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Prevalence of antibiotics and antibiotic resistance genes in a wastewater effluent-receiving river in the Netherlands

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ABSTRACT

Antibiotics are being used intensively for humans and livestock worldwide and have led to the presence of antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) in the environment. Wastewater treatment plants (WWTPs) have been identified as a point source for ARB&Gs, and water catchments consequently are potential receptors of ARB&Gs. The objective of this study was to investigate the occurrence of antibiotics (macrolides, sulfonamides, tetracyclines), ARGs (ermB, sul1, sul2, tetW), and class 1 integron (targeting the integrase gene), in a Dutch river that receives wastewater treatment plant effluent. Sediment and water samples were collected during one year along the river. The WWTP significantly increased the amounts of antibiotics and ARGs in the river as compared to the upstream samples, of which the antibiotics decreased once they entered the river. ARGs were persistent in the water and sediment from the WWTP effluent discharge point until 20 km downstream. This study provides insight in the prevalence of antibiotics and ARGs in a wastewater effluent-receiving river system in the Netherlands. Even though human antibiotic usage is low in the Netherlands, antibiotics, residues of antibiotics, and ARGs are detected in the river surface water-sediment system, which shows that a river has the potential to act as a reservoir of ARGs.

1. Introduction

For decades, antibiotics have been used to cure human or animal infectious diseases by either killing or inhibiting the growth of bacteria. Antibiotics have given a major contribution to the medical field for decades [1]. As antibiotics are not fully degraded within animals or humans, approximately 30–90% of the antibiotics used for animals are excreted through urine and faeces [2]. Through the sewage system, WWTPs also receive water that can contain a number of pollutants, including nutrients, metals, antibiotics, and chemicals from different sources [3]. However, our current WWTPs are not designed to remove micropollutants such as antibiotics and antibiotic resistant bacteria and genes, and those biological components might end up in the WWTP effluent [4] as they are not fully removed by current treatment technologies [5–7].

WWTPs have been suggested as potential hotspots for antibiotic resistance and antibiotic resistance genes (ARGs) [8]. Antibiotics, ARB and ARGs are frequently detected in WWTPs [9,10]. Antibiotic resistance is developing faster than new antibiotics are being developed, whereas identifying new antibiotics is becoming increasingly challenging and costly [11,12]. As a result, new antibiotics are hardly introduced, and antibiotic resistanceis found in hospital settings, and also in the natural environment. In addition, antibiotic resistance is also a natural phenomenon, as bacteria have evolved resistance to naturally present antibiotics [13].

As a result, wastewater treatment plant effluent can contain antibiotics, ARB and ARGs, and once this effluent is discharged to the surface water, these contaminants enter the environment. ARGs are spread in the surface water by ARB, that possibly acquired these ARGs through horizontal gene transfer. Even though the proliferation of ARGs in the environment is assumed to be low, ARB are also an important contributor in transporting and spreading of antibiotic resistance in the microbial community [14].

Recently, dissemination of ARGs in the environments has been highlighted as an emerging problem [15,16], especially if contaminated water resources are reused for cattle, irrigation, or drinking water

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production [17,18]. Such water reuse is gaining more attention for overcoming water scarcity and achieving sustainable water management in especially arid regions [19]. World population growth and draughts are the main factors that will increase the demand of water reuse. Therefore, the need for clean and safe water, free of emerging wastewater contaminants, antibiotics, antibiotics residues and ARGS are needed.

The role of WWTP as sources of antibiotics and ARGs and their dissemination in rivers is an active field of research [20–25]. An increase in concentrations of antibiotics and ARG in rivers has repeatedly been found to be caused by emissions of WWTP effluent [21,24], but also and by a variety of other activities such as increasing density of population along the river, industrial operations, agricultural and/or aquacultural activities [20,26]. For example, in India, Devarajan et al. [10] concluded that beta-lactamase genes (blaSHV and blaNDM) were identified in sediments contaminated by hospital and urban wastewaters in Cauvery River. Lekunberri et al. [27] also observed the same trend in Spain. They observed that concentrations of antibiotic and ARGs (macrolides gene (ermB), fluoroquinolones gene (qnrS) and tetracyclines gene (tetW)) in the Ter River showed a significant difference upstream and downstream of WWTP effluent discharge. Chen et al. [28] reported that tetracycline genes (tetC, tetB, tetM, tetO, and tetW) were detected in the Pearl River in China, which is heavily influenced by human activities. Meanwhile in China, Ling et al. [29] found out that sulfonamide genes (sul1 and sul2) and tetracycline genes (tetG, tetA, tetO, tetC, tetX, tetM and tetQ) were frequently observed in the Beijiang River, in locations with the highest degree of urbanisation. Dissemination of ARGs in the river has not only been shown in water and sediment, but also in biofilms [30] and aquatic animal guts [31]. With the dissemination of antibiotics and ARGs to rivers, ARGs finally enter marine environments including marine water or sponge species [28,32,33].

This paper describes the occurrence of emerging wastewater contaminants (antibiotics and ARGs) and nutrients along a Dutch river, tracking the effect of discharge of WWTP effluent. We performed the sampling in the river from 0.5 km upstream until 20 km downstream with only one small side stream at 2 km. Sediment and water samples were collected in repeated samplings during one year to identify correlations between ARGs and other environmental factors (pH, temperature, dissolved oxygen (DO), chemical oxygen analysis (COD), total phosphate (TP), ammonium (NH_4^+) and nitrate (NO_3^-))

2. Material and methods

2.1. Sampling site and sample collection

Field samples upstream and downstream a WWTP plant (Hapert, the Netherlands) were collected from February 2016 until January 2017. The WWTP treats 78% of domestic and 22% of industrial wastewater via a conventional system consisting of bar screens, grit removal, and an oxidation ditch. The WWTP effluent was split for two additional post treatments, namely two wetlands with a hydraulic retention time of 1 day or 3 days.

Sampling was performed in the Grote Beerze river, the Netherlands (51°22′N 5°14′E). Sediment (n = 390) and water (n = 390) samples were collected from 13 sampling points for one year. Sampling in the river started 0.5 km upstream (U1) and continued until 20 km downstream (D9) of the WWTP in the Grote Beerze river, and included two effluents from the wetlands (E1 and E2) that were discharged into this river (Fig. 1). For season comparison, water temperature during sampling was used to classify the season. Temperatures above 15 °C are classified as summer season, and below 15 °C are classified as winter season. Therefore, the summer months are May, June, July and August, and the winter months are September, October, November, December, January and February.

Water grab samples were collected into 1 l sterile green glass bottles

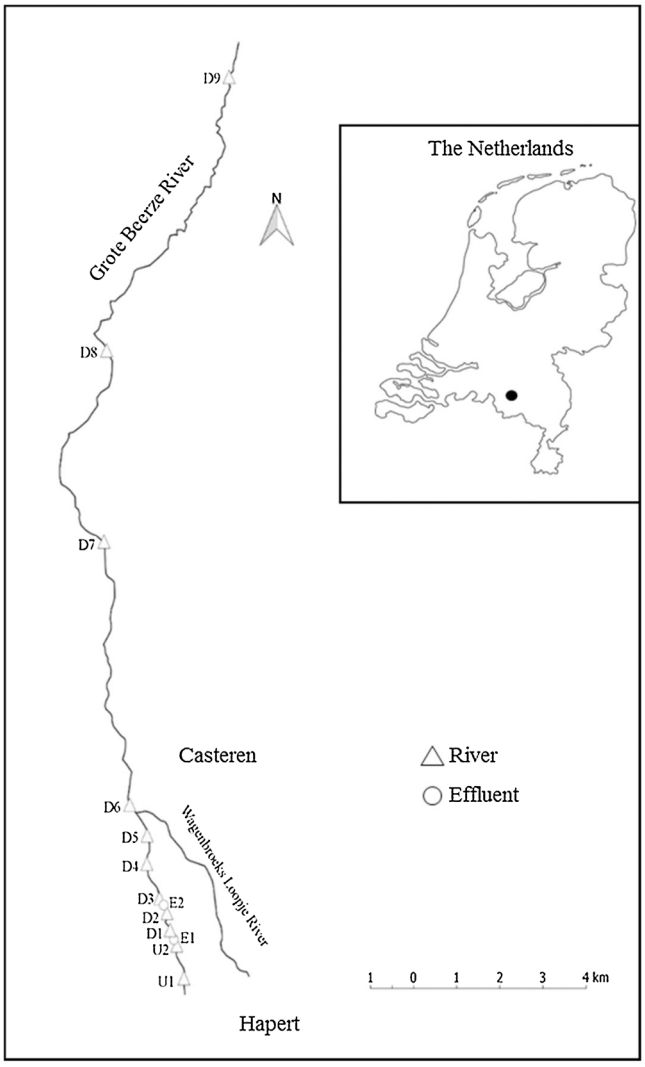

Fig. 1. Map showing the 13 sampling points along Grote Beerze River, The Netherlands. E1 = effluent of wetland with HRT 1-day. E2 = effluent of wetland with HRT 3-day. U1 = 0.5 km before E1, U2 = 0.1 km before E1, D1 = 0.1 km after E1, D2 = between E1 and E2, D3 = 0.1 km after E2, D4 = 0.5 km after E2, D5 = 1 km after E2, D6 = 1.5 km after E2, D7 = 5 km after E2, D8 = 10 km after E2, D9 = 20 km after E2.

with screw caps from each sampling point, and transported to the laboratory for analysis. 100 ml was stored at −20 °C for residue analysis of antibiotics and the rest of the water samples was stored at 4 °C for chemical and qPCR analysis. Chemical analyses were performed within 1–3 days of collection. Sediment samples were collected in 50 ml plastic tubes by using a grab sampler, transported to the laboratory, and stored at −20 °C for qPCR analysis. All samples were taken in triplicate.

2.2. General water quality and chemical analysis

General water quality parameters including pH, temperature, dissolved oxygen (DO), were measured in-situ using a pH and DO portable probe (Hach, USA). Chemical oxygen analysis (COD), total phosphate (TP), ammonium (NH_4^+) and nitrate (NO_3^-) were measured using Hach kits (USA; LCK 1414, LCK 349, LCK 304 and LCK 349, respectively) for each triplicate sample. COD and TP were measured directly in the stored samples, whereas NH_4^+ and NO_3^- were filtered using a 4–7 μm filter paper (Whatman, United Kingdom), prior to analysis. All samples were processed within 2 days after sampling.

2.3. Sample preparation for the analysis of antibiotics

Water samples for October and November were analysed for 18 sulfonamides, trimethoprim, 6 tetracyclines, 12 quinolones and 15 macrolides. The analyses were performed in single measurement. All antibiotics were listed in Table S1. The antibiotics were chosen from human and veterinary antibiotics that are frequently used and that have been detected in water [34,35]. We included antibiotics that correspond to the resistance gene classes analysed. Sulfonamides are corresponding to sul1 and sul2, tetracyclines are corresponding to tetW and macrolides are corresponding to ermB. Prior to chemical analysis by LC–MS/MS, the samples were concentrated by solid phase extraction (SPE). All chemicals were purchased from Sigma.

Liquid samples were taken from the freezer and thawed. Duplicate aliquots of 10 ml of each sample were transferred to two plastic tubes of 30 ml each. To both aliquots, 10 µl of a mixture of internal standard solution (500 µg/L) was added. To one aliquot, 100 µl of a mixture of all antibiotics (2.5 µg/L for the sulfonamides and 10 µg/L for the tetracyclins, quinolones and macrolides) was added. The samples were mixed and 4 ml of McIlvain buffer (0.1 M citric acid, 0.2 M phosphate buffer and Na_2EDTA; pH 4) was added. The samples were horizontally shaken for 5 min at 120 rpm, followed by centrifugation at 4000*g* for 10 min.

200 mg Strata-X (Phenomenex, USA) SPE columns were washed with 5 ml methanol (MeOH), followed by 5 ml McIlvain buffer. Thereafter, the sample extract was loaded onto the column, followed by a washing step with 5 ml purified water (Milli-Q, Merck, USA). Vacuum pressure was applied to extract the liquid from the SPE columns. Hereafter, the columns were eluted with 5 ml of MeOH and the eluting liquid was collected in clean collection glass tubes. The collection tubes were placed in a nitrogen evaporator of 40 °C to evaporate the MeOH. After complete evaporation, the samples were redissolved in 100 µl of MeOH and vortexed for 5 s. Finally, 400 µl of purified water was added and vortexed for another 5 s. The homogenized samples were transferred to LC-vials with insert. The vials were stored at −20 °C until further analysis using LC–MS/MS.

2.4. Liquid chromatography mass spectrometry (LC–MS/MS)

The samples were analysed for 18 sulfonamides, trimethoprim, 6 tetracyclines, 12 quinolones and 15 macrolides using LC–MS/MS. A standard calibration curve was prepared with at levels of 0–500 ng/l for the sulfonamides and trimethoprim and 0–2000 ng/l for the tetracyclines and macrolides. The chromatographic mobile phase was consisted of ammonium formate (1 M)/formic acid/water (2/0.16/1000) (V/V/V) (A) and ammonium formate (1 M)/formic acid/methanol (2/0.16/1000) (V/V/V) (B). The mobile phase gradient was ramped at a flow rate of 0.3 ml/min from 1% (A) to 25% in 2.5 min, 25% to 70% in 5.4 min, and 70%–100% in 0.1 min, kept for 1 min, then ramped to 0% in 7.5 min and kept for 2.6 min. The LC–MS/MS consisted of with an Acquity™ UPLC (Waters, USA) and AB Sciex QTrap 5500 (Applied Biosystem, USA) with a positive electrospray ionization (ESI) interface. The analytical column used was BEH C18 (Waters, 100 mm × 2.1 mm, 1.7 µm) at a temperature of 30 °C. Injection volume was 10 µl and the flow rate was 0.3 ml/min. The results were recorded by MultiQuant (Applied Biosystems, version 3.0.2). The specific instrument conditions are summarized in Table S2.

2.5. DNA extraction and quantitative PCR (qPCR)

For qPCR analyses, 100 ml water samples were filtered using 0.2 µm membrane filters (isopore filters polycarbonate, 0.2 um, 47 mm, Merck Millipore, Ireland) within 24 h. One extraction was prepared per sampling point per month. The filter was stored at −20 °C before DNA extraction. DNA extraction of water samples was conducted using a PowerWater DNA Isolation Kit (MoBio Laboratories, USA) and sediment samples with a PowerSoil DNA Isolation Kit (MoBio Laboratories, USA) according to the manufacturer's protocols. The extracted DNA was stored at −80 °C until further analysis.

The 16SrDNA gene, the class 1 integrase gene (IntI1) and four ARGs of interest, including sul1 and sul2 (sulfonamide resistance genes), tetW (tetracycline resistance genes) and ermB (macrolide resistance gene) were quantified by qPCR on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Canada). A reaction with total volume of 10 µl was set up by adding 3 µl of DNA to 7 µl of master mix including IQ supermix (iTaq™ DNA Polymerase) or IQ sybrgreen supermix iTaq™ DNA polymerase (Biorad), the appropriate primers (Eurogentec, Belgium), molecular-grade water, and precision blue (Biorad, USA). Details of qPCR conditions and primers are shown in Table S3. All samples were run in duplicates. Serially diluted DNA of a synthetic standard of known quantity was used as a standard and molecular-grade water was used as a negative control. The detection limit is in the range of 4.81–4.70 × 10^7 gene copy/µl for 16SrDNA, IntI1 and all ARGs. These quantifications were validated with high R^2 values (average 0.98) and high efficiencies (from 94 to 108%) (data not shown).

All samples were diluted 50 times before performing qPCR to avoid qPCR inhibition by humic acids, biological contaminants or proteins. The necessary degree of dilution had been determined in preliminary experiments with a range of dilutions of selected samples. The results were recorded by CFXManager (Biorad, version 3.0).

2.6. Statistical analysis

The effect of the WWTP discharge was tested in a linear model with location, season and sample type as factors in R (Version 3.4.0, USA) with Bonferroni correction for multiple testing of several genes. Linear regression was used to measure the effect between ARG concentration (log transformed) and river distance (log transformed) from D3 until D9. Sampling months were grouped in seasons in order to prevent overparametrisation of the model. Total load gene copies of ARGs per month were calculated by multiplying concentration of ARGs (total gene copies/ml) and water flow (m^3/day) of the sampling day. Precipitation data were provided by The Royal Netherlands Meteorological Institute (KNMI).

3. Results

3.1. WWTP discharge to the river

3.1.1. Fate of antibiotics

General characteristics (DO, pH, temperature, COD, NH_4^+, NO_3^-, and TP) of the effluents (E1 and E2) and along the river are summarized in Text S1 and Figs. S1–S7. The antibiotic concentrations were measured during two sampling campaigns, October and November 2016. The fate of 52 selected antibiotics was investigated in this study. Out of the 40 target antibiotic compounds, only sulfamethoxazole (SMX), sulfapyridine (SP), and trimethoprim (TMP) were detected (range 1–150 ng/l) in the WWTP effluents and along the river (Fig. 2). The other antibiotics were not detected. Of these three detected antibiotics, only sulfonamide was detected upstream of the WWTP (Fig. 2). Only sulphonamides were found at the upstream of the WWTP, but at lower concentrations than in the downstream samples. Concentrations of sulphonamides were highest in effluent of the wetlands, and remained relatively stable along the river in October and slowly decreased in concentration in November.

3.1.2. Fate of ARGs

In this study, 4 ARGs (ermB, sul1, sul2, and tetW) as well as the 16SrDNA gene and intI1 were quantified by qPCR in the sediment and water samples. The concentration of ARGs and intI1 in sediment and water samples upstream and downstream of the WWTP effluent are

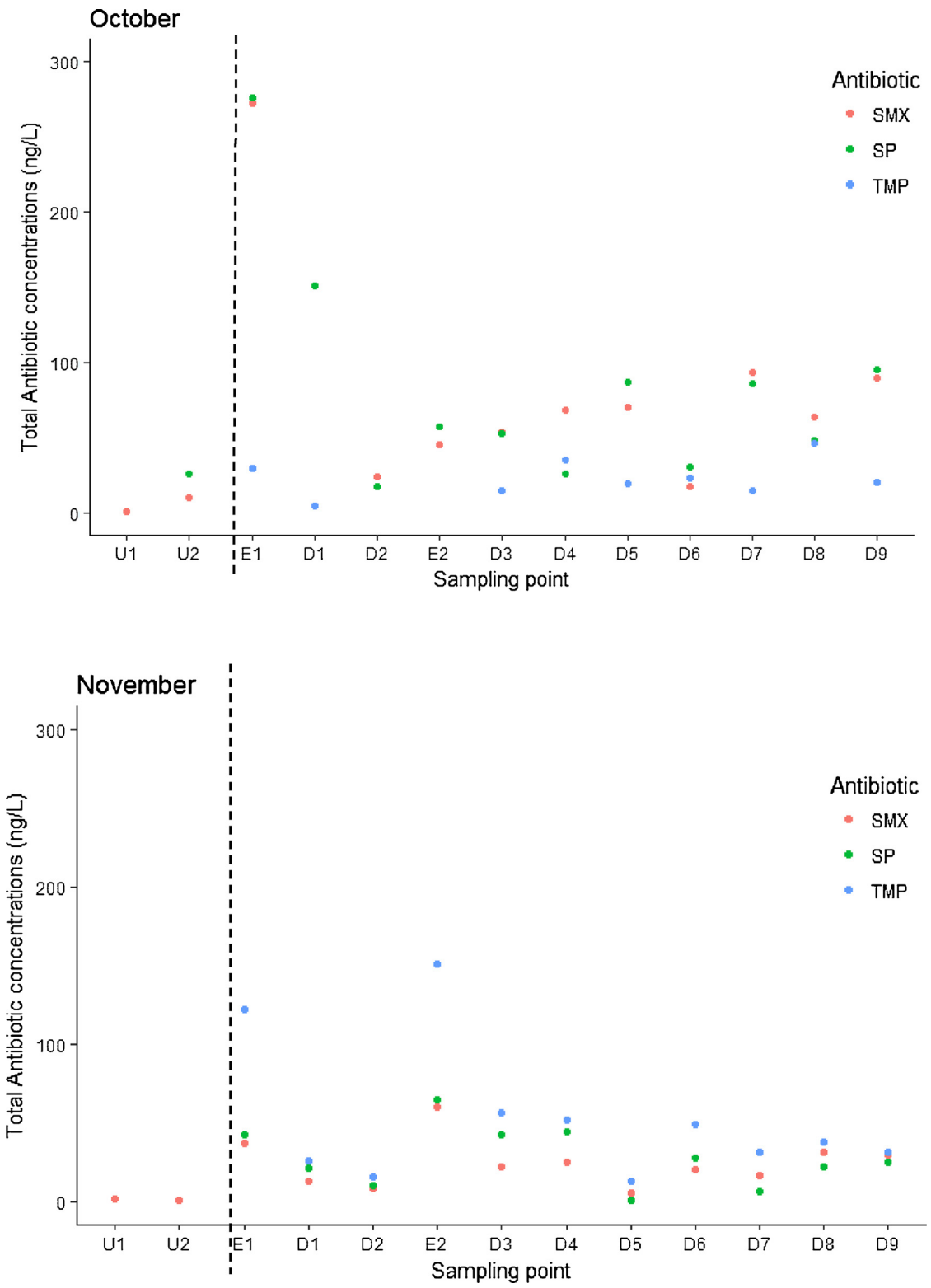

Fig. 2. Profile of antibiotics (ng/l) upstream and downstream of a WWTP for October and November. The details of the sampling points are given in Fig. 1. SMX = sulfamethoxazole, SP = sulfapyridine, TMP = trimethoprim. Dotted line distinguishes upstream and downstream of the WWTP.

presented in Fig. 3. The absolute concentration of 16SrDNA and the relative concentrations (calculated relative to the 16SrDNA data) are presented in Figs. S8–S13. Upstream data (U1) were compared to downstream data (D1) to determine the influence of the WWTP effluent discharge on the river. The data show elevated concentrations in the downstream locations as compared to the upstream locations. Downstream concentrations were more than two orders of magnitude higher ($2.34 \times 10^2 \pm 1.66$ gene copies from U1 to D1 ($p < 0.01$) for sul1). The same trend was observed in all ARGs and intI1 except for tetW. From U1 to D1, ermB increased $2.88 \times 10^1 \pm 1.48$ genes copies, $3.98 \times 10^2 \pm 0.23$ genes copies for sul2 and $3.02 \times 10^2 \pm 0.21$ genes copies for intI1, all showing a statistical significant difference

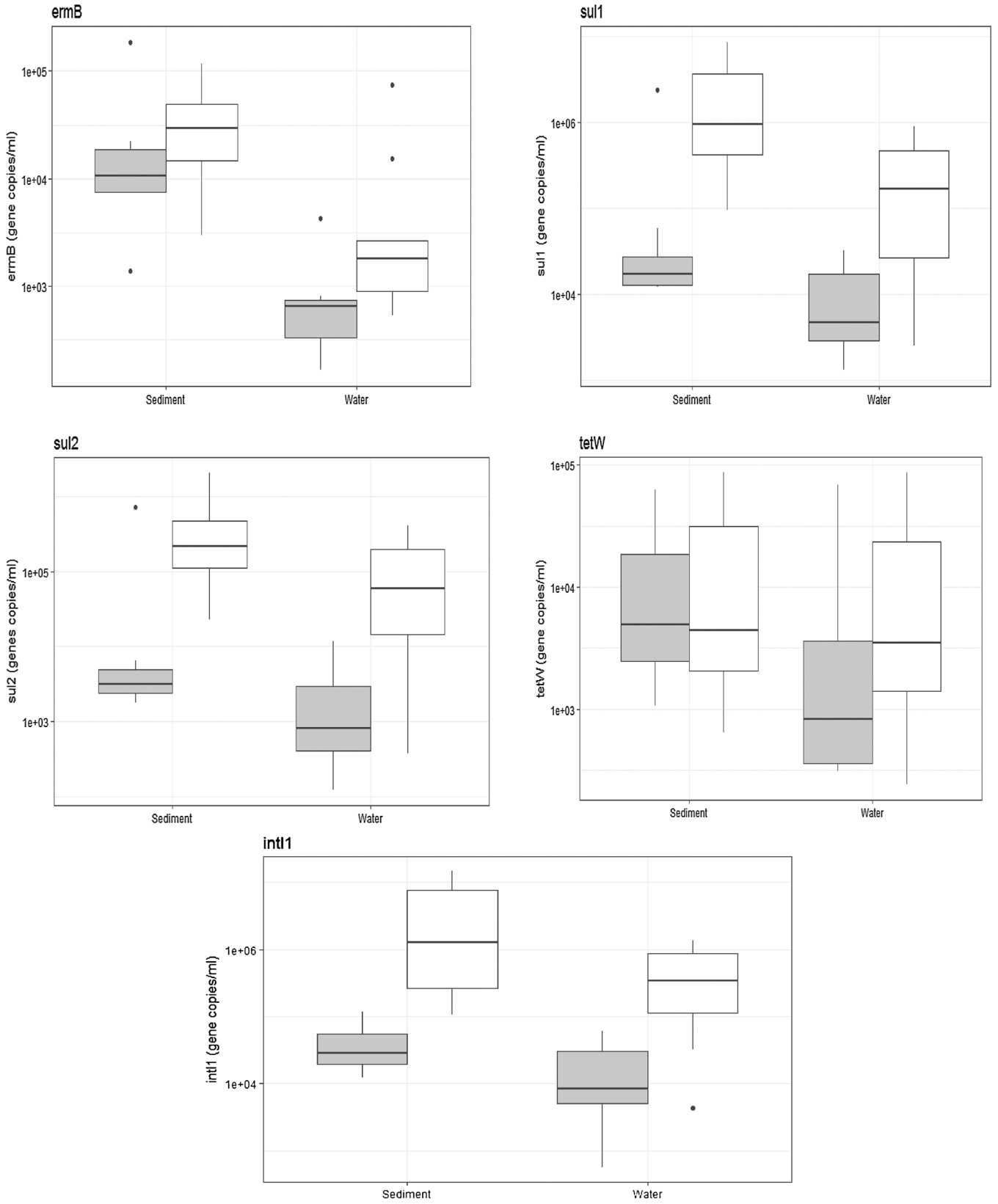

Fig. 3. Concentration of ARGs and intI1 in sediment and water samples upstream and downstream of the WWTP effluent. Within the box plot chart, each box plot represents maximum, upper-quartile, median (black bar), lower-quartile, and minimum values. Grey boxes represent U1 and white boxes represent D1. The black dots represent outliers, individual points of the data that do not fall within the mean value.

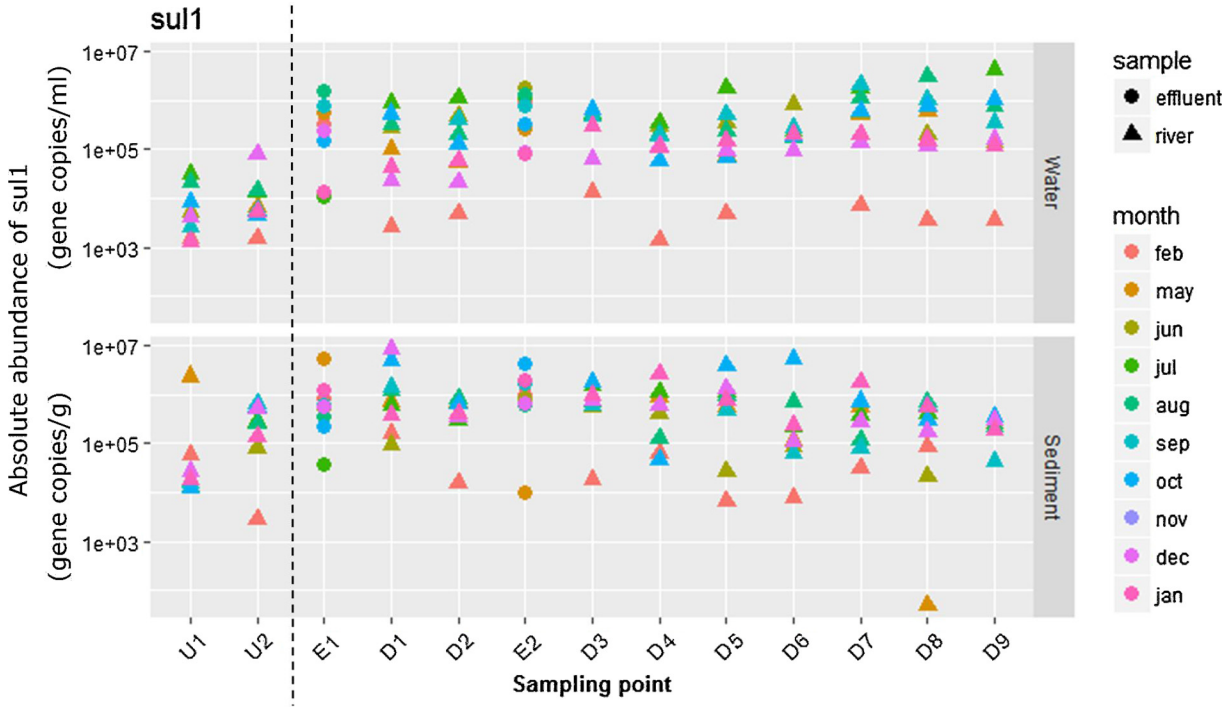

Fig. 4. Profile of sul1 for one year in sediment and water samples from 0.5 km upstream until 20 km downstream. The details of the sampling points are given in Fig. 1. Dotted line distinguishes upstream and downstream of the WWTP.

($p < 0.01$). The gene copy number of tetW did not increase significantly ($1.86 \times 10^1 \pm 1.73$ genes copies).

In general, all ARGs were detected at all sampling points in both sediment and water samples throughout the year. The fate of sul1 along the river is presented in Fig. 4, as a representative to show the fate and dissemination of ARGs along the river since the trend is similar for the other tested ARGs. Other ARGs and intI1 are presented in Figs. S15–S18. Significant difference in sul1 were observed between upstream (U1 and U2) and 0.1 km downstream from the WWTP discharge point (D1) in both sediment and water samples. Sul1 ranged from 3.16×10^3 until 3.16×10^6 copies/g in the sediment samples, whereas sul1 ranged from 1.00×10^3 until 1.00×10^6 copies/L in the water samples. However, between two sampling points (U1 and U2) upstream, we observed that the concentrations of ARGs and intlI1 in water already showed an increasing trend at a short distance (100m) upstream of the effluent of wetland HRT 1 day (U2). Furthermore, once sul1 was discharged with the WWTP effluent into the river, the concentration of sul1 remained constant until 20 km downstream (D9).

Statistical analysis revealed that there was no significant increase or decrease of ARGs along the river passage for both water and sediment, except for ermB that showed a slight decrease at D8 ($3.72 \times 10^1 \pm 1.58$ copies/ml). Wagenbroekloopje river, a side stream located after sampling point D5, did not significantly influence concentrations of ARGs from D6 until D9.

3.2. Fate of ARGs during the seasons

Linear regression analysis was conducted to explore the correlation between ARGs and seasons. ARG concentrations of the samples during winter differed from summer (Figs. S19–S23). Total load gene copies of ARGs were shown in S24–S28. This difference was most visible in February and December. Results showed that the concentration of ermB increased to $1.66 \times 10^1 \pm 1.29$ gene copies/ml during winter, whereas most of the other ARGs decreased; sul1 decreased $1.95 \times 10^1 \pm 1.35$ gene copies/ml, sul2 decreased $2.24 \times 10^1 \pm 1.35$ gene copies/ml and intI1 decreased $2.14 \times 10^1 \pm 1.32$ gene copies/ml. The only exception was tetW, which remained constant.

3.3. Correlation between ARGs and water quality data

Multivariate analysis was conducted to explore the correlation between ARGs and water quality data (Fig. 5). The ARG patterns of all samples clustered together across the year, except for February, which had a lower concentration of ARGs. The genes sul1, sul2 and intI1 were highly correlated, and independent from the genes ermB and tetW. With respect to water quality parameters, a positive correlation of ermB and tetW with NH_4^+ is seen, and a negative correlation between ARG and DO, both indicating that the influence of WWTP effluent on water quality is correlated to elevated levels of these ARGs.

4. Discussion

4.1. Role of WWTP effluent for presence of antibiotics along the river

As shown in Fig. 2, antibiotics were measured in the discharged wastewater effluent and along the river at concentrations in the range of 1 ng/l up to 275 ng/l. Out of 52 antibiotics analysed, two sulfonamides and trimethoprim were detected in the water. Sulfonamides in combination with trimethoprim are widely used in veterinary practice and in human medicine [36]. Previous studies have shown that antibiotics in treated municipal wastewater are typically present in low concentrations, in the range of ng/l [37,38]. In treated clinical or industrial wastewater, the concentration of antibiotics is higher, up to mg/l [39,14,40]. However, WWTP discharge is not the only source of antibiotics. Different contamination sources such as manure fertilization might contribute directly or indirectly to the concentration of antibiotics in a river Such sources can result in higher concentrations of antibiotics (up to 10^{10} mg/l) in the river, compared to the discharged wastewater [41,42]. As a result, the concentration of antibiotics can vary along the river. A study carried out by Chen et al. [28] showed that different anthropogenic activities such as aquaculture and animal farm can highly influence the concentration of antibiotics and ARGs along a trajectory from an inland river to the coast.

The river investigated in the present study was a suitable study area to investigate the fate of antibiotics from WWTP effluent discharge, and

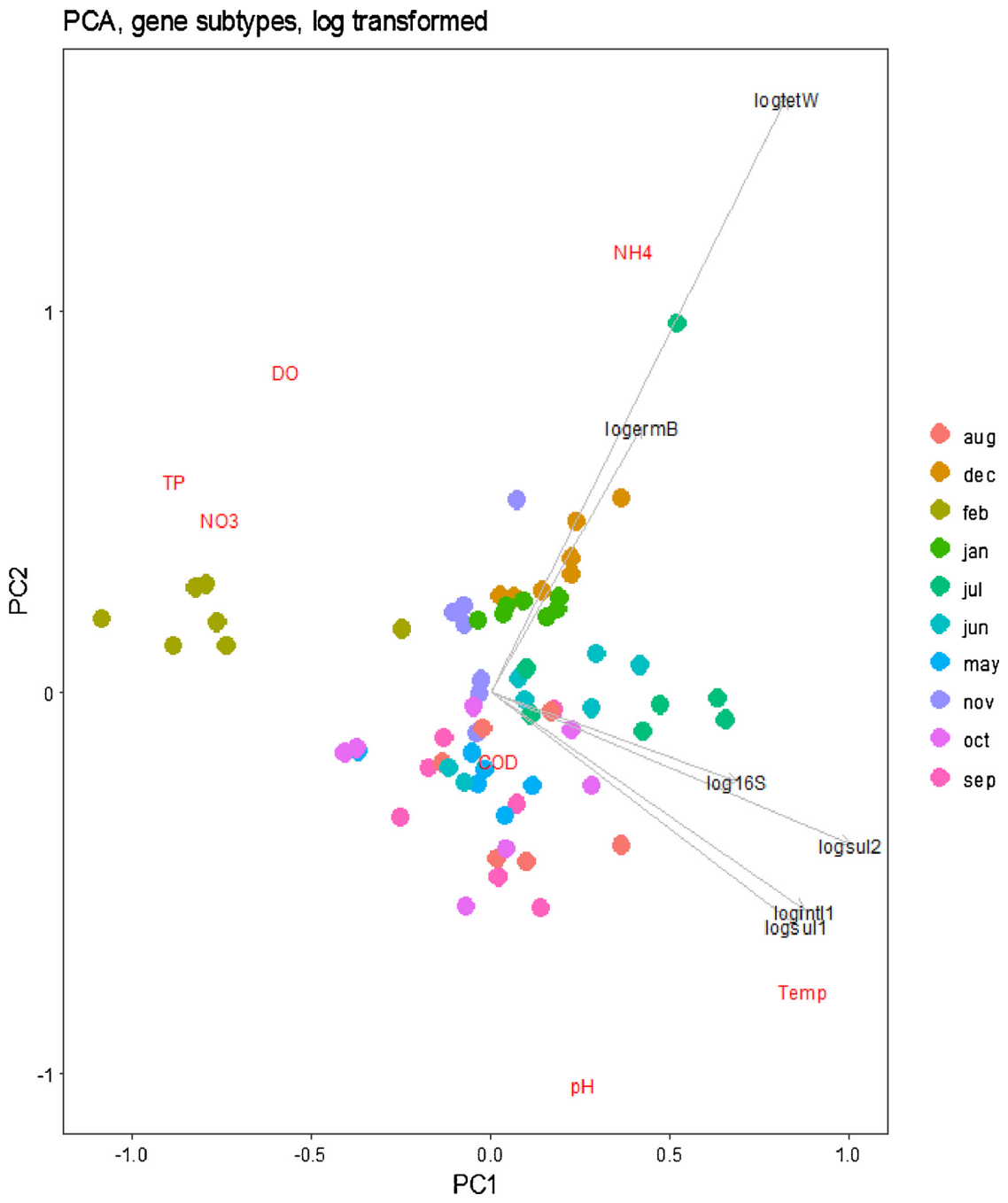

Fig. 5. Principle component analysis between ARGs and water quality parameters. Sampling months are indicated in circles. ARGs and water quality parameters are shown as vectors.

to detect antibiotic run off from the agricultural land surrounding the area. The presence of antibiotics upstream the WWTP shows that WWTP are not the only source of antibiotics in the catchment. As manure application is not allowed between September and February, alternative sources might come from the effluent of other WWTP's treating domestic and industrial wastewater, which can explain the difference between October and November. The WWTP effluents describe the role of effluent for the antibiotic concentrations. The concentration of antibiotics decreased once the antibiotics reached the river (November) or remained relatively stable (October). Other dominant sources of antibiotics or ARG downstream the WWTP were not identified in sediment or water, making the selected river segment a good study area. Other studies have shown before that the concentration of antibiotic residues in water is strongly influenced by the type of antibiotic, water level, water quality, flow conditions, and precipitation [43,44]. Dilution and dissolved organic carbon in the river influences the attenuation of macrolides, quinolones, and sulfonamides [45,46]. In addition, other processes, such as photodegradation by sun irradiation, contribute to the natural removal of antibiotics in the environment. This process was shown for the antibiotics fluoroquinolone and sulfonamide [46,47]. The presence of dissolved organic carbon, chloride ions, nitrate and a suitable pH will enhance the reaction rate of photodegradation [48–51]. This photodegradation might result in the formation of stable metabolites which can increase the sensitivity or preserve the biological activity of bacterial strains and promote ARB and ARGs proliferation [52,53].

In addition to photodegradation, other factor such as microorganisms, plants, temperature and adsorption also plays an important

pathway in the environment for the removal of antibiotics [54,55]. For example, photosynthetic and nitrifying bacteria are able to degrade antibiotics via metabolic and/or co-metabolic pathways [56]. Plants are able to adsorb antibiotics or excrete enzymes to degrade antibiotics. However, each antibiotic will have a different removal rate, e.g. related to natural lighting conditions, and physical characteristics of the compound. Antibiotics are also able to adsorb to soil or sediment, which reduces the concentration in the water phase [57]. From the compounds that we detected in the WWTP effluent, sulfonamide is known to be removed from the water-phase by photodegradation and biodegradation [58,46,49], and trimethoprim by sediment adsorption [58]. Therefore, dilution, photodegradation, adsorption and biodegradation are factors that all affect the concentration of antibiotics in a river.

A highly antibiotic contaminated environment can promote antibiotic resistance [59,60]. However, long term exposure to low concentrations of antibiotics and their transformation products can also promote the development and spreading of ARB and ARGs [39,61]. During long term exposure, the selective pressure remains present and can thus stimulate bacterial metabolism and proliferation of ARB [9,62]. Bacteria are able to adapt to antibiotic pressure either by gene mutations or horizontal gene transfer [63]. However, it is still unknown whether concentrations in the ng/l range as observed in this study are sufficient to pose a selective pressure.

4.2. Emission of ARGs into the river

We detected all ARGs and intI1 in our samples, including the upstream sampling points. Similar findings were observed by Marti et al. [64] and Lekunberri et al. [27]. We also observed that the influx of water from E1 to the river affect the river segment upstream the effluent discharge point of the WWTP (U2). Various months showed an increasing trend in ARGs before reaching E1 (Fig. S14). For example, sul1 concentration at U2 already showed an increasing trend before reaching E1 (Fig. 4).

Concentration that are detected upstream of a WWTP could have different origins, including run-off of faeces from pasture animals or wild animals, and also natural antibiotic resistance. Previous research has indeed shown that ARGs were identified in areas without exposure to contaminants and antibiotics, such as the deep terrestrial subsurface [65], pristine arctic wetland [66], pristine river [67], deep ocean sediment [68] and pristine creek [69]. This implies that antibiotic resistance also exists naturally, further showing the protective nature of microorganisms themselves.

We found a significant contribution of ARGs and intI1 in the WWTP effluent on the total concentration of ARGs and int1 in the river: our data showed an increasing trend for all ARGs except for tetW from U1 to D1 ($p < 0.01$). The wastewater source originated from a municipal WWTP. Antibiotic and the corresponding gene; macrolide (ermB), sulphonamide (sul1 and sul2) and tetracycline (tetW) are commonly used in livestock production for example swine and cattle farms [70,71]. This finding shows that human activities are the major driver of the spreading of ARGs in this particular watershed, thus increasing the prevalence of antibiotic resistance in the environment [72]. A significant increase of ARGs and intI1 from WWTP effluent to the receiving river was also observed by other researchers in the tested matrices, for example in water [29,73] and sediment [74,73]. In a study of Koczura et al. [73], they observed 1.2×10^4–2.7×10^4 gene copies/ml of sul1. In this study, we also observed similar concentrations of sul1, ranging from 9.30×10^4 to 4.25×10^6. The significant increase of these ARGs can originate from resistance genes present in human faeces, and other faeces present in the sewage, such as (domestic) animals. This confirms that discharged WWTP effluents are an important route for the dissemination of ARB and ARGs into the environment as also found by other authors [75].

4.3. Fate of ARGs along the river

In this study, the Grote Beerze river was selected to investigate the prevalence of antibiotics and ARGs. The Grote Beerze is a suitable model for studying the occurrence of antibiotics and ARGs in a wastewater effluent receiving river, since the river is in a 20 km-scale river segment with only one small side stream at 2 km. Therefore, changes in concentration of ARGs can only be attributed to processes in the river itself, and not by dilution of water along the river. This study not only provides information on concentrations of antibiotics and ARGs in water and in sediment, but also contributes to understanding the role of WWTP discharge on a river system. Macrolide, sulfonamide and tetracycline antibiotics with corresponding resistance genes were selected in this study as these antibiotics and ARGs are referred to as adequate for environmental monitoring of antibiotic resistance [76]. This study adds a perspective of the Netherlands as a country with a low background of resistance, since human antibiotic use in the Netherlands is relatively low [77] and the use of antibiotics in livestock has been reduced by 50% from 2008 until 2012 [78].

Limited studies describe the impact of antibiotic resistance genes and compounds along the distance of the river without side streams (20 km) in the water and sediment across a whole year, as we have done. The presence of ARGs in a WWTP effluent-receiving river between summer and winter or single month has been demonstrated in many studies [64,23,79,80,30,27]. However, these studies were performed either on a river with more than one discharge (WWTP, farming or mining discharges), side river inputs along the main river, or a relatively short downstream distance (0.1 km until 5 km), and were focused on either sediment or the water phase.

As observed in our study, once the ARGs enter the environment, the ARGs were persistent for 20 km. We also observed that ARGs in sediment are showing similar trends as ARGs in water. For example, concentrations of sul1, sul2 and intI1 for both water and sediment samples are lower in February than compared to those in other months. However, total concentration of ARGs in sediment were higher than the total concentration of ARGs in water. A possible explanation for this is that the sediment acts as reservoir for resident organisms, antibiotics, ARB and ARGs and shield them from sunlight and other degradative inactivation [54,81,68,82–84]. Moreover, extracellular and intracellular DNA in the sediment corroborates the presence of DNA in the sediment, with extracellular DNA being more stable in the sediment. This was shown by Mao et al. [85], who observed that ARGs were present at higher concentration in extracellular DNA with most of them present in the sediment.

Other than ARGs, intI1 has been suggested as an indicator for the spreading and disseminating of ARGs, because most ARGs are carried by mobile genetic elements such as plasmids, transposons and or integrons which are associated to mobile elements, like insertion elements [86]. Our results also revealed high concentration of intlI1, Hence, with the consistent contribution of ARGs and intlI1 from sediment to water, they remain in the river once they have entered the water body, even though there is no input of contaminants along the river itself.

4.4. Fate of ARGs over the seasons

In this study, we observed concentrations of ARG to vary an order of one to three between different months. With respect to seasons, a slight decrease in concentration was found for all tested ARGs, except tetW during winter. This was mainly due to lower concentrations of sul1, sul2, and IntI1 in February (coinciding with higher phosphate and DO concentrations in that month). Different studies have shown ARB and ARGs profiles in different seasons. Some of them reported that summer is the optimal season for ARGs proliferation [87,28,88], meanwhile others reported higher ARGs or intI1 concentrations in winter [73]. Furthermore, there are also studies that report only slightly variation or

inconsistent between the seasons [28,89]. This might indicate that the effect of season depends on the source, the sampling period (variation in precipitation and temperature), antibiotic usage, geographical location, hydrodynamic conditions and disposal practice [90,10]. In summer, Dutch rivers have a higher nutrient input and variations in precipitation occurs that facilitate microbial growth, but not of faecal microorganisms [28,91]. Precipitation also plays an important role in the spreading of ARGs within the microbial community of a river. In a study of Di Cesare et al. [92], absolute concentrations of ARG during a rain event were 8.6 times higher compared to the yearly average of rain. Storm water also has higher potential to transfer ARGs in the environment [93]. This effect was not relevant in our study, as the precipitation was 0 mm/h throughout the sampling days except in February 2016 (1.5 mm/h). One day before sampling, a small amount of precipitation (0–2 mm) was observed in February, August, September, November and December.

In this study, the water flow in the river is higher in winter (average 1000 m^3/h) than in summer (average 600 m^3/h). The ratio between the flowrate of the effluent and the flowrate of the river was different at each sampling campaign, and therefore there was not a consistent dilution of ARGs (Fig. S29). This change in flow changes the dilution factor, which might be the reason why the concentrations of ARGs and intI1 decreased during winter even though the total load was very similar between months, for example in February. We also see a clear separation in total loads between ermB and sul1/sul2/intI1 (Figs. S17–S21). For ermB, the upstream location contributes clearly to the concentration of the gene and as a result, ermB is independent from sul1/sul2/intI1 in the multivariate analyses (Fig. 5). However, sul1/sul2/intI1, E2 is the main source of ARG load in most months. This shows that other factors than just the season influences the ARG concentration in a river. Therefore, further studies to find the correlation between variation of antibiotic resistance release in different seasons need to be performed.

4.5. Correlation between ARGs and water quality data

Our results show that ARGs were positively correlated to some nutrients and negatively correlated to DO, which indicates a co-occurrence of nutrients and resistance genes in effluents, which also have lower DO. A positive correlation of ermB and tetW with NH_4^+ and a negative correlation between ARG and DO, both indicating a direct link between ARGs and discharged WWTP effluent. According to Novo and Manaia [94], processes and conditions in a WWTP influences the concentration of ARGs. However, water quality data cannot be used to estimate the concentration of ARGs, as the fate of nutrients and resistance genes in water will differ, as also shown by others [95]. Even so, WWTP is still a dominant source of antibiotics and ARGs in the river system. We also saw a strong correlation between sul1, sul2 and intI1. Sul genes are commonly associated with intI1, since the sul1 gene is located at 3′-conserved segments in intI1 [86].

5. Conclusion

In conclusion, our data show that WWTP effluents are a relevant source of antibiotics and ARGs, once the wastewater effluent is discharged to a river. This shows that a WWTP cannot eliminate these contaminants from the wastewater. The study gives a comprehensive profile of antibiotic resistance in a Dutch river, showing the ARGs are persistent in the water and sediment from the WWTP effluent discharge point until 20 km downstream. Wastewater indicatory effluent parameters, such as DO, nitrate, phosphate, were all below the allowed discharge levels, and showed no significant correlation with the fate of antibiotics, but did correlate to the concentrations of selected ARGs. A seasonal effect was observed for the presence of almost all ARGs in the river, except tetW, but could not explain all variations for all ARGs tested. The exact mechanism behind this is unclear, as ours and other studies showed different effects. Once the antibiotics and ARGs enter the river, the concentration of antibiotics only slightly decrease, and ARGs show persistence until 20 km downstream in the water as well as in the sediment. Simultaneous monitoring in water and sediment samples from the river system is therefore recommended. Our results show that the river attenuates antibiotics, but should be considered as a reservoir of antibiotic resistant bacteria and ARGs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jece.2018.03.004.

References

[1] I.M. Gould, F.M. MacKenzie, M.J. Struelens, J.M.W.v.d. Meer, Towards a European strategy for controlling antibiotic resistance, Clin. Microbiol. Infect. 6 (12) (2000) 670–674, http://dx.doi.org/10.1046/j.1469-0691.2000.00161.x.
[2] P. Gao, M. Munir, I. Xagoraraki, Correlation of tetracycline and sulfonamide antibiotics with corresponding resistance genes and resistant bacteria in a conventional municipal wastewater treatment plant, Sci. Total Environ. 421 (Suppl. C) (2012) 173–183, http://dx.doi.org/10.1016/j.scitotenv.2012.01.061.
[3] A. Naquin, A. Shrestha, M. Sherpa, R. Nathaniel, R. Boopathy, Presence of antibiotic resistance genes in a sewage treatment plant in Thibodaux, Louisiana, USA, Bioresour. Technol. 0 (2015), http://dx.doi.org/10.1016/j.biortech.2015.01.052.
[4] W. Giger, A.C. Alder, E.M. Golet, H.P.E. Kohler, C.S. McArdell, E. Molnar, H. Siegrist, M.J.F. Suter, Occurrence and fate of antibiotics as trace contaminants in wastewaters, sewage sludges, and surface waters, CHIMIA Int. J. Chem. 57 (9) (2003) 485–491, http://dx.doi.org/10.2533/000942903777679064.
[5] M. Hijosa-Valsero, G. Fink, M.P. Schlüsener, R. Sidrach-Cardona, J. Martín-Villacorta, T. Ternes, E. Bécares, Removal of antibiotics from urban wastewater by constructed wetland optimization, Chemosphere 83 (5) (2011) 713–719, http://dx.doi.org/10.1016/j.chemosphere.2011.02.004.
[6] N.J. Rowan, Defining established and emerging microbial risks in the aquatic environment: current knowledge, implications, and outlooks, Int. J. Microbiol. 2011 (2011) 15, http://dx.doi.org/10.1155/2011/462832.
[7] L. Rizzo, C. Manaia, C. Merlin, T. Schwartz, C. Dagot, M.C. Ploy, I. Michael, D. Fatta-Kassinos, Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review, Sci. Total Environ. 447 (0) (2013) 345–360, http://dx.doi.org/10.1016/j.scitotenv.2013.01.032.
[8] I. Michael, L. Rizzo, C.S. McArdell, C.M. Manaia, C. Merlin, T. Schwartz, C. Dagot, D. Fatta-Kassinos, Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review, Water Res. 47 (3) (2013) 957–995, http://dx.doi.org/10.1016/j.watres.2012.11.027.
[9] R. Szczepanowski, B. Linke, I. Krahn, K.H. Gartemann, T. Gützkow, W. Eichler, A. Pühler, A. Schlüter, Detection of 140 clinically relevant antibiotic-resistance genes in the plasmid metagenome of wastewater treatment plant bacteria showing reduced susceptibility to selected antibiotics, Microbiology 155 (7) (2009) 2306–2319, http://dx.doi.org/10.1099/mic.0.028233-0.
[10] N. Devarajan, A. Laffite, C.K. Mulaji, J.P. Otamonga, P.T. Mpiana, J.I. Mubedi, K. Prabakar, B.W. Ibelings, J. Poté, Occurrence of antibiotic resistance genes and bacterial markers in a tropical river receiving hospital and urban wastewaters, PLoS One 11 (2) (2016) e0149211, http://dx.doi.org/10.1371/journal.pone.0149211.
[11] E. Power, Impact of antibiotic restrictions: the pharmaceutical perspective, Clin. Microbiol. Infect. 12 (2006) 25–34, http://dx.doi.org/10.1111/j.1469-0691.2006.01528.x.
[12] L.L. Ling, T. Schneider, A.J. Peoples, A.L. Spoering, I. Engels, B.P. Conlon, A. Mueller, T.F. Schäberle, D.E. Hughes, S. Epstein, M. Jones, L. Lazarides, V.A. Steadman, D.R. Cohen, C.R. Felix, K.A. Fetterman, W.P. Millett, A.G. Nitti, A.M. Zullo, C. Chen, K. Lewis, A new antibiotic kills pathogens without detectable resistance, Nature 517 (2015) 455, http://dx.doi.org/10.1038/nature14098.
[13] L.M. Durso, D.A. Wedin, J.E. Gilley, D.N. Miller, D.B. Marx, Assessment of selected antibiotic resistances in ungrazed native Nebraska prairie soils, J. Environ. Qual. 45

(2) (2016) 454–462, http://dx.doi.org/10.2134/jeq2015.06.0280.
[14] E. Larson, Community factors in the development of antibiotic resistance, Annu. Rev. Public Health 28 (1) (2007) 435–447, http://dx.doi.org/10.1146/annurev.publhealth.28.021406.144020.
[15] WHO, Antimicrobial Resistance: Global Report on Surveillance 2014, (2014) Switzerland.
[16] B. Berglund, J. Fick, P.-E. Lindgren, Urban wastewater effluent increases antibiotic resistance gene concentrations in a receiving Northern European river, Environ. Toxicol. Chem. 34 (1) (2015) 192–196, http://dx.doi.org/10.1002/etc.2784.
[17] N. Fahrenfeld, Y. Ma, M. O'Brien, A. Pruden, Reclaimed water as a reservoir of antibiotic resistance genes: distribution system and irrigation implications, Front. Microbiol. 4 (2013) 130, http://dx.doi.org/10.3389/fmicb.2013.00130.
[18] H.C. Su, C.G. Pan, G.G. Ying, J.L. Zhao, L.J. Zhou, Y.S. Liu, R. Tao, R.Q. Zhang, L.Y. He, Contamination profiles of antibiotic resistance genes in the sediments at a catchment scale, Sci. Total Environ. 490 (Suppl. C) (2014) 708–714, http://dx.doi.org/10.1016/j.scitotenv.2014.05.060.
[19] M.L. Luprano, M. De Sanctis, G. Del Moro, C. Di Iaconi, A. Lopez, C. Levantesi, Antibiotic resistance genes fate and removal by a technological treatment solution for water reuse in agriculture, Sci. Total Environ. 571 (2016) 809–818, http://dx.doi.org/10.1016/j.scitotenv.2016.07.055.
[20] R. Pei, S.-C. Kim, K.H. Carlson, A. Pruden, Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (arg), Water Res. 40 (12) (2006) 2427–2435, http://dx.doi.org/10.1016/j.watres.2006.04.017.
[21] H. Storteboom, M. Arabi, J.G. Davis, B. Crimi, A. Pruden, Tracking antibiotic resistance genes in the South Platte River basin using molecular signatures of urban, agricultural, and pristine sources, Environ. Sci. Technol. 44 (19) (2010) 7397–7404, http://dx.doi.org/10.1021/es101657s.
[22] A. Pruden, M. Arabi, H.N. Storteboom, Correlation between upstream human activities and riverine antibiotic resistance genes, Environ. Sci. Technol. 46 (21) (2012) 11541–11549, http://dx.doi.org/10.1021/es302657r.
[23] G.C.A. Amos, L. Zhang, P.M. Hawkey, W.H. Gaze, E.M. Wellington, Functional metagenomic analysis reveals rivers are a reservoir for diverse antibiotic resistance genes, Vet. Microbiol. 171 (3–4) (2014) 441–447, http://dx.doi.org/10.1016/j.vetmic.2014.02.017.
[24] G.C.A. Amos, E. Gozzard, C.E. Carter, A. Mead, M.J. Bowes, P.M. Hawkey, L. Zhang, A.C. Singer, W.H. Gaze, E.M.H. Wellington, Validated predictive modelling of the environmental resistome, ISME J. 9 (2015) 1467, http://dx.doi.org/10.1038/ismej.2014.237 https://www.nature.com/articles/ismej2014237#supplementary-information.
[25] J. Zheng, R. Gao, Y. Wei, T. Chen, J. Fan, Z. Zhou, T.B. Makimilua, Y. Jiao, H. Chen, High-throughput profiling and analysis of antibiotic resistance genes in East Tiaoxi River, China, Environ. Pollut. 230 (Suppl. C) (2017) 648–654, http://dx.doi.org/10.1016/j.envpol.2017.07.025.
[26] H. Jiang, R. Zhou, Y. Yang, B. Chen, Z. Cheng, M. Zhang, J. Li, G. Zhang, S. Zou, Characterizing the antibiotic resistance genes in a river catchment: influence of anthropogenic activities, J. Environ. Sci. (2017), http://dx.doi.org/10.1016/j.jes.2017.08.009.
[27] I. Lekunberri, M. Villagrasa, J.L. Balcázar, C.M. Borrego, Contribution of bacteriophage and plasmid DNA to the mobilization of antibiotic resistance genes in a river receiving treated wastewater discharges, Sci. Total Environ. 601 (Suppl. C) (2017) 206–209, http://dx.doi.org/10.1016/j.scitotenv.2017.05.174.
[28] B. Chen, X. Liang, X. Huang, T. Zhang, X. Li, Differentiating anthropogenic impacts on args in the Pearl River estuary by using suitable gene indicators, Water Res. 47 (8) (2013) 2811, http://dx.doi.org/10.1016/j.watres.2013.02.042.
[29] Z. Ling, Y. Yang, Y. Huang, S. Zou, T. Luan, A preliminary investigation on the occurrence and distribution of antibiotic resistance genes in the Beijiang River, South China, J. Environ. Sci. 25 (8) (2013) 1656–1661, http://dx.doi.org/10.1016/S1001-0742(12)60223-X.
[30] L. Proia, D. von Schiller, A. Sànchez-Melsió, S. Sabater, C.M. Borrego, S. Rodríguez-Mozaz, J.L. Balcázar, Occurrence and persistence of antibiotic resistance genes in river biofilms after wastewater inputs in small rivers, Environ. Pollut. 210 (2016) 121–128, http://dx.doi.org/10.1016/j.envpol.2015.11.035.
[31] J. Fu, D. Yang, M. Jin, W. Liu, X. Zhao, C. Li, T. Zhao, J. Wang, Z. Gao, Z. Shen, Z. Qiu, J.-W. Li, Aquatic animals promote antibiotic resistance gene dissemination in water via conjugation: role of different regions within the zebra fish intestinal tract, and impact on fish intestinal microbiota, Mol. Ecol. 26 (19) (2017) 5318–5333, http://dx.doi.org/10.1111/mec.14255.
[32] S.M. Hatosy, A.C. Martiny, The ocean as a global reservoir of antibiotic resistance genes, Appl. Environ. Microbiol. 81 (21) (2015) 7593–7599, http://dx.doi.org/10.1128/aem.00736-15.
[33] M.S. Laport, P.V.M. Pontes, D.S. dos Santos, J.d.F. Santos-Gandelman, G. Muricy, M. Bauwens, M. Giambiagi-deMarval, I. George, Antibiotic resistance genes detected in the marine sponge petromica citrina from Brazilian coast, Braz. J. Microbiol. 47 (3) (2016) 617–620, http://dx.doi.org/10.1016/j.bjm.2016.04.016.
[34] P. Huovinen, L. Sundström, G. Swedberg, O. Sköld, Trimethoprim and sulfonamide resistance, Antimicrob. Agents Chemother. 39 (2) (1995) 279–289.
[35] Z. Ye, H.S. Weinberg, M.T. Meyer, Trace analysis of trimethoprim and sulfonamide, macrolide, quinolone, and tetracycline antibiotics in chlorinated drinking water using liquid chromatography electrospray tandem mass spectrometry, Anal. Chem. 79 (3) (2007) 1135–1144, http://dx.doi.org/10.1021/ac060972a.
[36] S. de Greeff, J. Mouton, A. Schoffelen, Nethmap: Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in The Netherlands in 2016, (2017) The Netherlands.
[37] C. Yan, Y. Yang, J. Zhou, M. Liu, M. Nie, H. Shi, L. Gu, Antibiotics in the surface water of the Yangtze estuary: occurrence, distribution and risk assessment, Environ. Pollut. 175 (Suppl. C) (2013) 22–29, http://dx.doi.org/10.1016/j.envpol.2012.12.008.
[38] K. Chen, J.L. Zhou, Occurrence and behavior of antibiotics in water and sediments from the Huangpu River, Shanghai, China, Chemosphere 95 (Suppl. C) (2014) 604–612, http://dx.doi.org/10.1016/j.chemosphere.2013.09.119.
[39] K.D. Brown, J. Kulis, B. Thomson, T.H. Chapman, D.B. Mawhinney, Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico, Sci. Total Environ. 366 (2–3) (2006) 772–783, http://dx.doi.org/10.1016/j.scitotenv.2005.10.007.
[40] T.H. Le, C. Ng, H. Chen, X.Z. Yi, T.H. Koh, T.M.S. Barkham, Z. Zhou, K.Y.H. Gin, Occurrences and characterization of antibiotic-resistant bacteria and genetic determinants of hospital wastewater in a tropical country, Antimicrob. Agents Chemother. 60 (12) (2016) 7449–7456, http://dx.doi.org/10.1128/aac.01556-16.
[41] A. Pruden, R. Pei, H. Storteboom, K.H. Carlson, Antibiotic resistance genes as emerging contaminants: studies in Northern Colorado, Environ. Sci. Technol. 40 (23) (2006) 7445–7450, http://dx.doi.org/10.1021/es060413l.
[42] N. Wang, X. Guo, Z. Yan, W. Wang, B. Chen, F. Ge, B. Ye, A comprehensive analysis on spread and distribution characteristic of antibiotic resistance genes in livestock farms of Southeastern China, PLoS One 11 (7) (2016) e0156889, http://dx.doi.org/10.1371/journal.pone.0156889.
[43] S.C. Kim, K. Carlson, Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices, Environ. Sci. Technol. 41 (1) (2007) 50–57, http://dx.doi.org/10.1021/es060737+.
[44] Y.S. Ok, S.C. Kim, K.R. Kim, S.S. Lee, D.H. Moon, K.J. Lim, J.K. Sung, S.O. Hur, J.E. Yang, Monitoring of selected veterinary antibiotics in environmental compartments near a composting facility in Gangwon Province, Korea, Environ. Monit. Assess. 174 (1) (2011) 693–701, http://dx.doi.org/10.1007/s10661-010-1625-y.
[45] Y. Luo, L. Xu, M. Rysz, Y. Wang, H. Zhang, P.J.J. Alvarez, Occurrence and transport of tetracycline, sulfonamide, quinolone, and macrolide antibiotics in the Haihe River Basin, China, Environ. Sci. Technol. 45 (5) (2011) 1827–1833, http://dx.doi.org/10.1021/es104009s.
[46] D. Zhang, L. Lin, Z. Luo, C. Yan, X. Zhang, Occurrence of selected antibiotics in Jiulongjiang River in various seasons, South China, J. Environ. Monit. 13 (7) (2011) 1953–1960, http://dx.doi.org/10.1039/C0EM00765J.
[47] R.M. Baena-Nogueras, E. González-Mazo, P.A. Lara-Martín, Photolysis of antibiotics under simulated sunlight irradiation: identification of photoproducts by high-resolution mass spectrometry, Environ. Sci. Technol. 51 (6) (2017) 3148–3156, http://dx.doi.org/10.1021/acs.est.6b03038.
[48] J.M. Burns, W.J. Cooper, J.L. Ferry, D.W. King, B.P. DiMento, K. McNeill, C.J. Miller, W.L. Miller, B.M. Peake, S.A. Rusak, A.L. Rose, T.D. Waite, Methods for reactive oxygen species (ros) detection in aqueous environments, Aquat. Sci. 74 (4) (2012) 683–734, http://dx.doi.org/10.1007/s00027-012-0251-x.
[49] S.R. Batchu, V.R. Panditi, K.E. O'Shea, P.R. Gardinali, Photodegradation of antibiotics under simulated solar radiation: implications for their environmental fate, Sci. Total Environ. 470–471 (Suppl. C) (2014) 299–310, http://dx.doi.org/10.1016/j.scitotenv.2013.09.057.
[50] P. Sun, S.G. Pavlostathis, C.-H. Huang, Photodegradation of veterinary ionophore antibiotics under uv and solar irradiation, Environ. Sci. Technol. 48 (22) (2014) 13188–13196, http://dx.doi.org/10.1021/es5034525.
[51] X. Bian, J. Zhang, Photodegradation of sulfadiazine in aqueous solution and the affecting factors, J. Chem. 2016 (2016) 5, http://dx.doi.org/10.1155/2016/8358960.
[52] A.C. Palmer, E. Angelino, R. Kishony, Chemical decay of an antibiotic inverts selection for resistance, Nat. Chem. Biol. 6 (2) (2010) 105–107 http://www.nature.com/nchembio/journal/v6/n2/suppinfo/nchembio.289_S1. html.
[53] F. Bonvin, J. Omlin, R. Rutler, W.B. Schweizer, P.J. Alaimo, T.J. Strathmann, K. McNeill, T. Kohn, Direct photolysis of human metabolites of the antibiotic sulfamethoxazole: evidence for abiotic back-transformation, Environ. Sci. Technol. 47 (13) (2013) 6746–6755, http://dx.doi.org/10.1021/es303777k.
[54] T.E. Doll, F.H. Frimmel, Fate of pharmaceuticals—photodegradation by simulated solar uv-light, Chemosphere (2003) 1757–1769, http://dx.doi.org/10.1016/S0045-6535(03)00446-6.
[55] Z. Yi, L. Haibo, Research and progress of antibiotic elimination in the environment, Paper Read at International Conference on Materials for Renewable Energy Environment (2011).
[56] A. Barra Caracciolo, E. Topp, P. Grenni, Pharmaceuticals in the environment: biodegradation and effects on natural microbial communities. A review, J. Pharm. Biomed. Anal. (2015) 25–36, http://dx.doi.org/10.1016/j.jpba.2014.11.040.
[57] S. Wang, H. Wang, Adsorption behavior of antibiotic in soil environment: a critical review, Front. Environ. Sci. Eng. 9 (4) (2015) 565–574, http://dx.doi.org/10.1007/s11783-015-0801-2.
[58] B. Li, T. Zhang, Biodegradation and adsorption of antibiotics in the activated sludge process, Environ. Sci. Technol. 44 (9) (2010) 3468–3473, http://dx.doi.org/10.1021/es903490h.
[59] E. Kristiansson, J. Fick, A. Janzon, R. Grabic, C. Rutgersson, B. Weijdegård, H. Söderström, D.G.J. Larsson, Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements, PLoS One 6 (2) (2011) e17038, http://dx.doi.org/10.1371/journal.pone.0017038.
[60] X. Guo, Z. Yan, Y. Zhang, W. Xu, D. Kong, Z. Shan, N. Wang, Behavior of antibiotic resistance genes under extremely high-level antibiotic selection pressures in pharmaceutical wastewater treatment plants, Sci. Total Environ. 612 (Suppl. C) (2018) 119–128, http://dx.doi.org/10.1016/j.scitotenv.2017.08.229.
[61] D.G.J. Larsson, Antibiotics in the environment, Ups. J. Med. Sci. 119 (2) (2014) 108–112, http://dx.doi.org/10.3109/03009734.2014.896438.
[62] E. Gullberg, S. Cao, O.G. Berg, C. Ilbäck, L. Sandegren, D. Hughes, D.I. Andersson, Selection of resistant bacteria at very low antibiotic concentrations, PLoS Pathog. 7

(7) (2011) e1002158, http://dx.doi.org/10.1371/journal.ppat.1002158.
[63] J.M. Munita, C.A. Arias, Mechanisms of antibiotic resistance, Microbiol. Spectr. 4 (2) (2016), http://dx.doi.org/10.1128/microbiolspec.VMBF-0016-2015.
[64] E. Marti, J. Jofre, J.L. Balcazar, Prevalence of antibiotic resistance genes and bacterial community composition in a river influenced by a wastewater treatment plant, PLoS One 8 (10) (2013) e78906, http://dx.doi.org/10.1371/journal.pone.0078906.
[65] M.G. Brown, D.L. Balkwill, Antibiotic resistance in bacteria isolated from the deep terrestrial subsurface, Microb. Ecol. 57 (3) (2008) 484, http://dx.doi.org/10.1007/s00248-008-9431-6.
[66] K.S. Diaz, V.I. Rich, J.E. McLain, Searching for antibiotic resistance genes in a pristine Arctic Wetland, J. Contemp. Water Res. Educ. 160 (1) (2017) 42–59, http://dx.doi.org/10.1111/j.1936-704X.2017.03239.x.
[67] H. Storteboom, M. Arabi, J.G. Davis, B. Crimi, A. Pruden, Identification of antibiotic-resistance-gene molecular signatures suitable as tracers of pristine river, urban, and agricultural sources, Environ. Sci. Technol. 44 (6) (2010) 1947–1953, http://dx.doi.org/10.1021/es902893f.
[68] B. Chen, Y. Yang, X. Liang, K. Yu, T. Zhang, X. Li, Metagenomic profiles of antibiotic resistance genes (args) between human impacted estuary and deep ocean sediments, Environ. Sci. Technol. 47 (22) (2013) 12753–12760, http://dx.doi.org/10.1021/es403818e.
[69] A.L. Barkovskii, M. Thomas, D. Hurley, C. Teems, Environmental factors responsible for the incidence of antibiotic resistance genes in pristine crassostrea virginica reefs, Mar. Pollut. Bull. 64 (12) (2012) 2692–2698, http://dx.doi.org/10.1016/j.marpolbul.2012.10.006.
[70] S. Giguère, Macrolides, azalides, and ketolides, Antimicrobial Therapy in Veterinary Medicine, John Wiley & Sons, Inc, 2013, pp. 211–231.
[71] L.J. Zhou, G.G. Ying, S. Liu, R.Q. Zhang, H.J. Lai, Z.F. Chen, C.G. Pan, Excretion masses and environmental occurrence of antibiotics in typical swine and dairy cattle farms in China, Sci. Total Environ. 444 (Suppl. C) (2013) 183–195, http://dx.doi.org/10.1016/j.scitotenv.2012.11.087.
[72] Q.Q. Zhang, G.G. Ying, C.G. Pan, Y.S. Liu, J.L. Zhao, Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance, Environ. Sci. Technol. 49 (11) (2015) 6772–6782, http://dx.doi.org/10.1021/acs.est.5b00729.
[73] R. Koczura, J. Mokracka, A. Taraszewska, N. Łopacinska, Abundance of class 1 integron-integrase and sulfonamide resistance genes in river water and sediment is affected by anthropogenic pressure and environmental factors, Microb. Ecol. 72 (4) (2016) 909–916, http://dx.doi.org/10.1007/s00248-016-0843-4.
[74] N. Czekalski, T. Berthold, S. Caucci, A. Egli, H. Buergmann, Increased levels of multiresistant bacteria and resistance genes after wastewater treatment and their dissemination into Lake Geneva, Switzerland, Front. Microbiol. 3 (2012), http://dx.doi.org/10.3389/fmicb.2012.00106.
[75] R. Aali, M. Nikaeen, H. Khanahmad, A. Hassanzadeh, Monitoring and comparison of antibiotic resistant bacteria and their resistance genes in municipal and hospital wastewaters, Int. J. Prev. Med. 5 (7) (2014) 887–894.
[76] T.U. Berendonk, C.M. Manaia, C. Merlin, D. Fatta-Kassinos, E. Cytryn, F. Walsh, H. Burgmann, H. Sorum, M. Norstrom, M.-N. Pons, N. Kreuzinger, P. Huovinen, S. Stefani, T. Schwartz, V. Kisand, F. Baquero, J.L. Martinez, Tackling antibiotic resistance: the environmental framework, Nat. Rev. Microbiol. 13 (5) (2015) 310–317.
[77] OECD, Antimicrobial Resistance: Policy Insights, OECD, 2016.
[78] C.M.J.E. Vandenbroucke-Grauls, Antimicrobial resistance in the Netherlands: a natural experiment? Front. Public Health 2 (2014) 5, http://dx.doi.org/10.3389/fpubh.2014.00005.
[79] T.M. LaPara, M. Madson, S. Borchardt, K.S. Lang, T.J. Johnson, Multiple discharges of treated municipal wastewater have a small effect on the quantities of numerous antibiotic resistance determinants in the upper Mississippi River, Environ. Sci. Technol. 49 (19) (2015) 11509–11515, http://dx.doi.org/10.1021/acs.est.5b02803.
[80] J. Xu, Y. Xu, H. Wang, C. Guo, H. Qiu, Y. He, Y. Zhang, X. Li, W. Meng, Occurrence of antibiotics and antibiotic resistance genes in a sewage treatment plant and its effluent-receiving river, Chemosphere 119 (2015) 1379–1385, http://dx.doi.org/10.1016/j.chemosphere.2014.02.040.
[81] L. Haller, J. Poté, J.L. Loizeau, W. Wildi, Distribution and survival of faecal indicator bacteria in the sediments of the Bay of Vidy, Lake Geneva, Switzerland, Ecol. Indic. 9 (3) (2009) 540–547, http://dx.doi.org/10.1016/j.ecolind.2008.08.001.
[82] H. Chen, M. Zhang, Effects of advanced treatment systems on the removal of antibiotic resistance genes in wastewater treatment plants from Hangzhou, China, Environ. Sci. Technol. 47 (15) (2013) 8157–8163, http://dx.doi.org/10.1021/es401091y.
[83] P.M. Mwanamoki, N. Devarajan, F. Thevenon, E.K. Atibu, J.B. Tshibanda, P. Ngelinkoto, P.T. Mpiana, K. Prabakar, J.I. Mubedi, C.G. Kabele, W. Wildi, J. Poté, Assessment of pathogenic bacteria in water and sediment from a water reservoir under tropical conditions (Lake Ma Vallée), Kinshasa Democratic Republic of Congo, Environ. Monit. Assess. 186 (10) (2014) 6821–6830, http://dx.doi.org/10.1007/s10661-014-3891-6.
[84] W. Calero-Cáceres, J. Méndez, J. Martín-Díaz, M. Muniesa, The occurrence of antibiotic resistance genes in a Mediterranean river and their persistence in the riverbed sediment, Environ. Pollut. 223 (Suppl. C) (2017) 384–394, http://dx.doi.org/10.1016/j.envpol.2017.01.035.
[85] D. Mao, Y. Luo, J. Mathieu, Q. Wang, L. Feng, Q. Mu, C. Feng, P.J.J. Alvarez, Persistence of extracellular DNA in river sediment facilitates antibiotic resistance gene propagation, Environ. Sci. Technol. 48 (1) (2014) 71–78, http://dx.doi.org/10.1021/es404280v.
[86] S.R. Partridge, G. Tsafnat, E. Coiera, J.R. Iredell, Gene cassettes and cassette arrays in mobile resistance integrons, FEMS Microbiol. Rev. 33 (2009).
[87] Q. Sui, J. Huang, S. Deng, W. Chen, G. Yu, Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in different biological wastewater treatment processes, Environ. Sci. Technol. 45 (8) (2011) 3341–3348, http://dx.doi.org/10.1021/es200248d.
[88] D. Mao, S. Yu, M. Rysz, Y. Luo, F. Yang, F. Li, J. Hou, Q. Mu, P.J.J. Alvarez, Prevalence and proliferation of antibiotic resistance genes in two municipal wastewater treatment plants, Water Res. 85 (2015) 458–466, http://dx.doi.org/10.1016/j.watres.2015.09.010.
[89] Q.B. Yuan, M.T. Guo, J. Yang, Monitoring and assessing the impact of wastewater treatment on release of both antibiotic-resistant bacteria and their typical genes in a Chinese municipal wastewater treatment plant, Environ. Sci. Processes Impacts 16 (8) (2014) 1930–1937, http://dx.doi.org/10.1039/C4EM00208C.
[90] Y.M. Awad, K.R. Kim, S.C. Kim, K. Kim, S.R. Lee, S.S. Lee, Y.S. Ok, Monitoring antibiotic residues and corresponding antibiotic resistance genes in an agroecosystem, J. Chem. 2015 (2015) 7, http://dx.doi.org/10.1155/2015/974843.
[91] E. Franz, J. Schijven, A.M. de Roda Husman, H. Blaak, Meta-regression analysis of commensal and pathogenic escherichia coli survival in soil and water, Environ. Sci. Technol. 48 (12) (2014) 6763–6771, http://dx.doi.org/10.1021/es501677c.
[92] A. Di Cesare, E.M. Eckert, M. Rogora, G. Corno, Rainfall increases the abundance of antibiotic resistance genes within a riverine microbial community, Environ. Pollut. 226 (Suppl. C) (2017) 473–478, http://dx.doi.org/10.1016/j.envpol.2017.04.036.
[93] S. Zhang, S. Pang, P. Wang, C. Wang, N. Han, B. Liu, B. Han, Y. Li, K. Anim-Larbi, Antibiotic concentration and antibiotic-resistant bacteria in two shallow urban lakes after stormwater event, Environ. Sci. Pollut. Res. 23 (10) (2016) 9984–9992, http://dx.doi.org/10.1007/s11356-016-6237-9.
[94] A. Novo, C.M. Manaia, Factors influencing antibiotic resistance burden in municipal wastewater treatment plants, Appl. Microbiol. Biotechnol. 87 (3) (2010) 1157–1166, http://dx.doi.org/10.1007/s00253-010-2583-6.
[95] M. Laht, A. Karkman, V. Voolaid, C. Ritz, T. Tenson, M. Virta, Abundances of tetracycline, sulphonamide and beta-lactam antibiotic resistance genes in conventional wastewater treatment plants (wwtps) with different waste load, PLoS One 9 (8) (2014) e103705, http://dx.doi.org/10.1371/journal.pone.0103705.