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Occurrence of antibiotics and antibiotic resistance genes in soils from wastewater irrigation areas in the Pearl River Delta region, southern China

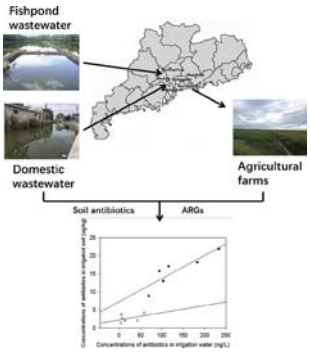
Min Pan [1], L.M. Chu *

School of Life Sciences, The Chinese University of Hong Kong, Shatin, NT, Hong Kong SAR, China

HIGHLIGHTS

- The concentration and abundance of antibiotics and ARGs in irrigation water and soils were investigated.
- Long-term wastewater irrigation increased the abundance of ARGs in soil.
- Antibiotic concentrations had a positive correlation with the abundances of ARGs.
- Antibiotic concentrations and abundances of ARGs decreased with increasing soil depth.

GRAPHICAL ABSTRACT



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ABSTRACT

The occurrence and distribution of tetracycline (TC) and sulfamethazine (SMZ), and the corresponding antibiotic resistance genes (ARGs) were investigated in six agricultural sites in the Pearl River Delta (PRD) region in southern China. Irrigation water and irrigated soils at two different depths (0–10 and 10–20 cm) were analyzed. The total concentrations of TC and SMZ in irrigation water ranged from 69.3 to 234 ng/L and from 4.00 to 58.2 ng/L, respectively, while the total concentrations of TC and SMZ in irrigated soils ranged from 5.00 to 21.9 μg/kg and from 1.30 to 4.20 μg/kg, respectively. After long-term irrigation with domestic and fishpond wastewater in the field, the residual TC and SMZ and their corresponding ARGs in soils were significantly higher in fishpond-irrigated soils (Dongguan and Shenzhen) than in domestic wastewater-irrigated soils (Foshan, Guangzhou, Huizhou and Zhongshan). The concentrations of antibiotics and their ARGs were significantly higher in irrigation water than in irrigated soils, which indicated that wastewater was the primary source of antibiotics in the soil environments. The domestic and fishpond wastewater were important repositories of antibiotics and their ARGs, which require effective treatment before their discharge into the environment. Other factors such as soil physicochemical properties, manure application, irrigation water sources and cropping patterns also affect the antibiotic concentrations and ARG abundances. The residual antibiotic concentrations statistically correlated with the corresponding ARGs in irrigation water and irrigated soils, both of which decreased with increasing soil depth, indicating that the concentration of antibiotics in the environment exerted a selection pressure on the microorganisms in the environment.

* Corresponding author.
E-mail address: leemanchu@cuhk.edu.hk (L.M. Chu).
[1] Present Address: Sino-Forest Applied Research Centre for Pearl River Delta Environment and Department of Biology, Hong Kong Baptist University, Hong Kong SAR, China.

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1. Introduction

Antibiotics are widely used in human medicine as well as in animal and fish farming for illness prevention, disease treatment and growth promotion (USEPA, 2012). They are pseudo-persistent compounds because of their continuous input into the environment and permanent presence (Sarmah et al., 2006; Van Boeckel et al., 2015). The Pearl River Delta (PRD) is a major agricultural region in southern China where large quantities of vegetables and edible crops are produced and exported from. Because of water supply shortages that result from climate change, urbanization, regional drought and pollution, untreated wastewater is commonly used to irrigate agricultural land in arid and semi-arid regions (IPCC, 2009; WWAP, 2012). The use of wastewater for irrigation in agricultural land carries multiple economic and environmental benefits. However, the broad agricultural application of wastewater faces a new challenge: more than 10 million hectares of arable land in China have been contaminated by heavy metals and organic pollutants, while 3.3 million hectares have been contaminated as a result of wastewater irrigation (DAGDP, 2013). Studies from the past two decades showed that antibiotics are present in increasing concentrations in untreated wastewater (Awad et al., 2014; Watkinson et al., 2009), and most of these agents are biologically active and create potential risks to the environment (Chen et al., 2011; Pan and Chu, 2017a; Papadopoulos et al., 2009; Yan et al., 2013). Antibiotics are frequently detected in the soil in recent years, with concentrations varying from ng/g to the low μg/g (Li et al., 2015; Li et al., 2011b; Pan et al., 2014). Therefore, wastewater irrigation may be strongly associated with the significant increases in residual antibiotic concentrations in agricultural soils in the Pearl River Delta Region (Pan and Chu, 2015; Pan and Chu, 2016; Pan and Chu, 2017b).

Tetracyclines constitute one of the most important antibiotic groups used as veterinary and human medicine and as feed additives in the agricultural sector (Li et al., 2011a; Simon, 2005). Annual worldwide production of tetracyclines is estimated to be in the thousands of tons (Michalova et al., 2004), which ranks second among antibiotics. Moreover, it is the most produced and frequently used antibiotic in China (Cheng, 2005; Xie et al., 2010). For instance, the estimated total amount of tetracyclines used in China in 2013 was approximately 12,000 tons; human consumption and veterinary application accounted for 1770 tons and 4430 tons, respectively (Zhang et al., 2015). Sulfonamides are widely used in the therapeutic treatment of animals and humans, but they also pose environmental contamination risks. In 2013, the total amount of sulfonamides used in China was estimated to be approximately 7920 tons, of which human consumption and veterinary application accounted for 905 tons and 5970 tons, respectively (Zhang et al., 2015). In addition, tetracycline and sulfamethazine are the most extensively used compounds of tetracyclines and sulfonamides, which are frequently found in the terrestrial and aquatic environment (Pan et al., 2014; Zhang et al., 2015). Tetracycline has been reported to persist in surface water and soil for more than one year (Zuccato et al., 2000).

In recent years, the occurrence of antibiotics and antibiotic resistance genes (ARGs) in urban water has become an important issue for the protection of the terrestrial environment and public health, as domestic or fishpond wastewater are frequently used for agricultural activities (e.g., irrigation and cultivation). The widespread occurrence of antibiotics in wastewater and agricultural soils may exert selection pressure on environmental microorganisms, resulting in the proliferation of antibiotic resistance in microorganisms. ARGs are considered new contaminants and may pose potential health risks to humans worldwide (Zhu et al., 2013). ARGs in microorganisms could be transferred to indigenous environmental bacteria by horizontal gene transfer (Gillings et al., 2014). The usage of antibiotics in agricultural soil was recognized as an important factor in the generation and propagation of ARGs (Kristiansson et al., 2011). ARGs could migrate to deeper soil layers and possibly contaminate groundwater by vertical transport (Huang et al., 2013). Joy et al. (2014) reported that the abundance of ARGs in surface soils was in the orders of magnitude higher than that in subsoil after manure amendment. However, only a limited number of studies examined the occurrence of antibiotics and relative ARGs in wastewater-irrigated agricultural soils. Tamtam et al. (2011) determined antibiotic concentrations in soils after long-term wastewater irrigation, but they did not examine the ARGs in soils; Negreanu et al. (2012) assessed the long-term effect of wastewater irrigation on antibiotic resistance in soils, but they did not measure the concentrations of antibiotics in soils, nor did they explore the possible correlations between antibiotic concentrations and ARG abundances. Further study is therefore needed to better understand the impact of wastewater irrigation on the occurrence of antibiotics and ARGs, as well as their relationships in agricultural soils.

In this study, soils from six agricultural sites with long-term wastewater irrigation in southern China were sampled and the levels of two typical antibiotics, tetracycline (TC) and sulfamethazine (SMZ), as well as eleven ARGs (*tet* A, *tet* B, *tet* C and *tet* E, which code for energy-dependent efflux proteins that export tetracyclines out of the cell; *tet* M, *tet* O and *tet* S, which code for ribosomal protection proteins; *tet* X, which encodes tetracycline-inactivating enzymes; and *sul* I, *sul* II and *sul* III, which code for dihydropteroate synthases) were quantified using LC-MS/MS and real-time qPCR. The objectives were (1) to assess the effects of long-term wastewater irrigation on the occurrence of antibiotics and relative ARGs in agricultural soils; (2) to verify the vertical distribution of antibiotics and ARG abundances at different soil depths (0–10 and 10–20 cm); and (3) to investigate the relationships between antibiotic concentrations and ARG levels in wastewater and irrigated agricultural soils. This study is the first to investigate the effects of long-term wastewater irrigation on the occurrence of antibiotics and ARGs in agricultural soils in southern China.

2. Materials and methods

2.1. Sampling sites and sample collection

Agricultural sites from six districts, namely Huizhou (HZ), Foshan (FS), Zhongshan (ZS), Guangzhou (GZ), Dongguan (DG) and Shenzhen (SZ), in the PRD region were sampled. This region contains more than 11 million hectares of arable land, and the sampling sites were representative of typical farming practices and had a long history (>20 y) of wastewater reuse. The irrigation water in HZ, FS, ZS and GZ is mainly domestic wastewater, which is mostly untreated and discharged directly into open ditches or nearby rivers, while fishpond water is commonly used for irrigation in DG and SZ (Fig. 1).

Irrigation water samples were collected in 1 L amber glass bottles; 50 mL of methanol was immediately added, and the solution was adjusted to pH 3.0 using 4 M H_2SO_4 to preserve the aqueous samples (five replicates per sampling site). Soil samples were collected from five random plots (100 m × 100 m) at each sampling site. Soil samples were collected at two depths: 0–10 cm and 10–20 cm. An adjacent plot with no antibiotic contamination and without cultivation served as the control. The samples were delivered to the laboratory on ice and processed within 24 h. Soil samples were characterized, and their physicochemical properties are given in Table 1.

2.2. Antibiotic analysis

TC and SMZ were examined in this study; standards and sulfamethazine-d_4 were obtained from Sigma-Aldrich (USA), while tetracycline-d_6 was obtained from Toronto Research Chemicals (Canada). Oasis HLB extraction cartridges (6 mL, 500 mg) (Waters Corporation, USA) were used to extract and purify the target compounds. All organic solvents used were of HPLC grade and purchased from Merck Corporation (Germany). Individual stock solutions and internal