# Determination of selected antibiotics in the Victoria Harbour and the Pearl River, South China using high-performance liquid chromatography–electrospray ionization tandem mass spectrometry

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Antibiotics were found at high concentration levels in an urban reach of Pearl River in southern China with contrast diurnal variations between the high and low water seasons.

## Abstract

Nine selected antibiotics in the Victoria Harbour and the Pearl River at Guangzhou, South China, were analyzed using high-performance liquid chromatography–electrospray ionization tandem mass spectrometry. The results showed that the concentrations of antibiotics were mainly below the limit of quantification (LOQ) in the marine water of Victoria Harbour. However, except for amoxicillin, all of the antibiotics were detected in the Pearl River during high and low water seasons with the median concentrations ranging from 11 to 67 ng/L, and from 66 to 460 ng/L, respectively; and the concentrations in early spring were about 2-15 times higher than that in

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summer with clearer diurnal variations. It was suggested that the concentrations of antibiotics in the high water season were more affected by wastewater production cycles due to quick refreshing rate, while those in the low water season may be more sensitive to the water column dynamics controlled by tidal processes in the river.

## 1. Introduction

In recent years, there has been an increasing interest in the study of occurrence and fate of pharmaceuticals in the aquatic environment because of their potential function toward the spread and maintenance of resistance in bacterial pathogens and post-therapeutic effects. More than 3000 different chemical substances have been used as human medicines, and in aquaculture and farming applications (Ternes et al., 2004), in which antibiotic is one of the most important groups of common pharmaceuticals in our daily lives. It is well known that the principal pathway of antibiotics into the aquatic environment is through wastewater systems following consumption and excretion by humans, and via effluents from landfills, farms and abattoirs (Daughton and Ternes., 1999; Simon et al., 2005). However, conventional wastewater treatment plants (WWTPs) were designed without consideration of antibiotics removal in wastewater. Many previous studies have shown that, while some antibiotics may be eliminated in the WWTPs, some may be hard to be removed in the process, and can therefore reach the aquatic environment. It was reported that more than 16 categories of antibiotics were observed in river water (Hirsch et al., 1998; Golet et al., 2001; Sacher et al., 2001; Ternes et al., 2002; Kolpin et al., 2002; Lindberg et al., 2004; Miao et al., 2004), sludge and soils (Golet et al., 2002; Hamscher et al., 2002; Michael et al., 2003; Golet

50 et al., 2003).

51 The annual usage of antibiotics has been estimated between 100,000-200,000 tons globally (Kü

52 mmerer., 2003), with more than 25,000 tons in China. In this study, a total nine mostly common

53 antibiotics used in China, selected from five important categories, including quinolones (ofloxacin

54 and norfloxacin), macrolides (roxithromycin and erythromycin), sulfonamides (sulfadiazine，

55 sulfadimidine and sulfamethoxazole) , $\beta$-lactam (amoxicillin) and chloramphenicol

56 (chloramphenicol), were analyzed by HPLC-MS-MS technology in water samples collected from

57 the Victoria Harbour, Hong Kong, and the Pearl River at Guangzhou, South China. Located in

58 South China, the Pearl River Delta (PRD) is one of the fast developing and most urbanized regions

59 in China. With a population of more than 40 millions (including Hong Kong), and a wastewater

60 treatment ratio of lower than 50%, it is estimated that pharmaceuticals and personal care products

61 (PPCPs) in the surface water in the Pearl River Delta may deserve a great concern. However, there

62 has been very little research so far on antibiotics in the environment of the region (Richardson et

63 al., 2005).

64 The aims of the present study were (1) to develop a sensitive method for the determination of

65 selected common antibiotics in various water samples;(2) to determine the selected antibiotics in

66 seawater from the Victoria Harbour of Hong Kong, and the Pearl River water at Guangzhou for an

67 understanding the occurrence of common antibiotics in a subtropical region; (3) to study the

68 diurnal and seasonal variations of antibiotic in the Pearl River water.

69

70 ## 2. Materials and methods

71 *2.1. Chemicals and standards*

72  Ofloxacin, norfloxacin, roxithromycin, erythromycin, sulfadiazine, sulfadimidine, sulfamethoxazole,

73  amoxicillin and chloramphenicol were purchased from Sigma-Aldrich Co. $^{13}C_3$-caffeine solution was obtained

74  from Cambridge Isotope Labs (1 mg/ml in methanol, USA）. All the antibiotics were dissolved in methanol and

75  stored in a freezer. Erythromycin-$H_2O$, major degradation of erythromycin, was obtained by acidification from

76  erythromycin using the method described by Christa et al (2003). Methanol and acetonitrile (HPLC grade) were

77  obtained from Merck (Darmstadt, Germany). Ultra-pure water was prepared with a Milli-Q water purification

78  system (Millipore, Bedford, MA, USA). Unless otherwise indicated, chemicals used were purer than the analytical

79  grade.

80

81  *2.2. Sampling*

82  Seawater samples were collected in the Victoria Harbour of Hong Kong in December 2004 and February 2005

83  at five different sites (B1, B2, B3, B4 and B5) (see Fig. 1). Surface and bottom water (1 m above the sediment and

84  water interface) samples were obtained during the sampling cruise and stored in a precleaned distilled water

85  container.   The river water samples were collected every two hours over one whole day during the representative

86  high (June, 2005) and low (March, 2005) water seasons at a sampling station located at the urban section of the

87  Pearl River at Guangzhou. All the samples were collected (about 1 m below the surface) using a water grab

88  sampler and stored in pre-cleaned brown glass bottles.   All samples (seawater and river water) were kept in the

89  dark at 5 ℃ in a cold storage room before further treatment and analysis.

90

91  *2.3. Preparation for analysis*

92  The water samples were filtered through 0.45 μm glass fiber filters (Millipore, MA, USA). About one liter of

93  water sample was acidified to pH = 3.0 by adding 3.0 M $H_2SO_4$, followed by addition of 0.2 g disodium

4

94  ethylenediamine tetraacetate (Na$_2$EDTA). Before the samples were subjected to extraction, 100 ng $^{13}$C$_3$-caffeine

95  was added to each sample as a surrogate to monitor the recovery. An Oasis HLB cartridge (6 mL, 500 mg, Waters)

96  used for the solid-phase extraction (SPE) was preconditioned sequentially with 6.0 mL of methanol, 6.0 mL of

97  ultra-pure water and 6.0 mL of 10 mmol/L Na$_2$EDTA buffer (pH 3.0). Thereafter, the samples were passed through

98  the SPE columns at a flow rate of approximately 10 mL/min. The HLB column was then rinsed with 10 mL of

99  ultra-pure water pH 3.0, and dried under nitrogen gas for 1 h. After drying, each cartridge was eluted with 2 mL (×

100 3) of methanol. The analytes were collected in a 10 mL brown glass vial, volume-reduced under nitrogen purge to

101 about 20 μl, and then dissolved in 40% aqueous methanol to a final volume of 1.0 mL.

102     For the recovery experiments, one L of filtered groundwater, artificial seawater and river water were fortified

103 separately with 100 ng of target analytes, and 100 ng of surrogate ($^{13}$C$_3$-caffeine). The solutions were treated in the

104 same procedure as the field samples described above.

105 *2.4. HPLC system*

106     The LC system was an HP 1100 LC (Agilent Technologies, Palo Alto, CA, USA) controlled gradient system. It

107 was equipped with an auto sampler, a pump and a thermostated column oven. An ODS-P (Dikma, USA. 4.6

108 mm×250 mm i.d., 3.5 μm) chromatograph column was employed and was operated at 35℃. Optimum separation

109 was achieved using gradient elution. Mobile phase consisted of A: acetonitrile; B: water with 0.2% (v/v) formic

110 acid. The gradient was set up as follows: 0–8 min 40% A, 8–10 min a linear gradient to 60% A, 10–25 min 60% A,

111 25–30 min linear gradient to 40% A, and kept at 40% for 5 min. The injection volume was 20 μl, and the flow-rate

112 was 0.4 mL/min. All the compounds were eluted out of the column within 22 min.

113

114 *2.5. MS-MS system*

115     Mass spectrometric measurements were performed on a Sciex API 4000$^{TM}$ (Applied Biosystems, Foster City,

116    CA, USA) equipped with an electrospray ionization source (ESI). The analysis was performed in negative mode

117    for amoxicillin and chloramphenicol, in positive mode for the other compounds. Both positive and negative ions

118    were acquired in multiple reaction monitoring (MRM) mode with a dwell time of 200 ms. For the positive model,

119    the temperature of the heated capillary was 450℃, and the source voltage was set to 5.5 kV. For the negative

120    model, they were set as 400℃ and 4.5 kV, respectively. Nebulizer gas, curtain gas, collision gas, entrance

121    potential and collision cell exit potential were set at the following values: 10, 15, 6 psi, −10 and 13V, respectively.

122    Declustering potential and collision energy were two key elements which influenced the abundance of the

123    product ion. In order to achieve the highest possible sensitivity of the instrument, declustering potential and

124    collision energy were optimized by direct infusion of the pure analytes to the MS-MS compartment (see Table 1).

125

## 3. Results and discussion

126

*3.1. Calibration, recoveries and limits of quantification*

127

128    Calibration solutions (from 0.1 to 2000 ng/L in six points) were prepared by spiking

129    groundwater with each of the targeted compounds. The reported mean correlation coefficients ($r^2$)

130    of the calibration curves were higher than 0.998 (Table 2), and the relatively standard deviations

131    for all analytes were lower than 6.5%. To obtain the limits of quantification (LOQ) for the

132    groundwater and seawater samples in the current study, the groundwater and artificial seawater

133    were spiked with different amounts of the analytes (n = 5), and the LOQs were calculated as 3 X

134    the standard deviations of the actual measurements of the groundwater and artificial seawater. For

135    the river water samples, LOQ was difficult to determine because the samples already contained

136    some of the selected analytes and the matrix interference was serious. Therefore, LOQs in the

137    river water were defined as a signal-to-noise (S/N) ratios of 10.

138     The recovery rates of groundwater, artificial seawater and river water are shown in Table 2. The

139    mean recoveries for these spiked antibiotics in groundwater, artificial seawater and river water

140    were higher than 67%, 64% and 61%, respectively. Each compound was quantified under the

141    MRM mode using two highest characteristic precursor ion/product ion transitions. Together with

142    the retention times, the characteristic ions were used to ensure correct peak assignment and peak

143    purity. $^{13}C_3$-caffeine was added as a surrogate standard to all samples prior to the enrichment of

144    the control to avoid possible losses during the analytical procedure. It should be noted that

145    erythromycin was determined in the form of its dehydration product, erythromycin-$H_2O$.

146    Erythromycin exhibited strong pH sensitivity. Hirsch et al (1998) and Christa et al (2003) showed

147    that erythromycin-$H_2O$ was the predominant form of erythromycin in the aquatic environment. In

148    addition, the degraded product, erythromycin-$H_2O$, does not exhibit the original antibiotics

149    properties because the orally applied erythromycin has to pass through strongly acidic conditions

150    in the stomach, (Yang et al., 2004).

151

152    *3.2. Occurrence of the selected antibiotics in the Victoria Harbour*

153    Only macrolides and quinolones were detected in a few sampling sites from the Victoria

154    Harbour of Hong Kong. Other antibiotics in all sampling sites were lower than the limit of

155    quantification. Table 3 contains a summary of the results with observed maximum and median

156    concentrations.

157    In Hong Kong, the population is estimated to be 6.9 millions. The wastewater of the city is

158    estimated to be more than 2,000,000 $m^3$ a day. The Victoria Harbour was one of the main venues

159    for WWTP effluent outfalls in Hong Kong. The absence or low concentrations of antibiotics in the

160      seawater samples from Victoria Harbour implies that the environmental conditions in the Harbour

161      has been recovered significantly with the implementation of Harbour Area Treatment Scheme

162      (HATS).　 In addition, the exchange of the marine water between the Harbour and the outside sea

163      is highly efficient for the dispersion of many water pollutants.　 The present results also showed

164      that there were not remarkable differences among selected antibiotics in water between different

165      seasons (Fig. 2). Human-use pharmaceuticals have been shown to reach surface waters primarily

166      via discharge of treated wastewater effluents (Hirsch et al., 1999). In our study, the concentrations

167      of antibiotics in the surface seawater were also obviously higher than that in the bottom water in

168      both seasons.

169      It is also worthy to note that Site B1 and B2 are close to a large fish farming area, Lei Yue Mun

170      (a lot of seafood restaurants there) (Fig. 1).　 Therefore, the elevated concentrations of some

171      antibiotics at these sampling sites may be mainly associated with the intensive fish farming

172      activities in the Harbour, rather than the sewage outfalls.

173

174      *3.3. Occurrence of the selected antibiotics in the Pearl River*

175      The results from the two sampling campaigns (March and June, 2005) of the Pearl River at

176      Guangzhou are presented in Table 4. Except for amoxicillin, all the selected antibiotics were

177      detected in both the high and low water seasons. The median concentrations of antibiotics in the

178      high and low seasons ranged from 11 to 67 ng/L, and from 66 to 460 ng/L, respectively.

179      Guangzhou has a population of more than 10 millions at the end of 2004, and it is estimated that

180      more than 1.7 million tons of domestic wastewater were produced per day in the city. The Pearl

181      River is the sole receiving water body for the wastewater, treated or untreated. The wastewater

182  treatment ratio for domestic sewage was only 61.7% in Guangzhou at the end of 2004, leaving

183  about 0.65 million ton of wastewater per day discharged directly to the Pearl River without any

184  treatment. More new WWTPs are under construction in order to achieve a wastewater treatment

185  ratio of 70% by the end of 2005, and 100% by 2007.

186  Our data showed that the urban section of the Pearl River in Guangzhou was still seriously

187  contaminated by some common antibiotic. It would be of great interest to monitoring these

188  pollutants in river water with the continuous implementation of new WWTPs in the city in the

189  next few years.

190  Significant differences were observed for all the antibiotics under study between the two

191  sampling times. Considerably higher concentrations were found during the sampling campaign in

192  March 2005 (low water season) than June 2005 (high water season with storm water and flooding).

193  Storm water and flooding may either input additional antibiotics from agricultural land and fish

194  ponds to the river, or dilute the concentrations of these pollutants in river water. In the Pearl River

195  Delta region, June is one of the most typical months in the rainy seasons. In June 2005, especially

196  from the 20$^{th}$ to the 29$^{th}$, due to the concurrence of continuous rainfall and astronomical tides, the

197  water level of the urban section of the Pearl River at Guangzhou reached its 100-year record (13 m,

198  63,200 m$^3$/s), which was 1.5 m higher than the security water level in the city. In contrast, the

199  water level in March 2005 was only 2 - 3 m, with a water flow of 2500-3000 m$^3$/s. The lower

200  concentrations of antibiotics in the June 2005 suggested a dominant process of dilution in the Pearl

201  River in high water season, and further indicated that domestic sewages, irrespective of rainfall,

202  may be the principal source for antibiotics in the river water.

203  Ofloxacin and norfloxacin were only detected at two sampling times during the 24 h

204  sampling cycle in the high water season. However, they were found at all the sampling times in

205  the low water season, with the concentrations ranging from 53 to 108 ng/L, and from 117 to 251

206  ng/L, respectively. Quinolone concentrations in the low water season were much high than those

207  in the river water of the Glatt Valley Watershed, Switzerland (Golet et al., 2002), and were

208  comparable and within the range of those previously reported in wastewater in Switzerland and

209  Sweden (Golet et al., 2003; Lindberg et al., 2005), which might reflect the relatively high use rate

210  of quinolones in China.

211      Macrolides were detected in both the high and low water seasons. Erythromycin is readily

212  dehydrated by loss of one water molecule, and its dehydration product erythromycin-$H_2O$ has

213  been detected predominantly in the environment (Gobel et al, 2005). Macrolides were detected in

214  the high water season in similar concentration ranges of the Poudre River in USA (Yang et al.,

215  2004), with concentrations ranging from 5 to 105 ng/L for roxithromycin, and from 13 to 423 ng/L

216  for erythromycin-$H_2O$. In Germany and Canada, macrolides have been detected in all WWTP

217  effluents with high concentrations of roxithromycin and erythromycin-$H_2O$. Roxithromycin, was

218  detected in the Pearl River with a median concentration of 66 ng/L in the low water season.

219  Erythromycin-$H_2O$ concentrations (median 460 ng/L, maximum 636 ng/L) in the low water season

220  were comparable and within the range of normal WWTPs in Canada (Miao et al., 2004).

221      In the high water season, the concentrations (median) of sulfadiazine, sulfadimidine and

222  sulfamethoxazole were 38, 67 and 37 ng/L, respectively. The maximum concentrations of

223  sulfadiazine, sulfadimidine in the low water season were 336 and 323 ng/L, respectively. The

224  sulfamethoxazole concentrations (111-193 ng/L) in the low water season were much higher than

225  that in the Höje River, Sweden (David et al., 2005) and the surface water in German (Hartig et al.,

226  1999). The extensive usage of sulfonamides in various applications contributes to the frequent

227  detection of them in the environment. In addition, sulfonamides have a high potential to resist

228  degradation, and are thus hydrophilic enough to be transported into the aquatic environment

229  (Zuccato et al., 2001).

230  Amoxicillin, the most frequently prescribed antibiotic in China, was not found in both high

231  and low water seasons. The potential explanation could be that the lactam class of antibiotics

232  readily undergoes hydrolysis shortly after excretion due to the chemically unstable lactam ring

233  (Gáspár et al., 2002), and this degradation processes may be enhanced in the subtropical water

234  environment, such as Hong Kong and Guangzhou.

235  Chloramphenicol has widely been used to treat animals since the 1950s. Besides, the external

236  use of chloramphenicol in aquaculture and human application has been rather extensive in China.

237  Chloramphenicol was detected in both the high and low water seasons in the Pearl River, with

238  concentrations ranging from 11 to 266 ng/L, and from 54 to 187 ng/L, respectively.

239

240  *3.4. Diurnal variations of antibiotics concentrations in the Pearl River*

241  The discharge of domestic wastewater in an urban area may display a diurnal pattern as a

242  sequence of daily life cycles of the residents. The Guangzhou section of the Pearl River is also

243  tidal, with two flood tides and ebb tides every day.   In the present study, diurnal variations of the

244  antibiotics concentrations in the Pearl River were monitored by sampling the water every two

245  hours through a full day (24 h cycle). Fig. 3 and 4 show the diurnal variations of antibiotics

246  concentrations in the high water season and the low water season, respectively. Also depicted in

247  the figures are the relative water levels recorded every two hours at the water sampling station.

248        Macrolides, sulfonamides and chloramphenicol were detected most frequently, while

249     quinolones were only detected in a few sampling times in the high water season. However, all the

250     compounds were detected in the river water samples during the low water season. The antibiotics

251     concentrations in the high water season showed a strong diurnal pattern. It is interesting to note

252     that much higher concentrations of antibiotics in the river water were observed, despite of the high

253     water level, in 20:00 hrs, 22:00-01:00 hrs and 08:00 hrs, which corresponded with the major

254     domestic sewage production times (via untreated outfalls).

255        On the contrary, the antibiotics concentrations in the low water season displayed only weak

256     diurnal variations. However, it was found that the concentrations tended to increase with the rising

257     of the water level as a result of the flood tide twice a day. When a flood tide comes from the lower

258     reach, the relatively dense saline water would probably enhance the resuspension of bed sediments

259     and/or the mixing processes in the water column. Previous studies indicated that the amount of

260     antibiotics sorbed to suspended solids and bed sediments can not be neglected (Hektoen et al.,

261     1995; Halling-Sorensen et al., 1998; Löffler et al., 2003; Beausse, 2004; Lalumera et al., 2004).

262     Therefore, it is suggested that, in the low water season, the enhanced resuspension of bed

263     sediments and the mixing processes in the water column by tidal intrusion may be responsible for

264     the relatively high concentrations of antibiotics in the Pearl River at Guangzhou.

265        The interesting contrast diurnal variations of antibiotics concentrations in the river water

266     during the high and low water seasons may deserve more detailed study. As in the high water

267     season, the flow rate in the river was much higher than that in the low water season, due to the

268     rainfalls and surface runoffs from the watershed; this will by no means enable a quick

269     refresh/replacement of water in the urban river section. In the meantime the dynamic processes

such as mixing and resuspension in the river water body may be less affected by the tidal processes. Therefore, the antibiotics concentrations in the water may be more depended on the *in situ* discharge of wastewater and daily sewage production cycles. In the low water season, the refreshing rate of the river water is lower, leading to a relatively longer residence time of antibiotics in the urban river section, as may mask the variations caused by daily sewage production cycles, while the tidal processes may strongly affect the dynamics of the water body in the absence of significant contribution from storm/rain waters.

## 4. Conclusions

The method developed for the determination of antibiotics was successfully applied to the analysis of seawater and river water samples. The use of the MRM model can improve the precision and sensitivity of the analysis. In the Victoria Harbour of Hong Kong, the concentrations of antibiotics were mainly below the limit of quantification (LOQ). However, the selected antibiotics were found in the Pear River during the high and low water seasons at 10s to 100s ng/L levels. The concentrations of antibiotics in the low water season were much higher than those in the high water season, but with a less significantly diurnal variation. It is suggested that the antibiotics concentrations in the high water season was more controlled by daily sewage discharge patterns due to quick refreshing rates, while those in the low water season may be more sensitive to the water column dynamics as enhanced by tidal processes.

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375     **Table 1**

376     **Optimized MS/MS parameters for the target antibiotics**

| Compound | Precursor ion | Product ion | Declustering potential ($V$) | Collision energy ($V$) |
|---|---|---|---|---|
| Ofloxacin | 362 $[M+H]^+$ | 318 $[M-CO_2+H]^+$ | 130 | 24 |
| Norfloxacin | 320 $[M+H]^+$ | 302 $[M-H_2O+H]^+$ | 120 | 25 |
| Roxithromycin | 837.1 $[M+H]^+$ | 158.2[desosamine $+H]^+$ | 120 | 54 |
| Erythromycin-$H_2O$ | 716.1 $[M+H]^+$ | 558.3$[M+H-desosamine-H_2O]^+$ | 120 | 25 |
| Sulfadiazine | 250.9 $[M+H]^+$ | 92.2 | 70 | 40 |
| Sulfadimidine | 279.1 $[M+H]^+$ | 186.1$[M-aminophonyl]^+$ | 75 | 25 |
| Sulfamethoxazole | 253.9 $[M+H]^+$ | 156.1[Sulfonamidyl cation]$^+$ | 68 | 26 |
| Amoxicillin | 363.8 $[M-H]^-$ | 205.6 | -80 | -25 |
| Chloramphenicol | 321 $[M-H]^-$ | 151.6 | -100 | -25 |
| $^{13}C_3$-caffeine | 198.1 $[M+H]^+$ | 140.1 | 80 | 25 |

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378    **Table 2**

379    **Linearity of calibration, recoveries and limits of quantification of the analytes in groundwater (GW),**

380    **artificial seawater (ASW) and river water (RW).**

| Compound | $R^2$ | Recovery ± SD (%) | | | LOQ[a] (ng/L) | | |
|---|---|---|---|---|---|---|---|
| | | GW | ASW | RW | GW | ASW | RW |
| Ofloxacin | 0.998 | 88±10 | 84±12 | 81±12 | 1.5 | 2.6 | 10 |
| Norfloxacin | 0.998 | 86±4 | 77±7 | 80±5 | 2.2 | 3.2 | 10 |
| Roxithromycin | 0.998 | 85±12 | 78±9 | 78±11 | 0.4 | 2.0 | 5 |
| Erythromycin-$H_2O$ | 0.998 | 91±11 | 72±6 | 81±9 | 0.4 | 2.0 | 5 |
| Sulfadiazine | 0.999 | 91±4 | 88±4 | 86±4 | 0.2 | 0.5 | 1 |
| Sulfadimidine | 0.999 | 90±5 | 90±3 | 79±5 | 0.2 | 0.5 | 1 |
| Sulfamethoxazole | 0.999 | 88±7 | 85±10 | 84±9 | 1.0 | 0.8 | 1 |
| Amoxicillin | 0.998 | 67±15 | 64±11 | 61±10 | 3.2 | 5.0 | 20 |
| Chloramphenicol | 0.998 | 74±8 | 78±18 | 80±12 | 2.4 | 4.1 | 5 |
| $^{13}C_3$-caffeine | 0.999 | 93±6 | 88±10 | 83±8 | 0.3 | 0.4 | 1 |

381    [a] Limits of quantification.

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383   **Table 3**

384   **Summary results for the selected antibiotics in seawater from the Victoria Harbour, Hong Kong**

| Compound | No. of samples[a] | Concentrations of the antibiotics in seawater in December 2004 (ng/L) | | | Concentrations of the antibiotics in seawater in February 2005 (ng/L) | | |
|---|---|---|---|---|---|---|---|
| | | No.>LOQ[b] | Median | Maximum | No.>LOQ | Median | Maximum |
| Ofloxacin | 10 | 5 | 5.2 | 8.1 | 6 | 10 | 16.4 |
| Norfloxacin | 10 | 4 | 9.4 | 28.1 | 4 | 12.3 | 20.1 |
| Roxithromycin | 10 | 3 | 6.1 | 21.1 | 5 | 5.1 | 30.6 |
| Erythromycin-$H_2O$ | 10 | 2 | 3.3 | 5.2 | 3 | 3.4 | 4.2 |
| Sulfadiazine | 10 | 0 | nd[c] | nd | 0 | nd | nd |
| Sulfadimidine | 10 | 0 | nd | nd | 0 | nd | nd |
| Sulfamethoxazole | 10 | 0 | nd | nd | 0 | nd | nd |
| Amoxicillin | 10 | 0 | nd | nd | 0 | nd | nd |
| Chloramphenicol | 10 | 0 | nd | nd | 0 | nd | nd |

385   [a] Samples from the five sampling sites (including surface and bottom water)

386   [b] Limits of quantification.

387   [c] Not detected.

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401    **Table 4**

402    **Summary results for the selected antibiotics in the Pearl River water samples during the high and low water**

403    **seasons**

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| Compound | No. of samples[a] | Concentrations of the antibiotics in river water (high water season, ng/L) | | | Concentrations of the antibiotics in river water (low water season, ng/L) | | |
|---|---|---|---|---|---|---|---|
| | | No.>LOQ[b] | Median | Maximum | No.>LOQ | Median | Maximum |
| Ofloxacin | 12 | 2 | 11 | 16 | 12 | 77 | 108 |
| Norfloxacin | 12 | 2 | 12 | 13 | 12 | 150 | 251 |
| Roxithromycin | 12 | 11 | 16 | 105 | 12 | 66 | 169 |
| Erythromycin-$H_2O$ | 12 | 12 | 30 | 423 | 12 | 460 | 636 |
| Sulfadiazine | 12 | 12 | 38 | 141 | 12 | 209 | 336 |
| Sulfadimidine | 12 | 12 | 67 | 179 | 12 | 184 | 323 |
| Sulfamethoxazole | 12 | 12 | 37 | 165 | 12 | 134 | 193 |
| Amoxicillin | 12 | 0 | nd[c] | nd | 0 | nd | nd |
| Chloramphenicol | 12 | 12 | 41 | 266 | 12 | 127 | 187 |

405    [a] Samples each campaign from the Pearl River during every two hours over one whole day.

406    [b] Limits of quantification.

407    [c] Not detected.

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409 **Figures Captions**

410

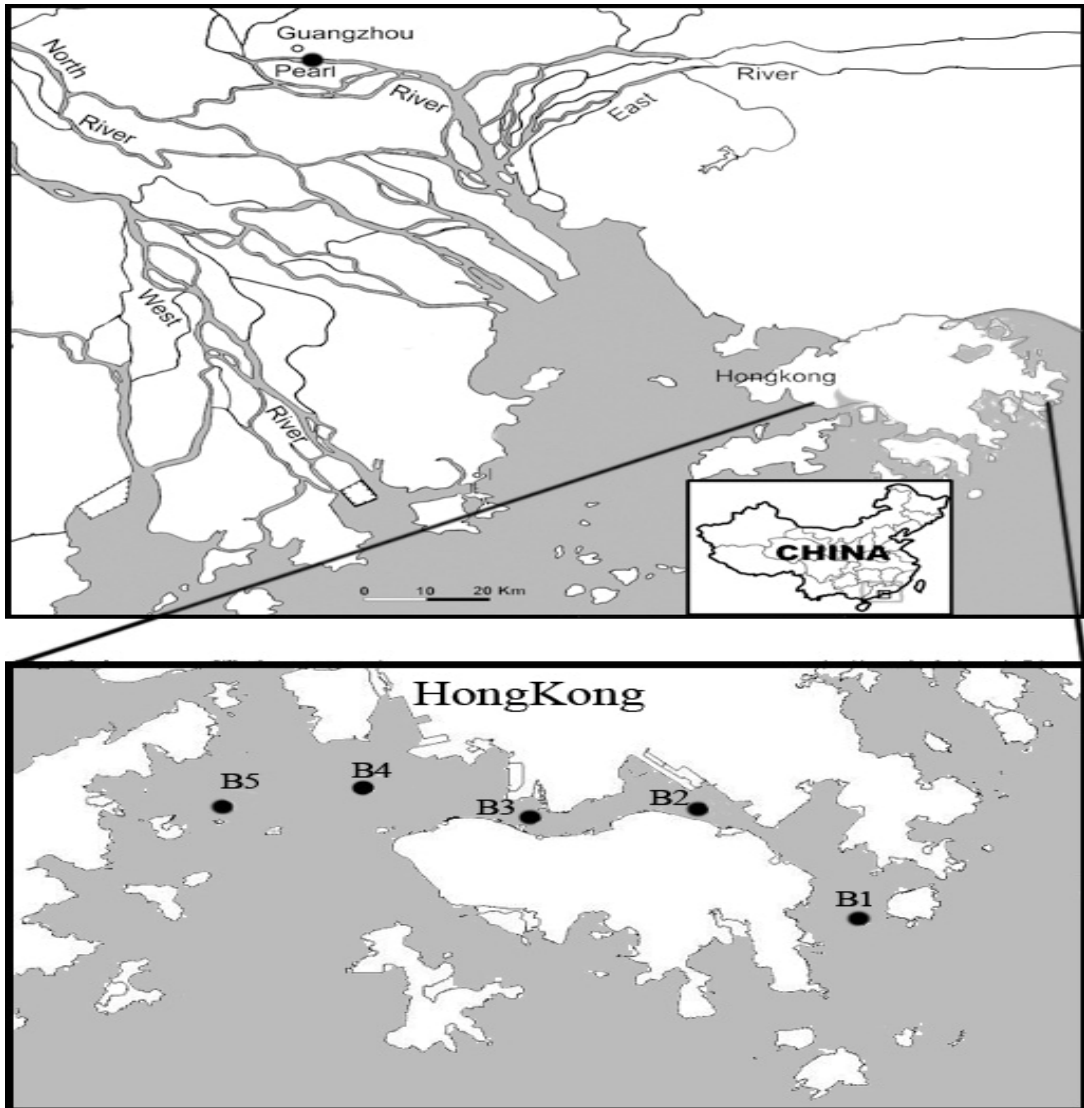411 Fig. 1. Sketch map showing the sampling sites

412 Fig. 2. Concentrations of the selected antibiotics at each sampling location of the Victoria Harbour

413 in December 2004 and February 2005.

414 Fig. 3. Mean concentrations (n = 3) of the selected antibiotics in each sampling time during one

415 day in the low water season of the Pearl River.

416 Fig. 4. Mean concentrations (n = 3) of the selected antibiotics in each sampling time during one

417 day in the high water season of the Pearl River.

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422    **Fig. 1.    Sketch map showing the sampling sites**

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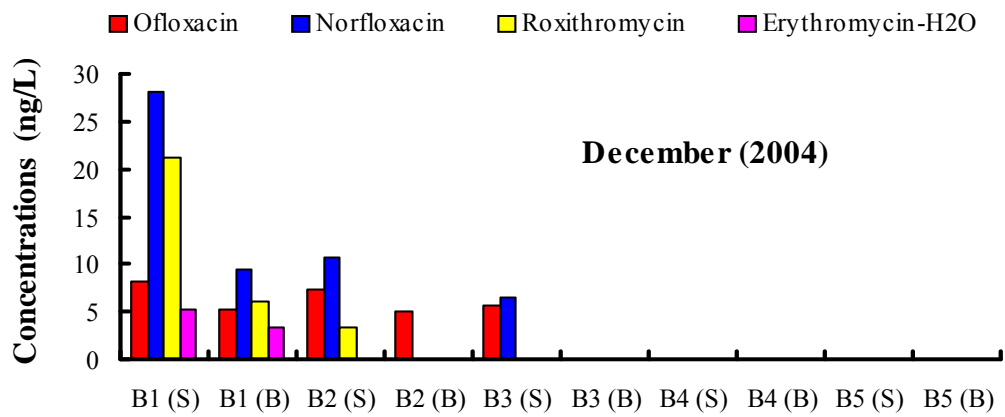
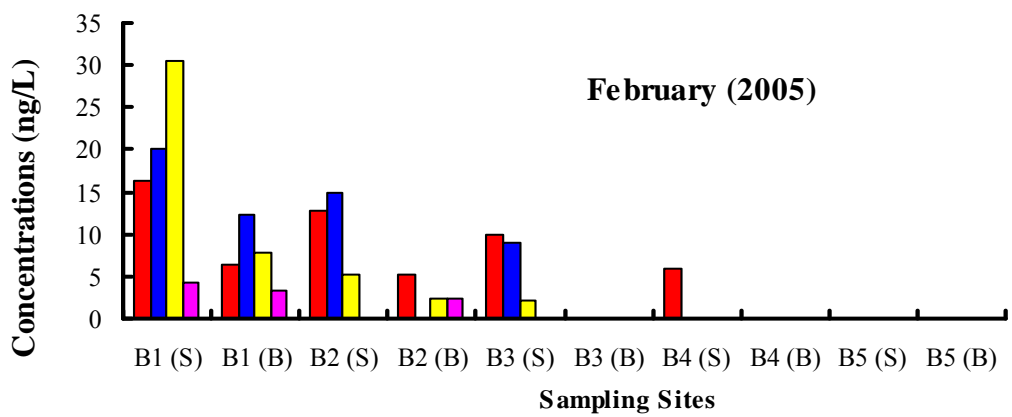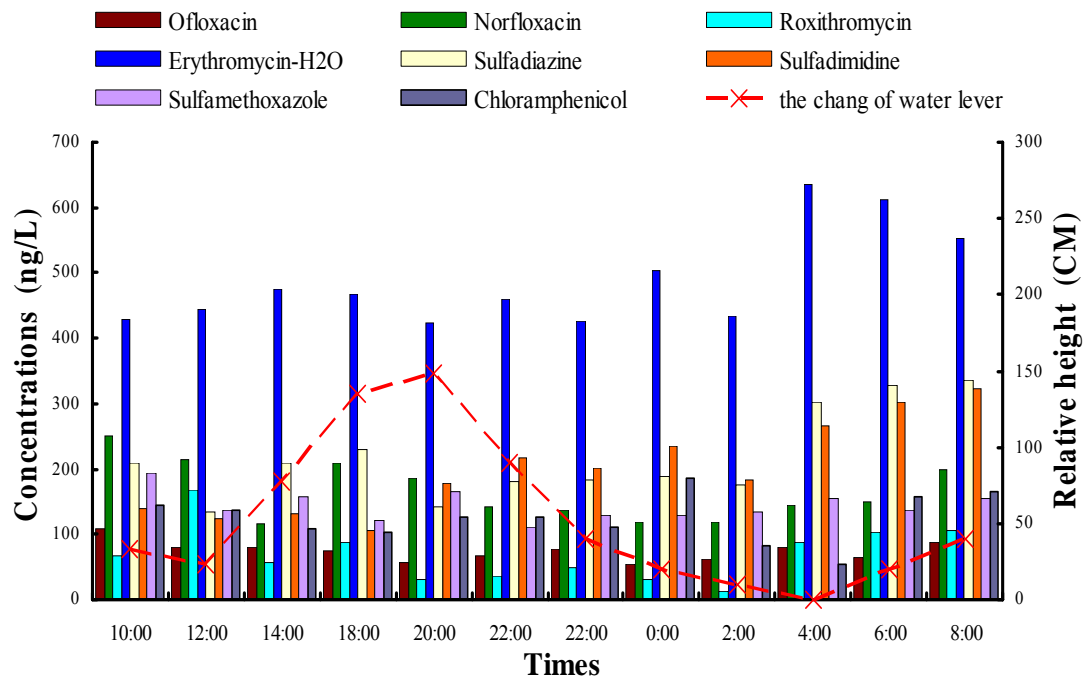**Fig. 2. Concentrations of the selected antibiotics in each sampling location of the Victoria Harbour in December 2004 and February 2005.**

**(S) - surface water**

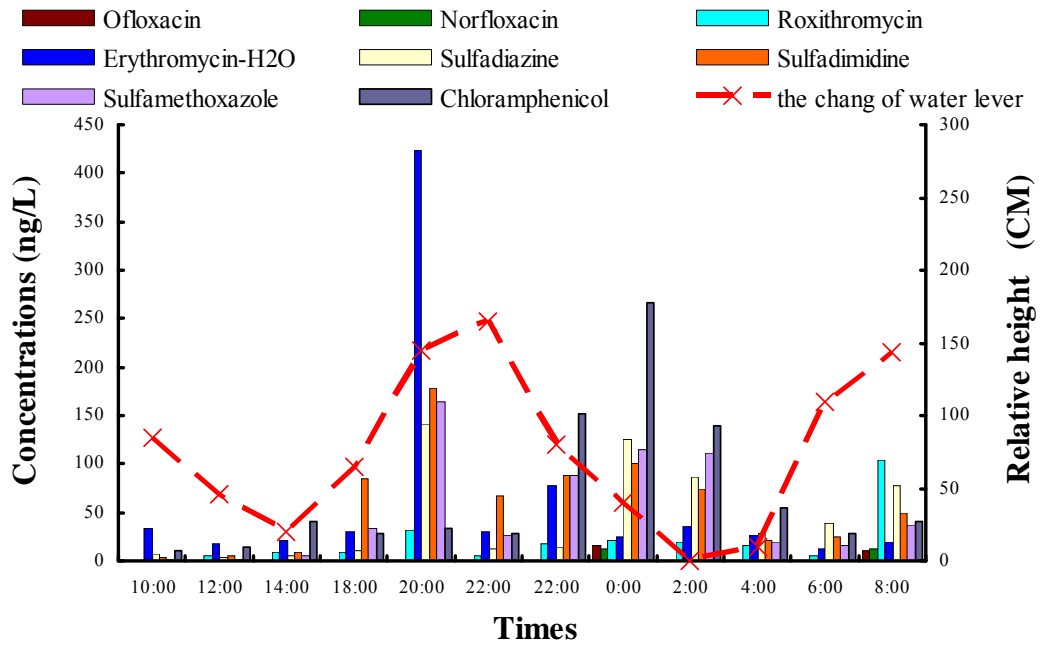**(B) - bottom water**

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449 **Fig. 3. Mean concentrations (n=3) of the selected antibiotics in each sampling time during one day in the low**
450 **water season of the Pearl River.**

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Fig. 4. Mean concentrations (n=3) of the selected antibiotics in each sampling time during one day in the high water season of the Pearl River.

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