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Antibiotic residues in the cirata reservoir, Indonesia and their effect on ecology and the selection for antibiotic-resistant bacteria

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ABSTRACT

Antibiotic residues, their mixture toxicity, and the potential selection for antibiotic-resistant bacteria could pose a problem for water use and the ecosystem of reservoirs. This study aims to provide a comprehensive understanding of the occurrence, concentration, distribution, and ecological risks associated with various antibiotics in the Cirata reservoir, Indonesia. In our water and sediment samples, we detected 24 out of the 65 antibiotic residues analyzed, revealing a diverse range of antibiotic classes present. Notably, sulphonamides, diaminopyrimidine, and lincosamides were frequently found in the water, while the sediment predominantly contained tetracyclines and fluoroquinolones. Most antibiotic classes reached their highest concentrations in the water during the dry season. However, fluoroquinolones and tetracyclines showed their highest concentrations in the water during the wet season. Ecotoxicological risk assessments indicated that the impact of most antibiotic residues on aquatic organisms was negligible, except for fluoroquinolones. Looking at the impact on cyanobacteria, however, varying risks were indicated, ranging from medium to critical, with antibiotics like sulfamethoxazole, ciprofloxacin, norfloxacin, and lincomycin posing substantial threats. Among these, ciprofloxacin emerged as the antibiotic with the strongest risk. Furthermore, fluoroquinolones may have the potential to contribute to the selection of antibiotic-resistant bacteria. The presence of mixtures of antibiotic residues during the wet season significantly impacted species loss, with Potentially Affected Fraction of Species (msPAF) values exceeding 0.75 in almost 90% of locations. However, the impact of mixtures of antibiotic residues in sediment remained consistently low across all locations and seasons. Based on their occurrences and associated risks, 12 priority antibiotic residues were identified for monitoring in the reservoir and its tributaries. Moreover, the study suggests that river inflow serves as the most significant source of antibiotic residues in the reservoir. Further investigations into the relative share attribution of antibiotic sources in the reservoir is recommended to help identify effective interventions.

1. Introduction

The high dependence of humans on renewable electricity production has contributed to a dramatic increase in the number of hydroelectric reservoirs. More than 50,000 large dams have been constructed worldwide (Lehner et al., 2011), with 12,000 of these reservoirs contributing to over 85% of global renewable electricity production (Ho and Goethals, 2019). However, these reservoirs constantly face threats from anthropogenic stress due to their multiple uses, jeopardising services provision, including energy and food production, drinking water, and irrigation water provision. Main anthropogenic influences are extensive aquaculture, the discharge of untreated sewage due to the absence of wastewater treatment plants, and untreated livestock discharges, which can all potentially contain antibiotic residues.

Ecosystem services and ecological biodiversity are known to have a synergistic relationship (Grizzetti et al., 2019). The introduction of antibiotic residues into the reservoir might disrupt aquatic organisms responsible for the stability of services, such as primary producers and

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microbial communities, leading to the loss of additional ecosystem services (Grenni et al., 2018; Rinke et al., 2019). Nutrient cycling and food web dynamics are supporting systems for other ecosystem services provided by the reservoir. Previous studies have shown that antibiotic residues through ecological risk-assessment endpoints interfere with green algae growth, promoting mortality even at low concentrations, and thereby altering the balance of the aquatic ecosystem (Chen et al., 2018, 2020; Cui et al., 2018; Jiang et al., 2018). However, most of the studies still rely on the individual risk assessment of residues of each individual antibiotic present, leaving a gap in understanding their mixture effect on the reservoir ecosystem. The potential ecological risk posed by specific combinations of antibiotic residues to representative photosynthetic aquatic organisms has been investigated (González-Pleiter et al., 2013; Guo et al., 2016; Magdaleno et al., 2015; Marx et al., 2015). However, most of these studies focused on binary effects (two types of pharmaceuticals) and used predicted environmental concentrations as a basis for ecotoxicological risk determination. The Potentially Affected Fraction of Species (msPAF) can be used to assess the potential impact of broad mixtures of antibiotic residues on species inhabiting the river and reservoir. Furthermore, prolonged exposure to antibiotic residues in the reservoir may contribute to selection of antibiotic-resistant bacteria, posing risks related to water use for drinking (Huang et al., 2019; Lu et al., 2018; Xu et al., 2020), irrigation purposes (Gekenidis et al., 2018; Iwu et al., 2020; Sun et al., 2020), aquaculture purposes (Klase et al., 2019; Santos and Ramos, 2018), and the biological self-purification process through shifts in biogeochemical processes mediated by microorganisms (Chen et al., 2018; Roose-Amsaleg and Laverman, 2016). Therefore, exploring the impacts of individual and mixtures of antibiotic residues present on the ecology and the selection for antibiotic-resistant bacteria in the reservoir is necessary.

Various classes of antibiotic residues have been detected in water systems worldwide, including Indonesia's urban sewage, rivers (Shimizu et al., 2013; Wilkinson et al., 2022), and sea bays (Sudaryanto et al., 2023). These studies predominantly focus on antibiotic residues originating from treated and untreated domestic sewage, overlooking other significant contributors of antibiotic pollution to the reservoirs such as livestock manure and aquaculture. Moreover, there is a notable gap in research focused on artificial ecosystems like reservoirs. The Cirata reservoir is facing an unknown risk of exposure to antibiotic residues from extensive aquaculture, untreated sewage and livestock discharges. Exploring the current antibiotic residues present in the reservoir is an essential step towards understanding the potential ecological risk of antibiotic residues on the supporting services provided by the reservoir.

Therefore, this study explores the spatial and temporal distribution of antibiotic residues, and assesses their ecological risks in water and sediment samples collected from the Cirata reservoir and its tributaries located in Indonesia. This study also explores the selection for antibiotic-resistant bacteria along with the individual and mixtures toxicity of all antibiotic residues detected. The analysis encompassed 65 antibiotic residues, including fluoroquinolones, sulphonamides, sulphones, macrolides, tetracyclines, lincosamides, diaminopyrimidines, and amphenicols. To address the different knowledge gaps, this study aims to: (i) assess the spatial and seasonal distribution of antibiotic residues in the water and sediment of inflowing rivers, outflow, and inside the Cirata reservoir, (ii) evaluate the ecotoxicological risk of antibiotic residues both as individual and mixture, and determine their potential to select for antibiotic-resistant bacteria, and (iii) determine a list of priority antibiotics based on their occurrence and risk to aquatic organisms as well as their potential to select for antibiotics-resistant bacteria.

2. Material and methods

2.1. Sampling and preparation

For water a total of six sampling campaigns were conducted during the wet and dry seasons which were determined by rainfall patterns over the last 30 years (see Table S.1). Three sampling campaigns in wet season, (February–March) and three sampling campaigns in dry season (August) were conducted at 24 locations as illustrated in Fig. 1, representing the upstream area, inlets, inside, and outlet of the reservoir. For each sampling campaign 24 samples were taken, resulting in a total of 144 water samples. Water samples from the reservoir were collected separately from the surface layer (0.5 m below the surface), middle layer (5 m below the surface), and bottom layer (55–60 m below the surface), and were thoroughly mixed to create one representative composite sample per sampling location. Additionally, for the rivers representing the inflow and outflow of the reservoir, surface water samples were collected. Since sediment is less prone to variation in antibiotic residue concentrations than a water grab sample, for sediment, one sampling campaign for each season was conducted at the same locations as the water samples, resulting in a total of 48 samples. Surface sediments were collected from all locations using an Ekman grab. The samples were labelled, stored in a freezer at −18 °C, and air-transported in cooled condition in boxes filled with ice packs to the laboratory of Wageningen Food Safety Research, The Netherlands. Upon arrival, all samples were below 10 °C and immediately stored at −18 °C until analysis.

2.2. Physicochemical properties of water and sediment samples

Different parameters can affect the distribution and occurrence of antibiotics. In this study several parameters were measured. The Standard Methods for examination of water and wastewater 23rd edition (Rice et al., 2012) were used to quantify the basic environmental parameters during sampling. Water pH and temperature were measured using a pH meter, turbidity was measured according to the Nephelometric method (2130-B), total dissolved solid was measured according to the Standard Methods 2540-C. For sediment, total organic carbon was measured according to the Standard Methods 5310-B meanwhile the percentage of dry matter of sediment was estimated by gravimetric method (CEN/BT TF 151 WI CSS 99022/2007).

2.3. Apparatus and reagents

Methanol (MeOH), Acetonitrile (ACN), citric acid monohydrate, disodium hydrogen phosphate, ethylenediaminetetraacetic acid (EDTA) were purchased at Witega (Darmstadt, Germany). Lead acetate trihydrate, trifluoro acetic acid (TFA) were purchased at Sigma-Aldrich (St Louis, MO, USA).

McIlvain EDTA buffer was made by mixing 500 mL 0.1 M citric acid, 280 mL 0.2 M di-sodium hydrogen phosphate, and 74.4 g Na2EDTA to 1 L of water. The pH was adjusted to 4.0 and diluted with water until the final volume of 2 L of volumetric flask. A solution of 0.125% TFA in ACN was freshly prepared by adding 1.25 mL of TFA in 1 L of ACN.

2.3.1. Reference standards

The reference standards used in this study is listed in oxytetracycline (OTC, 99%), chlortetracycline (CTC, 93%), tetracycline (TC, 98%), doxycycline (DC, 97%), minocycline (MNC, 96%), methacycline (MTC, 95%), demeclocycline (DMC, 93%), marbofloxacin (MAR, 98%), norfloxacin (NOR, 98%), ciprofloxacin (CIP, 99%), danofloxacin (DFX, 99%), enrofloxacin (ENR, 100%), sarafloxacin (SAR, 89%), difloxacin (DIF, 93%), oxolinic acid (OXO, 100%), nalidixic acid (NAL, 100%), ofloxacin (OFX, 99%), trovafloxacin (TVA, 100%), moxifloxacin (MOX, >98%), pefloxacin mesylate (PFX, >97%), erythromycin (ERY, 97%), tylosine (TYL, 86%), josamycine (JOS, 98%), spiramycine (SPI, 4674 IU/mg), lincomycin (LIN, 95%), tiamulin (TIA, 100%), tilimicosine (TIL, 85%), valnemulin (VAL, 95%), azithromycin (AZM, 100%), clarithromycin (CLA, 98%), vancomycin (VAN), rifamycin (RMP, >97%), sulfadiazine (SDZ, 99%), sulfathiazole (STZ, 92%), sulphapyridine (SPD, >99%), sulfamerazine (SMR, 100%), sulfamoxole (SMO, 98%), sulfadimidine (SDD, 100%), sulfamethizole (SMT, 99%),

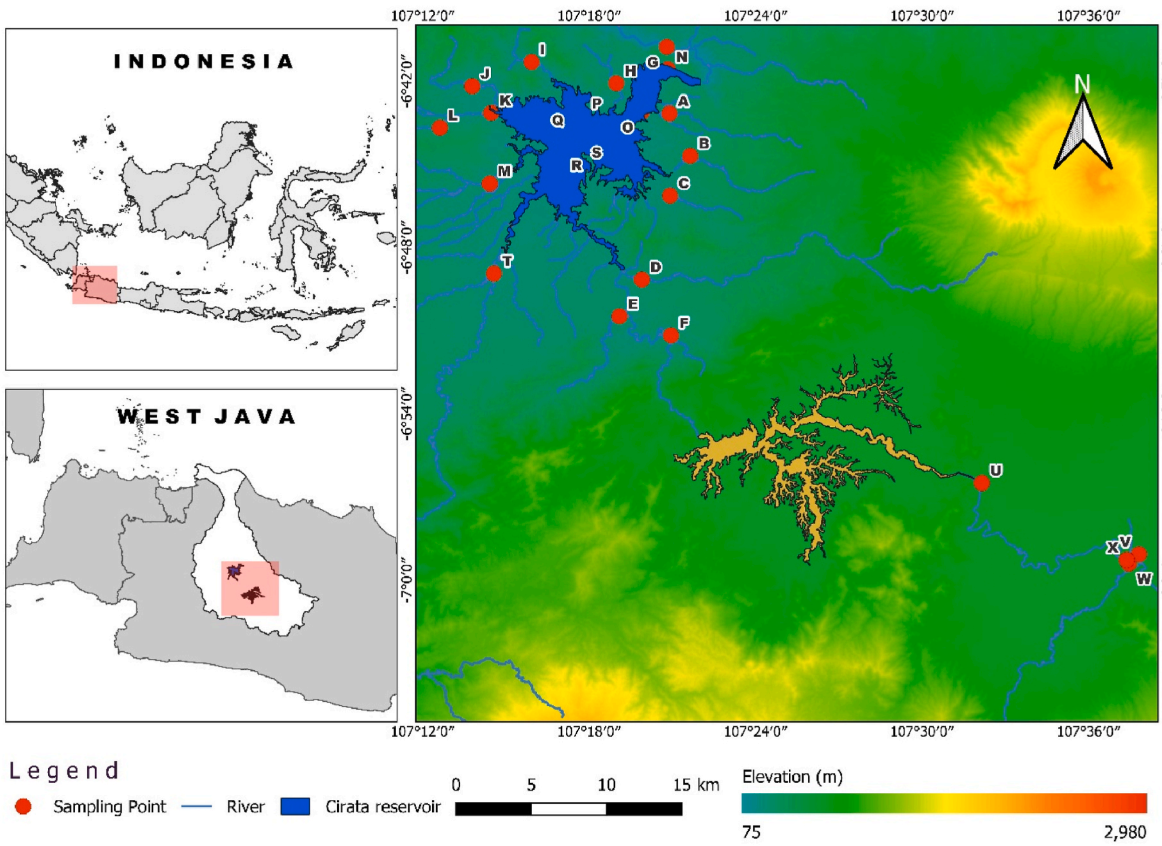

Fig. 1. Map of sampling points (inlet: A-F, H-M, T; outlet: G; inside: N-S) Additional sampling points located even further upstream (upstream of both reservoirs; U, V, W, X), serve to comprehensively capture the dynamics of antibiotic concentrations in the study area. A more detailed assessment of the upstream reservoir (Saguling) falls outside the scope of this study.

sulfamethoxypyridazine (SMP, 100%), sulfamonomethoxine (SMM, 95%), sulfachloropyridazine (SCP, 99%), sulfadoxine (SDX, 98%), sulfamethoxazole (SMZ, 99%), sulfisoxazole (SSX, 100%), sulfadimethoxine (SDM, 99%), sulfaquinoxaline (SQX, 91%), sulfacetamide (SCM, 100%), trimethoprim (TMP, 100%), dapsone (DDS, 100%), chloramphenicol (CAP, 100%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Flumequine (FLU, 99%), sulfaphenazole (SFZ, 99%) were purchased from Dr. Ehrenstorfer GMBH (Augsburg, Germany). Sparfloxacin (SFX, 98%), neospiramycine 1 (NEO, 96%), pirlimycin (PIR, 96%), natamycin (NAT, 99%), gamithromycin (GAM, 99%), clindamycin (CLI, 96%), rifapentine (RFP, 96%), roxithromycin (ROX, 98%), sulfalene (SFL, 98%) were purchased from Toronto Research Chemicals (Toronto, ON, Canada). Tylvalosin (TVS, 100%) was purchased at ECO Animal Health (London, UK), tildipirosin (TLD, 100%) at BOC sciences (New York, USA), and tulathromycin (TUL, 99%) at Santa Cruz Biotechnology (DALLAS, TX, USA).

The internal standards TC-d6, DC-d3, MAR-d8, DFX-d3, FLU-13C3, ERY-13C-d3, Tyl-d3, SPI-d3, LIN-d3, TIA-d10-HCl, TIL-d3, VAL-d6, TVS-d9, GAM-d4, TLD-d10, SDZ-d4, SMZ-d4, SCM-d4, DDS-d8 were purchased at Toronto Research Chemicals (Toronto, ON, Canada). NOR-d5, CIP-d8, ENR-d5, SAR-d8, DIF-d3, OXO-d5, NAL-d5, STZ-13C6, SPD-13C6, SMR-13C6, SDD-13C6, SMT-13C6, SMP-d3, SCP-13C6, SDX-d3, SIZ-13C6, SDM-d6, SQX-13C6, CAP-d5 were purchased at Witega (Berlin, Germany). TMP-d9 was purchased at Sigma-Aldrich (St. Louis, MO, USA).

A mixed solution of reference standards was prepared in methanol at a concentration of 4 mg/L for tetracyclines, quinolones and fluoroquinolones, macrolides, and amphenicol, and at 1 mg/L for sulphonamides. This was achieved by diluting stock solutions of tetracyclines, macrolides, and sulphonamides at levels of 1000 mg/L, and 100 mg/L for quinolones and amphenicol. Simultaneously, a mixed solution containing all internal standards was prepared in methanol with the same concentration as the mixed solution of reference standards. All mixed solutions were stored in the ultrafreezer at −70 °C until analyses.

2.4. Sample preparation and clean-up

In this study, a total of 144 water samples and 48 of sediments samples were analyzed for sixty-five antibiotic residues from eight class (fluoroquinolones, sulphonamides, sulfone, macrolides, tetracycline, lincosamide, diaminopyrimidine, and amphenicol). These antibiotics were selected based on the antibiotics used by humans listed in the government database (National Food and Drµg Agency; https://cekbpom.pom.go.id/all produk, accessed March 10, 2022), antibiotic residue detection in wastewater treatment plant nearby the reservoir (Astuti et al., 2023) and upstream part of Citarum river where the water flows into the reservoir (Wilkinson et al., 2022), government regulations, antibiotics consumption figures by livestock, and results from interviews with aquaculture farmers.

Each 40 mL water sample was weighted into a 50 mL polypropylene (PP) tubes (Greiner Bio-One, Alphen aan de Rijn, The Netherlands). Thereafter, 10 µL of internal standard solution was added to all aliquots. After vortexing, 4 mL of McIlvain-EDTA buffer was added as extraction solvent and samples were shaken thoroughly by hand to then be extracted using head-over-head (Heidolph REAX-2, Schwabach, Germany) for 5 min. The aliquots were centrifuged (Biofuge Stratos centrifuge, Heraeus instruments, Germany) for 10 min at 3500 g. The supernatant was added into a 12 mL glass tube before further sample clean-up using solid phase extraction (SPE).

The sediment samples were prepared according to a previous study (Jansen et al., 2019). Briefly: 2 g of wet sediment was weighed in duplicate into 50 mL PP tubes. Fifty µL of internal standard was added to all samples, whereas 50 μL of mixed standard solution was only added to one of the tubes. Samples were then vortexed and left at room temperature for 20 min. For extraction, 4 mL of freshly prepared 0.125% TFA in ACN solution was added and samples were shaken thoroughly by hand. Subsequently, 4 mL of McIlvain-EDTA buffer was added and samples were extracted using head-over-head for 15 min. Then 2 mL of lead acetate solution was added, and samples were centrifuged for 10 min at 3500 g. The supernatant was brought into a 12 mL glass tube and the ACN was evaporated (40 °C, N2) using a mild nitrogen flow (TurboVap LV Evaporator Zymark, Hopkinton, MA, USA). The samples were then diluted by adding 13 mL of 0.2 EDTA solution before further sample clean-up using SPE.

After extraction of the water and sediment samples a reverse-phase polymeric SPE cartridge 33μ, 200 mg, 6 mL (Strata-X, Phenomenex. Torrance, CA, USA) was conditioned with 5 mL of methanol and 5 mL of McIlvain-EDTA buffer, consecutively. The entire extract was then transferred into the SPE cartridge. The cartridge was washed with 5 mL of milliQ water. Afterwards, the antibiotic residues were eluted using 5 mL methanol. The eluates were evaporated until dry (40 °C, N2), reconstituted in 100 μL of methanol by vortex mixing and diluted with 400 μL of miliQ water. The final extracts were analyzed immediately or stored at −18 °C before being injected into LC-MS/MS.

2.5. LC-MS/MS method

The targeted antibiotic residues both in water and sediments matrices were detected by Acquity UPLC system (Waters, Place, Country) coupled with an AB Sciex Q-trap 6500 mass spectrometer (Sciex, Place, Country), operating in positive electrospray ionization mode except for chloramphenicol which was analyzed using negative electrospray ionization. Separations were performed with a HSS-T3 2.1 × 100 mm 1.8 μm analytical column (Waters). The mobile phases used were 2 mM ammonium formate and 0.16% formic acid diluted in water for mobile phase A, while the same chemicals were diluted in methanol for mobile phase B. The injection volume was 5 μL. The water samples were analyzed using a flow rate of 0.4 mL/min and a column temperature of 30 °C. The used gradient was 0–1.0 min, 0% B; 1.0–2.5 min, a linear increase to 25% B; 2.5–5.4 min, a linear increase to 70%; 5.4–5.5, a linear increase to 100% B with a final hold of 1.0 min and an equilibration time of 1.0 min. Meanwhile, the sediment samples were analyzed using a flow rate of 0.3 mL/min and a column temperature of 40 °C, the used gradient was 0–0.5 min, 1% B; 0.5–2.5 min, a linear increase to 25% B; 2.5–5.4 min, a linear increase to 70%; 5.4–5.5, a linear increase to 100% B with a final hold of 1.0 min and an equilibration time of 0.5 min.

The detection mode used was selected reaction monitoring (SRM) with collision-induced dissociation (CID). Transition characteristic for each antibiotics can be seen in Table S.2 and S.3. Data processing was performed using SCIEX OS-MQ 2.1.6 software.

2.6. Quality assurance and control

To evaluate the performance of the methods, the maximum relative deviation of ion ratio between the first and second product ion, maximum relative deviation of retention time, linearity, and limit of detection and quantitation were assessed. To ensure compliance with the confirmation criteria stated in EC 2021/808 (Commision, 2021), the ion ratio and relative retention time of the analyte in the sample were compared to matrix-fortified standards. The maximum deviation of the ion ratio was accepted if it was within ±40% relative deviation. For the maximum relative deviation of retention time a deviation of less than 1% was deemed acceptable. To assess linearity, matrix fortified calibration curves were constructed for each series on the same day as the sample analysis. Twelve aliquots of blank water samples (40 mL) were spiked at levels ranging from 0.25 to 2500 ng/L for sulphonamides and from 1 to 10,000 ng/L for the other antibiotics. Meanwhile, 10 aliquots of blank sediment samples (2 g) were spiked at levels ranging from 0.25 to 250 μg/kg for sulphonamides and from 1 to 1000 μg/kg for the other antibiotics. At least 5 concentration points were used for each antibiotic to estimate the correlation coefficient, with a high correlation coefficient ($r \geq 0.9800$) being accepted as evidence of goodness of fit. The limit of detection (LOD) was determined at the concentration where the most abundant product ion showed a signal-to-noise ratio of at least 6, while the limit of quantitation (LOQ) values were based on a signal-to-noise ratio of at least 6 for the least abundant product ion (Jansen et al., 2019). A sample was considered positive if the antibiotic residue was found above the determined LOQ and complied with the confirmation criteria stated above.

2.7. Environmental risk assessment: individual risk

The ecological risk assessment in this study was quantified using risk quotient assessment (Liu et al., 2019) using the following Eqs. (1) and (2); below:

$$RQi = \frac{MECi}{PNECi} \quad (1)$$

$$PNECi = \frac{ChV}{AF} \quad (2)$$

Where RQ_i is the risk quotient of specific antibiotic (i), MEC_i is the measured concentration of antibiotic residues (ng/L or μg/kg), $PNEC_i$ is the predicted no effect concentration of specific antibiotic derived from dividing the chronic values (ChV) of the most sensitive organisms by assessment factor (AF). The assessment factor was chosen depending on the availability of test endpoints from a technical guidance document on risk assessment (European Commission, 2003). The test endpoints of no observed effect concentration (NOEC), or median effect (lethal) concentration (E(L)C50) are listed in Table S.4.1 – S.4.3. In this study, the AF for each antibiotic residue present was ranging from 10 to 1000 as listed in Table S.5. The toxicity data based for each species inhabiting in the reservoir were retrieved from previous published studies, ECOTOX (http://cfpub.epa.gov/ecotox/), and ECOSAR (ECOSAR ver 1.1, EPA) (see Table S.4.1-S.4.3).

Meanwhile, the risk assessment of antibiotic residues found in the sediment were assessed by converting the MEC in the sediment to the concentration of antibiotic residues in pore water (Cpore), using the following Eq. (3) and Eq. (4) (Chen et al., 2022; European Commission, 2003) below:

$$Cpore = \frac{10^3 \ x \ Csi \ x \ \%TOC}{K_{OC}} \quad (3)$$

$$Log \ K_{OC} = 0.6231 \ Log \ K_{OW} + 0.873 \quad (4)$$

Where Csi is concentration of antibiotic residues (i) in sediment (μg/kg), TOC is total organic carbon in sediment (%), whereas KoC and KoW are the adsorption coefficient and octanol/water partition coefficient of antibiotics respectively. The risk is quantified using the equations for water (Eq. (1) – Eq. (2)). TOC and other water and sediment characteristics are listed in detail in Table S.6.1-S.6.2.

To understand the distribution of antibiotic residues in water and sediment, the pseudo-partitioning coefficient (P-PC, L/kg) was calculated. P-PCs are calculated by dividing the concentration of antibiotic residues found in the sediment by their corresponding concentration in the water, representing the dynamics of antibiotics between sediment and water matrices (Cheng et al., 2014; Du et al., 2017; Xu et al., 2009).

To estimate the potential for the selection for antibiotic-resistant bacteria in the reservoir environment, each antibiotic residue detected in water and sediments was analyzed using Eq. (1). However, the PNEC for estimating the impact on the selection for antibiotic-resistant bacteria was generated using the upper minimum selective concentration

(MSC) boundaries proposed by Bengtsson-Palme and Larsson (2016) as listed in the supplementary materials (Table S5). The MSC refers to the lowest antibiotic residues concentration that may select for antibiotic-resistant bacteria (Grenni et al., 2018).

The critical risk for aquatic organisms in the water and sediment compartment is indicated by RQ $\geq$ 1 meanwhile the selection for antibiotic-resistant bacteria is indicated by $RQ_{ARB} \geq 0.1$ (Almeida et al., 2023).

2.8. Environmental risk assessment: mixture toxicity

The mixture toxicity of the antibiotic residues found in the reservoir was quantified using Potentially Affected Fraction of Species (msPAF) (De Zwart and Posthuma, 2005; Lindim et al., 2019; Wang et al., 2021) and was based on their mode of action. The toxicity of antibiotics with the same mode of actions (MOA) were calculated using concentration addition (CA) (Eq. (5)). Meanwhile the effect contributions for antibiotics with different modes of action were calculated using response additivity (RA) (Eq. (6)).

$$msPAF_{CA} = \text{NORM.DIST}\left(\log\left(\sum \frac{MECi}{HC50i}\right)\right), 0, \sigma_I, 1 \tag{5}$$

$$msPAF_{RA} = 1 - \prod_{1}^{n}(1 - msPAF_{CA}) \tag{6}$$

Where MECi is the concentration (ng/L or μg/kg) of antibiotic residues (i) with specific MOA, HC50i is the mid hazardous concentration for the antibiotic residues within the same MOA, $\sigma_{i,}$ is the average of species sensitivity distributions (SSD) slope of each antibiotic residues. The formulation of SSD values involves multiple species of organisms, including fish, invertebrates, and plants, as collected from RIVM e-toxbase (Posthuma et al., 2019) (see Table S.7). Values of 0 resulting from msPAF calculations indicate locations that were not affected, while a value of 1corresponds to the most affected area with a mixture of antibiotic residues present (Posthuma et al., 2019).

3. Result and discussion

3.1. Quality assurance of analytical procedure

In this study 144 water and 48 sediment samples were analyzed. A total of 24 antibiotic residues from various classes were detected in both water and sediment matrices, as they complied with the confirmation criteria as stated in EC 2021/808 and exceeded the determined LOQs. It should be noted that in comparison with other antibiotic classes, most of the macrolides did not meet the criteria for maximum relative deviation of ion ratio and retention time, as well as linearity (Table S.8. & S.9.). However, these antibiotics were not reported to be used in the study area by humans or livestock. Therefore, we considered these antibiotics to be of minimal significance for our study. Sample quantification was conducted using matrix fortified calibration curves, yielding correlation coefficients ranging from 0.985 to 1 for the antibiotic residues detected, which is considered acceptable. Linearity for each antibiotic residue present is shown in Table S.8. LOQs for tetracyclines in water ranged from 4 to 400 ng/L, for fluoroquinolones from 1 to 40 ng/L, for macrolides from 1 to 1000 ng/L, for sulphones and sulphonamides from 0.25 to 5 ng/L, for lincosamides, diaminopyrimidine, and amphenicol from 1 to 4 ng/L. LOQs for sediments were higher than for water, since sediment was measured at ppb (μg/kg) level as opposed to water, which was measured at ppt (ng/L) level. For sediment LOQs for tetracycline ranged from 1 to 200 μg/kg, for fluoroquinolones from 1 to 40 μg/kg, for macrolides 1–200 μg/kg, for sulphones and sulphonamides 0.25–1 μg/kg, and for lincosamides, diaminopyrimidine, and amphenicol from 0.25 to 4 μg/kg. The LOD and LOQ for all individual compounds can be found in Table S.9.

The samples in this study were collected to represent both seasons and were taken from various locations to improve our understanding of sources of antibiotic residues. To obtain a full understanding of temporal and spatial variability in antibiotic residues presence, concentrations and risks, ideally antibiotic residues are measured continuously, for a longer time period of time, known as measuring at a time-weighted average. To measure antibiotic residues in a time-weighted average manner, our study employed grab sampling with six repetitive sampling campaigns. Instead of grab sampling, some studies have applied passive samplers to measure antibiotic residues in water (Chen et al., 2013; Yu et al., 2024). However, obtaining reliable quantitative data through passive sampling is still recognized as a problem, because the water sampled needs to be corrected for variations in field conditions, such as flow rate, using performance reference compounds (PRCs) (Alvarez et al., 2004). These PRCs, however, are not suitable for quantifying unknown compounds and mixtures in the sampled water (de Weert et al., 2020). Since mixtures of antibiotic residues were expected grab samples at six different time points were taken in this study in order to have the best time-weighted average possible in the timeframe of this project.

3.2. Spatial distribution and seasonal variations of antibiotics

3.2.1. Antibiotic residues in water samples

As much as 21 of the 65 antibiotic residues analyzed from six classes (tetracyclines, fluoroquinolones, sulphonamides, diaminopyrimidines, lincosamides, and amphenicols) were detected in water samples with detection frequencies between 4 and 79%. The chemical properties of each detected antibiotic is listed in Table S.10. The total concentration of antibiotic residues (sum of all antibiotic residues detected) in almost all locations was higher in the wet season and lower in the dry season (see Fig. 2). More in detail, the highest total concentration of antibiotic residues during wet season was observed in the inlet reaching 440 ng/L (location A in Fig. 2A). The higher total concentration of antibiotic residues in the inlet during the wet season is likely attributed to the higher use of fluoroquinolones by livestocks (iSIKHNAS, 2023), as well as a higher soil erosion rate. Fluoroquinolones tend to bind to soil particles (Chen et al., 2022) and a higher soil erosion rate will probably increase antibiotic concentrations in water.

In both seasons, the number of antibiotic residues present in the reservoir area was lower than in the inlet and upstream area, and their concentrations decreased from the river to the reservoir system. This implies that river input is a potentially important source of antibiotic residues in the reservoir as also shown in some studies (Chen et al., 2018, 2019). Furthermore, in this study some antibiotic residues such as tetracycline, enrofloxacin, difloxacin, ofloxacin, flumequine, sulphapyridine, sulfadimethoxine, sulfadimidine, dapsone, lincomycin, pirlimycin, and chloramphenicol were detected in either the inlet or upstream area, but none of these antibiotics were found above the LOQ in the reservoir area (see Table S.11). This suggests that most antibiotic residues may have either diluted, degraded, or adsorb onto suspended particles in the reservoir water. Which contradicts previous research in which antibiotic residues were accumulated or enriched in the reservoir, and thereby resulting in higher antibiotic concentration higher in the reservoirs compared to the inlet (Li et al., 2020). On the other hand, compared to other antibiotics classes found in the reservoir, in this research some fluoroquinolones were observed with the highest concentration in the reservoir during the wet season (see Table S11). This may indicate the application of fluoroquinolones in human activities including aquaculture inside the reservoir, since fluoroquinolones are the most frequently prescribed antibiotics in global aquaculture (Caneschi et al., 2023). Compared to the antibiotic concentration found inside of the reservoir, some classes such as sulphonamides and lincosamides, were unexpectedly found in the reservoir's outlet at higher concentrations than in the reservoir itself in both seasons. This contradicts a previous study conducted in a reservoir with a single inlet and

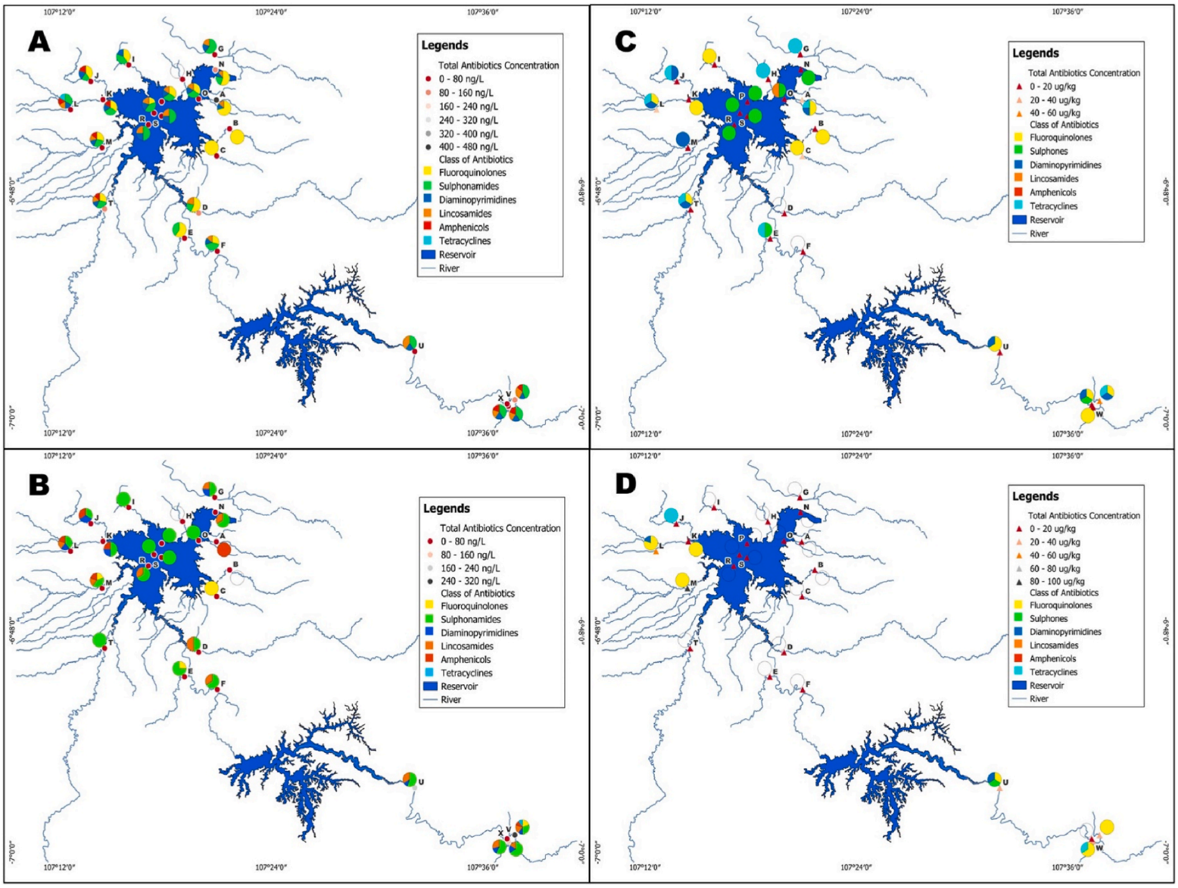

Fig. 2. Spatial distribution and total concentration of each antibiotic residues in the water during wet (A) and dry (B) season and in the sediment during wet (C) and dry (D) season.

outlet, where the concentrations of sulfonamides showed a declining trend from the inlet to the outlet site (Cui et al., 2018). The Cirata reservoir has many inlets but a single outlet. The unexpected higher concentrations of antibiotic residues found in the outlet might indicate that additional antibiotic residues come from the area right after the outlet and sampling locations (G) (see Fig. 1). As the samples were taken at locations where the water from the outlet was already mixing with the water from the upstream part of the outlet which may contain antibiotic residues, this might explain the unexpectedly higher concentrations of antibiotic residues found in the outlet.

When looking at the presence of the individual antibiotic classes, sulphonamides such as sulfamethoxazole and sulfadiazine as well as

Table 1
Concentration (ng/L) and detection frequency (DF, %) of antibiotic residues in the water samples.

Class	Type of Antibiotics	Range (ng/L)		Mean (ng/L)		Median (ng/L)		DF (%)	
		Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Tetracyclines	**Tetracycline**	<LOQ-5.0	<LOQ-10	5.0	1.8	5.0	1.8	4.2	4.2
Fluoroquinolones	**Norfloxacine**	<LOQ-84		37		25		8.3	
	Ciprofloxacine	<LOQ-103	<LOQ-30	20	3.1	20	3.1	8.3	4.2
	Danofloxacine	<LOQ-97		27		12		21	
	Enrofloxacine	<LOQ-27		23		23		8.3	
	Sarafloxacine	<LOQ-112	<LOQ-7.0	16	5.7	11	5.3	58	13
	Difloxacine	<LOQ-9.5		8.3		8.2		17	
	Flumequine	<LOQ-3.0		2.1		2.1		8.3	
	Sparfloxacine	<LOQ-9.4		7.0		6.5		25	
	Ofloxacin		<LOQ-22		22		22		4.2
Sulphonamides	**Sulfadiazine**	<LOQ-9.5	<LOQ-16	2.6	3.2	2.1	2.2	71	75
	Sulfapyridine	<LOQ-2.8	<LOQ-5.4	2.8	4.8	2.8	4.8	4.2	17
	Sulfadimidine		<LOQ-4.8		4.4		4.6		8.3
	Sulfamethoxazole	<LOQ-68	<LOQ-109	8.2	15	5.2	7.5	79	79
	Sulfadimethoxine	<LOQ-1.6		1.5		1.5		21	
Sulphone	**Dapsone**		<LOQ-8.0		5.0		3.4		17
Diaminopyrimidine	**Trimethoprim**	<LOQ-15	<LOQ-36	5.0	9.5	4.0	5.7	79	33
Lincosamides	**Lincomycin**	<LOQ-23	<LOQ-46	11	14	9.5	10	13	25
	Clindamycin	<LOQ-28	<LOQ-43	7.0	14	5.7	9.3	71	33
	Pirlimycin		<LOQ-6.0		6.3		6.3		4.2
Amphenicol	**Chloramphenicol**	<LOQ-11	<LOQ-29	3.3	5.8	2.9	2.5	33	25

diaminopyrimidines such as trimethoprim were most often detected. In both seasons, sulphonamides, and trimethoprim were frequently detected in water as illustrated in Fig. 2. Sulfamethoxazole was present in 79% of sampling locations (Table 1), exhibiting the highest concentration among all sulphonamides at 68 ng/L and 109 ng/L in the wet and dry season, respectively (location T and U in Table S11). Additionally, trimethoprim, often used in combination with sulfamethoxazole (Prescott, 2013), was detected at the same frequency as sulfamethoxazole in the wet season. Sulfadiazine, another antibiotic belonging to the sulphonamides, was also detected frequently (75% of the sampling locations). Compared to other antibiotic classes, sulphonamides have a smaller pseudo-partitioning coefficient (P-PCs), mostly below 100 L/kg (Harrower et al., 2021), making them more likely to be present in the water environment (Thiele-Bruhn, 2003) than attached to particles or sediment. The mean concentration of sulphonamides and diaminopyrimidine was higher during the dry season. The lower dilution due to lower rainfall in dry season (63 mm) may promote higher concentrations of sulphonamides and diaminopyrimidines, as low water flow could lead to higher antibiotic residue concentrations (Ding et al., 2017). In this study sulphonamides — particularly sulfadiazine, sulphapyridine, sulfamethoxazole, as well as trimethoprim — were detected with relatively high concentrations up to 109 ng/L in all locations in the upstream area during the dry season (see Table 1 and Table S.11). This could probably originate from the ruminant farms which are especially located in the upstream area since certain sulphonamides and diaminopyrimidines are predominantly consumed by ruminants. These results are in line with previous studies where sulfamethoxazole, sulfadiazine, and trimethoprim were the most analyzed and detected antibiotic residues due to their widespread usage by human and veterinary practices and their resistance to various degradation processes in the aqueous environment (Grenni et al., 2018; Chen et al., 2022).)

Looking at the class of lincosamides, clindamycin and lincomycin were detected. Clindamycin was present at a relatively higher frequency in the wet season (71%) compared to the dry season (33%). However, during the dry season, it was found at slightly higher concentrations than in the wet season, reaching 43 ng/L compared to 28 ng/L in the wet season (see Table 1). Our results align with previous studies where clindamycin was prominently found in surface water with concentrations up to more than 500 ng/L (Voigt et al., 2020; Wu et al., 2014). Lincomycin was present in 13% and 25% of locations during wet and dry seasons respectively, with highest concentration present up to 46 ng/L in the wet season mostly in the upstream area similar to clindamycin. Clindamycin is primarily used in human medicine, as evidenced by its prevalence in the downstream waters of municipal sewage treatment plants (Voigt et al., 2020). In contrast, lincomycin is the second most abundant antibiotic found in livestock wastewater, especially in tropical Asian waters, due to its extensive use following sulfamethoxazole (Shimizu et al., 2013). The extensive use of these antibiotics is likely to lead to its presence in the reservoir (Li et al., 2022; Tran et al., 2019; Zhang et al., 2015).

Meanwhile, fluoroquinolones recorded their highest concentrations in the wet season (Table 1). Fluoroquinolones were present with highest concentrations (112 ng/L) among all antibiotics, especially in the inlet area (see Table S11). The higher concentration of fluoroquinolones in the wet season may be caused by a combination of the fluoroquinolones usage and environmental factors such as erosion rate, temperature, rate of photolysis and biodegradation (Luo et al., 2011). First of all, fluoroquinolones are primarily consumed by broilers (broiler farms are especially located at the inlet area based on field observation) and consumption is known to be higher in the wet season compared to the dry season (iSIKHNAS, 2023). Moreover, during the wet season, higher water flow rates resulting from increased rainfall (265 mm) may accelerate soil erosion containing antibiotic residues and the direct discharges of antibiotic residues through runoff, thereby probably increasing the concentration of less mobile and persistent antibiotics such as fluoroquinolones in the inlet area.

In contrast to fluoroquinolones, tetracycline recorded its highest concentrations during the dry season in the upstream area, reaching up to 10 ng/L, which was found in only one location in both seasons. As observed in our study, other studies also show that tetracycline is rarely detected in water (Hu et al., 2018; Kovalakova et al., 2020; Zhang et al., 2020). The lower detection frequency of tetracycline in water may be due to its greater affinity for sediment compared to water (Felis et al., 2020). Unlike fluoroquinolones, tetracycline is mainly used for human medical purposes in Indonesia (Limato et al., 2022), which results in its presence in upstream areas where the downstream of sources such as municipal sewage treatment plant is located.

Amphenicols such as chloramphenicol were mostly found in the upstream location. Chloramphenicol was present in 25–33% of sampling locations. In contrast to our research it was found that due to its high lipid solubility, chloramphenicol is rarely present in the aqueous phase, but is preferentially found in suspended solids (Carvalho and Santos, 2016). In this study, however, chloramphenicol was not found in the sediment samples, but was found in the water samples during both seasons, mostly in the upstream area. Our observation indicates that the total dissolved solid (TDS, mg/L) present in the upstream area was relatively higher up to 485 mg/L which may explain why chloramphenicol was present in the water (see Table S.6.2). Chloramphenicol was often detected in higher concentrations (up to 30 ng/L) in the upstream locations compared to the concentrations (up to 5.4 ng/L) in the inlet. The higher concentration of chloramphenicol in the upstream areas further supports the notion of human consumption being a significant source of chloramphenicol in the upstream area, since chloramphenicol is prohibited for use in livestock in Indonesia. However, the exact allocation of the contribution of antibiotic residues from various sources was not further explored in this study and is recommended for future research.

3.2.2. Antibiotic residues in sediment

The number of different antibiotic residues present in the sediment was lower than the number of residues present in the water samples (15 out of 65) but their concentrations were higher, measured at the ppb level (see Fig. 2). The lower number present could probably be attributed to the higher LOQ in sediment (μg/kg) compared to water (ng/L), which is in line with previous studies (Kim and Carlson, 2007; Wei et al., 2014; Yang et al., 2010).

As much as five classes of antibiotics (diaminopyrimidines, lincosamides, tetracyclines, fluoroquinolones, and sulphone) were detected in the sediment with their detection frequencies ranging from 4 to 38%. Enrofloxacin, a fluoroquinolone being the most prevalent, observed in 38% of the locations (Table 2). The highest total concentration of antibiotic residues was observed in the inlet area during the dry season reaching 88 μg/kg. This suggests that river input is a potential source for antibiotics. Looking at the different seasons, the number of different antibiotics present were lower during the wet season, yet most of them were distributed across nearly all locations except for specific inlets (C, D). The number of antibiotics present in the dry season was higher than in the wet season, but they were mostly detected with lower frequencies (up to 13%) compared to the wet season.

Looking at the presence of the individual antibiotic classes, none of the sulphonamides were present in the sediment during both seasons (see Fig. 2). This suggests that despite their frequent use, the low persistence (a half-life of approximately one day) in sediment and the high mobility (log KoC of 0.5–0.7 L/kg) of sulphonamides prevents them from being present in the sediment (Berendsen et al., 2021). Unlike in water, where the presence of trimethoprim is typically associated with the presence of sulphonamides, because trimethoprim is mostly consumed together with sulfadiazine or sulfamethoxazole by livestock in the study area(iSIKHNAS, 2023), trimethoprim was found alone in the sediment at 33% of the locations. The highest concentration, 0.6 μg/kg, was observed at an inlet (M) during the wet season The presence of trimethoprim in sediment without the presence of sulphonamides is

Table 2
Concentration (μg kg^{-1}) (dry weight) and detection frequency (DF, %) of antibiotic residues in the sediment.

Class	Type of Antibiotics	Range (μg/kg)		Mean (μg/kg)		Median (μg/kg)		DF (%)	
		Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
Tetracyclines	**Tetracycline**	<LOQ-3.0	<LOQ-3.3	2.3	2.3	1.3	2.3	33	8.3
Fluoroquinolones	**Marbofloxacine**	<LOQ-33	<LOQ-11	14	7.3	7.7	7.3	13	13
	Norfloxacine		<LOQ-4.7		4.7		4.7		4.2
	Ciprofloxacine	<LOQ-38	<LOQ-34	22	25	15	23	13	13
	Danofloxacine		<LOQ-16		16		16		4.2
	Enrofloxacine	<LOQ-25	<LOQ-18	9.0	10	6.1	7.0	38	13
	Sarafloxacine		<LOQ-23		23		23		4.2
	Difloxacine		<LOQ-17		11		11		8.3
	Oxolinic acid		<LOQ-1.1		1.1		1.1		4.2
	Nalidixic acid		<LOQ-1.0		1.0		1.0		4.2
	Ofloxacin	<LOQ-9.2	<LOQ-6.6	9.2	6.6	9.2	6.6	4.2	4.2
	Sparfloxacine		<LOQ-6.0		3.8		3.0		13
Sulphone	**Dapsone**	<LOQ-0.3	<LOQ-0.2	0.2	0.2	0.2	0.2	33	4.2
Diaminopyrimidine	**Trimethoprim**	<LOQ-0.6	<LOQ-0.3	0.3	0.2	0.3	0.3	33	8.3
Lincosamides	**Lincomycin**	<LOQ-0.4		0.4		0.4		4.2	

probably caused by the longer half-life and higher KoC value of trimethoprim compared to sulphonamides, which is approximately 75 days and more than 4.3 L/kg, respectively (Berendsen et al., 2021).

In contrast to the water, only lincomycin was detected in the sediment among lincosamides. It was found at one location in the reservoir area, with a concentration of 0.4 μg/kg. Although the number of detected lincosamides was higher in water (probably due to lower LOQs), the concentration of lincomycin detected in sediment was much higher, This is probably caused by the moderate to high sorption potential to particulate matter (Carvalho and Santos, 2016). The presence of lincomycin in the reservoir area in this study may originate from its administration to livestock (such as broilers, cattle, goats, and sheep) in the inlet and upstream areas, where it was also detected in the water.

Meanwhile, the antibiotic class fluoroquinolones exhibited a higher detection frequency particularly during the wet season in the sediment compared to the water, as illustrated in Fig. 2. The (fluor)quinolones: enrofloxacin, ciprofloxacin, and marbofloxacin were identified in sediment at various inlets and upstream locations, but were in most locations absent in the corresponding water samples in the wet season. A possible explanation could be the relative high pseudo-partitioning coefficient, which reflects the relationship between solid and water phases. According to a previous study in a lake in China, the pseudo-partitioning coefficient value for fluoroquinolones were between 4493 and 47,093 L/kg, suggesting strong adsorption on sediment compared to other antibiotics (Cheng et al., 2014). The pseudo-partitioning coefficient (L/kg) for fluoroquinolonones such as enrofloxacin and ciprofloxacin from our study were 274–529 L/kg and 762 L/kg respectively (see Table S5). This suggest that fluoroquinolones are strongly absorbed to sediments, as also indicated by the higher average concentration present in the sediment in our study (Table 2). Ciprofloxacin, a fluoroquinolone, was detected in the highest average concentration among all antibiotic residues present in sediment, reaching more than 38 μg/kg in wet season, followed by other fluoroquinolones including marbofloxacin (33 μg/kg) and enrofloxacin (25 μg/kg). The higher content of organic carbon in the sediment during the wet season, which was one to two orders of magnitude higher compared to dry season in most sampling locations might have resulted in higher concentrations of these antibiotic residues in the wet season (Table S.6.1), as existing studies have shown that the partitioning of some fluoroquinolones has a positive correlation with the organic carbon content of sediment. Moreover, the high concentration of ciprofloxacin in the sediment may represent the high consumption of this clinically important antibiotic by humans in the study area. Finally, the high concentration of ciprofloxacin could also be the result of the biotransformation of enrofloxacin to ciprofloxacin through photolysis mechanism. Enrofloxacin is often used as a veterinary antibiotic (Sukul and Spiteller, 2007; Voigt et al., 2020; Wammer et al., 2013).

Similar to the fluoroquinolones, the antibiotic tetracycline was predominantly detected in sediment. It was found in 33% of the locations during the wet season and was less frequently detected during the dry season (8.3 %). The highest concentrations were observed mainly at the inlet and upstream, reaching up to 3.3 μg/kg and 3 μg/kg, respectively. Tetracycline has a moderate pseudo-partitioning coefficient ranging from 138 to 288 L/kg in this study (see Table S5) and is known to easily adsorbs to sediment through cation exchange and bridging (Li et al., 2020). This probably explains the higher detection frequency in the sediments than in the water in this study.

Finally, dapsone, a sulphone antibiotic which was present in all sediment samples in the reservoir during the wet season, with concentrations ranging from 0.1 to 0.22 μg/kg. Dapsone was found in only one location at the inlet (E) during the wet season and upstream (U) during the dry season, with the same concentration range as in the reservoir (see Table S.11). On the other hand, dapsone was detected only once in the water samples (see Table S11). Since the pseudo-partitioning coefficient value of dapsone from previous studies is unknown and our study could not determine it due to the absence of dapsone in the water, the octanol/water partition coefficient was used in this study to identify the solubility of dapsone in the water. The low solubility characteristics of dapsone (Log Kow = 0.97) (see Table S.10) Table A.10 results probably in greater presence of this antibiotic residue in sediment. As in Europe, dapsone is prohibited for use in livestock and aquaculture in Indonesia. Therefore, the presence of dapsone in sediment samples is likely to originate from human sources, as it is used to treat leprosy (Krismawati et al., 2020).

Overall it can be seen that the analysis indicated seasonal variations in the presence and concentration of antibiotics in sediment samples (see Table 2). However, capturing these seasonal differences is challenging as the sampling time was too limited to adequately capture seasonal variations. Therefore, future studies should include multi-year sampling for a more comprehensive assessment.

3.3. Risk associated with antibiotic residues

3.3.1. Individual risk to aquatic organisms and for selecting antibiotic-resistant bacteria

The impact of most antibiotic residues present in the water on aquatic organisms in our study, such as phytoplankton and daphnia, is negligible. Except for ofloxacin, which poses a medium risk in the upstream area during the dry season (Fig. 3). Ofloxacin is known as an inhibitor of nucleic acid synthesis, which may inhibit the DNA gyrases working during the DNA synthesis process and finally affect cell growth (Fu et al., 2017). As algae play crucial roles as primary producers, the adverse effect of antibiotic residues might disrupt the existence of higher trophic levels.

Several antibiotics, including sulfamethoxazole, ciprofloxacin,

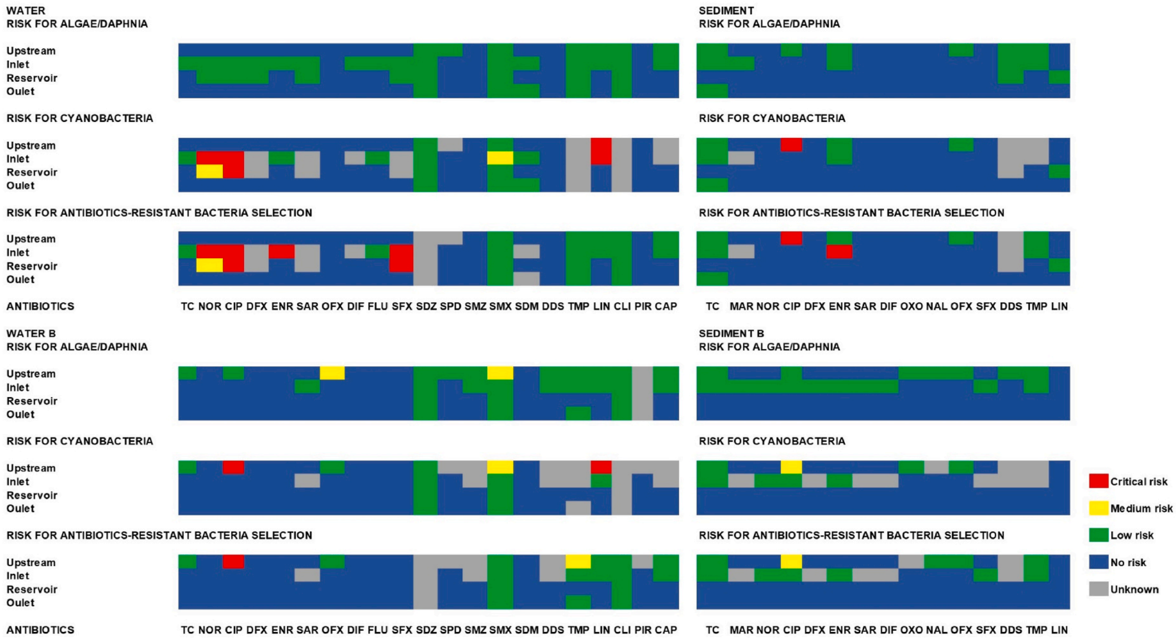

Fig. 3. Spatial ecotoxicological risk to algae/daphnia (RQ_{Ecotox}), cyanobacteria ($RQ_{Cyanobacteria}$) and antibiotics -resistant bacteria (RQ_{ARBs}) of individual antibiotic residues in the water and sediment during wet (A) and dry season (B). Critical ($RQ \geq 1$ for aquatic organisms and $RQ \geq 0.1$ for ARBs), medium ($0.5 \leq RQ < 1$ for aquatic organisms and $0.05 \leq RQ < 0.1$ for ARB, low ($RQ < 0.5$ for aquatic organisms and $RQ < 0.05$ for ARBs), no risk (no antibiotic residues present), unknown (antibiotic residues present but the risk data are unavailable).

norfloxacin, and lincomycin, pose a medium to critical risk to cyanobacteria in water (Fig. 3). Among them, only ciprofloxacin, poses a critical risk to cyanobacteria both in water and sediment. The impact of ciprofloxacin to cyanobacteria can vary depending on the concentrations which can influence its mode of action. Ciprofloxacin has a potential to decrease cell densities and chlorophyll-a at concentrations exceeding 200 μg/L, which may alter photosynthesis processes and pose a serious ecological threat (Yisa et al., 2021). The decrease of cell densities in cyanobacteria can alter ecosystem dynamics by inhibiting cyanobacteria growth. Cyanobacteria are important primary producers, and therefore ciprofloxacin concentrations might affect the primary and secondary consumers (Rico et al., 2014). However, the presence of ciprofloxacin in relatively low concentration (10–200 μg/L) may induce a hormesis effect for *Microcystis aeruginosa* which could lead to the proliferation and formation of cyanobacteria toxin-producing strains, thereby suppressing the non toxin producing strains, thereby suppressing the non toxin-microcystin-producing strains (Yisa et al., 2021). Furthermore, under eutrophic conditions, the presence of ciprofloxacin at an even lower concentration of 0,3 μg/L can accelerate bloom density and promote the dominance of cyanobacteria, including Microcystis, Synecoccus, and Oscillatoria, while reducing biodiversity (Xu et al., 2021). Furthermore, In addition, the long-term exposure to ciprofloxacin and enrofloxacin, which was also detected in this study, is likely to lead to an outbreak of *Microcystis aeruginosa* while accelerating its toxin production (Xia et al., 2023).

In our study area, fluoroquinolones (ciprofloxacin, enrofloxacin, norfloxacin, sparfloxacin) identified in water during the wet season may create selective conditions for antibiotic-resistant bacteria in inlet and reservoir locations, as well as ciprofloxacin in the upstream area during the dry season (see Fig. 3). Furthermore, we found that ciprofloxacin and enrofloxacin residue concentrations are higher in the sediment during the wet season, which may provide selective conditions for ARBs in the upstream and inlet locations (see Fig. 3). Finally, it was shown in this study that the concentration of fluoroquinolones decreased from the river system (inlet) to the reservoir. However, this result does not necessarily make the reservoir less susceptible to providing selective conditions for antibiotic-resistant bacteria, as the mechanisms for selecting ARBs and ARGs in aquatic environments vary and do not solely depend on the type, number, and concentration of antibiotic residues present (Aminov et al., 2021; Lima et al., 2020).

The presence of antibiotic residues can affect local bacterial communities, providing a selective advantage to resistant bacteria or inducing horizontal transfer of ARGs between bacteria. As it is shown that exposure to antibiotic residues can enrich and maintain antibiotic-resistant bacteria (ARBs), or promote the selection of ARBs via horizontal transfer mechanisms, even at concentrations far below the minimal inhibitory concentrations (MICs) (Jutkina et al., 2016; Gullberg et al., 2011). Several studies explored the correlation between residue concentrations and ARBs prevalence. The concentration of fluoroquinolones and the number of antibiotics present in the river-reservoir have a strong correlation with the abundance of intl-1 (integron gene), known as the proxy for ARGs distribution through horizontal gene transfer (Chen et al., 2019). Furthermore, a previous study involving rivers in 48 different countries showed that the prevalence of fluoroquinolone resistance in *Escherichia coli* was positively correlated with the concentration of ciprofloxacin (Kenyon, 2022). Meanwhile in sediment, a high concentration of fluoroquinolones and a high abundance of quinolones resistance (qnr) genes were found in Indian river (Rutgersson et al., 2014). However, it should be noted that results do not always show an unambiguous correlation, because the local ARB prevalence may very well reflect conditions and interactions that occurred upstream.

In this study, the risk quotient calculation was based on conventional endpoints using standard species rather than site-specific risk assessments, which may affect the accuracy of the risk evaluation. Additionally, the lack of risk data for certain antibiotics (see unknown results in Fig. 3), particularly concerning cyanobacteria and the selection of antibiotic-resistant bacteria, could lead to an underestimation of the risk. Using MSCs as a basis for determining the risk of antibiotic resistance without further analysis to confirm the type and abundance of ARBs may also impact risk evaluation. To better assess the impact of antibiotic residues on the selection of antibiotic-resistant bacteria, future studies could incorporate experimental data. Despite these limitations, this study offers a risk evaluation of antibiotics in the river-reservoir, serving as a reference for more comprehensive future risk assessments.

3.3.2. Mixture risk to aquatic organisms

To assess the impact of mixtures of antibiotic residues on species loss inhabiting the water and sediment of the river and reservoir, the Potentially Affected Fraction of Species (msPAF) was determined. High PAF values express critical effects which leads to a higher fraction of species loss (Posthuma et al., 2019). During the wet season, a substantial impact on species is observed in almost 90% of the locations, as was reflected by msPAF values exceeding 0.75 (Fig. 4). Only on three inlet locations (B, C, E) a low to moderate effect on the species living in the water was observed. In contrast to the wet season, the presence of the mixtures of antibiotic residues in the dry season generally has a low to moderate effect, particularly in the inlets (Fig. 4).

In our study, the msPAF values generated for each antibiotic class with the same mode of action ($msPAF_{CA}$) revealed that during the wet season, sulphonamides and fluoroquinolones were the primary contributors to toxicity in the water. In contrast, during the dry season, lincosamides, along with sulphonamides were the major contributors to toxicity (see Table S.12.1). Previous research using the msPAF approach indicated severe impacts on three sites, while 16 others sites were considered moderately impacted by mixture toxicities of 11 pharmaceuticals classes, including antibiotics in a Mediterranean river in Eastern Spain (Fonseca et al., 2020). The toxicity was predominantly associated with the analgesic class (e.g. diclofenac) followed by the antibiotic classes. The antibiotic residues contributing to toxicity varied with the seasons; macrolides dominated in winter and autumn, while fluoroquinolones dominated in summer (Fonseca et al., 2020).

In contrast to the water, the impact of mixtures of antibiotic residues in the sediment was generally low to moderate in most locations during the wet season. However, exceptions were noted, with three locations in the upstream (U, V, X) and in the inlet area (A, L, T) showing a potentially substantial impact. During the dry season, the impact of mixtures of antibiotic residues in sediment remained consistently low across all locations. The antibiotic residues contributing the most to toxicity in the sediment as represented by $msPAF_{CA}$ values were tetracyclines followed by fluoroquinolones in the wet season. In contrast, during the dry season, only fluoroquinolones were observed as the dominant contributors to toxicity (see Table S.12.2).

Using msPAF to determine the impact of joint toxicity of antibiotics provides an insight in the potential impact of mixture toxicity on species loss. However, despite the SSD used in this calculation are generated from a large amount of toxicity data across various taxa (Posthuma et al., 2019), information about the type of local species loss in the msPAF calculation derived from local environmental toxicity data is needed for more accurate determination of msPAF values.

Based on the results obtained with this study, 10 individual antibiotics were selected as most important for monitoring their occurrence and potential risks in the water of the river-reservoir system in Cirata. These include fluoroquinolones (ciprofloxacin, enrofloxacin, norfloxacin, sarafloxacin, sparfloxacin), sulphonamides (sulfamethoxazole, sulfadiazine), diaminopyrimidine (trimethoprim), and lincosamides (clindamycin, lincomycin). Meanwhile, five antibiotics were suggested for sediment monitoring which include tetracylines (tetracycline), fluoroquinolones (ciprofloxacin, enrofloxacin), sulphone (dapsone), and diaminopyrimidine (trimethoprim). Although the detection frequency of tetracycline, dapsone, and trimethoprim in sediment matrices was more than 30%, we have deemed it necessary to include these antibiotics in the priority list, emphasizing the importance of monitoring them

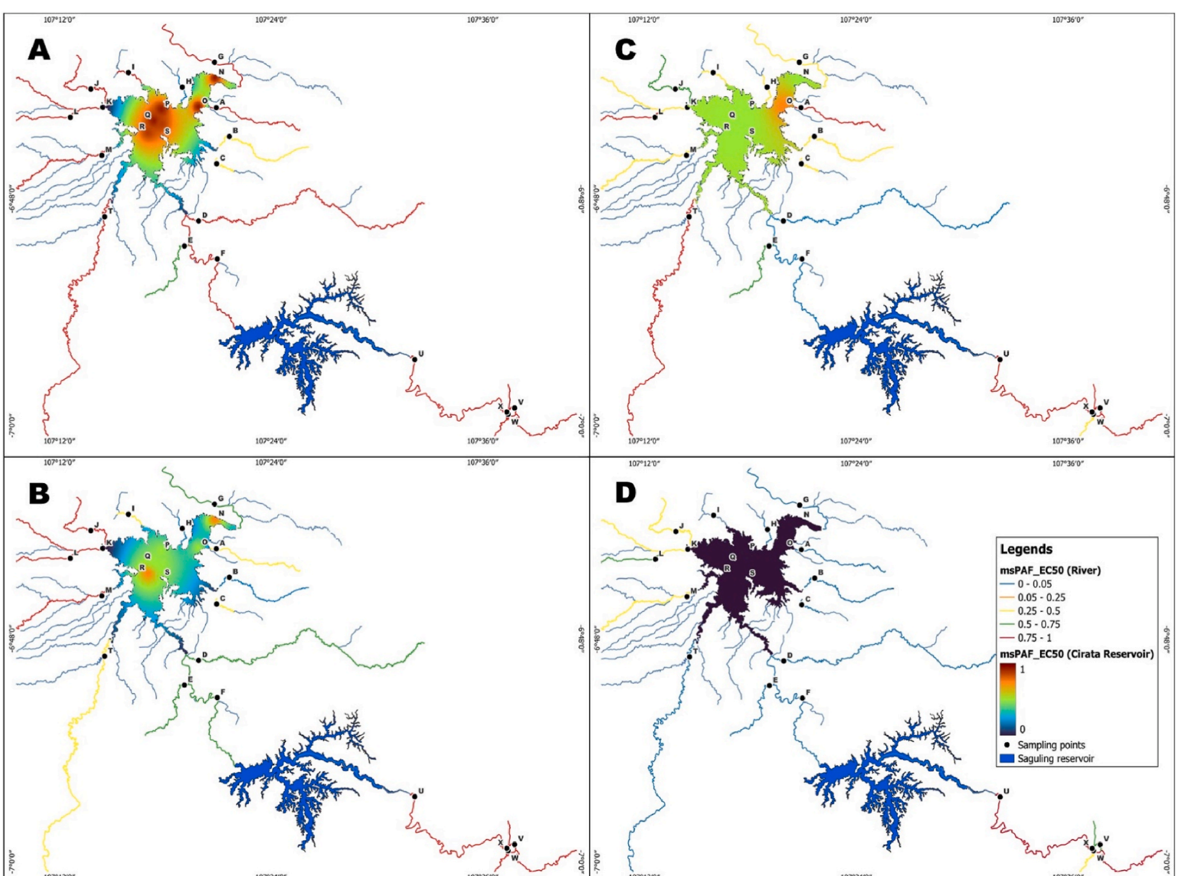

Fig. 4. Potentially Affected Fraction of Species (msPAF) of mixture antibiotic residues in the water during wet (A) and dry season (B) and in the sediment during wet (C) and dry (D) season.

closely. Together this makes a priority list of 12 different antibiotics.

4. Conclusions

The comprehensive analysis of water and sediment samples revealed a diverse range of 24 out of 65 analyzed antibiotic residues, with seasonal variations in their numbers and concentrations. Notably, the study highlights the presence of antibiotics residues which varied seasonally, reaching the highest concentrations during the dry season in water for three antibiotic classes: sulphonamides, lincosamides, and diaminopyrimidines. Meanwhile, the two antibiotic classes showed their highest concentrations in the water during the wet season: fluoroquinolones, and tetracyclines. The presence of the antibiotics sulfamethoxazole, ciprofloxacin, norfloxacin, and lincomycin pose substantial threats to cyanobacteria. Meanwhile, based on the RQ value, certain fluoroquinolones including norfloxacin, ciprofloxacin, enrofloxacin, and sparfloxacin, may have the potential to contribute to the selection of antibiotic-resistant bacteria. It is therefore recommended to validate this estimation through actual experiments in future studies. The impact of mixtures of antibiotic residues, particularly during the wet season, significantly influenced species loss, emphasizing the need for comprehensive monitoring and management strategies. Based on these results 12 priority antibiotics for monitoring in and around the Cirata reservoir were defined, offering valuable insights for future environmental protection initiatives. Further investigations into the relative share attribution of antibiotics sources in the river-reservoir and the impact of antibiotic residues on water-related ecosystem services providing by the reservoir are recommended.

CRediT authorship contribution statement

Miranti Ariyani: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Conceptualization. **Larissa J.M. Jansen:** Writing – review & editing, Validation, Methodology. **Paula Balzer-Rutgers:** Validation, Methodology, Formal analysis. **Nynke Hofstra:** Writing – review & editing, Supervision, Conceptualization. **Pieter van Oel:** Writing – review & editing, Supervision, Conceptualization. **Milou G.M. van de Schans:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.119992.

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