the levels of coliform bacteria in surface water often peak after a rain event, and are higher in commercial, residential, pasture and agricultural lands, but lower in forest areas (Kistemann et al., 2002; Tong and Chen, 2002; Mehaffey et al., 2005). Internally, the physicochemical and biological conditions in reservoirs also play an important role in controlling the

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coliform bacteria (Curtis et al., 1992; Davies et al., 1995). The coliform decay rate often increases with elevated water temperature, pH and nutrient scarcity as well as the increasing predation and parasitism (Flint, 1987; Curtis et al., 1992; Davies et al., 1995). In addition, the survival of coliform bacteria can be prolonged or they can sometimes even grow under certain environmental conditions such as proper pH, proper temperature, rich nutrients and abundant suspended particles (Davies et al., 1995; LeChevallie 2003; Juhna et al., 2007).

The Dongjiang River, a tributary of the Pearl River, is an important source of drinking water for Shenzhen, Hong Kong as well as the adjacent areas. During the period of 1996–2001, the Dongjiang River had experienced water quality deterioration due to untreated domestic and industrial discharges, agricultural chemical applications and soil erosion (Ho and Hui, 2001; Ho et al., 2003). A microbiological survey was carried out during the period of 2000–2001, showing that levels of total coliform bacteria in the lower reach of the Dongjiang River water ranged between 4.1×10^4 and 1.0×10^8 CFU/100 mL, and pathogens, such as *Salmonella* spp., *Vibro* spp., *Giardia lamblia* and *Cryptosporidium parvum*, were occasionally detected (Ho et al., 2003). In recent years, with a great deal of work undertaken by authorities in Guangdong) and Hong Kong to control contamination, water quality in the Dongjiang River has been improved (Hong Kong Water Supplies Department, 2009a, 2009b; Yang, 2006, 2007). Monitoring data showed that the Dongjiang River water is now generally classified into the 2nd class of the surface water, according to the State Environmental Quality Standards of China (Hong Kong Water Supplies Department, 2009a). However, it is not known what determine the spatial and temporal changes in the abundance of waterborne pathogens in many water storage reservoirs fed by the Dongjiang River.

The main objective of present study was to identify environmental factors determining total and fecal coliform levels within the Dongjiang River water system. It was hoped that information from this study might help facilitate source water protection and establish more effective water treatment strategies.

1 Material and methods

1.1 Field sampling

Water sampling was conducted in wet season (August 2006) and dry season (December 2006). Six water storage reservoirs were selected, including: (1) reservoir A, storing the upstream Dongjiang River water, located in rural areas and surrounded mostly by forests with limited human activities; (2) reservoirs B and C, storing the downstream water, surrounded by cities with dense population and various industries, such as food processing factories, domestic sewage works; (3) reservoirs D and E, storing the downstream water, surrounded by forests with little human activities; (4) reservoir F, storing local rain water (no input of the Dongjiang River water), surrounded by forests with little human activities.

Water samples were collected from approximately 50 cm below the surface with an open-mouthed bottle (for analysis of physicochemical parameters) and a sterile 1000 mL glass vessel (for total and fecal coliform analyses). For each reservoir, 2–4 sampling points were selected according to the location or shape, and all of the samplings was conducted during 9:30–11:30 am. Water pH and temperature were determined. All water samples were transported to the laboratory in coolers on ice within the same day.

1.2 Determination of total and fecal coliform bacteria

The enumeration of total coliforms (TC) and fecal coliforms (FC) in surface water samples was conducted using membrane filtration according to standard method (APHA, 1998). In brief, water samples was filtered through 0.45 μm pore-sized filters. For TC measurement, the prepared filters were directly placed onto absorbent pads soaked with M-Endo medium on culture dishes, and the inverted dishes were incubated for 22 to 24 hr at (35 ± 0.5)°C. Colonies showing a pink to dark-red color with a metallic surface sheen were defined as typical coliforms, while the dark red, mucoid, or nucleated without sheen are considered as atypical coliforms. Both the typical and atypical coliform colonies were verified using lauryl tryptose broth and brilliant green lactose broth. For FC counting, M-FC medium was used instead of M-Endo medium, and the dishes were incubated at (44.5 ± 0.5)°C for (24 ± 2) hr. Colonies showing various shades of blue on M-FC medium and confirmed with lauryl tryptose broth and EC broth were identified as FC and enumerated.

1.3 Analysis of physicochemical parameters

Each water sample was filtered using a pre-weighted glass fiber filter (GC-50) (1.2 μm), the retained matter on the glass filter was dried at 105°C to a constant weight, and the difference in weights of the filter before and after filtration was the total suspended solids (TSS) (APHA, 1998). The filtrate was used to determine total dissolved phosphorus (TDP), and biodegradable dissolved organic carbon (BDOC). TDP was detected by the molybdate-blue method (APHA, 1998). BDOC was determined according to method described by Servais et al. (1989). Briefly, the filtrate was inoculated with surface water bacteria and then incubated at (20 ± 0.5)°C in the dark for 28 days. DOC levels were determined at the beginning of the inoculation (average DOC value is 1.79 mg/L, ranging from 1.23 to 2.7 mg/L) and at the end of incubation (average DOC value is 1.38 mg/L, ranging from 1.01 to 1.85 mg/L), respectively. The BDOC was calculated as the difference between initial and final DOC.

1.4 Data analysis

An independent *t*-test was applied to compare the levels of physicochemical parameters between the dry and wet seasons. One-way ANOVA followed by the Duncan multiple comparisons test were used to compare coliform levels or physicochemical parameters among the reservoirs. Pearson linear correlations and Principal Components Analysis (PCA) were used to study the relationship between microbiological and physicochemical parameters, employing Statistical Package for the Social Sciences (version 16.0). In PCA analysis, factors were identified via varimax rotation with eigenvalue > 1.

2 Results and discussion

2.1 Seasonal and temporal changes in distribution of total and fecal coliforms

Most reservoirs exhibited one to two orders of magnitude higher levels of coliforms in the wet season than that in the dry season (except FC level in reservoir A) (Table 1). Since the wet season in this area has much higher precipitation than in the dry season (Hong Kong Observatory, 2009), the result suggests that storm water runoff during the wet season serves as an importance carrier of the coliforms flushed into the reservoirs. Usually, rainstorms not only introduce fecal materials from contamination sources to surface waters through runoff or storm water discharge, but also greatly increase water flow (Medema et al., 2003; George et al., 2004; Djuikom et al., 2006). Under rapid water flow, self-purification mechanisms that may remove pathogens in water, such as sedimentation, dilution, sunlight inactivation, predation and starvation, become much less significant as compared to that under slower water flow (Medema et al., 2003). Resuspension of sediments, which may contain much greater load of coliform bacteria than overlaying water, becomes important during rainy season due to a flushing effect of fast flowing water (An et al., 2002; Servais et al., 2007). In addition, there were significantly ($p < 0.05$) higher levels of TC and FC in reservoirs B and C than in other reservoirs (Table 1). This supports the results of previous studies that reservoirs surrounded by densely-populated urban areas are more contaminated by fecal bacteria than those located in sparsely populated areas with forests in the vicinity (Tong and Chen, 2002; Mehaffey et al., 2005).

Table 1 Levels of total coliforms and fecal coliforms in six water storage reservoirs

Indicator bacteria	Reservoir	Wet season	Dry season
Total coliforms	A	2050.0 ± 70.7 e	630.0 ± 28.3 b
(CFU/100 mL)	B	17800.0 ± 283.0 b	1850.0 ± 354.0 a
	C	2500.0 ± 1414.0 a	2800.0 ± 849.0 a
	D	1900.0 ± 141.0 e	190.0 ± 42.4 b
	E	6000.0 ± 566.0 d	700.0 ± 283.0 b
	F	11500.0 ± 424.0 c	550.0 ± 70.7 b
Fecal coliforms	A	3.5 ± 2.1 c	22.5 ± 5.0 b
(CFU/100 mL)	B	7600.0 ± 848.6 a	80.0 ± 28.3 a
	C	4150 ± 1485.0 b	76.0 ± 22.6 a
	D	54.5 ± 3.5 c	1.5 ± 0.7 b
	E	74.0 ± 48.1 c	6.0 ± 0.0 b
	F	51.0 ± 5.7 c	9.5 ± 0.7 b

Each datum represents the mean ± standard deviation. Among the six reservoirs (A–F) within the same season for the same group of indicator bacteria, means with the same letters are not significantly different ($p > 0.05$) according to one-way ANOVA.

2.2 Physicochemical parameters associated with distribution of total and fecal coliforms

Physicochemical properties of the water in the reservoirs also varied seasonally and spatially (Table 2). Particularly, water temperature and pH were all higher in the wet season than in the dry season, and levels of TSS (reservoirs A, B, D and F), TDP (reservoirs A and F) and BDOC (reservoirs E and F) in the wet season were significantly different ($p < 0.05$) compared to dry season. Spatial difference was most obviously reflected in TSS and TDP, with significantly ($p < 0.05$) high levels found in reservoirs B and C in both the wet and dry seasons. It shows that high nutrients due to the great runoff in the wet season and urban areas (Tong and Chen, 2002; Faithful and Finlayson, 2005; Park et al., 2009), may have stimulated the algal growth and result in high pH in the wet season (van der Steen et al., 2000).

Physicochemical properties of the water influence the survival, decay or growth rate of coliform bacteria. For example, suspended solids may facilitate the coliform survival or growth by adsorbing coliform and protecting them from the adverse factors, such as UV-radiation, metal toxicity and attack by bacteriophage (Davies et al., 1995; An et al., 2002; Medema et al., 2003). Suspended solids may also provide coliform bacteria organic and inorganic nutrients in the particles (Gerba and Mcleod, 1976; Davies et al., 1995). On the other hand, coliform death rate increased with elevated water temperature (Flint, 1987), while temperature was positively correlated with occurrence of coliforms (LeChevallie, 2003). In addition, excessively high pH was harmful for the survival of coliform (Curtis et al., 1992; van der Steen et al., 2000), whereas BDOC

Table 2 Physicochemical parameters in six water storage reservoirs

Reservoir	Temperature (°C)		pH		Total suspended solid (mg/L)		Total dissolved phosphorus (μg/L)		Biodegradable dissolved organic carbon (mg/L)	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
A	29.9	18.6	7.57	6.91	1.50 ± 0.30 c*	0.33 ± 0.21 c*	6.67 ± 0.00 c*	4.17 ± 0.83 d*	0.23 ± 0.02 b	0.33 ± 0.27 a,b
B	28.7	15.9	7.72	6.41	10.00 ± 0.80 a*	3.77 ± 0.21 a*	26.67 ± 0.00 a	26.67 ± 0.00 a	0.33 ± 0.22 b	0.31 ± 0.07 a,b
C	32.8	18.9	8.9	6.85	6.40 ± 1.60 b	3.67 ± 0.64 a	9.17 ± 0.83 b*	15.83 ± 0.83 b*	0.83 ± 0.27 a	0.54 ± 0.29 a
D	30.7	18.9	6.79	6.41	1.77 ± 0.25 c*	0.53 ± 0.12 c*	5.83 ± 0.83 c	5.00 ± 0.00 d	0.35 ± 0.20 b	0.14 ± 0.19 b
E	32	19	7.22	6.53	2.40 ± 0.17 c	1.50 ± 0.62 b	8.33 ± 0.00 b	6.67 ± 0.00 c	0.78 ± 0.29 a*	0.21 ± 0.03 b*
F	28.7	18.9	6.19	5.86	2.53 ± 0.35 c*	0.83 ± 0.21 b,c*	6.67 ± 0.00 c*	2.50 ± 0.83 e*	0.82 ± 0.24 a*	0.06 ± 0.17 b*

Among the six reservoirs (A–F) within the same season for the same physicochemical parameter, means with the same letters are not significantly different ($p > 0.05$) according to one-way ANOVA.

* means significantly difference ($p < 0.05$) between wet and dry season in light of independent t-test.

Wet and Dry are abbreviations of wet season and dry season, respectively.

and TDP were the organic and inorganic nutrients available for coliform survival/growth (LeChevallie, 2003; Juhna et al., 2007). It is suggested that factors such as seasonal rainfall and the surrounding environment not only directly influenced the TC and FC levels in the reservoirs (Table 1), but also indirectly influenced the TC and FC levels through influencing physicochemical properties of the water (Table 2).

The relationships among physicochemical parameters and coliform levels were analyzed (Table 3). TC and FC were closely correlated ($p < 0.05$), same observation was obtained in Table 1. The results also showed that TC was positively ($p < 0.05$) correlated with pH, temperature, TSS and BDOC, whereas FC was positive ($p < 0.05$) correlated with pH, TSS and TDP ($p < 0.05$). These relationships were generally expected as proper temperature, TSS, TDP and BDOC are beneficial factors for the coliform survival and/or growth (LeChevallie, 2003; Medema et al., 2003). While the positive relationship between pH and coliform was opposite to the common notion that high pH was harmful for the coliform survival (Curtis et al., 1992; van der Steen et al., 2000). This may be due to: (1) except for two extreme pH values, most pH values in the investigated reservoirs ranged between 6.41–7.72, which should not exert adverse effects on coliform survival/growth (Curtis et al., 1992); (2) the harmful effects to the coliform survival derived from high pH was relieved by the beneficial effects derived from other favorable conditions such as the high TSS level, algal growth (Davies et al., 1995; van der Steen et al., 2000).

Greater correlations ($p < 0.05$) were also observed between pH and temperature, and pH and TSS (Table 3), another effect resulting from algal activities. With higher water temperature and sunlight, algal photosynthesis and growth increased, which led to increased pH and TSS levels (van der Steen et al., 2000; Wetzel, 2001). The relationships between BDOC and temperature may reflect a seasonal change in bioavailability of organic matter, while the close relationship between TDP and TSS was expected as the phosphorus is often transported by attaching to particles (Jonge et al., 2004).

2.3 Identification of factors influencing occurrence of total and fecal coliforms

In order to clarify factors affecting the occurrence of coliforms, Principal Components Analysis (PCA) was conducted (Fig. 1). According to the PCA results, 83.9% of the total variance was explained by two factors. The first factor, accounting for 45.5% of the data variance, had a high positive loading of TC (0.807), pH (0.726), temperature (0.913), and BDOC (0.824). Apparently, this factor may represent the internal physicochemical factors affecting the number of total coliforms.

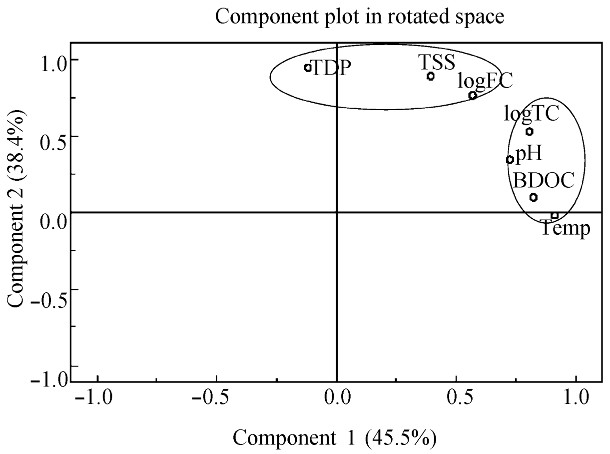

Fig. 1 Factor analysis following varimax rotation for coliform bacterial and physicochemical parameters.

The second factor was responsible for 38.4% of the total variance, predominantly contributed by FC (0.766), TSS (0.891), and TDP (0.947). Generally, elevated TSS in surface water is often closely associated with external causes such as receiving the runoff from drainage basin, re-suspension of sediments due to the dredging, rainfall, boating activity etc. (An et al., 2002; George et al., 2004; Liang et al., 2008). Moreover, both phosphorus and fecal bacteria tend to be bounded by particles, and they are often transported in a particle-facilitated manner (Medema et al., 2003; Jonge et al., 2004). Thus, this factor generally suggests an external influence on the number of fecal coliforms in water.

Overall, the microbiological water quality within Dongjiang water system in 2006 (total coliforms: 1.9×10^2–1.78×10^4 CFU/100 mL) (this study) was much better than those in 2000-01 (total coliforms: 4.1×10^4 –1.0×10^8 CFU/100 mL) (Ho et al., 2003). The marked improvement of microbiological water quality was mainly due to a great deal of protection measures carried out during this period, including constructing sewage treatment plants along the Dongjiang to reduce pollution, removal of factories which polluted the Dongjiang, and using a dedicated aqueduct from the Dongjiang intake to

Table 3 Correlation matrix for coliform levels and physicochemical parameters in reservoir water samples

	logTC	logFC	pH	Temp	TSS	TDP	BDOC
logTC	1.000						
logFC	**0.851**	1.000					
pH	**0.647**	**0.657**	1.000				
Temp	**0.723**	0.469	**0.641**	1.000			
TSS	**0.778**	**0.912**	**0.611**	0.376	1.000		
TDP	0.426	**0.596**	0.197	–0.101	**0.754**	1.000	
BDOC	**0.776**	0.545	0.428	**0.592**	0.325	0.031	1.000

Values in bold letters indicate significant correlation ($p < 0.05$).
TC, FC, Temp, TSS, TDP and BDOC is the abbreviation of total coliform, fecal coliform, temperature, total suspended solid, total dissolved phosphorus and biodegradable dissolved organic carbon, respectively.

the storage reservoirs and completed works to intercept wastewater flowing into the reservoir etc. (Hong Kong Water Supplies Department, 2009b). This study shows that seasonal urban runoff was the main cause affectting the coliform level within Dongjiang water system, while domestic discharges and agricultural wastes were the main cause of microbiological water quality deterioration for Dongjiang water during 2000–2001 (Ho et al., 2003). The PCA results further demonstrate that TC was more related to internal water physicochemical parameters, and FC was mainly attributed to external input through seasonal runoff. Since most pathogens are contained in fecal materials and FC is a better indicator for fecal contamination than TC (WHO, 2006), it seems that reducing the influence from seasonal precipitation, such as interception of seasonal urban runoff or developing buffer zones around the water bodies (Norris, 1993), may serve as important measures to control the microbiological water quality in the reservoirs.

3 Conclusions

This work indicated that seasonal precipitation and the surrounding urban environment not only directly increased the TC and FC levels in the investigated reservoirs, but also indirectly influenced the TC and FC distribution through affecting physicochemical properties of the water. Statistical analysis demonstrated that TC and FC distribution were closely related to physicochemical factors such as pH, temperature, TSS, BDOC and TDP. Principle Component Analysis further indicated that TC was mainly associated with water physicochemical properties, while FC was more linked to the external source. The present results indicate that reducing the influence from seasonal urban runoff may be the key to an effective management of the microbiological water quality in the source water.

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