Science of the Total Environment 571 (2016) 974–982

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Removal of antibiotics and antibiotic resistance genes from domestic sewage by constructed wetlands: Effect of flow configuration and plant species

Jun Chen [a], Guang-Guo Ying [a,*], Xiao-Dong Wei [a], You-Sheng Liu [a], Shuang-Shuang Liu [a], Li-Xin Hu [a], Liang-Ying He [a], Zhi-Feng Chen [b], Fan-Rong Chen [a], Yong-Qiang Yang [a]

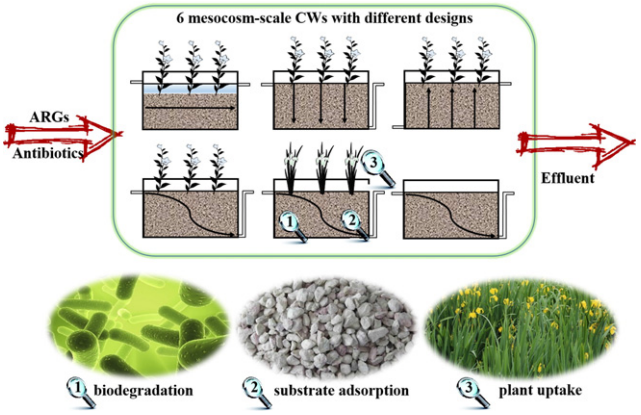
[a] State Key Laboratory of Organic Geochemistry, CAS Research Centre for Pearl River Delta Environment Pollution and Control, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China

[b] Institute of Environmental Health and Pollution Control, School of Environmental Science and Engineering, Guangdong University of Technology, Guangzhou 510006, China

HIGHLIGHTS

- Six mesocosm-scale CWs differed in their flow configuration and plant species.
- Nutrients, antibiotics and ARGs in wastewater were efficiently reduced by the CWs.
- The HSSF-CWs and VSSF-CWs showed higher removals of pollutants than the SF-CWs.
- Planting in the CWs was beneficial to pollutant removal.
- Mass removals attributed to biodegradation, substrate adsorption, and plant uptake.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 June 2016
Received in revised form 12 July 2016
Accepted 12 July 2016
Available online 18 July 2016

Editor: J Jay Gan

Keywords:
Antibiotics
Antibiotic resistance genes
Constructed wetland
Substrate adsorption

ABSTRACT

This study aims to investigate the removal of antibiotics and antibiotic resistance genes (ARGs) in raw domestic wastewater by various mesocosm-scale constructed wetlands (CWs) with different flow configurations or plant species including the constructed wetland with or without plant. Six mesocosm-scale CWs with three flow types (surface flow, horizontal subsurface flow and vertical subsurface flow) and two plant species (*Thalia dealbata* Fraser and *Iris tectorum* Maxim) were set up in the outdoor. 8 antibiotics including erythromycin-H_2O (ETM-H_2O), monensin (MON), clarithromycin (CTM), leucomycin (LCM), sulfamethoxazole (SMX), trimethoprim (TMP), sulfamethazine (SMZ) and sulfapyridine (SPD) and 12 genes including three sulfonamide resistance genes (*sul1*, *sul2* and *sul3*), four tetracycline resistance genes (*tetG*, *tetM*, *tetO* and *tetX*), two macrolide resistance genes (*ermB* and *ermC*), two chloramphenicol resistance genes (*cmlA* and *floR*) and 16S rRNA (bacteria) were determined in different matrices (water, particle, substrate and plant phases) from the mesocosm-scale systems. The aqueous removal efficiencies of total antibiotics ranged from 75.8 to 98.6%, while those of total ARGs varied between 63.9 and 84.0% by the mesocosm-scale CWs. The presence of plants was beneficial to the removal of pollutants, and the subsurface flow CWs had higher pollutant removal than the surface flow CWs, especially for

* Corresponding author.
E-mail addresses: guangguo.ying@gmail.com, guang-guo.ying@gig.ac.cn (G.-G. Ying).

http://dx.doi.org/10.1016/j.scitotenv.2016.07.085

Plant uptake
Biodegradation

antibiotics. According to the mass balance analysis, the masses of all detected antibiotics during the operation period were 247,000, 4920–10,600, 0.05–0.41 and 3500–60,000 μg in influent, substrate, plant and effluent of the mesocosm-scale CWs. In the CWs, biodegradation, substrate adsorption and plant uptake all played certain roles in reducing the loadings of nutrients, antibiotics and ARGs, but biodegradation was the most important process in the removal of these pollutants.

1. Introduction

As emerging contaminants, antibiotics and related antibiotic resistance genes (ARGs) have received increasing attentions due to their potential impact on human health and ecosystem (Costanzo et al., 2005; Pruden et al., 2006; Kotzerke et al., 2008; Liu et al., 2009; Underwood et al., 2011). Previous studies have reported wide detection of various antibiotics and ARGs in effluent and sludge from wastewater treatment plants (WWTPs), which could be a major cause for the ubiquitous occurrence of antibiotics and ARGs in various environmental compartments (Pruden et al., 2006; Durham et al., 2010; Li and Zhang, 2010; Tamminen et al., 2011; Gao et al., 2012; Jia et al., 2012; Su et al., 2012; Cheng et al., 2013; Coleman et al., 2013; Zhou et al., 2013). Therefore, a better wastewater treatment technology is needed for removing antibiotics and ARGs.

Constructed wetlands (CWs) are artificial wetlands designed and constructed to simulate the natural processes to treat domestic and livestock wastewaters (Nurk et al., 2005; Keffala and Ghrabi, 2005; Reyes-Contreras et al., 2012; Liu et al., 2013; Adrados et al., 2014; Younger and Henderson, 2014). Previous studies demonstrated that CWs are capable of removing a majority of environmental pollutants including nitrogen, phosphorous, chemical oxygen demand (COD) (Lin et al., 2002a; Hu et al., 2012; Li et al., 2013; Wang et al., 2013), and emerging contaminants such as antibiotics (Hijosa-Valsero et al., 2011; Liu et al., 2013; Berglund et al., 2014; Chen et al., 2015) and ARGs (Liu et al., 2013; Chen et al., 2015). Their performance depends on the design parameters such as plant species, flow types, substrates, hydraulic loading rates, hydraulic retention time and applied pollutants loadings (Hijosa-Valsero et al., 2010; Hijosa-Valsero et al., 2011; Saeed and Sun, 2012; Weerakoon et al., 2013; Wu et al., 2014; Wu et al., 2015). The removal of pollutants may involve substrate adsorption, plant uptake, photolysis, volatilization and biodegradation (Zhang et al., 2012; Arroyo et al., 2013; Chen et al., 2014; Li et al., 2014). However, the exact contributions by different removal mechanisms depend on wetland design and characteristics of pollutants themselves. Our previous study (Chen et al., 2016) optimized wetland substrate and hydraulic loading for the removal of antibiotics and ARGs. As emerging contaminants with completely different properties, the fate of antibiotics and ARGs in different CWs requires to be investigated further in terms of flow configuration and plant species in wetland design.

The main objective of this study was to compare the efficiencies of the six mesocosm-scale CWs with different design parameters (mainly flow configuration and plant species) in removing antibiotics and ARGs as well as conventional wastewater quality parameters (COD, TN, NH_4-N, TP and TOC). In addition to aqueous removal of antibiotics and ARGs, mass balance analysis approach was applied to assess the mass loadings of antibiotics in mesocosm-scale CWs to understand the removal mechanism by these CWs.

2. Materials and methods

2.1. Setup of the mesocosm-scale constructed wetlands

In February 2015, six mesocosm-scale CWs were constructed outdoors inside the campus of Guangzhou Institute of Geochemistry (GIG), Chinese Academy of Sciences, in Guangzhou City, China. All of the CWs were containers made of stainless steel, with a size of 80 cm in height, 80 cm in length and 60 cm in width. The CWs differed from each other in their flow configuration or plant species (including the constructed wetland with or without plant), which are shown in Fig. 1, and briefly described as follows: CW1 is a surface flow constructed wetland planted with *Thalia dealbata* Fraser. (SF-CW), CW2 is a vertical subsurface flow constructed wetland (VSSF-CW) planted with *Thalia dealbata* Fraser., which wastewater entering from top and leaving through the bottom, CW3 is another vertical subsurface flow constructed wetland planted with *Thalia dealbata* Fraser., which wastewater entering from bottom and leaving through the top, CW4 is a horizontal subsurface flow constructed wetland (HSSF-CW) planted with *Thalia dealbata* Fraser., CW5 is also a horizontal subsurface flow constructed wetland but planted *Iris tectorum* Maxim., while CW6 is a horizontal subsurface flow constructed wetland without plants.

The substrates of 6 mesocosm-scale CWs were zeolite (the grain size of zeolite is 2–3 cm, and the void fraction is 44.7%) based on the optimization results of our previous study (Chen et al., 2016). CW1 had 40 cm layer of substrate (approximately 3.8×10^5 g) and CW2-CW6 had 65 cm layer of substrate (approximately 5.5×10^5 g), while all the six mesocosm-scale CWs had 60 cm layer of water. All CWs (except CW6) planted 6 plants with two rows and three columns. Raw domestic sewage from the GIG residential buildings with a population of 330 people was transferred to a stainless steel regulating pool of 4.3 m^3 before being pumped to the mesocosm-scale CWs. The hydraulic loading rates (HLRs) of the CW systems were 20 cm/d according to our previous study (Chen et al., 2016). All 6 mesocosm-scale CWs had been stably operated for 300 days before sampling. The stability of the CWs was defined by stable water quality within each CW based on weekly measured physicochemical parameters. The experiment work started in November 2015.

2.2. Sample collection

In the sampling campaign, we collected 7 samples of 24-h composite wastewaters (sampling every 8 h and sampling campaign lasts 24 h) (Influent, W0; CW1 effluent, W1; CW2 effluent, W2; CW3 effluent, W3; CW4 effluent, W4; CW5 effluent, W5 and CW6 effluent, W6), 6 composite solid samples with 3-point sampling approach (CW1 substrate, S1; CW2 substrate, S2; CW3 substrate, S3; CW4 substrate, S4; CW5 substrate, S5 and CW6 substrate, S6) (there were 3 sampling spots located in the diagonal of each CW every 0.25 m, and each sampling spot had 3 sample depths, including the bottom one (at the bottom of the CW), the middle one (0.25 m from the bottom) and the top one (0.5 m from the bottom)), and harvested all six plants in each CW to form 5 plant samples (CW1 plants, P1; CW2 plants, P2; CW3 plants, P3; CW4 plants, P4; CW5 plants, P5) (Fig. 1).

There were 3 water outlets in every CW including the bottom one (at the bottom of the CW), the middle one (0.25 m from the bottom) and the top one (0.5 m from the bottom). The wastewater samples for analysis of antibiotics were collected from the water outlet 3 in 1-L precleaned brown glass bottles (1 L each), then approximately 50 mL of methanol was added to each bottle (1 L) of the water samples and the pH values of the samples were adjusted to 3 by using 4 M H_2SO_4. For analysis of ARGs and bacterial biomass, the wastewater samples were collected as the composite samples from the three water outlets in 0.5-L sterile polypropylene bottles (0.5 L each wastewater sample).

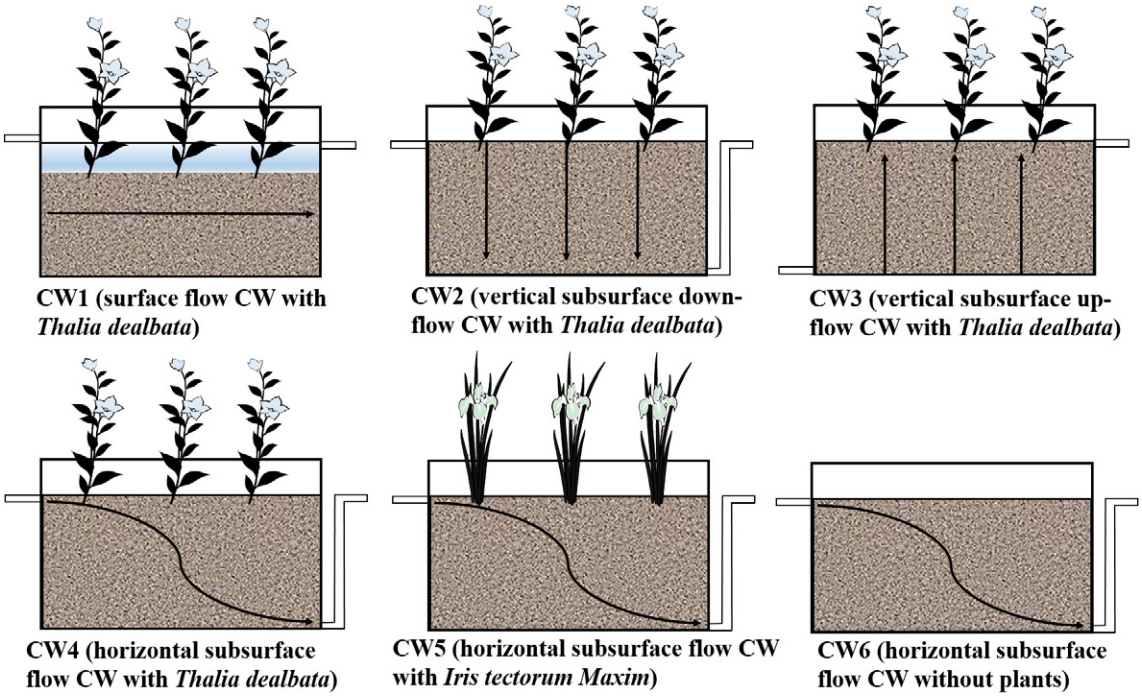


Fig. 1. Schematic design of the mesocosm-scale constructed wetlands.

Following the sampling of wastewater samples, the substrate samples were collected in 1-L glass bottles for analysis of antibiotics, then one gram of sodium azide was added to each substrate sample to suppress microbial activity. For analysis of ARGs, the substrate samples were collected in 1-L sterile brown glass bottles. Then the plant samples were collected by harvesting all six plants including the above ground and underground parts in each CW.

All the samples were then kept refrigerated and transported to the laboratory as soon as possible, where they were stored at 4 °C before analysis (within 24 h). Substrate and plant samples were freeze-dried, homogenized, and passed through a 60-mesh standard sieve and then kept at −20 °C in the dark until extraction.

2.3. Chemical analysis

2.3.1. Physicochemical parameters and conventional wastewater quality parameters

The YSI meter (YSI-Pro2030; YSI Incorporated, Yellow Springs, OH, USA) was used to monitor physicochemical parameters (pH, DO: dissolved oxygen, temperature, conductivity and redox potential) of the mesocosm-scale CWs. Conventional wastewater quality parameters (COD: chemical oxygen demand, TP: total phosphorus, TOC: total organic carbon, TN: total nitrogen and NH_3-N: ammonium nitrogen) were determined according to Chinese standard methods. COD was measured using the potassium dichromate method (GB 11914-89). TP was measured using ammonium molybdate spectrophotometric method (HJ 671-2013). TOC was measured using combustion oxidation nondispersive infrared absorption method (HJ 501-2009). TN (HJ 636-2012) and NH_3-N (HJ 536-2009) were determined by a UV–vis spectrophotometer (Shimadzu Instrument Co. Ltd., UV-2450, Japan).

2.3.2. Antibiotics extraction and instrumental analysis

Based on our previous study (Zhou et al., 2012), 50 antibiotics of 11 classes were selected for analysis in the present experiment: sulfonamides, diaminopyrimidines, tetracyclines, fluoroquinolones, macrolides, polyether ionophores, aminocoumarins, polypeptides, lincosamides, chloramphenicol derivatives, and β-lactams. Detailed information about target standards and internal standards used in the analysis and the physicochemical properties of the target compounds, as well as the analytical method can be found in the Supporting Information (SI Text1) and previous study (Zhou et al., 2012).

Briefly, the water samples were extracted by Oasis HLB cartridges (6 mL, 500 mg), while the solid samples including substrate, particle phase and plant samples were extracted by the method of ultrasonic-assisted extraction with solvents (acetonitrile and citric acid buffer), followed by an enrichment and clean-up step with solid-phase extraction using SAX-HLB cartridges in tandem. Rapid-resolution liquid chromatography-tandem mass spectrometry (RRLC-MS/MS) was used to analyze target antibiotics. The RRLC-MS/MS used was Agilent liquid chromatography 1200 series RRLC system coupled to an Agilent 6460 triple quadrupole MS equipped with an electrospray ionization (ESI) source (Agilent, Palo Alto, CA, USA). The quantitative analysis of the target compounds was carried out in dynamic multiple reaction monitoring (DMRM) mode. Laboratory blanks and laboratory controls were also analyzed along with the samples as quality controls.

2.4. ARGs analysis

DNA extraction and ARG determination methods can be referred to our previous study (Su et al., 2014), which were briefly described as follows.

2.4.1. DNA extraction and purification

Each water sample (0.5 L) was filtered through a sterile membrane filter (0.45-μm pore diameter) with a vacuum filtration apparatus. Then, the membrane filter was aseptically removed by using a sterile forceps, cut into pieces by a sterile scissor and put into the tube provided by the PowerSoil DNA Isolation Kit (MoBio Laboratories, USA). The DNA extraction and purification procedures used here followed the protocol provided by the manufacturer.

10 g of each homogeneous substrate sample were used to extract total DNA. The 0.85% sterile stroke-physiological saline was used to wash each substrate sample with the purpose of getting almost all of the microorganism, then the saline containing microorganisms was filtered through a sterile membrane filter (0.45-μm pore diameter) with a vacuum filtration apparatus. The following steps were the same as the

DNA extraction of the water sample. Then, DNA was further purified using the DNA Spin Kit (Tiangen Biotech, China) to minimize PCR inhibition.

2.4.2. ARGs quantification

Real-Time quantitative PCR (qPCR) were used to quantify 12 target genes including sulfonamide (*sul1*, *sul2* and *sul3*), tetracycline (*tetG*, *tetM*, *tetO* and *tetX*), quinolone (*ermB* and *ermC*) and chloramphenicol genes (*cmlA* and *floR*), and 16S rRNA. The specific primers and external reference methods used in this study for RTFQ PCR are listed in SI Table S1. The ViiA 7 Real-Time fluorescence quantitative PCR System (ABI, USA) using SYBR Green qPCR Kit (TAKARA, Japan) was used to quantitatively determine the abundance of target genes. 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 qPCR 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 (some primers of ARGs have different annealing temperatures, see Table S1), 72 °C for 30 s, and a final step for melting curve. Calibration curves were generated using plasmids carrying target genes. The external reference method (SI Text S2) 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 between 95 and 110%.

2.5. Mass balance analysis

Mass balance analysis was used to assess mass removal capacity of the mesocosm-scale CWs in different conditions. It was assumed that concentrations of the pollutants in influents and conditions of six mesocosm-scale CWs were stable during the operation period, since the plant samples can only be collected once. The mass flow of a pollutant entering and leaving each mesocosm-scale CW was assessed by mass balance analysis.

$$M_i = C_i \times Q \text{ (or M)} \times T \quad (1)$$

where M_i is the mass loading of the pollutant i in the water, particle, substrate and plant, C_i represents the concentration of pollutant i in the water, particle, substrate and plant, Q is the average daily water flow in the mesocosm-scale CWs and M is the dry weight of the substrates or plants, and T is operation time of the mesocosm-scale CWs.

$$M_{removal} = M_{influent} - M_{effluent} \quad (2)$$

where $M_{influent}$ and $M_{effluent}$ are the mass loadings of a pollutant in the influent and each mesocosm-scale CW effluent, respectively (including water phase and particle phase), and $M_{removal}$ on behalf of the mass removal of the pollutant after mesocosm-scale CW treatment.

Meanwhile,

$$M_{removal} = M_{substrate} + M_{plant} + M_{loss} \quad (3)$$

where $M_{substrate}$ and M_{plant} are the mass loading of a pollutant adsorbed by substrates and plants, M_{loss} is the mass loadings of a pollutant degraded by microorganism or removed by other ways.

2.6. Statistical analysis

Basic data analysis was performed with Microsoft Excel 2010 to obtain averages and standard deviations of concentrations of target contaminants. One-way ANOVA with Duncan test was used to evaluate the statistical significance of difference and Pearson correlation analysis was used to investigate the statistical correlation between antibiotic removal and microbial biomass using SPSS version 20.0 (IBM, NY).

3. Results

3.1. Operational performance of the mesocosm-scale CWs

The general wastewater quality parameters (temperature, pH, DO, conductivity and redox potential) related to unfiltered water and conventional wastewater control parameters (COD, TN, NH_3-N, TP and TOC) of the samples collected from CWs are summarized in Table 1 and SI Table S2. The results showed that all six CWs had variable removal efficiencies. Conventional wastewater control parameters decreased and removal rates ranged from 25.8% to 83.6% (Table 2). In the influent (W0), COD, TN, NH_3-N, TP and TOC were detected at the concentrations of 109, 60.7, 34.7, 4.67 and 19.7 mg/L respectively, while in the final effluents (W1–W6), these parameters were detected at 18.9–37.8, 27.3–38.5, 19.3–24.3, 1.94–2.61 and 3.90–11.4 mg/L respectively (SI Table S2).

3.2. Occurrence and removal of antibiotics

Among the 50 antibiotics of 11 classes, 8 antibiotics including erythromycin-H_2O (ETM-H_2O), monensin (MON), clarithromycin (CTM), leucomycin (LCM), sulfamethoxazole (SMX), trimethoprim (TMP), sulfamethazine (SMZ) and sulfapyridine (SPD) were detected in influent and effluents, whereas 3 of them (ETM-H_2O, TMP and SPD) were found in particle phase, 2 of them (ETM-H_2O and CTM) were found in substrate phase and 1 of them (ETM-H_2O) was found in plant. In influent, ETM-H_2O had the highest concentration of 8370 ng/L, followed by MON, CTM, LCM and SMX with their concentrations of 80.5, 70.3, 37.5 and 30.6 ng/L respectively, while TMP, SMZ and SPD were detected at the lowest concentrations of 12.2, 9.48 and 6.09 ng/L respectively (Fig. 2 and SI Table S3). In particle phase, ETM-H_2O, TMP and SPD were detected at the concentrations of 14.4–329, 2.20–2.26 and 2.52–2.61 ng/L respectively. In substrate phase, ETM-H_2O and CTM were detected at the concentrations of 7.70–17.6 and 1.58–1.70 ng/g respectively. And in plant phase, only ETM-H_2O was detected, with its concentrations ranging between 0.70 and 4.02 ng/g (Fig. 2 and SI Table S3). The analytical results showed that ETM-H_2O was the predominant analyte in the four different types of samples.

After treatment by mesocosm-scale CWs, all detected antibiotics were decreased to different degrees. Fig. 3 showed the aqueous removal rates (including water phase and particle phase) of different antibiotics by the mesocosm-scale CWs. For LCM and SMZ, the aqueous removal rates of all six mesocosm-scale CWs reached 100%. For ETM-H_2O, MON, CTM and TMP, the removal rates by all six CWs were mostly >67.2% (76.0–97.2%, 79.4–86.3, 67.2–87.3% and 84.3–84.6%, respectively), while SMX and SPD were removed from 22.1%–69.2% and 11.1%–13.9%, respectively. It should be noted that aqueous removal efficiencies for all detected antibiotics by the six mesocosm-scale CWs were in general the following order: CW5 > CW4 > CW3 > CW6 > CW2 > CW1 (Fig. 3 and SI Table S4).

3.3. Occurrence and removal of ARGs

All selected 12 genes including three sulfonamide resistance genes (*sul1*, *sul2* and *sul3*), four tetracycline resistance genes (*tetG*, *tetM*, *tetO* and *tetX*), two macrolide resistance genes (*ermB* and *ermC*), two chloramphenicol resistance genes (*cmlA* and *floR*) and 16S rRNA (bacteria) were detected in both water and substrate samples from the mesocosm-scale systems (Fig. 4; SI Tables S5 and S6). In general, among the 11 target ARGs of 4 classes, *sul1*, *sul2*, *tetG* and *floR* were relatively abundant (1.96×10^8, 1.60×10^8, 2.30×10^7 and

Table 1
Wastewater physicochemical parameters of the mesocosm-scale constructed wetlands.

	Physicochemical parameters				
	Temperature (°C)	pH	DO[a] (mg/L)	Conductivity (μs/cm)	Redox potential (mV)
Influent	25.6 ± 0.35	7.78 ± 0.09	1.00 ± 0.08	626 ± 7.16	−169 ± 3.68
CW1	24.7 ± 0.20	7.66 ± 0.02	0.36 ± 0.24	607 ± 6.11	−130 ± 3.24
CW2	24.9 ± 0.07	7.78 ± 0.01	0.17 ± 0.06	614 ± 2.36	−189 ± 9.07
CW3	25.3 ± 0.05	7.79 ± 0.01	0.27 ± 0.13	624 ± 2.22	−181 ± 4.80
CW4	25.1 ± 0.02	7.81 ± 0.01	0.12 ± 0.03	609 ± 0.19	−202 ± 6.90
CW5	25.0 ± 0.05	7.84 ± 0.01	0.12 ± 0.03	607 ± 4.10	−211 ± 5.00
CW6	24.9 ± 0.14	7.80 ± 0.02	0.16 ± 0.09	608 ± 18.2	−188 ± 8.02

[a] Dissolved oxygen.

4.37×10^7 copies/mL, respectively) in all water samples (Fig. 4 and SI Table S5), while the highest copy number was found for *sul3* and *ermC* (1.34×10^6 and 1.72×10^6 copies/g, respectively) in most CWs in all substrate samples (Fig. 4 and SI Table S6).

Following wetland treatment, the concentrations of the detected ARGs were decreased in the six mesocosm-scale systems (Fig. 5 and Table S7). For the dominant ARGs, the 6 removal rates of *sul1*, *sul2*, *tetG* and *floR* in the mesocosm-scale CWs were 70.0–86.7%, 47.2–79.1%, 79.7–92.9% and 82.8–94.6%, respectively. The aqueous removal rates of all target ARGs (∑ ARGs) ranged from 63.9% to 84.0% (SI Table S7). The removal efficiencies for all detected ARGs by the 6 mesocosm-scale CWs were in the following order: CW3 > CW6 > CW5 > CW2 > CW4 > CW1.

3.4. Correlation between pollutant removal and bacterial biomass

Pearson correlation analysis was performed to study the correlations between pollutants removal amounts (COD, TN, NH_3-N, TP, TOC and ∑ antibiotics) and bacterial biomasses. In order to estimate bacterial biomasses in different mesocosm-scale CWs, the concentrations of 16S rRNA (bacteria) in both water and substrate, the volume of water and the weight of substrate were determined. The total bacterial biomass of the mesocosm-scale CWs ranged from 1.01×10^{13} to 6.36×10^{13} copies/unit (SI Table S8). As shown in Table 5, strong and significant positive correlations existed between the total bacterial biomass and COD, TN, NH_3-N and ∑ antibiotics ($R = 0.82$–0.91, $p < 0.05$), suggesting the removal efficiencies of pollutants were linked to microbial activities in the mesocosm-scale CWs.

3.5. Mass loadings of pollutants in the mesocosm-scale CWs

Mass loadings of pollutants in the influent and effluent could reflect the treatment capacity of the CWs. The mass removals of pollutants including the 8 detected antibiotics and general wastewater quality parameters (COD, TN, NH_3-N, TP and TOC) by the mesocosm-scale CWs are summarized in Table 3. The calculated total mass removals of COD, TN, NH_3-N, TP and TOC by the mesocosm-scale CWs were 6.81–8.38 g/d, 2.48–3.20 g/d, 0.86–1.46 g/d, 0.20–0.26 g/d and 0.80–1.58 g/d, respectively. The mass removal of total antibiotics by the mesocosm-scale CWs was 652–846 μg/d.

Removal mechanism was elucidated from the mass loading in influent, effluent, plant, substrate and biodegradation. The mass loadings of each antibiotic in different media of the CWs during the operation period are given in SI Table S9. The mass loading of all detected antibiotics

Table 2
Removal rates (%) of conventional quality parameters by the mesocosm-scale constructed wetlands.

	COD[a]	TN[b]	NH_3-N[c]	TP[d]	TOC[e]
CW1	68.6	43.0	31.6	48.3	63.8
CW2	71.1	52.0	25.8	44.0	83.6
CW3	77.1	55.0	38.7	50.6	67.1
CW4	77.4	54.3	44.0	48.7	75.3
CW5	80.2	54.7	41.9	58.4	80.3
CW6	65.2	42.0	29.9	44.4	42.4

[a] Chemical oxygen demand.
[b] Total nitrogen.
[c] Ammonia nitrogen.
[d] Total phosphorus.
[e] Total organic carbon.

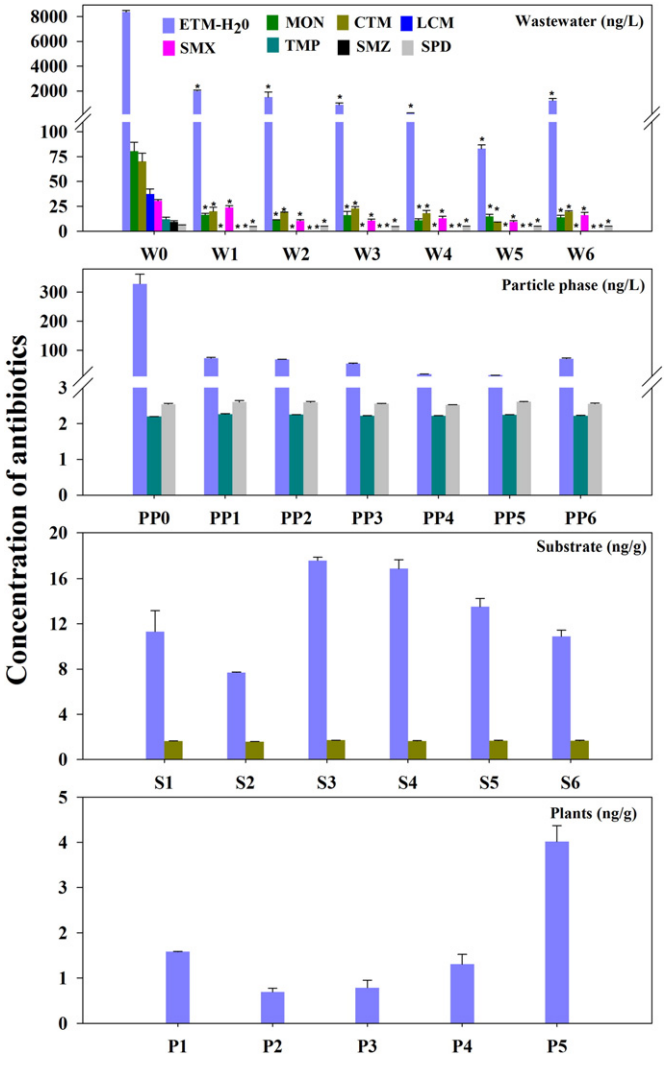


Fig. 2. Concentrations (ng/L) of detected antibiotics in different phases of the mesocosm-scale constructed wetlands (asterisk indicates the significant differences between influent and effluent from different CWs). Influent (W0 and PP0), CW1 (W1, PP1, S1 and P1), CW2 (W2, PP2, S2 and P2), CW3 (W3, PP3, S3 and P3), CW4 (W4, PP4, S4 and P4), CW5 (W5, PP5, S5 and P5), CW6 (W6, PP6 and S6).

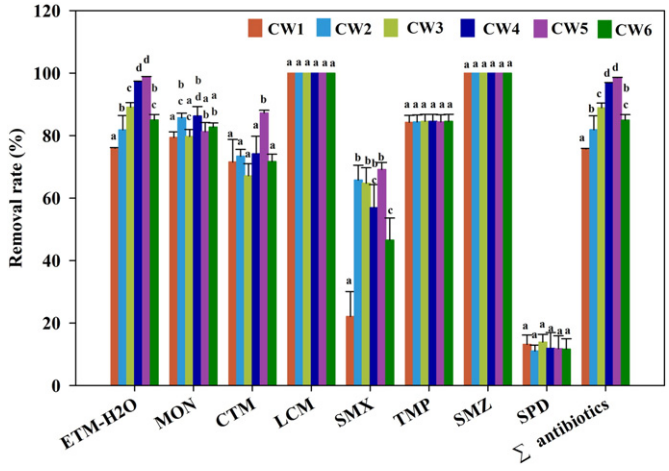


Fig. 3. Removal rates (%) of the total antibiotics in the mesocosm-scale constructed wetlands (letters (a, b, c, d) indicates the significant differences between different CWs).

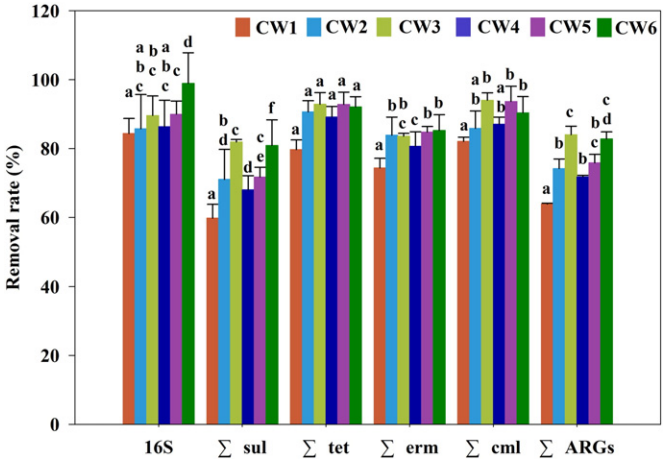


Fig. 5. Removal rates (%) of the total ARGs in the mesocosm-scale constructed wetlands (letters (a, b, c, d, e, f) indicates the significant differences between different CWs).

in influent was up to 247,000 μg, which was reduced to 3500–60,000 μg in effluent of the six CWs. The mass loadings of plant uptake and substrate adsorption were 0.05–0.41 μg and 4920–10,600 μg. Table 4 showed that plant uptake and substrate adsorption accounted for small percentages of mass removal for antibiotics by CWs, while dissipation due to biodegradation and other processes accounted for a majority of the mass removal.

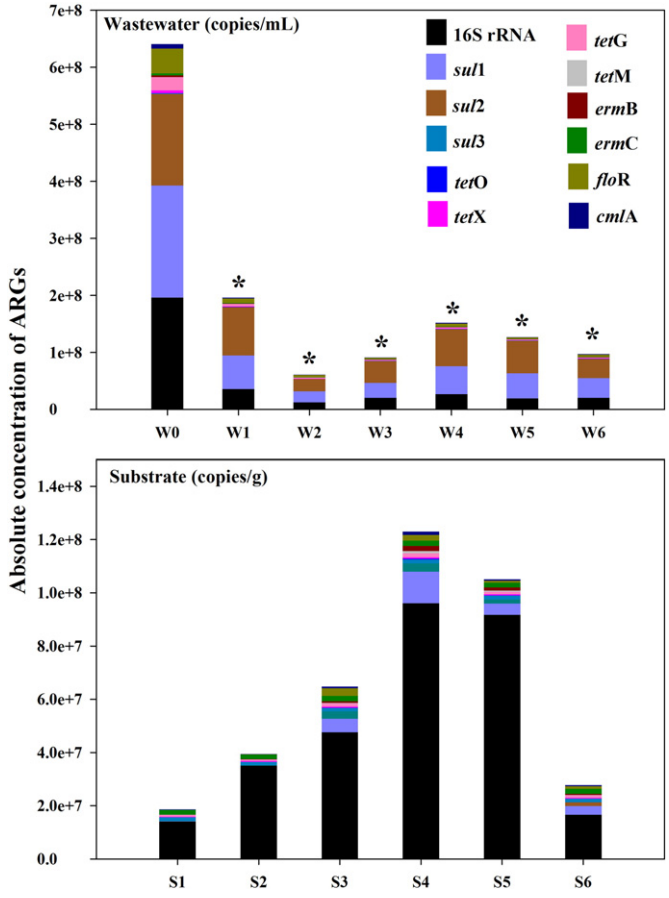


Fig. 4. Absolute concentrations of ARGs in the influent and effluents (copies/mL) and in different substrates (copies/g) of the mesocosm-scale constructed wetlands (asterisk indicates the significant differences between influent and effluent from different CWs). Influent (W0), CW1 (W1 and S1), CW2 (W2 and S2), CW3 (W3 and S3), CW4 (W4 and S4), CW5 (W5 and S5), CW6 (W6 and S6).

4. Discussion

4.1. Performance comparison among the mesocosm-scale CWs

The results from the present study showed variable removals of the contaminants including conventional wastewater quality parameters (COD, TN, NH_3-N, TP and TOC), 8 detected antibiotics and target 11 ARGs in the raw domestic wastewater by the six mesocosm-scale CWs with different design parameters. With regard to COD, TN and TOC, it was found that the CW1 (SF-CW) and CW6 (HSSF-CW without plants) performed worse than the other four CWs including two HSSF-CWs and two VSSF-CWs. Subsurface flow and planting were beneficial to the removal of nutrients, which is consistent to some previous studies (Brix, 1994; Lin et al., 2002b; Wen et al., 2010; Vymazal, 2011; Carvalho et al., 2014). For 8 detected antibiotics, CW4 and CW5 (HSSF-CWs with different plants) performed better than the other four CWs, with CW1 being the worst (Fig. 3 and SI Table S4). It suggests the HSSF-CWs are the better choice than both SF-CWs and VSSF-CWs for eliminating antibiotics. In addition, the presence of plants in CWs was also helpful to remove antibiotics. In contrast, CW3 (VSSF-CW with down flow) and CW6 (HSSF without plants) performed better for the ARGs. This requires further investigation into the removal mechanism of CWs for ARGs.

4.2. Removal mechanism for antibiotics and ARGs

Eight antibiotics including 3 sulfonamides (SPD, SMZ and SMX), one sulfonamides potentiator (TMP), 3 macrolides (ETM-H_2O, LCM and CTM) and an antibiotic of ionophores (MON) were detected in wastewater samples, while only 2 antibiotics (ETM-H_2O and CTM) were detected in the substrate samples and only one antibiotic (ETM-H_2O) in the plant samples. The detection of ETM-H_2O and CTM was consistent with their higher K_{ow} and lower solubility values than the others (Zhou et al., 2012). There were no sulfonamides and ionophores detected in the substrate and plant samples, suggesting degradation (and transformation) played a more important role in the aqueous removal of these two classes of antibiotics than substrate adsorption and plant uptake. The results are in good agreement with the previous reports that sulfonamides (including TMP used as sulfonamides potentiator) and MON are easily biodegradable (Xu et al., 2007; Mohring et al., 2009; Li and Zhang, 2010; García-Galán et al., 2011; Sun et al., 2014). For the detected 3 macrolides, adsorption onto substrates and uptake by plants only accounted for a minor percentage of the total mass loading into each CW. Previous reports showed that the removal of

Table 3
Removal of the mass loadings of antibiotics and conventional quality parameters every day by the mesocosm-scale constructed wetlands.

	Antibiotics (μg/d)									Conventional quality parameters (g/d)				
	ETM-H_2O[a]	MON[b]	CTM[c]	LCM[d]	SMX[e]	TMP[f]	SMZ[g]	SPD[h]	∑ antibiotics[i]	COD[j]	TN[k]	NH_3-N[l]	TP[m]	TOC[n]
CW1	634 ± 7.93	6.14 ± 0.78	4.83 ± 0.87	3.60 ± 0.48	0.65 ± 0.25	1.16 ± 0.18	0.91 ± 0.12	0.11 ± 0.03	652 ± 9.33	7.17	2.50	1.05	0.22	1.21
CW2	689 ± 39.4	6.62 ± 0.86	4.95 ± 0.64	3.60 ± 0.48	1.93 ± 0.21	1.17 ± 0.18	0.91 ± 0.12	0.09 ± 0.02	703 ± 40.1	7.43	3.03	0.86	0.20	1.58
CW3	744 ± 2.45	6.16 ± 0.53	4.53 ± 0.69	3.60 ± 0.48	1.91 ± 0.20	1.17 ± 0.18	0.91 ± 0.12	0.12 ± 0.03	762 ± 2.24	8.05	3.20	1.29	0.23	1.27
CW4	812 ± 11.9	6.66 ± 0.94	5.00 ± 0.83	3.60 ± 0.48	1.67 ± 0.27	1.17 ± 0.18	0.91 ± 0.12	0.10 ± 0.05	831 ± 13.3	8.09	3.16	1.46	0.22	1.43
CW5	826 ± 13.3	6.28 ± 0.84	5.89 ± 0.65	3.60 ± 0.48	2.03 ± 0.03	1.17 ± 0.18	0.91 ± 0.12	0.10 ± 0.04	846 ± 14.6	8.38	3.18	1.39	0.26	1.52
CW6	710 ± 14.2	6.39 ± 0.70	4.84 ± 0.64	3.60 ± 0.48	1.37 ± 0.21	1.17 ± 0.18	0.91 ± 0.12	0.10 ± 0.03	728 ± 14.6	6.81	2.45	1.00	0.20	0.80

[a] Erythromycin-H_2O.
[b] Monensin.
[c] Clarithromycin.
[d] Leucomycin.
[e] Sulfamethoxazole.
[f] Trimethoprim.
[g] Sulfamethazine.
[h] Sulfapyridine.
[i] All detected antibiotics.
[j] Chemical oxygen demand.
[k] Total nitrogen.
[l] Ammonia nitrogen.
[m] Total phosphorus.
[n] Total organic carbon.

macrolides was mainly attributed to biological treatment (Li and Zhang, 2010; Zhou et al., 2013). In addition, photolysis might play a certain role in the removal of antibiotics in the surface flow CWs (Cardinal et al., 2014), although their mass removal rate was the lowest (Table 4).

As biological contaminants, ARGs exhibit different behavior and fate in various environmental media since their mass and composition may change with biological activities (Auerbach et al., 2007; Chen et al., 2015). It is noteworthy that the removal efficiencies of antibiotics and ARGs by different mesocosm-scale CWs in the present study were even better than conventional WWTPs (Li and Zhang, 2010; Jia et al., 2012; Chen and Zhang, 2013; Zhou et al., 2013; Xu et al., 2015). The present and previous studies (Liu et al., 2013) implied that sorption and biological processes could be the two main mechanisms for ARGs elimination. The biological process within CWs could play a complex role in ARG removal as it may lead to ARG transmission and proliferation, while it may also involve in ARG degradation (Ghosh and LaPata, 2007; Diehl and LaPara, 2010; Guo et al., 2014; Yang et al., 2014). Redox conditions are the important factor in the degradation of organic compounds (Ying et al., 2008; Liu et al., 2011), thus further research is needed to understand the role of biological process in ARG removal under different redox conditions.

In the present study, not only the conventional pollutants (TN, NH_3-N, TP and TOC), but also 2 antibiotics (ETM-H2O and CTM) and all of the 11 target ARGs were found accumulated in the substrates of the six mesocosm-scale CWs, which suggested that substrate adsorption is an important removal process from wastewater for nutrients, antibiotics and ARGs. Meanwhile, detection of ETM-H_2O in the plant samples of the five CWs (Fig. 2) implies that plant uptake is another process for some antibiotics. Previous studies documented that substrate adsorption and plant uptake were the important ways to reduce the nutrient loadings and other environmental pollutants such as antibiotics and ARGs by constructed wetlands (Truu et al., 2009; Vymazal, 2011; Carvalho et al., 2014). Although the mass removal percentages by substrate adsorption and plant uptake were relatively low in comparison to the degradation losses (Table 4), these two processes could be important for the biodegradation process by providing retention sites and increasing microbial activities in CWs. It should also be noted that the different masses accumulated in plants were different in the two HSSF-CWs with two different plant species (CW4 and CW5), suggesting different uptake capacity of different species. In addition, previous studies reported that the removal of general pollutants was primarily related to microbial activity (Nurk et al., 2005; Tao et al., 2007; Sundberg et al., 2007; Truu et al., 2009). The present study shows that the microbial activities in the mesocosm-scale CWs can be positively correlated to the removal efficiency of the chemical pollutants (Table 5). Some previous studies also proved that microbes may play a more important role in pollutants removal in large scale CWs (Chen et al., 2015; Ávila et al., 2015). This further suggests that biodegradation plays a more important role when compared to substrate adsorption and plant uptake in terms of mass removal.

Table 4
The mass removal percentages of antibiotics by different mechanisms in the mesocosm-scale constructed wetlands during the operation period.

Percentage (%)		CW1	CW2	CW3	CW4	CW5	CW6
∑ antibiotics[a]	$M_{removal}$[b]	75.7	81.9	88.9	96.8	98.6	85.0
	$M_{substrate}$[c]	1.99	2.07	4.29	4.12	3.38	2.79
	M_{plant}[d]	3.75×10^{-5}	1.86×10^{-5}	1.91×10^{-5}	3.58×10^{-5}	1.65×10^{-4}	–
	M_{loss}[e]	73.7	79.8	84.7	92.7	95.2	82.2

[a] All detected antibiotics.
[b] Total removal percentage of all detected antibiotics mass loading.
[c] Removal percentage of all detected antibiotics mass loading by substrates.
[d] Removal percentage of all detected antibiotics mass loading by plants.
[e] Removal percentage of all detected antibiotics mass loading by microorganism or other ways.

5. Conclusion

The results from this study showed the nutrient loadings, antibiotics and ARGs could be efficiently reduced by mesocosm-scale CWs with different designs. The HSSF-CWs and VSSF-CWs showed higher removal rates of pollutants than SF-CWs, and the presence of plants is beneficial to pollutant removal. The reduction of antibiotics and ARGs by the CWs could be achieved at relatively similar or even higher rates than conventional wastewater treatment plants. Therefore, this study demonstrated that CWs is a promising technology for treatment of domestic sewage to remove various contaminants like antibiotics and ARGs. In terms of removal mechanism, substrate adsorption, plant uptake and biodegradation contribute to the reduction of various wastewater contaminants including the nutrients, antibiotics and ARGs, while the biodegradation plays a very important role. However, further research is still needed to explore the fate and removal mechanism of antibiotics and ARGs in CWs under different design conditions in large scale CWs.

Table 5
Correlations between the pollutants removal amounts and bacterial biomass in the CWs by Pearson correlation analysis.

		The removal amounts					
		COD[b]	TN[c]	NH_3-N[d]	TP[e]	TOC[f]	∑antibiotics[g]
Bacterial biomass		0.91*,[a]	0.82*,**	0.85*	0.66	0.67	0.88*
The removal amounts	COD	–	0.92*	0.83*	0.80	0.70	0.81
	TN	–	–	0.61	0.52	0.77	0.71
	NH_3-N	–	–	–	0.73	0.27	0.84*
	TP	–	–	–	–	0.41	0.63
	TOC	–	–	–	–	–	0.35
	∑antibiotics	–	–	–	–	–	–

* Means that the correlation is significant at the 0.05 level (2-tailed).
** Means that the correlation is significant at the 0.01 level (2-tailed).
[a] Values indicate the Pearson correlation coefficient (r).
[b] Chemical oxygen demand.
[c] Total nitrogen.
[d] Ammonia nitrogen.
[e] Total phosphorus.
[f] Total organic carbon.
[g] All detected antibiotics.

Acknowledgement

The authors would like to acknowledge the financial support from Guangzhou municipal government (20150401007 and PC2015), and the Chinese Academy of Sciences (KZZD-EW-09). This is a Contribution No. IS-2269 from GIGCAS.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.07.085.

References

Adrados, B., Sánchez, O., Arias, C.A., Becares, E., Garrido, L., Mas, J., Brix, H., Morató, J., 2014. Microbial communities from different types of natural wastewater treatment systems: vertical and horizontal flow constructed wetlands and biofilters. Water Res. 55, 304–312.
Arroyo, P., Ansola, G., Sáenz de Miera, L.E., 2013. Effects of substrate, vegetation and flow on arsenic and zinc removal efficiency and microbial diversity in constructed wetlands. Ecol. Eng. 51, 95–103.
Auerbach, E.A., Seyfried, E.E., McMahon, K.D., 2007. Tetracycline resistance genes in activated sludge wastewater treatment plants. Water Res. 41, 1143–1151.
Ávila, C., Bayona, J.M., Martín, I., Salas, J.J., García, J., 2015. Emerging organic contaminant removal in a full-scale hybrid constructed wetland system for wastewater treatment and reuse. Ecol. Eng. 80, 108–116.
Berglund, B., Khan, G.A., Weisner, S.E.B., Ehde, P.M., Fick, J., Lindgren, P.E., 2014. Efficient removal of antibiotics in surface-flow constructed wetlands, with no observed impact on antibiotic resistance genes. Sci. Total Environ. 476-477, 29–37.
Brix, H., 1994. Functions of macrophytes in constructed wetlands. Water Sci. Technol. 29 (4), 71–78.
Cardinal, P., Anderson, J.C., Carlson, J.C., Low, J.E., Challis, J.K., Beattie, S.A., Bartel, C.N., Elliott, A.D., Montero, O.F., Lokesh, S., Favreau, A., Kozlova, T.A., Knapp, C.W., Hanson, M.L., Wong, C.S., 2014. Macrophytes may not contribute significantly to removal of nutrients, pharmaceuticals, and antibiotic resistance in model surface constructed wetlands. Sci. Total Environ. 482-483, 294–304.
Carvalho, P.N., Basto, M.C.P., Almeida, C.M.R., Brix, H., 2014. A review of plant–pharmaceutical interactions: from uptake and effects in crop plants to phytoremediation in constructed wetlands. Environ. Sci. Pollut. Res. 21, 11729–11763.
Chen, H., Zhang, M., 2013. Effects of advanced treatment systems on the removal of antibiotic resistance genes in wastewater treatment plants from Hangzhou, China. Environ. Sci. Technol. 47, 8157–8163.
Chen, Y., Wen, Y., Tang, Z., Li, L., Cai, Y., Zhou, Q., 2014. Removal processes of disinfection byproducts in subsurface-flow constructed wetlands treating secondary effluent. Water Res. 51, 163–171.
Chen, J., Liu, Y.S., Su, H.C., Ying, G.G., Liu, F., Liu, S.S., He, L.Y., Chen, Z.F., Yang, Y.Q., Chen, F. R., 2015. Removal of antibiotics and antibiotic resistance genes in rural wastewater by an integrated constructed wetland. Environ. Sci. Pollut. Res. 22, 1794–1803.
Chen, J., Wei, X.D., Liu, Y.S., Ying, G.G., Liu, S.S., He, L.Y., Su, H.C., Hu, L.X., Chen, F.R., Yang, Y. Q., 2016. Removal of antibiotics and antibiotic resistance genes from domestic sewage by constructed wetlands: optimization of wetland substrates and hydraulic loading. Sci. Total Environ. 565, 240–248.
Cheng, W.X., Chen, H., Su, C., Yan, S.H., 2013. Abundance and persistence of antibiotic resistance genes in livestock farms: a comprehensive investigation in eastern China. Environ. Int. 61, 1–7.
Coleman, B.L., Louie, M., Salvadori, M.I., McEwen, S.A., Neumann, N., Sibley, K., Irwin, R.J., Jamieson, F.B., Daignault, D., Majury, A., Braithwaite, S., Crago, B., McGeer, A.J., 2013. Contamination of Canadian private drinking water sources with antimicrobial resistant *Escherichia coli*. Water Res. 47, 3026–3036.
Costanzo, S.D., Murby, J., Bates, J., 2005. Ecosystem response to antibiotics entering the aquatic environment. Mar. Pollut. Bull. 51, 218–223.
Diehl, D.L., LaPara, T.M., 2010. Effect of temperature on the fate of genes encoding tetracycline resistance and the integrase of class 1 integrons within anaerobic and aerobic digesters treating municipal wastewater solids. Environ. Sci. Technol. 44, 9128–9133.
Durham, L., Ge, M., Cuccia, A., Quinn, J., 2010. Modeling antibiotic resistance to project future rates: quinolone resistance in *Escherichia coli*. Eur. J. Clin. Microbiol. Infect. Dis. 29, 353–356.
Gao, L.H., Shi, Y.L., Li, W.H., Niu, H.Y., Liu, J.M., Cai, Y.Q., 2012. Occurrence of antibiotics in eight sewage treatment plants in Beijing, China. Chemosphere 86, 665–671.
García-Galán, M.J., Rodríguez-Rodríguez, C.E., Vicent, T., Caminal, G., Díaz-Cruz, M.S., Barceló, D., 2011. Biodegradation of sulfamethazine by *Trametes versicolor*: removal from sewage sludge and identification of intermediate products by UPLC–QqTOF-MS. Sci. Total Environ. 409, 5505–5512.
Ghosh, S., LaPata, T.M., 2007. The effects of subtherapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. ISME J. 1, 191–203.
Guo, X.P., Li, J., Yang, F., Yang, J., Yin, D.Q., 2014. Prevalence of sulfonamide and tetracycline resistance genes in drinking water treatment plants in the Yangtze River Delta, China. Sci. Total Environ. 493, 626–631.
Hijosa-Valsero, M., Sidrach-Cardona, R., Martín-Villacorta, J., Bécares, E., 2010. Optimization of performance assessment and design characteristics in constructed wetlands for the removal of organic matter. Chemosphere 81, 651–657.
Hijosa-Valsero, M., Fink, G., Schlüsener, M.P., Sidrach-Cardona, R., Martín-Villacorta, J., Ternes, T., Bécares, E., 2011. Removal of antibiotics from urban wastewater by constructed wetland optimization. Chemosphere 83, 713–719.
Hu, Y.S., Zhao, Y.Q., Zhao, X.H., Kumar, J.L.G., 2012. High rate nitrogen removal in an alum sludge-based intermittent aeration constructed wetland. Environ. Sci. Technol. 46, 4583–4590.
Jia, A., Wan, Y., Xiao, Y., Hu, J.Y., 2012. Occurrence and fate of quinolone and fluoroquinolone antibiotics in a municipal sewage treatment plant. Water Res. 46, 387–394.
Keffala, C., Ghrabi, A., 2005. Nitrogen and bacterial removal in constructed wetlands treating domestic waste water. Desalination 185, 383–389.
Kotzerke, A., Sharma, S., Schauss, K., Heuer, H., Thiele-Bruhn, S., Smalla, K., Wilke, B.M., Schloter, M., 2008. Alterations in soil microbial activity and *N*-transformation processes due to sulfadiazine loads in pig-manure. Environ. Pollut. 153, 315–322.
Li, B., Zhang, T., 2010. Biodegradation and adsorption of antibiotics in the activated sludge process. Environ. Sci. Technol. 44, 3468–3473.
Li, H.B., Li, Y.H., Gong, Z.Q., Li, X.D., 2013. Performance study of vertical flow constructed wetlands for phosphorus removal with water quenched slag as a substrate. Ecol. Eng. 53, 39–45.
Li, F.M., Lu, L., Zheng, X., Zhang, X.W., 2014. Three-stage horizontal subsurface flow constructed wetlands for organics and nitrogen removal: effect of aeration. Ecol. Eng. 68, 90–96.
Lin, Y.F., Jing, S.R., Lee, D.Y., Wang, T.W., 2002a. Nutrient removal from aquaculture wastewater using a constructed wetlands system. Aquaculture 209, 169–184.
Lin, Y.F., Jing, S.R., Wang, T.W., Lee, D.Y., 2002b. Effects of macrophytes and external carbon sources on nitrate removal from groundwater in constructed wetlands. Environ. Pollut. 119, 413–420.
Liu, F., Ying, G.G., Tao, R., Zhao, J.L., Yang, J.F., Zhao, L.F., 2009. Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. Environ. Pollut. 157, 1636–1642.
Liu, Y.S., Ying, G.G., Shareef, A., Kookana, R.S., 2011. Biodegradation of three selected benzotriazoles under aerobic and anaerobic conditions. Water Res. 45, 5005–5014.

Liu, L., Liu, C.X., Zheng, J.Y., Huang, X., Wang, Z., Liu, Y.H., Zhu, G.F., 2013. Elimination of veterinary antibiotics and antibiotic resistance genes from swine wastewater in the vertical flow constructed wetlands. Chemosphere 91, 1088–1093.
Mohring, S.A., Strzysch, I., Fernandes, M.R., Kiffmeyer, T.K., Tuerk, J., Hamscher, G., 2009. Degradation and elimination of various sulfonamides during anaerobic fermentation: a promising step on the way to sustainable pharmacy? Environ. Sci. Technol. 43, 2569–2574.
Nurk, K., Truu, J., Truu, M., Mander, Ü., 2005. Microbial characteristics and nitrogen transformation in planted soil filter for domestic wastewater treatment. J. Environ. Sci. Health A 40, 1201–1214.
Pruden, A., Pei, R., Storteboom, H., Carlson, K.H., 2006. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environ. Sci. Technol. 40, 7445–7450.
Reyes-Contreras, C., Hijosa-Valsero, M., Sidrach-Cardona, R., Bayona, J.M., Bécares, E., 2012. Temporal evolution in PPCP removal from urban wastewater by constructed wetlands of different configuration: a medium-term study. Chemosphere 88, 161–167.
Saeed, T., Sun, G., 2012. A review on nitrogen and organics removal mechanisms in subsurface flow constructed wetlands: dependency on environmental parameters, operating conditions and supporting media. J. Environ. Manag. 112, 429448.
Su, H.C., Ying, G.G., Tao, R., Zhang, R.Q., Zhao, J.L., Liu, Y.S., 2012. Class 1 and 2 integrons, sul resistance genes and antibiotic resistance in *Escherichia coli* isolated from Dongjiang River, South China. Environ. Pollut. 169, 42–49.
Su, H.C., Pan, C.G., Ying, G.G., Zhao, J.L., Zhou, L.J., Liu, Y.S., Tao, R., Zhang, R.Q., He, L.Y., 2014. Contamination profiles of antibiotic resistance genes in the sediments at a catchment scale. Sci. Total Environ. 490, 708–714.
Sun, P.Z., Cabrera, M.L., Huang, C.H., Pavlostathis, S.G., 2014. Biodegradation of veterinary ionophore antibiotics in broiler litter and soil microcosms. Environ. Sci. Technol. 48, 2724–2731.
Sundberg, C., SundbladTonderski, K., Lindgren, P.E., 2007. Potential nitrification and denitrification and the corresponding composition of the bacterial communities in a compact constructed wetland treating landfill leachates. Water Sci. Technol. 56 (3), 159–166.
Tamminen, M., Karkman, A., Lõhmus, A., Muziasari, W.I., Takasu, H., Wada, S., Suzuki, S., Virta, M., 2011. Tetracycline resistance genes persist at aquaculture farms in the absence of selection pressure. Environ. Sci. Technol. 45, 386–391.
Tao, W.D., Hall, K.J., Ramey, W., 2007. Effects of influent strength on microorganisms in surface flow mesocosm wetlands. Water Res. 41, 4557–4565.
Truu, M., Juhanson, J., Truu, J., 2009. Microbial biomass, activity and community composition in constructed wetlands. Sci. Total Environ. 407, 3958–3971.
Underwood, J.C., Harvey, R.W., Metge, D.W., Repert, D.A., Baumgartner, L.K., Smith, R.L., Roane, T.M., Barber, L.B., 2011. Effects of the antimicrobial sulfamethoxazole on groundwater bacterial enrichment. Environ. Sci. Technol. 45, 3096–3101.
Vymazal, J., 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia 674, 133–156.
Wang, Z., Dong, J., Liu, L., Zhu, G.F., Liu, C.X., 2013. Study of oyster shell as a potential substrate for constructed wetlands. Water Sci. Technol. 67 (10), 2265–2272.
Weerakoon, G., Jinadasa, K., Herath, G., Mowjood, M., Van Bruggen, J., 2013. Impact of the hydraulic loading rate on pollutants removal in tropical horizontal subsurface flow constructed wetlands. Ecol. Eng. 61, 154–160.
Wen, Y., Chen, Y., Zheng, N., Yang, D.H., Zhou, Q., 2010. Effects of plant biomass on nitrate removal and transformation of carbon sources in subsurface-flow constructed wetlands. Bioresour. Technol. 101, 7286–7292.
Wu, S.B., Kuschk, P., Brix, H., Vymazal, J., Dong, R.J., 2014. Development of constructed wetlands in performance intensifications for wastewater treatment: a nitrogen and organic matter targeted review. Water Res. 57, 40–55.
Wu, H.M., Zhang, J., Ngo, H.H., Guo, W.S., Hu, Z., Liang, S., Fan, J.L., Liu, H., 2015. A review on the sustainability of constructed wetlands for wastewater treatment: design and operation. Bioresour. Technol. 175, 594–601.
Xu, W.H., Zhang, G., Li, X.D., Zou, S.C., Li, P., Hu, Z.H., Li, J., 2007. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. Water Res. 41, 4526–4534.
Xu, J., Xu, Y., Wang, H.M., Guo, C.S., Qiu, H.Y., He, Y., Zhang, Y., Li, X.C., Meng, W., 2015. Occurrence of antibiotics and antibiotic resistance genes in a sewage treatment plant and its effluent-receiving river. Chemosphere 119, 1379–1385.
Yang, Y., Li, B., Zou, S.H., Fang, H.H.P., Zhang, T., 2014. Fate of antibiotic resistance genes in sewage treatment plant revealed by metagenomic approach. Water Res. 62, 97–106.
Ying, G.G., Toze, S., Hanna, J., Yu, X.Y., Dillon, P.J., Kookana, R.S., 2008. Decay of endocrine-disrupting chemicals in aerobic and anoxic groundwater. Water Res. 42, 1133–1141.
Younger, P.L., Henderson, R., 2014. Synergistic wetland treatment of sewage and mine water: pollutant removal performance of the first full-scale system. Water Res. 55, 74–82.
Zhang, D.Q., Tan, S.K., Gersberg, R.M., Zhu, J.F., Sadreddini, S., Li, Y.F., 2012. Nutrient removal in tropical subsurface flow constructed wetlands under batch and continuous flow conditions. J. Environ. Manag. 96, 1–6.
Zhou, L.J., Ying, G.G., Liu, S., Zhao, J.L., Chen, F., Zhang, R.Q., Peng, F.Q., Zhang, Q.Q., 2012. Simultaneous determination of human and veterinary antibiotics in various environmental matrices by rapid resolution liquid chromatography-electrospray ionization tandem mass spectrometry. J. Chromatogr. A 1244, 123–138.
Zhou, L.J., Ying, G.G., Liu, S., Zhao, J.L., Yang, B., Chen, Z.F., Lai, H.J., 2013. Occurrence and fate of eleven classes of antibiotics in two typical wastewater treatment plants in South China. Sci. Total Environ. 452–453, 365–376.