Performance of full scale constructed wetlands in removing antibiotics and antibiotic resistance genes

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HIGHLIGHTS

- TIA, TYL, OTC, SMX and TRI were the most abundant antibiotics in winter.
- ARGs except *ermB* did not show a significant increase or decrease between seasons.
- Antibiotic removal for each CW ranges from 13% to 100%.
- Log removal of ARGs in the water phase is low (0.8 to 1.5 log).
- HRT does not influence the removal of either antibiotics or ARGs.

GRAPHICAL ABSTRACT
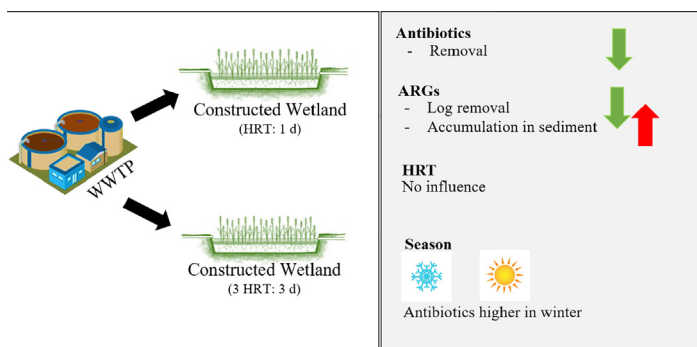
ABSTRACT

Additional treatment of wastewater, such as constructed wetlands (CWs), is a possible solution to reduce the discharge of antibiotics and antibiotic resistance genes (ARGs) from households and industry to the environment. This study aims to investigate the occurrence and removal of antibiotics and ARGs by two full scale CWs operated at different hydraulic retention times (HRT), namely 1 day and 3 days. Both CWs were receiving the same wastewater treatment plant (WWTP) effluent. Temporally and spatially distributed sampling of water and sediment was conducted for one year and samples were analyzed for antibiotics and ARGs by using LC-MS/MS and qPCR. Results showed that both CWs removed antibiotics significantly with a comparable overall removal of 28%–100%, depending on the type of antibiotics. However, some of the antibiotics showed higher concentration after the CW treatment. Five antibiotics (tiamulin, tylosin, oxytetracycline, sulfamethoxazole and trimethoprim) were the most abundant (>1500 ng/l on average) in winter. Meanwhile, *ermB* was the most abundant (average of 5.0 log) in winter compared to summer (average of 3.5 log). Other ARGs did not show a significant increase or decrease between winter and summer. ARGs were removed from the wastewater by 0.8 to 1.5 log. The HRT did not influence the removal of either the antibiotics or the ARGs. A strong correlation was found between sul genes and *intI1*. The results also revealed a positive and a negative relationship from sampling point 1 to sampling point 5: a positive relation between abundance of antibiotics, ARGs, and of NO_3–N, NH_4–N, TP, COD and a negative relation between antibiotics, ARGs and temperature. This relationship showed the effect between antibiotics and ARGs concentrations with physicochemical parameters and nutrients. The ability of CWs to reduce the input of micropollutants into the environment makes CWs a potential post treatment to WWTP.

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1. Introduction

Antibiotics are widely used to treat and prevent diseases in humans and for livestock but has gained attention since antibiotic resistance is becoming a serious threat to global public health and the environment (Chee-Sanford et al., 2009; WHO, 2014). Approximately 30–70% of antibiotics are partially or not metabolized by the human body, and consequently, antibiotics enter WWTP via the sewage system (Bouki et al., 2013; Kümmerer, 2004). The use of antibiotics and possibly also their presence in wastewater might promote the proliferation of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs), which, as a consequence, can enter the environment (Barancheshme and Munir, 2018).

WWTPs are designed to treat wastewater to meet quality requirements before being discharged into the environment, by reducing the concentration of chemical oxygen demand (COD), total organic carbon (TOC), total nitrogen (TN) and total phosphate (TP). However, WWTPs are not sufficiently tailored to remove micropollutants such as pharmaceuticals, personal care products, and antibiotics (Margot et al., 2015). Therefore, these micropollutants are detected in the effluents in the range of ng/l (norfloxacin) (Dong et al., 2016) to mg/l (ciprofloxacin) (Larsson et al., 2007). Additional treatment is needed to ensure the high quality of wastewater effluent, before it is released into the surface water.

A possible solution to remove antibiotics and ARGs is the addition of tertiary treatment steps to existing WWTPs. Tertiary treatments, such as advanced oxidation technologies (ozonation, UV irradiation) and membrane technologies, have shown to remove antibiotics and ARGs (Zheng et al., 2010; Zhang et al., 2016; Radjenović et al., 2009). However, such treatment processes have high installation, operation, and maintenance costs and require much energy (Li et al., 2014; Hijosa-Valsero et al., 2011). This is a drawback, especially for developing countries that are looking for an alternative to remove antibiotics and ARGs (Gruchlik et al., 2018), but also in developed countries where treatment plant managers want to reduce their ecological footprint. Currently, there are no legal regulations or policy that define the permitted levels of antibiotics or antibiotic resistance that are allowed into the environment (Pazda et al., 2019). Due to the increased presence of these antibiotics or antibiotic resistance in water, their presence is of concern. Hence, alternative sustainable technologies are desired for all economic contexts.

Other than the tertiary treatments mentioned above, constructed wetlands (CWs) is an alternative tertiary treatment in treating wastewater. Lower costs, minimal use of mechanical/physical/chemical equipment, efficiency in removing nutrients, and environment-friendly features compared to other tertiary treatment make CWs an interesting alternative for treating domestic, agricultural, and industrial wastewater (Verlicchi and Zambello, 2014).

CWs are designed to imitate natural processes using plants and soil to treat wastewater in a controlled environment (Parde et al., 2021). CWs have a high rate of biological activity compared with other ecosystems, and they have the potential to transform several common pollutants into harmless by-products (Fernandes, 2014). In addition to the removal of organic matter, nutrients, and suspended solids (Lavrnić et al., 2020), CWs have also shown good removal of micropollutants such as pharmaceuticals, personal care products, detergents, hormones, veterinary medicines, surfactants, pesticides, flame retardants, plasticizers, and various industrial compounds (Kaur et al., 2020; Anderson et al., 2013; Sochacki et al., 2018; Santos et al., 2019; Hsieh et al., 2015).

Most of the published studies have been performed under controlled conditions, and limited data are available on the removal of antibiotics and ARGs in full scale CWs. Vymazal et al. (2017) studied 31 micropollutant compounds includes erythromycin, sulfamethoxazol, trimetoprim and clarithromycin in a full scale of horizontal subsurface flow CW in the Czech Republic. They reported the antibiotic removal efficiencies varied between 37 and 70%. Meanwhile, in the United Kingdom, Petrie et al. (2018) reported that the average removal of

clarithromycin, azithromycin, trimethoprim and sulfamethoxazole (out of 88 micropollutants) from a full scale CW horizontal subsurface flow ranged from 24 to 73%. Some studies only focusing on the removal of micropollutants without antibiotics (Matamoros et al., 2017) or reported that a CW may effectively attenuate nutrients, micropollutants and bacteria, but it does not specifically removed ARGs (Anderson et al., 2013). A full scale CW may or may not show efficient in reducing antibiotics and ARGs due to a variety of factors, including age of the wetland, seasonality, and plants (Hijosa-Valsero et al., 2012). Hence, more data to measure the effect of attenuation of antibiotics and ARGs in full scale CW are needed, including a better understanding of the relation between retention time and removal efficiency.

Therefore, the objective of this study was to investigate the occurrence and removal of antibiotics and ARGs in full scale CWs. To our knowledge, no studies have compared full scale CWs with different HRTs that receive wastewater from the same WWTP. The investigations aimed for identifying temporal and spatial trends. In addition, correlations between antibiotics, ARGs and general water qualities were analyzed to determine the relation between generic water qualities with the distribution of antibiotics and ARGs in both CWs.

2. Material and methods

2.1. Sampling site and collection of samples

Sampling was performed at two different surface-flow CWs at Hapert WWTP (the Netherlands) from February 2016, May until December 2016 and January 2017. One sampling campaign was performed for each month. This WWTP treats a mixture of domestic (78%) and industrial (22%) wastewater via a conventional system consisting of bar screens, grit removal, and an oxidation ditch. The industrial wastewater originates from meat processing industries, metal industries and food industries.

Effluent from the WWTP is channeled to two CWs, each with a different hydraulic retention time (HRT) located south and north of the WWTP, 1 day (HRT-1) and 3 days (HRT-3), respectively (Fleskens et al., 2016). Each wetland consists of a parallel horizontal subsurface flow CW with reed beds (*Phragmites australis* (*P. australis*)) and a horizontal subsurface flow CW with woody emergent plants also referred to as swamps (Fonder and Headley, 2013) (Fig. 1). Open water is available in CW with HRT-1 at sampling point 1 and CW with HRT-3, at sampling point 3. The total surface area of the CW with HRT-1 is 7010 m^3 and the size of the CW with HRT-3 is 17,820 m^3. The depth of the reed beds is 133 cm, and the depth of the swamp is 25 cm. The average water flow of the CWs is 718 m^3/h during dry weather and 2543 m^3/h during the raining season (Fleskens et al., 2016). The average CW water flow during our sampling is presented in Fig. S1. The CWs have been serving as a WWTP post-treatment for 15 years. This CW is designed to treat and improve the discharge of the WWTP to 5 mg/l nitrogen and 0.5 mg/l phosphate (reed bed) to meet the requirement of European Water Framework Directive and create an ecological connection between the nature reserves and the Beerze river (swamp) (Fleskens et al., 2016).

For seasonal comparison, the average daily air temperature during sampling was used to classify the season. The data of average daily air temperature was extracted from The Royal Netherlands Meteorological Institute (KNMI, the Netherlands). Temperatures above 15 °C are classified as summer season, and below 15 °C are classified as winter season. Therefore, the summer months in this study are May, June, July, August and September, and the winter months October, November, December, January and February.

The influent of the CW (sampling point 1) was sampled only in the CW with HRT-1 since the effluent from the WWTP is from the same source for both CWs (Fig. 1). Within each CW, three sampling points were determined. Sampling point 2 represents 30% of the length of
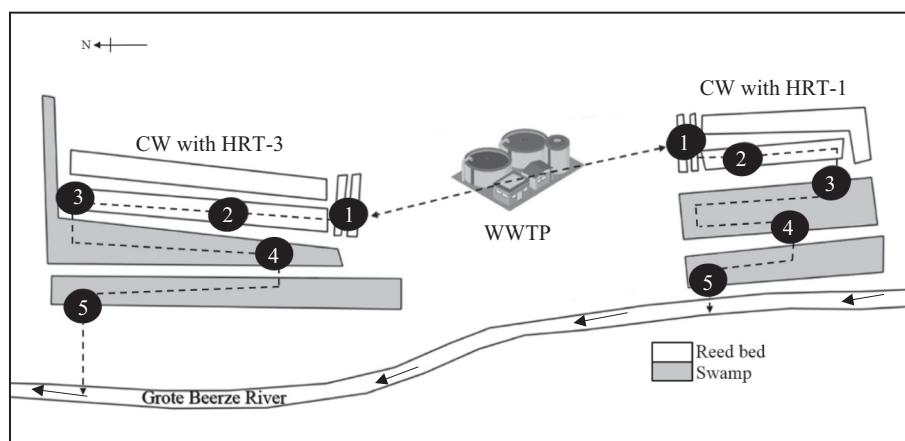
Fig. 1. Sampling point for CW with HRT-1 and CW with HRT-3. 1 = influent of both CWs. 2 = 30% of reed bed. 3 = influent of swamp. 4 = 30% of swamp. 5 = effluent of the CW.

the reed bed, sampling point 3 represents the influent of swamp, and sampling point 4 represents 30% of the length of the of swamp. 30% indicated the travel time of the water in the reed bed or swamp. The effluent of each CW (sampling point 5) was taken at the effluent of the CW before the water entered the river part. Water ($n = 270$) and sediment samples (n = 270) were collected from 9 sampling points. The sediment samples were taken as a core within the CW bed or swamp.

In total, at each of the ten sampling campaigns, triplicate samples were taken at five points in the CW with HRT-1 and four in the CW with HRT-3 and were transported back to the lab. Sediment samples were collected in 50 ml plastic tubes by using a grab sampler in the core of each CW. Samples for chemical analyses and DNA isolation were stored at 4 °C and processed within 24–48 h after sampling. The water and sediment samples for antibiotic analysis were stored at −20 °C until the samples were processed.

2.2. Physicochemical analysis

In the field, the pH, temperature, and dissolved oxygen (DO) were directly measured in the water using a pH and DO portable probe (Hach, USA). Groundwater samples were analyzed in the lab for chemical oxygen demand (COD), total phosphate (TP), ammonium (NH_4^+), nitrate (NO_3^-) using Hach kits (USA; LCK 1414, LCK 349, LCK 304 and LCK 339, respectively). The samples were filtered by using a 4–7 μm filter paper (Whatman, England) prior to analyses of NH_4^+ and NO_3^- analyses, but not for analyses of COD and TP. All water samples were manually mixed by shaking the sample bottles before analyses.

2.3. Antibiotics analysis

All water and sediment samples were extracted and concentrated by solid phase extraction (SPE) before performing LC-MS/MS analyses. The first water samples were measured in triplicate after which all samples were measured in a single measurement, as no significant difference was found in the triplicate (data not shown). Analyses were repeated for two times only for the samples with high concentration of detected antibiotics. Water samples and standards were prepared as previously described (Sabri et al., 2020). For sediment samples, matrix-matched standard samples were prepared in blank pot soil and spiked with sulfonamides and trimethoprim (0–400 μg/kg) and tetracyclines, quinolones, and macrolides (0–200 μg/kg). Sediment samples (2 g dry weight) were weighed in 50 ml tubes in duplicate. 80 μl of a mix-standard solution was added to duplicate samples for standard addition quantification. 20 μl of internal standard (Jansen et al., 2017) (5000 μg/kg) was added to all samples. After 20 min, 4 ml of pure acetonitrile (with freshly added 0.125% of trifluoroacetic acid) was added and

shaken manually for 30 s, followed by the addition of 4 ml of McIlvain buffer (0.1 M; pH 4.0) (Sabri et al., 2020). All samples were casted on a tube rotator for 15 min before adding 2 ml of lead acetate (200 g/l). The tubes were shaken vigorously before centrifuging for 10 min at 3500g. The supernatant was transferred in a glass tube, and 4 ml of acetonitrile was vaporised at 40 °C under nitrogen gas. After adding 13 ml of 0.2 M EDTA, the samples were ready for SPE purification as previously described by Sabri et al. (2020).

All samples were analyzed by LC-MS/MS on 18 sulfonamides, trimethoprim, 17 macrolides, 12 quinolones, and 6 tetracyclines (Table S1). These antibiotics are selected in this study because these antibiotics are referred for environmental monitoring of antibiotic resistance (Berendonk et al., 2015). The details of the method have been described in our previous study (Sabri et al., 2020). Briefly, antibiotics were analyzed using Acquity™ UPLC (Waters, USA) and AB Sciex QTrap 6500 (Sciex, USA) with electrospray ionization (ESI). The mobile phase was composed of eluent A (ammonium formate (1 M):formic acid:water (2 V:0.16 V:1000 V)) and eluent B (ammonium formate (1 M):formic acid:methanol (2 V:0.16 V:1000 V)). The antibiotics separation was acquired by a Waters C18 column (100 mm × 2.1 mm, 1.7 μm) at a flow rate of 0.3 ml/min. The limit of quantification (LOQ) of the target compounds in water was 5 ng/l for sulfonamides, 500 ng/l for tetracyclines, 25 ng/l for quinolones, and 50 ng/l for macrolides. Meanwhile, the LOQ of the target compounds in sediment was 2.5 μg/kg for sulfonamides and 10 μg/kg for tetracyclines, quinolones and macrolides. For quality control, a known amount (80 μg/l) and internal standard of each compound was spiked to every sample. The recovery percentages of compounds spiked to the sample ranged from 70 to 120%.

2.4. DNA extraction and ARG detection

Water samples were filtered by vacuum filtering through 0.2 μm membrane filters (isopore filters polycarbonate, 0.2 μm, 47 mm, Merck Millipore, Ireland), and stored at −20 °C until extraction. Sediment samples were used directly in the DNA isolation process. Total genomic DNA was extracted from water filters using the PowerWater® DNA Isolation Kit (MoBio Laboratories, USA) and from sediment using the PowerSoil® DNA isolation kit (MoBio Laboratories, USA) according to the manufacturer's instructions. The extracted DNA was stored at −80 °C until further analysis. Absolute quantification of genes was performed with quantitative PCR (qPCR) assays for the detection of 16S rRNA genes, the class 1 integrase gene (*intI1*) and four ARGs, including *sul1* and *sul2* (sulfonamide resistance genes), *tetW* (tetracycline resistance genes) and *ermB* (macrolide resistance gene), as previously described (Sabri et al., 2020). qPCR was carried out on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Canada) and was recorded by CFXManager (Biorad, version 3.0). The

results were expressed as genes per ml for water and genes per gram dry weight (DW) after adjusting for the dry weight of the sediment.

2.5. Statistical analysis

The removal percentage was calculated by comparing mean concentrations of antibiotics (ng/l) in sampling point 1 (influent of the CW) and sampling point 5 (effluent of the CW), according to Eq. (1).

$$Removal\ percentage = \left(\frac{Concentration\ influent - Concentration\ effluent}{Concentration\ influent} \right) \times 100\% \quad (1)$$

Statistical analyses were based on linear models, Pearson correlation and principal component analysis (PCA) on the R (Version 3.5.2). Antibiotic concentrations (ng/l and μg/kg) and ARGs abundance values (copies/ml and copies/g (dry weight)) were log-transformed prior to the linear model analysis. Statistical significance was determined at the 95% confidence level. The p-values for multiple testing were corrected using Bonferroni correction.

3. Results and discussions

3.1. General operational parameters

Measured concentrations of COD, DO, pH, water temperature, NH_4^+, NO_3^- and TP in all sampling points for the monitoring period are shown in Figs. S2–S8. The profile of each parameter is presented in Fig. S9. Temperature and precipitation on the sampling day are presented in Fig. S10. Generally, the effluent quality of both CWs was in a similar range. However, there was more variation and fluctuation within the sampling points in the CW with HRT-1 compared to the CW with HRT-3 for COD and TP. Also, NH_4^+, NO_3^- and TP values at sampling point 1–4 increased by 300% in both CWs in November and December, compared to other months. Other than these months, COD and all nutrients were reduced after the treatment of CWs. There was an occasionally high concentration of NO_3^- and TP at a certain sampling point within both CWs in May 2016 to August 2016 and January 2017 (Figs. S2, S6–S8).

The two studied CWs with a different HRT showed no significant difference ($p > 0.05$) in overall performance, and both showed high variability in nutrient removal of individual nutrients (Fig. S9). Both CWs significantly ($p < 0.05$) removed COD, NO_3^- and NH_4^+ and TP. This shows that both CWs able to reduce the COD and nutrients and meet the EU effluent requirement for urban wastewater by Council Directive 91/271/EEC (1991).

Regardless of the HRT, the COD concentration at the effluent of the CW with HRT-1 and the CW with HRT-3 was similar (20–30 mg/l). This indicated that the retention time in the CW with HRT-1 appeared to be sufficient for nutrient removal, and that additional retention time had no added value to improve the performance of the CW. The DO level was also affected by the degradation of excessive organic matters, and resulting DO level was lower in summer compared to winter (Zhou et al., 2019).

3.2. Occurrence and removal of antibiotics in the constructed wetlands

3.2.1. Fate of antibiotics in the water phase

The performance of CWs in removing antibiotics in two HRTs was evaluated. LC-MS/MS analysis revealed that 14 out of 54 analyzed antibiotics were detected in the water samples. Fig. 2(a) and (b) show the measured antibiotics in water during one year in both CWs. Fig. S11 (a) and (b) shows an enlarged segment of Fig. 2(a) and (b) from May to November with the antibiotics oxytetracycline (OTC), sulfadiazine (SF), sulfadimidine (SDM), sulfadoxine (SFX), sulfamethoxazole (SMX), sulfapyridine (SP), trimethoprim (TRI), azithromycin (AZI),

clarithromycin (CLA), lincomycin (LIN), tiamulin (TIA), tylosin (TYL), ciprofloxacin (CIP) and flumequine (FLU).

The performance of two CWs receiving wastewater from the same WWTP was evaluated during one year. Both CWs removed antibiotics significantly, and the two different HRTs showed a comparable overall removal. This indicates that the CWs can attenuate these compounds (Table S2) detected in this study, as demonstrated before (Hijosa-Valsero et al., 2011; Zhang et al., 2016; Dordio et al., 2010). However, in some cases, either at certain months or some locations within the CW, negative removal of specific antibiotics was observed. This might indicate either the release of antibiotics sorbed onto the sediments or the presence of deconjugates in the influent of the CW interfering with the biological transformation of the conjugated compounds, i.e. resulting in an apparent production of the compound in the wetland (Verlicchi and Zambello, 2014).

The total antibiotic concentrations in the influent of the CW per month were below 5000 ng/l except in February, December, and January. The total antibiotic concentration is here referred to as the sum of the selected antibiotics for this research. It cannot be ruled out that the wastewater contained other antibiotics that were not covered by our LC-MS/MS methodology and not added in the total antibiotic concentration. These three months showed higher concentrations than the rest of the months, with a total concentration up to 20,000 ng/l. Concentration levels of the antibiotics ranged from 200 to 20,000 ng/l in the influent of the CW, with tiamulin being the most abundant compound in the influent of the CW. After treatment by the CWs, the detected antibiotics decreased to only seven antibiotics, detected up to 16,000 ng/l in the effluent of the CW, with again tiamulin as the most abundant compound. The overall removal percentage per month for the CW with HRT-1 was from −1167 to 100%. Meanwhile, for the CW with HRT-3, the removal percentage was between −66 to 100%. The removal of each group of antibiotics in both CW effluents is illustrated in Fig. S12. The performance of the CWs in reducing antibiotics after the reed bed or swamp part is presented in Fig. S13. The concentration of macrolides (LIN) and sulfonamides (SDM, SF, SFX, SP, TRI) did not change after the reed bed and swamp. Meanwhile, quinolones (FLU, CIP) and tetracyclines (OTC) showed a decreasing trend after the reed bed and the swamp.

Other than that, the biological reaction rate is positively associated with higher temperatures, and enzyme-catalyzed reactions are most active within the range of optimal temperatures (Bruce and Perry, 2001). Sunlight photodegradation and temperature can also be involved in the removal of antibiotics in the CWs under open water spaces (Choi et al., 2016; Kim and Carlson, 2007; Jiang et al., 2018). Data from KNMI (Royal Netherlands Meteorological Institute) show that solar irradiation is 2.8 KWh/m² (average 1 h) in winter compared to 19.4 KWh/m² (average 10 h) in summer, thus confirming less photobiodegradation potential in winter. Therefore, antibiotics persist for a longer time during winter (Yang et al., 2011). As photodegradation rates are different per antibiotic, differences in removal were observed. For example, sulfathiazole degrades relatively quickly, followed by sulfisoxazole, sulfamethizole, and SMX (Boreen et al., 2004), whereas TRI was not susceptible to photodegradation (Nguyen Dang Giang et al., 2015). We also observed that concentration of SMX and TRI remained almost the same within the CW, in February (less photodegradation), and in August (most photodegradation). That explains lower antibiotics concentration in warmer months as we also can observe less total antibiotics were measured (average less than 1000 ng/l) in August. Another explanation for different concentration concentrations in summer compared to winter months is variation in antibiotics use over the year. Hence, the combination of slow photodegradation rates, a shorter time of sunlight in winter (average of 1 h) in winter, and high consumption and excretion of antibiotics by humans most likely contribute to a high concentration of sulfonamides in February.

Surprisingly, macrolides were detected at high concentrations in the water phase, especially in January. Tylosin, which was found at the

(a) CW with HRT-1
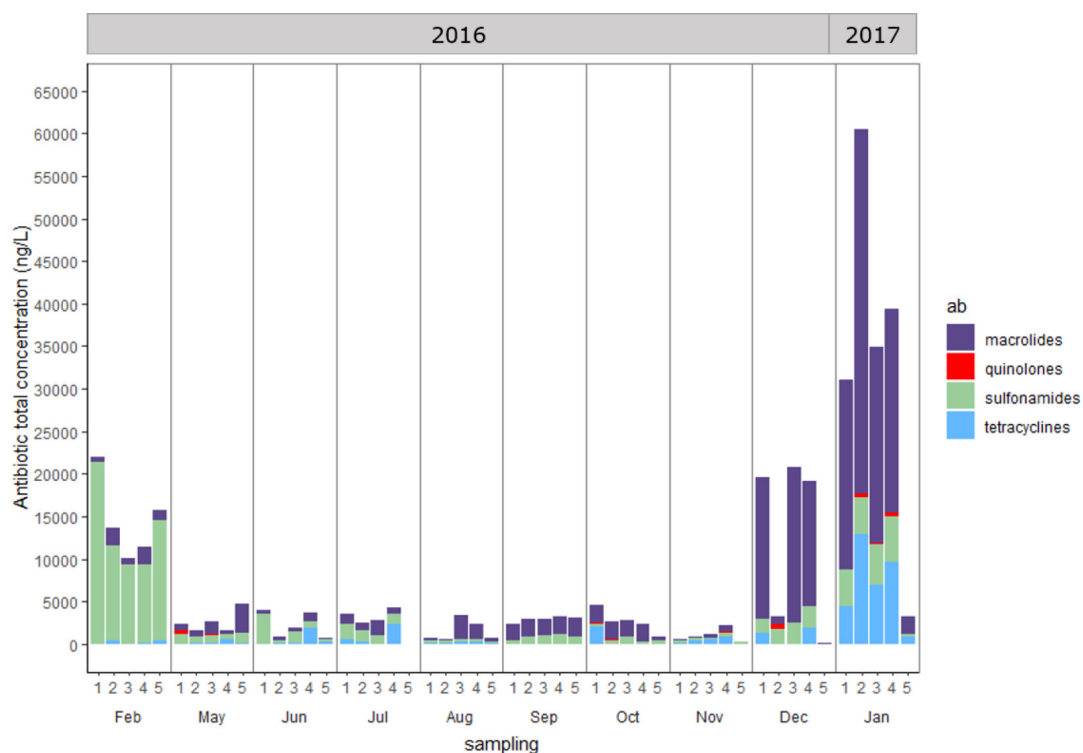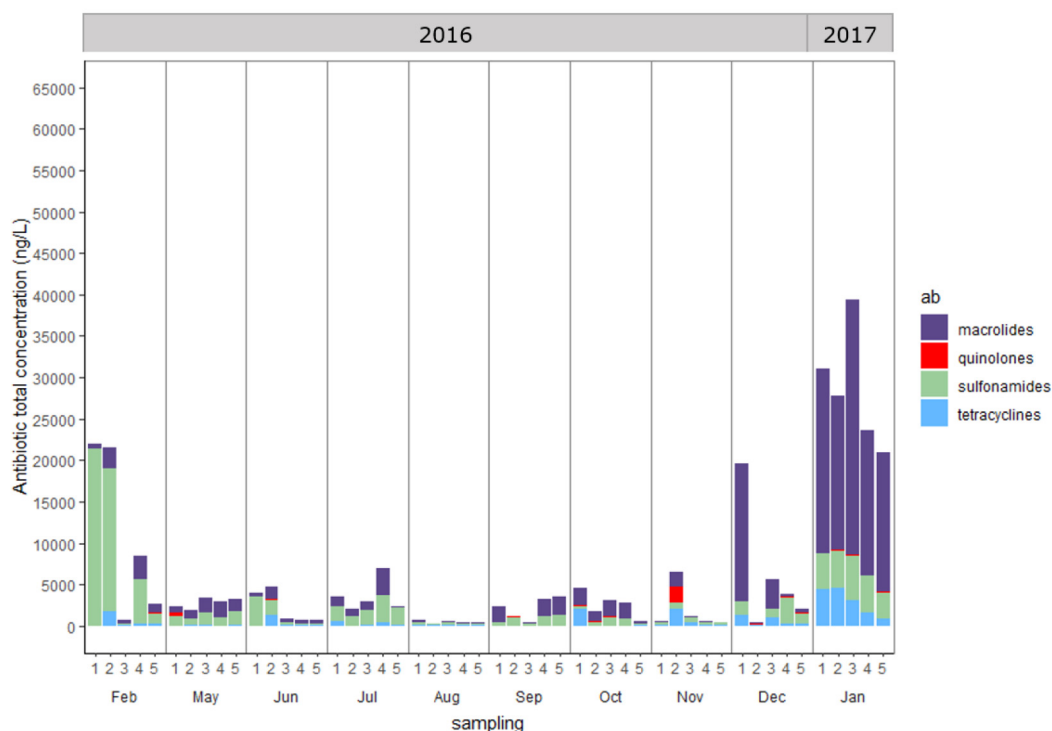

(b) CW with HRT-3

Fig. 2. Concentrations of antibiotics (ng/l) in water in a CW with (a) HRT-1 and (b) HRT-3. Numbers 1, 2, 3, 4, 5 refer to sampling points.

highest concentrations, is a veterinary antibiotic. These compounds are used for treatment and prophylaxis of dysentery, pneumonia, and mycoplasmal infections in pigs and poultry (Islam et al., 2009; Lewicki, 2006).

As manure application is prohibited between October and February, the usual higher flow of agricultural drainage in winter months and several other factors may cause flushing of some substances

from agricultural areas. The tylosin we detected, might result from tylosin residues that were not degraded or washed out and thus entered the WWTP.

3.2.2. Fate of antibiotics in the solid phase

Fig. 3(a) and (b) shows the antibiotics detected in the sediment. Among 50 antibiotics tested, 21 out of 50 antibiotics were detected;
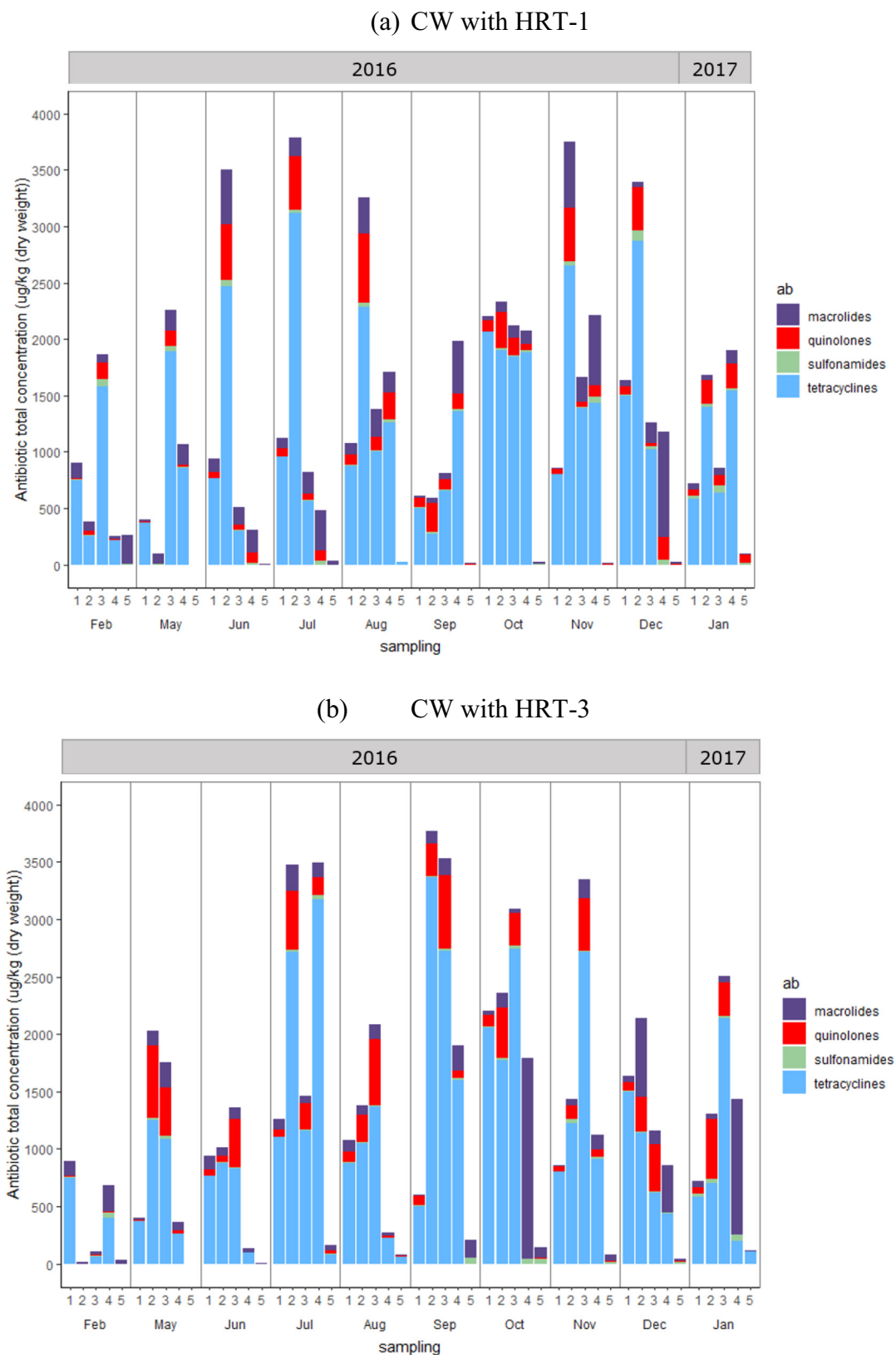
(a) CW with HRT-1

(b)   CW with HRT-3

Fig. 3. Concentrations (μg/kg (dry weight)) of antibiotics in sediment in a CW with (a) HRT-1 and (b) HRT-3. Numbers 1, 2, 3, 4, 5 refer to sampling points.

OTC, chlortetracycline (CTC), tetracycline (TET), doxycycline (DC), SF, sulfapyridine (SP), SDM, SFX, SMX, TRI, sulfadimethoxine (SMT), AZI, CLA, LIN, TIA, TYL, CIP, FLU, norfloxacin (NOR), enrofloxacin (ENRO), and levofloxacin (LEV).

The sum of the concentrations of antibiotics found per sample point was below 4000 μg/kg in each month. Concentration levels of the individual antibiotics ranged from 275 to 1220 μg/kg in the influent of the CW. In the sediments, mainly tetracyclines (OTC, CTC, TC, and DC) were detected. The total concentration of antibiotics in the sediment fluctuated within the CW, regardless of the CW with HRT-1 or the CW with HRT-3. The total concentration of antibiotics at sampling points 2, 3, and 4 were higher than the concentration in influent of the CW, whereas it decreased again at the effluent for both CWs. This trend is almost similar throughout the year. In the effluent of the CW, the number of detected antibiotics was decreased to only seven with concentrations ranging from <LOQ to 198 μg/kg. The removal percentage for the individual antibiotics was −7852 to 100% for the CW with HRT-1 and -1091% to 100% for the CW with HRT-3. The removal of each antibiotic group is illustrated in Fig. S14 and Table S3 for individual compound.

In the sediments, we observed a more stable trend of the total antibiotic concentrations throughout the year. However, within the CW, the total concentration of antibiotics fluctuated, especially at the sampling point 2. This can be explained by the difference in biological processes within the wetland as described before, but also relates to the properties of the antibiotics and sediment. Sediment properties such as organic carbon content, pH, ionic strength, clay content, and texture affect the extent of adsorption of organic compounds, such as antibiotics (Rabølle and Spliid, 2000), thus reducing the mobility, reactivity, bioavailability for microbial degradation and their presence in the water phase (Almeida et al., 2016; Liu et al., 2013; Pan and Chu, 2016; Choi et al., 2016).

Our data showed that sulfonamides were mostly found in the water phase, whereas tetracyclines were mostly found in the sediment phase. This is in accordance to the nature of the chemicals, reflected by the water distribution coefficient (K_d) and organic carbon normalized partition coefficient (K_{oc}) of these compounds (Tolls, 2001). Hence, the sulfonamides are hardly adsorbed to sediment and are usually detected in surface water, groundwater, and drinking water (Wegst-Uhrich et al., 2014). The presence of lower concentration sulfonamides in the sediment suggests that degradation in the water phase may play a more dominant role than substrate adsorption and plant uptake, like has been demonstrated by Chen et al. (2016). For example, SMX is expected to have high mobility based on the estimated K_{oc} of 72 (National Center for Biotechnology Information, 2020b). In contrast, tetracyclines and quinolones have higher K_{oc} tend to adsorb in the sediments due to their ability to form complexes with doubly charged cations (Tolls, 2001; Kümmerer, 2009). K_{oc} of oxytetracycline is ranging from 27,800 to 93,300 (Song and Guo, 2014) and ciprofloxacin is 6100, are expected to have moderate to no mobility in the sediment (National Center for Biotechnology Information, 2020a). Similar observations have been reported by previous research, who showed a low concentration of sulfonamides (8.67 μg/kg) in the sludge compared to macrolides (438.85 μg/kg) (Hu et al., 2018).

As a result, sediment acts as a potential sink, as demonstrated by our data and in line with prior studies. Furthermore, adsorption of antibiotics in the sediment is an important mechanism of antibiotics removal from water, as shown by the presence of antibiotics in the sediment during winter and summer in our study. This has also been demonstrated by other water-sediment investigations (Liu et al., 2019). Kerrigan et al. (2018) reported that the history of ten types of antibiotics in sediment cores for 100 years is increased rapidly since the 1950s with an accumulation rate of up to 20.5 ng cm^{-2}/yr. Meanwhile, Li et al. (2019) observed that the concentration of 60 types of antibiotics in the sediment of different wetlands was up to 118 ng/g.

The performance of the CWs in reducing antibiotics in the sediment after the reed bed and swamp part is presented in Fig. S15. After the

reed bed, quinolones and tetracyclines showed an increase followed by macrolides and sulfonamides. All antibiotics showed a decrease after the sampling point 5. Lastly, antibiotics did not show a significant accumulation or reduction in the water phase at sampling point 1 until sampling point 3. However, the antibiotics showed accumulation in the sediment phase (Table S4).

In addition, we also observed that the concentration of the antibiotics accumulated at sampling point 2 compared to sampling point 4. This is maybe due to the difference of the sediment materials, loamy soil (after the reed bed), and sandy loam soil (after the swamp/effluent of the CW). The availability and mobility of antibiotics in the sediment depend on conditions that prevail in the soil, such as soil type, pH and temperature (Sarmah et al., 2006). We did not further study the effect of the sediment type however, this merits more research in the future to understand the role of different sediment/soil type in ARGs removal.

3.3. Occurrence and removal of ARGs in the constructed wetlands

16S rRNA genes, integrase genes (*intI1*), and four ARGs (*ermB*, *sul1*, *sul2*, and *tetW*) were detected in both CWs in all water and sediment samples. Fig. 4(a) and (b) shows the gene abundance for *sul1*. The other genes in water are presented in Fig. S16(a–e) and in sediment are presented in Fig. S17(a–e).

At the influent of the CWs, the concentrations of ARGs in water varied from 4.9×10^2 copies/ml to 1.4×10^7 copies/ml. The concentration of ARGs ranged from 1×10^1 copies/ml to 2.4×10^8 copies/ml in the CW with HRT-1 and ranged from 4.2×10^1 copies/g to 9.5×10^7 copies/ml in the CW with HRT-3. *intI1* was the most abundant in both CWs, followed by *sul1*, *sul2*, *tetW*, and *ermB*. Higher variability of ARGs was observed in the water samples compared to sediment samples.

Meanwhile, at the sampling no 1 of the CW, the concentrations of ARGs in sediment, varied from 5.5×10^3 copies/ml to 4.2×10^7 copies/g DW. The concentration of ARGs ranged from 1.2×10^2 copies/g DW to 4.9×10^7 copies/g DW in the CW with HRT-1 and ranged from 1.2×10^2 copies/g DW to 4.2×10^7 copies/g DW in the CW with HRT-3. *intI1* was the most abundant gene in both CWs, followed by *sul1*, *sul2*, *tetW*, and *ermB*. All four ARGs were significantly reduced in the CWs by 0.2–3 orders of magnitude ($p < 0.05$).

Nevertheless, concentrations within the CWs varied. For example, at the CW with HRT-1 in February, *sul1* showed no significant difference from the sampling point 1 to sampling point 2, slightly increased at sampling point 3, then decreased at sampling point 4 and 5. Meanwhile, in June, *sul1* increased up to 1 log at sampling point 2, decreased at sampling point 3 and increased again at sampling point 4 and effluent of the CW with HRT-1.

The performance of the CWs in removing ARGs is presented in Fig. 5. The overall removal of the CW with HRT-1 (water) was 0.84 log and the overall removal of the CW with HRT-3 (water) 0.53 log. Meanwhile, the overall removal of the CW with HRT-1 (sediment) was 0.8 log, and the overall removal of the CW with HRT-3 (sediment) was 0.5 log. However, relative abundances (ARGs/16S rRNA genes) of *ermB*, *sul1*, *sul2*, and *tetW* were not significantly lower in the effluent of the CWs compared to in the influent of the CWs (Fig. S18).

The performance of the CWs after the reed bed (i.e., sampling point 1 until sampling point 3) and swamp (sampling point 3 until sampling point 5) for both water and sediment are presented in Fig. S19 (CW with HRT-1) and Fig. S20 (CW with HRT-3). After the reed bed, ARGs showed a decrease (e*rmB*), remained similar (*sul1*, *intI1*, *tetW*), or increased (*sul2*), with the exception of *sul2* (no changes) and *tetW* (decrease) in the CW with HRT-3. Meanwhile, in the sediment samples, ARGs remained the same or increased (*ermB*, *sul1*, *sul2*, *intI1*) or decreased (*tetW*) after the reed bed in the CW with HRT-1, with the exception of decreased *ermB* in the CW with HRT-3. Lastly, similar to antibiotics, ARGs did not show a significant accumulation or reduction in the sampling points within the CWs (Table S5), except at the sampling point 2 in the sediment.
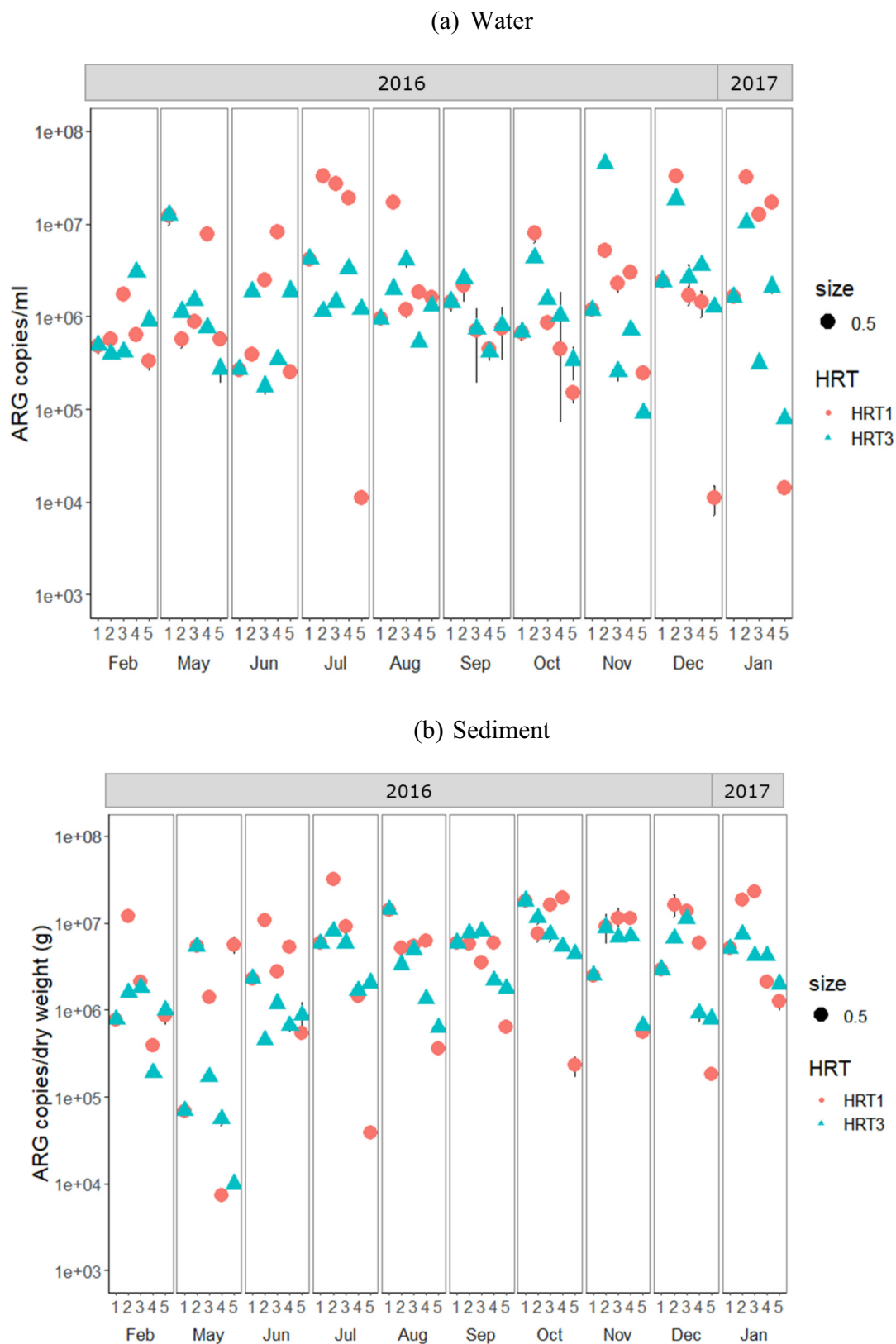
(a) Water

(b) Sediment

Fig. 4. Concentrations of *sul1* detected in the CWs for one year in (a) water and (b) sediment. Numbers 1, 2, 3, 4, 5 refer to sampling points.

In this study, we found that the analyzed genes, 16S rRNA gene, *intI1*, and 4 ARGs were reduced by 0.2 to 3 log in the water phase from the influent of the CW to the effluent of the CW, even though some of ARGs were increased in aqueous concentrations in the influent of the CW just for a few months. Like antibiotics, a variety of mechanisms is involved in ARGs removal in the CWs, such as biological, physical and chemical processes (Vacca et al., 2005; Dordio et al., 2010). The biological processes include plant uptake, and die-off of bacterial hosts (Diehl and Lapara, 2010). The plants in a CW can contribute to the removal of ARGs (Vacca et al., 2005; Sidrach-Cardona and Bécares, 2013; García
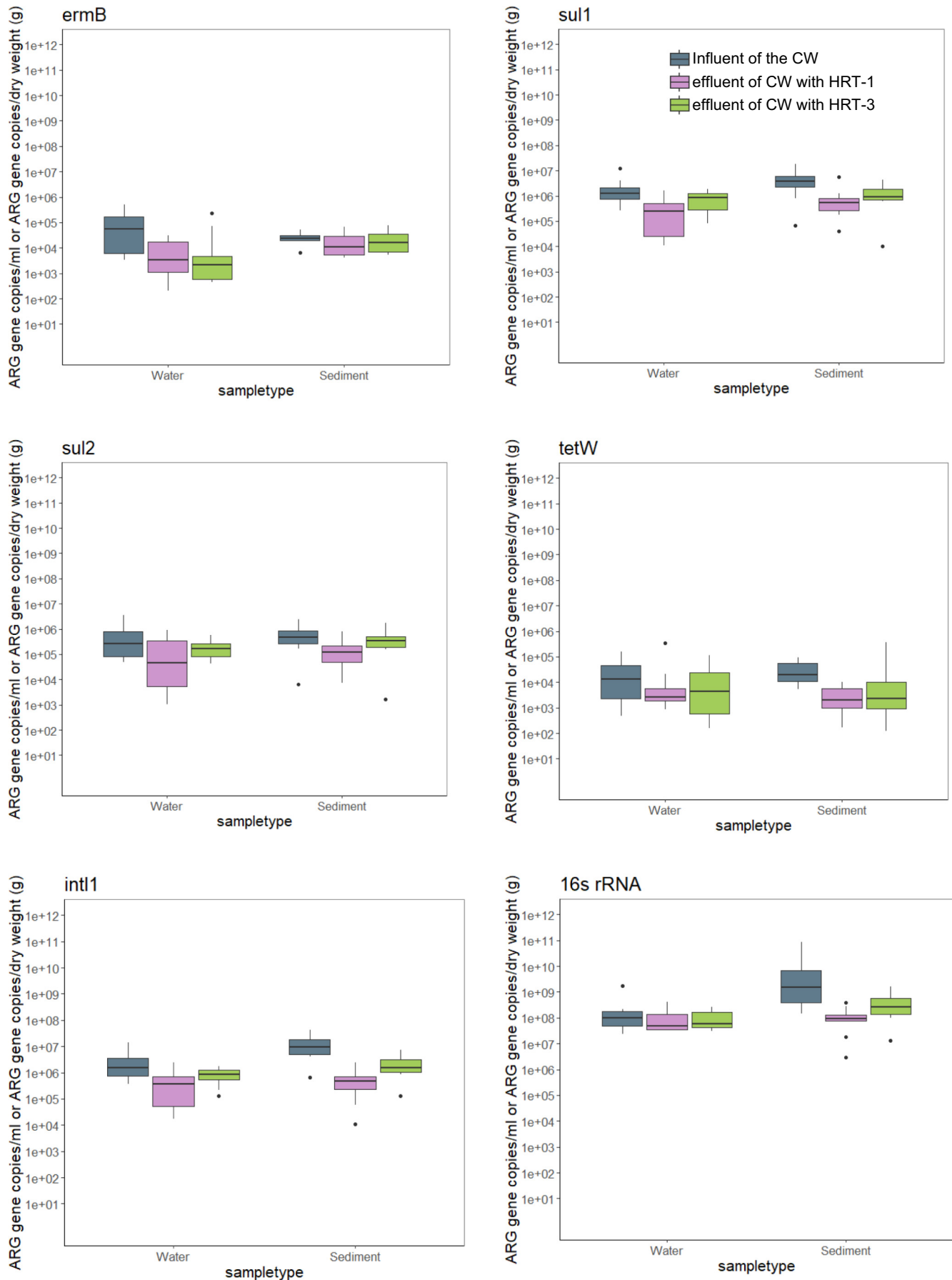
Fig. 5. Gene abundance for all ARGs in water and sediment samples measured in the constructed wetlands for one year.

et al., 2008). e.g. by uptake of the antibiotics and ARGs through roots to the stem, and through leaves via transpiration stream. (Carvalho et al., 2014). The microbial community at the roots may also help in accelerating the biodegradation process (Zhao et al., 2019). The physical processes include sorption to sediment or organic matter, mechanical filtration, or sedimentation. Of these mechanisms, biological processes such as plant uptake and sorption to organic material could be the main mechanisms for ARGs elimination (Chen et al., 2016; Toet et al., 2005).

The ARG abundance in both CWs suggests that a CW can act as sinks for ARGs. ARGs still can persist in sediments when associated with organic substances and clay particles. Sediments adsorb DNAses that hydrolyze free DNA, including ARGs, and as a result, the sediment contains more ARB and ARGs (Luo et al., 2010). In addition, the source of the extracellular ARGs can be derived from the secretion from live cells or the lysis of dead cells and also deposits in the sediments (Pietramellara et al., 2009).

We observed high concentrations of *intI1* and *sul1* in the water and sediment. The presence of *sul1* and *intI1* ($r = 0.92$) and *sul2* and *intI1* ($r = 0.89$) were strongly correlated ($p < 0.001$) in water. A similar observation was seen in the sediment, for *sul1* and *intI1* ($r = 0.67$, $p < 0.001$) and *sul2* and *intI1* ($r = 0.60$, $p < 0.001$). *Sul1* was measured at relatively high concentrations (10^6–10^7) compared to other ARGs in other studies CW (Anderson et al., 2013; Chen et al., 2015). The relatively high concentration of the genes in the CW can be explained by the continuous input of the genes into the CW and resulted in the low variability of ARGs in sediment. As a result, sediments may act as a reservoir where the inflowing ARGs are immobilized and maintained. At the same time, new ARGs may emerge, spread, and new and stored ARGs may be mobilized by resuspension depending on the flow and weather conditions (Heß et al., 2018).

The ARGs *ermB*, *sul2* and *tetW* showed increased concentrations (up to 1 log) in winter ($p < 0.05$) in water and sediment. The higher concentration of ARGs in winter can be due to a higher concentration of antibiotics, as demonstrated by their correlation in this study, and by others (Caucci et al., 2016). However, other studies reported that most of the ARGs in river sediments were higher in summer than in winter, especially *sul1* (Guo et al., 2018). The findings of lower ARG's in water in summer in this study may be related to elevated temperatures, providing a suitable condition for microorganisms and predators to proliferate and degrade ARGs and antibiotics.

Apart from that, ARGs removal efficiency is also related to operating conditions such as HRT. Similar to the antibiotics, a different HRT did not affect the removal of the ARGs ($p > 0.05$). Although a longer HRT was expected to have a positive effect on the removal of ARGs, this was not shown in our study. CW with HRT-3 may contribute to higher flux of all substrates and promote the adsorption and degradation of antibiotics or ARGs (Huang et al., 2017). This might relate to the unknown history of both CWs, differences in organic material, and complex biological activity between the CWs, which can not only be explained by the difference in HRT.

Even though the concentration for some ARGs in the effluent of the CWs was significantly higher than in the surface water of river Beerze that received the treated wastewater after the passage of the CW as we showed in our previous work (Sabri et al., 2020) and other work (Choi et al., 2016), CW improved the removal efficiency of ARGs compared to treatment by conventional WWTPs only (Zhou et al., 2013; Chen and Zhang, 2013). This shows that CW can be an effective additional treatment in removing antibiotics and ARGs from domestic sewage treatment plant effluents.

3.4. Temporal distribution of antibiotics and ARGs

Temporal variations of antibiotics and ARGs in water and sediment are shown in Fig. S21 and Fig. S22, respectively. Some antibiotics and ARGs showed an increase in winter compared to summer. All antibiotics showed significant temporal variation ($p < 0.05$) in water samples. Meanwhile, insignificant differences were found for antibiotic concentrations in sediment samples during summer and winter, except for sulfonamides ($p < 0.05$). Among the ARGs, *ermB*, *sul2* and *tetW* increased significantly in winter ($p < 0.05$) in water and sediment samples, whereas *sul1* demonstrated a significant temporal variation only for the sediment. In addition, *intI1* was not affected by the season throughout the year of study.

In general, both CWs showed a reduction in antibiotics from sampling point 1 to sampling point 5. Both antibiotics and ARGs did not show a significant increase or decrease in the sampling points within the CWs except for sampling point 2 in the sediment. Sampling point 2 is located in the reedbed, which indicates that the reedbed area is a potential area to accumulate antibiotics and ARGs, through particle sedimentation and adsorption to the sediment matrix. The plants and soil types in the reedbed play a role in this, as a higher concentration of antibiotics accumulated in the soil than in the media and vegetation as also observed by others (Liu et al., 2013).

3.5. Correlation between antibiotics, ARGs and general water qualities

The antibiotics, ARGs and general water qualities at different sampling points within the CWs demonstrated a broader variation in winter compared to summer, as shown in an ordination of antibiotics, ARGs, and general water qualities (Fig. 6).

Furthermore, our study shows that significant correlations exist between the abundances of antibiotics and ARGs from sampling point 1 to sampling point 5 (Fig. 6). The correlation between antibiotics and ARGs are presented in Table S6. The total concentration of antibiotics (macrolides, sulfonamides, tetracyclines and quinolones) were correlated to their corresponding and non-corresponding genes ($p < 0.05$), with a stronger correlation between antibiotics and non-corresponding ARGs. Second, correlations between antibiotics, ARGs and general water qualities in sampling point 1 to sampling point 5 were revealed. A significant temporal variation among different general water qualities was observed. The total concentration of all antibiotic groups (except sulfonamides) was significantly associated with concentrations of nutrients, such as NO_3-N, NH_4-N, COD, and TP ($p < 0.05$), and the positive or negative orientation of the correlation depends on the type of nutrient (Table S7). The ARGs (*sul1*, *sul2*, and *intI1*) were most strongly associated with TP, followed by COD ($p < 0.001$) (Table S8). Finally, temperature correlated negatively to antibiotics and ARGs, i.e. indicating higher temperatures made both less persistent.

A low concentration of antibiotics may promote antibiotic resistance and can therefore indirectly correlate with their corresponding ARGs (Bouki et al., 2013). In this study, we found correlation between the total concentration of antibiotics (sulfonamides, macrolides, quinolones, tetracyclines) and their corresponding or non-corresponding ARGs (*sul1*, *sul2*, *ermB*, *tetW*). A stronger correlation between the concentration of antibiotics and non-corresponding ARGs was found, compared to antibiotics and their corresponding ARGs. However, such a generic correlation is inconsistent with previous studies: i.e. Gao et al. (2012) observed a negative correlation between tetracyclines and tet genes but a significant correlation for sulfonamides and sul genes. No correlation was found between antibiotics and their corresponding genes once the wastewater reached the surface water (Guo et al., 2018; Yang et al., 2019). Therefore, our results suggest that the distribution of antibiotics, and their corresponding ARGs over the aquatic environmental compartments depends on many factors, such as their co-release with feces, and most importantly, differences in their fate. This can lead to a lack of correlation in surface waters, in contrast to correlations in sediments.

In this study, nutrients (NO_3-N, NH_4-N, TP and COD) correlated positively and temperature negatively with the occurrence of the antibiotic groups (except sulfonamides). Furthermore, TP and COD positively correlated to ARGs. These findings are in line with previous studies that
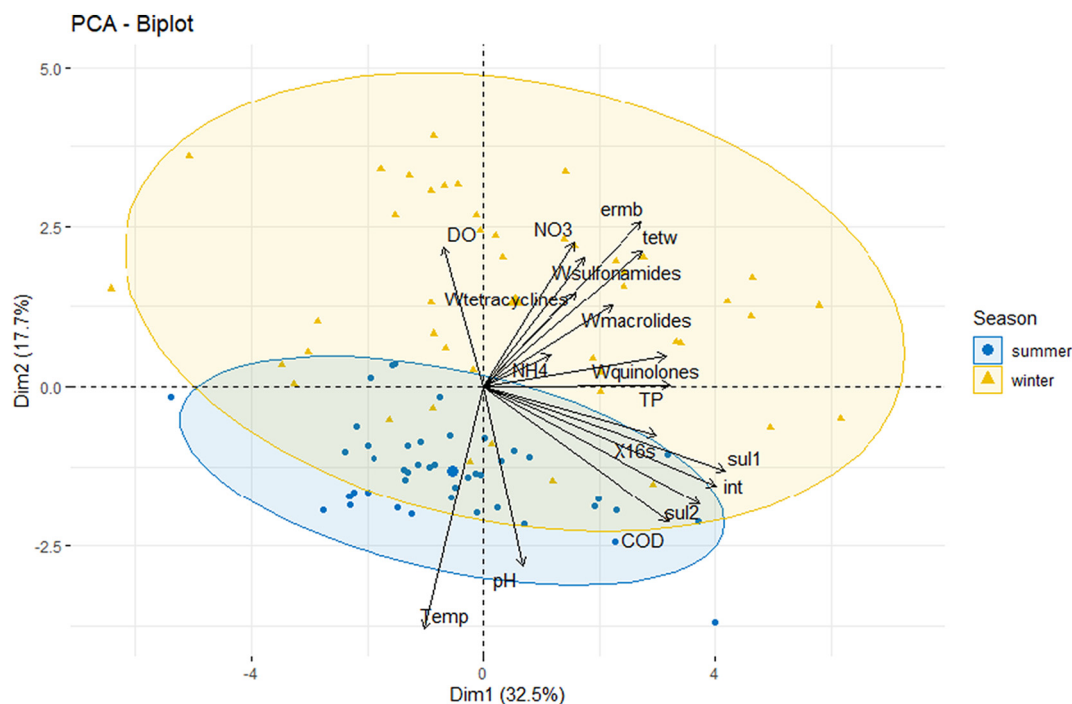
Fig. 6. Principal component analysis of antibiotics (log-transformed), ARGs (log-transformed), and general water qualities in water in both CWs. The first two PCs explain 50.2% of the variations of total antibiotics, ARGs abundance, and general water qualities. PC1 explains 32.5% of the variation.

observed a similar relationship between the concentration of antibiotics or ARGs with some of the general water qualities (Zhu et al., 2020; Novo and Manaia, 2010; Nõlvak et al., 2013). These results support the hypothesis that (at least a part of) the nutrients undergo similar changes as antibiotics and ARGs along the CWs. In this sense, improvements in general water qualities by WWTP or CW can go hand in hand with removal of antibiotics and ARGs (Yuan et al., 2018).

4. Conclusion

The present study evaluated the performance of two full scale CWs, receiving the same wastewater for the removal of antibiotics and ARGs from wastewater. The results of this study demonstrate that the CWs reduced the concentration of targeted antibiotics and ARGs from wastewater. Antibiotics were removed from 28% to 100% in both CWs, depending on the type of antibiotics. However, some of the antibiotics were increased in water and sediment phase after the CW treatment. Five antibiotics (tiamulin, tylosin, oxytetracycline, sulfamethoxazole and trimethoprim) were the most abundant at (>1500 ng/l on average) in February, December and January. Macrolides were most abundantly found in the effluent of the CWs, with strongly fluctuating concentrations in time.

ARGs were removed 1 to 3 log by the CWs. *ermB* was the most abundant (average of 5.0 log) in winter compared to summer (average of 3.5 log). Other ARGs did not show a significant increase or decrease between winter and summer. *intI1* as most abundantly found in the effluent of the CWs. Both sul genes were strongly correlated to *intI1*. The antibiotics removal may be attributed to microbial degradation, substrate adsorption and plant uptake. ARGs removal was more complex and variable, as ARGs mass and composition also depend on the biological activities in the CWs.

Results showed that different HRTs (1 day or 3 days) of the CWs did not affect the removal of the antibiotics or ARGs. No significant accumulation of antibiotics and ARGs was found within the CWs, in water and sediment. The total concentration of antibiotics (sulfonamides, macrolides, tetracyclines) correlated to the concentration of ARGs (*sul1*, *sul2*, *ermB*, and *tetW*). Our results also show a relationship

between concentrations of antibiotics and ARGs with general water qualities, antibiotics correlated with NO_3-N, NH_4-N, and TP, and ARGs correlated with TP and COD.

In general, CWs are easy to operate and to maintain, show a good removal of antibiotics and moderate removal of ARGs, making CWs a potential post treatment to WWTP to reduce antibiotics, and most likely also other micropollutants. CWs reduce the input of micropollutants into the environment and prevent the spreading of antibiotic resistance. However, CWs can accumulate antibiotics and ARGs in the sediment. A better understanding of the elimination processes of ARGs within a CW might result in adjusted designs for CWs, leading to higher removal efficiencies for antibiotics and ARGs in the future.

CRediT authorship contribution statement

N.A. Sabri: Conceptualization, Investigation, Data curation, Visualization, Writing – original draft. **H. Schmitt:** Conceptualization, Data curation, Visualization, Writing – review & editing. **B.M. van der Zaan:** Conceptualization, Data curation, Visualization, Funding acquisition, Writing – review & editing. **H.W. Gerritsen:** Validation, Writing – review & editing. **H.H.M. Rijnaarts:** Supervision, Writing – review & editing. **A.A.M. Langenhoff:** Conceptualization, Data curation, Visualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147368.

References

Almeida, C.M.R., Carvalho, P.N., Fernandes, J.P., Basto, M.C.P., Mucha, A.P., 2016. Constructed wetlands for livestock wastewater treatment: antibiotics removal and effects on CWs performance. In: Ansari, Abid A., Gill, Sarvajeet Singh, Gill, Ritu, Lanza, Guy R., Lee, Newman (Eds.), Phytoremediation: Management of Environmental Contaminants. vol. 4. Springer International Publishing, Cham, pp. 267–281.

Anderson, J., Carlson, J., Low, J., Challis, J., Wong, C., Knapp, C., Hanson, M., 2013. Performance of a constructed wetland in Grand Marais, Manitoba, Canada: removal of nutrients, pharmaceuticals, and antibiotic resistance genes from municipal wastewater. Chem. Central J. 7 (1), 54.

Barancheshme, F., Munir, M., 2018. Strategies to combat antibiotic resistance in the wastewater treatment plants. Front. Microbiol. 8 (2603). https://doi.org/10.3389/fmicb.2017.02603.

Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Burgmann, H., et al., 2015. Tackling antibiotic resistance: the environmental framework. 13 (5), 310–317.

Boreen, A.L., Arnold, W.A., McNeill, K., 2004. Photochemical fate of sulfa drugs in the aquatic environment: sulfa drugs containing five-membered heterocyclic groups. Environ. Sci. Technol. 38 (14), 3933–3940. https://doi.org/10.1021/es0353053.

Bouki, C., Venieri, D., Diamadopoulos, E., 2013. Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: a review. Ecotoxicol. Environ. Saf. 91 (Supplement C), 1–9. https://doi.org/10.1016/j.ecoenv.2013.01.016.

Bruce, E.R., Perry, L.M., 2001. Environmental Biotechnology: Principles and Applications. McGraw-Hill Education, New York.

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 (20), 11729–11763. https://doi.org/10.1007/s11356-014-2550-3.

Caucci, S., Karkman, A., Cacace, D., Rybicki, M., Timpel, P., Voolaid, V., Gurke, R., Virta, M., Berendonk, T.U., 2016. Seasonality of antibiotic prescriptions for outpatients and resistance genes in sewers and wastewater treatment plant outflow. FEMS Microbiol. Ecol. 92 (5). https://doi.org/10.1093/femsec/fiw060.

Chee-Sanford, J.C., Mackie, R.I., Koike, S., Krapac, I.G., Lin, Y.-F., Yannarell, A.C., Maxwell, S., Aminov, R.I., 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J. Environ. Qual. 38 (3), 1086–1108. https://doi.org/10.2134/jeq2008.0128.

Chen, H., Zhang, M., 2013. Occurrence and removal of antibiotic resistance genes in municipal wastewater and rural domestic sewage treatment systems in eastern China. Environ. Int. 55 (0), 9–14. https://doi.org/10.1016/j.envint.2013.01.019.

Chen, J., Liu, Y.S., Su, H.C., Ying, G.G., Liu, F., Liu, S.S., He, L.Y., et al., 2015. Removal of antibiotics and antibiotic resistance genes in rural wastewater by an integrated constructed wetland. Environ. Sci. Pollut. Res. 22 (3), 1794–1803. https://doi.org/10.1007/s11356-014-2800-4.

Chen, J., Ying, G.G., Wei, X.D., Liu, Y.S., Liu, S.S., Hu, L.X., He, L.Y., et al., 2016. Removal of antibiotics and antibiotic resistance genes from domestic sewage by constructed wetlands: effect of flow configuration and plant species. Sci. Total Environ. 571, 974–982. https://doi.org/10.1016/j.scitotenv.2016.07.085.

Choi, Y.J., Kim, L.H., Zoh, K.D., 2016. Removal characteristics and mechanism of antibiotics using constructed wetlands. Ecol. Eng. 91, 85–92. https://doi.org/10.1016/j.ecoleng.2016.01.058.

Council Directive 91/271/EEC, 1991. Council Directive 91/271/EEC of 21 May 1991 Concerning Urban Waste-water Treatment.

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 (23), 9128–9133. https://doi.org/10.1021/es102765a.

Dong, H., Yuan, X., Wang, W., Qiang, Z., 2016. Occurrence and removal of antibiotics in ecological and conventional wastewater treatment processes: a field study. J. Environ. Manag. 178, 11–19. https://doi.org/10.1016/j.jenvman.2016.04.037.

Dordio, A., Carvalho, A.J.P., Teixeira, D.M., Dias, C.B., Pinto, A.P., 2010. Removal of pharmaceuticals in microcosm constructed wetlands using Typha spp. and LECA. Bioresour. Technol. 101 (3), 886–892. https://doi.org/10.1016/j.biortech.2009.09.001.

Fernandes, J.P., 2014. Response of Microorganisms From Natural and Constructed Wetlands to Veterinary Drugs. University of Porto, Porto.

Fleskens, L., Matte, G., Zanten, O.v., 2016. Zuiver water komt tot leven Vijftien jaar waterharmonica's bij Waterschap De Dommel. the Netherlands: Watesrchap de Dommel.

Fonder, N., Headley, T., 2013. The taxonomy of treatment wetlands: a proposed classification and nomenclature system. Ecol. Eng. 51, 203–211. https://doi.org/10.1016/j.ecoleng.2012.12.011.

Gao, P., Munir, M., Xagoraraki, I., 2012. Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant. Sci. Total Environ. 421-422, 173–183. https://doi.org/10.1016/j.scitotenv.2012.01.061.

García, M., Soto, F., González, J.M., Bécares, E., 2008. A comparison of bacterial removal efficiencies in constructed wetlands and algae-based systems. Ecol. Eng. 32 (3), 238–243. https://doi.org/10.1016/j.ecoleng.2007.11.012.

Gruchlik, Y., Linge, K., Joll, C., 2018. Removal of organic micropollutants in waste stabilisation ponds: a review. J. Environ. Manag. 206, 202–214. https://doi.org/10.1016/j.jenvman.2017.10.020.

Guo, X.-p., Liu, X., Niu, Z.-s., Lu, D.-p., Zhao, S., Sun, X.-l., Wu, J.-y., et al., 2018. Seasonal and spatial distribution of antibiotic resistance genes in the sediments along the Yangtze Estuary, China. Environ. Pollut. 242, 576–584. https://doi.org/10.1016/j.envpol.2018.06.099.

Heß, S., Berendonk, T.U., Kneis, D., 2018. Antibiotic resistant bacteria and resistance genes in the bottom sediment of a small stream and the potential impact of remobilization. FEMS Microbiol. Ecol. 94 (9). https://doi.org/10.1093/femsec/fiy128.

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 (5), 713–719. https://doi.org/10.1016/j.chemosphere.2011.02.004.

Hijosa-Valsero, M., Sidrach-Cardona, R., Bécares, E., 2012. Comparison of interannual removal variation of various constructed wetland types. Sci. Total Environ. 430, 174–183. https://doi.org/10.1016/j.scitotenv.2012.04.072.

Hsieh, C.Y., Liaw, E.T., Fan, K.M., 2015. Removal of veterinary antibiotics, alkylphenolic compounds, and estrogens from the Wuluo constructed wetland in southern Taiwan. J. Environ. Sci. Health A 50 (2), 151–160. https://doi.org/10.1080/10934529.2015.975062.

Hu, Y., Jiang, L., Zhang, T., Jin, L., Han, Q., Zhang, D., Lin, K., Cui, C., 2018. Occurrence and removal of sulfonamide antibiotics and antibiotic resistance genes in conventional and advanced drinking water treatment processes. J. Hazard. Mater. 360, 364–372. https://doi.org/10.1016/j.jhazmat.2018.08.012.

Huang, X., Zheng, J., Liu, C., Liu, L., Liu, Y., Fan, H., 2017. Removal of antibiotics and resistance genes from swine wastewater using vertical flow constructed wetlands: effect of hydraulic flow direction and substrate type. Chem. Eng. J. https://doi.org/10.1016/j.cej.2016.09.110.

Islam, K.M.S., Klein, U., Burch, D.G.S., 2009. The activity and compatibility of the antibiotic tiamulin with other drugs in poultry medicine—a review. Poult. Sci. 88 (11), 2353–2359. https://doi.org/10.3382/ps.2009-00257.

Jansen, L.J.M., Bolck, Y.J.C., Rademaker, J., Zuidema, T., Berendsen, B.J.A., 2017. The analysis of tetracyclines, quinolones, macrolides, lincosamides, pleuromutilins, and sulfonamides in chicken feathers using UHPLC-MS/MS in order to monitor antibiotic use in the poultry sector. Anal. Bioanal. Chem. 409 (21), 4927–4941. https://doi.org/10.1007/s00216-017-0445-0.

Jiang, Y., Xu, C., Wu, X., Chen, Y., Han, W., Gin, K.Y.-H., He, Y., 2018. Occurrence, seasonal variation and risk assessment of antibiotics in Qingcaosha Reservoir. Water 10 (2), 115.

Kaur, R., Talan, A., Tiwari, B., Pilli, S., Sellamuthu, B., Tyagi, R.D., 2020. Chapter 5 - constructed wetlands for the removal of organic micro-pollutants. In: Varjani, Sunita, Pandey, Ashok, Tyagi, R.D., Ngo, Huu Hao, Larroche, Christian (Eds.), Current Developments in Biotechnology and Bioengineering. Elsevier, pp. 87–140.

Kerrigan, J.F., Sandberg, K.D., Engstrom, D.R., LaPara, T.M., Arnold, W.A., 2018. Sedimentary record of antibiotic accumulation in Minnesota Lakes. Sci. Total Environ. 621, 970–979. https://doi.org/10.1016/j.scitotenv.2017.10.130.

Kim, S.C., Carlson, K., 2007. Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices. Environ. Sci. Technol. 41 (1), 50–57. https://doi.org/10.1021/es060737+.

Kümmerer, K., 2004. Resistance in the environment. J. Antimicrob. Chemother. 54 (2), 311–320. https://doi.org/10.1093/jac/dkh325.

Kümmerer, K., 2009. The presence of pharmaceuticals in the environment due to human use – present knowledge and future challenges. J. Environ. Manag. 90 (8), 2354–2366. https://doi.org/10.1016/j.jenvman.2009.01.023.

Larsson, D.G.J., de Pedro, C., Paxeus, N., 2007. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J. Hazard. Mater. 148 (3), 751–755. https://doi.org/10.1016/j.jhazmat.2007.07.008.

Lavrnić, S., Nan, X., Blasioli, S., Braschi, I., Anconelli, S., Toscano, A., 2020. Performance of a full scale constructed wetland as ecological practice for agricultural drainage water treatment in northern Italy. Ecol. Eng. 154, 105927. https://doi.org/10.1016/j.ecoleng.2020.105927.

Lewicki, J., 2006. TYLOSIN: A Review of Pharmacokinetics, Residues in Food Animals and Analytical Methods. FAO.

Li, Y., Zhu, G., Ng, W.J., Tan, S.K., 2014. A review on removing pharmaceutical contaminants from wastewater by constructed wetlands: design, performance and mechanism. Sci. Total Environ. 468–469 (0), 908–932. https://doi.org/10.1016/j.scitotenv.2013.09.018.

Li, S., Zhang, R., Hu, J., Shi, W., Kuang, Y., Guo, X., Sun, W., 2019. Occurrence and removal of antibiotics and antibiotic resistance genes in natural and constructed riverine wetlands in Beijing, China. Sci. Total Environ. 664, 546–553. https://doi.org/10.1016/j.scitotenv.2019.02.043.

Liu, L., Liu, C., Zheng, J., Huang, X., Wang, Z., Liu, Y., Zhu, G., 2013. Elimination of veterinary antibiotics and antibiotic resistance genes from swine wastewater in the vertical flow constructed wetlands. Chemosphere 91 (8), 1088–1093. https://doi.org/10.1016/j.chemosphere.2013.01.007.

Liu, L., Li, J., Fan, H., Huang, X., Wei, L., Liu, C., 2019. Fate of antibiotics from swine wastewater in constructed wetlands with different flow configurations. Int. Biodeterior. Biodegradation 140, 119–125. https://doi.org/10.1016/j.ibiod.2019.04.002.

Luo, Y., Mao, D., Rysz, M., Zhou, Q., Zhang, H., Xu, L., Alvarez, P.J.J., 2010. Trends in antibiotic resistance genes occurrence in the Haihe River, China. Environ. Sci. Technol. 44 (19), 7220–7225. https://doi.org/10.1021/es100233w.

Margot, J., Rossi, L., Barry, D.A., Holliger, C., 2015. A review of the fate of micropollutants in wastewater treatment plants. Wiley Interdiscip. Rev. Water 2 (5), 457–487. https://doi.org/10.1002/wat2.1090.

Matamoros, V., Rodríguez, Y., Bayona, J.M., 2017. Mitigation of emerging contaminants by full-scale horizontal flow constructed wetlands fed with secondary treated wastewater. Ecol. Eng. 99, 222–227. https://doi.org/10.1016/j.ecoleng.2016.11.054.

National Center for Biotechnology Information, 2020a. PubChem database. Oxytetracycline, CID=54675779. cited Feb. 2, 2020. Available from. https://pubchem.ncbi.nlm.nih.gov/compound/Oxytetracycline.

National Center for Biotechnology Information, 2020b. PubChem database. Oxytetracycline. PubChem Database. Sulfamethoxazole, CID=5329. cited Feb. 2, 2020. Available from. https://pubchem.ncbi.nlm.nih.gov/compound/Sulfamethoxazole.

Nguyen Dang Giang, C., Sebesvari, Z., Renaud, F., Rosendahl, I., Hoang Minh, Q., Amelung, W., 2015. Occurrence and dissipation of the antibiotics sulfamethoxazole, sulfadiazine, trimethoprim, and enrofloxacin in the Mekong Delta, Vietnam. PLoS One 10 (7), e0131855. https://doi.org/10.1371/journal.pone.0131855.

Nõlvak, H., Truu, M., Tiirik, K., Oopkaup, K., Sildvee, T., Kaasik, A., Mander, Ü., Truu, J., 2013. Dynamics of antibiotic resistance genes and their relationships with system treatment efficiency in a horizontal subsurface flow constructed wetland. Sci. Total Environ. 461–462 (0), 636–644. https://doi.org/10.1016/j.scitotenv.2013.05.052.

Novo, A., Manaia, C.M., 2010. Factors influencing antibiotic resistance burden in municipal wastewater treatment plants. Appl. Microbiol. Biotechnol. 87 (3), 1157–1166. https://doi.org/10.1007/s00253-010-2583-6.

Pan, M., Chu, L.M., 2016. Adsorption and degradation of five selected antibiotics in agricultural soil. Sci. Total Environ. 545-546, 48–56. https://doi.org/10.1016/j.scitotenv.2015.12.040.

Parde, D., Patwa, A., Shukla, A., Vijay, R., Killedar, D.J., Kumar, R., 2021. A review of constructed wetland on type, treatment and technology of wastewater. Environ. Technol. Innov. 21, 101261. https://doi.org/10.1016/j.eti.2020.101261.

Pazda, M., Kumirska, J., Stepnowski, P., Mulkiewicz, E., 2019. Antibiotic resistance genes identified in wastewater treatment plant systems – a review. Sci. Total Environ. 697, 134023. https://doi.org/10.1016/j.scitotenv.2019.134023.

Petrie, B., Rood, S., Smith, B.D., Proctor, K., Youdan, J., Barden, R., Kasprzyk-Hordern, B., 2018. Biotic phase micropollutant distribution in horizontal sub-surface flow constructed wetlands. Sci. Total Environ. 630, 648–657. https://doi.org/10.1016/j.scitotenv.2018.02.242.

Pietramellara, G., Ascher, J., Borgogni, F., Ceccherini, M.T., Guerri, G., Nannipieri, P., 2009. Extracellular DNA in soil and sediment: fate and ecological relevance. Biol. Fertil. Soils 45 (3), 219–235. https://doi.org/10.1007/s00374-008-0345-8.

Rabølle, M., Spliid, N.H., 2000. Sorption and mobility of metronidazole, olaquindox, oxytetracycline and tylosin in soil. Chemosphere 40 (7), 715–722. https://doi.org/10.1016/S0045-6535(99)00442-7.

Radjenović, J., Petrović, M., Barceló, D., 2009. Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor (MBR) treatment. Water Res. 43 (3), 831–841. https://doi.org/10.1016/j.watres.2008.11.043.

Sabri, N.A., Schmitt, H., Van der Zaan, B., Gerritsen, H.W., Zuidema, T., Rijnaarts, H.H.M., Langenhoff, A.A.M., 2020. Prevalence of antibiotics and antibiotic resistance genes in a wastewater effluent-receiving river in the Netherlands. J. Environ. Chem. Eng. https://doi.org/10.1016/j.jece.2018.03.004.

Santos, F., Almeida, C.M.R., Ribeiro, I., Mucha, A.P., 2019. Potential of constructed wetland for the removal of antibiotics and antibiotic resistant bacteria from livestock wastewater. Ecol. Eng. 129, 45–53. https://doi.org/10.1016/j.ecoleng.2019.01.007.

Sarmah, A.K., Meyer, M.T., Boxall, A.B.A., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65 (5), 725–759. https://doi.org/10.1016/j.chemosphere.2006.03.026.

Sidrach-Cardona, R., Bécares, E., 2013. Fecal indicator bacteria resistance to antibiotics in experimental constructed wetlands. Ecol. Eng. 50, 107–111. https://doi.org/10.1016/j.ecoleng.2012.01.001.

Sochacki, A., Felis, E., Bajkacz, S., Nowrotek, M., Miksch, K., 2018. Removal and transformations of diclofenac and sulfamethoxazole in a two-stage constructed wetland system. Ecol. Eng. 122, 159–168. https://doi.org/10.1016/j.ecoleng.2018.07.039.

Song, W., Guo, M., 2014. Residual veterinary pharmaceuticals in animal manures and their environmental behaviors in soils. In: He, Zhongqi, Zhang, Hailin (Eds.), Applied Manure and Nutrient Chemistry for Sustainable Agriculture and Environment. Springer Netherlands, Dordrecht, pp. 23–52.

Toet, S., Logtestijn, R.S.P.V., Kampf, R., Schreijer, M., Verhoeven, J.T.A., 2005. The effect of hydraulic retention time on the removal of pollutants from sewage treatment plant effluent in a surface-flow wetland system. Wetlands 25 (375). https://doi.org/10.1672/13.

Tolls, J., 2001. Sorption of veterinary pharmaceuticals in Soils: a review. Environ. Sci. Technol. 35 (17), 3397–3406. https://doi.org/10.1021/es0003021.

Vacca, G., Wand, H., Nikolausz, M., Kuschk, P., Kästner, M., 2005. Effect of plants and filter materials on bacteria removal in pilot-scale constructed wetlands. Water Res. 39 (7), 1361–1373. https://doi.org/10.1016/j.watres.2005.01.005.

Verlicchi, P., Zambello, E., 2014. How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. Sci. Total Environ. 470-471 (Supplement C), 1281–1306. https://doi.org/10.1016/j.scitotenv.2013.10.085.

Vymazal, J., Dvořáková Březinová, T., Koželuh, M., Kule, L., 2017. Occurrence and removal of pharmaceuticals in four full-scale constructed wetlands in the Czech Republic – the first year of monitoring. Ecol. Eng. 98, 354–364. https://doi.org/10.1016/j.ecoleng.2016.08.010.

Wegst-Uhrich, S.R., Navarro, D.A.G., Zimmerman, L., Aga, D.S., 2014. Assessing antibiotic sorption in soil: a literature review and new case studies on sulfonamides and macrolides. Chem. Central J. 8, 5. https://doi.org/10.1186/1752-153X-8-5.

WHO, 2014. Antimicrobial Resistance: Global Report on Surveillance 2014 (Switzerland).

Yang, J.F., Ying, G.G., Zhao, J.L., Tao, R., Su, H.C., Liu, Y.S., 2011. Spatial and seasonal distribution of selected antibiotics in surface waters of the Pearl Rivers, China. J. Environ. Sci. Health B 46 (3), 272–280. https://doi.org/10.1080/03601234.2011.540540.

Yang, Y., Liu, Z., Xing, S., Liao, X., 2019. The correlation between antibiotic resistance gene abundance and microbial community resistance in pig farm wastewater and surrounding rivers. Ecotoxicol. Environ. Saf. 182, 109452. https://doi.org/10.1016/j.ecoenv.2019.109452.

Yuan, Q.-B., Zhai, Y.-F., Mao, B.-Y., Hu, N., 2018. Antibiotic resistance genes and intI1 prevalence in a swine wastewater treatment plant and correlation with metal resistance, bacterial community and wastewater parameters. Ecotoxicol. Environ. Saf. 161, 251–259. https://doi.org/10.1016/j.ecoenv.2018.05.049.

Zhang, S., Song, H.-L., Yang, X.-L., Yang, Y.-L., Yang, K.-Y., Wang, X.-Y., 2016. Fate of tetracycline and sulfamethoxazole and their corresponding resistance genes in microbial fuel cell coupled constructed wetlands. RSC Adv. 6 (98), 95999–96005. https://doi.org/10.1039/C6RA20509G.

Zhao, X., Li, X., Li, Y., Sun, Y., Zhang, X., Weng, L., Ren, T., Li, Y., 2019. Shifting interactions among bacteria, fungi and archaea enhance removal of antibiotics and antibiotic resistance genes in the soil bioelectrochemical remediation. Biotechnol. Biofuels 12 (1), 160. https://doi.org/10.1186/s13068-019-1500-1.

Zheng, S., Cui, C., Liang, Q., Xia, X., Yang, F., 2010. Ozonation performance of WWTP secondary effluent of antibiotic manufacturing wastewater. Chemosphere 81 (9), 1159–1163. https://doi.org/10.1016/j.chemosphere.2010.08.058.

Zhou, L.J., Ying, G.G., Liu, S., Zhang, R.Q., Lai, H.J., Chen, Z.F., Pan, C.G., 2013. Excretion masses and environmental occurrence of antibiotics in typical swine and dairy cattle farms in China. Sci. Total Environ. 444 (Supplement C), 183–195. https://doi.org/10.1016/j.scitotenv.2012.11.087.

Zhou, X., Wang, R., Liu, H., Wu, S., Wu, H., 2019. Nitrogen removal responses to biochar addition in intermittent-aerated subsurface flow constructed wetland microcosms: enhancing role and mechanism. Ecol. Eng. 128, 57–65. https://doi.org/10.1016/j.ecoleng.2018.12.028.

Zhu, N., Jin, H., Ye, X., Liu, W., Li, D., Shah, G.M., Zhu, Y., 2020. Fate and driving factors of antibiotic resistance genes in an integrated swine wastewater treatment system: from wastewater to soil. Sci. Total Environ. 721, 137654. https://doi.org/10.1016/j.scitotenv.2020.137654.