

Article

Linking Antibiotic Residues and Antibiotic Resistance Genes to Water Quality Parameters in Urban Reservoirs: A Seasonal Perspective

Sihan Li ¹, Raphinos Tackmore Murava ¹, Qiyue Zhang ¹, Tong Zhou ¹, Armstrong Ighodalo Omoregie ² , Adharsh Rajasekar ^{1,3,*}  and Tariq Ouahbi ^{4,*} 

¹ Key Laboratory of Meteorological Disaster, Ministry of Education (KLME)/Joint International Research Laboratory of Climate and Environmental Change (ILCEC)/Collaborative Innovation Centre on Forecast and Evaluation of Meteorological Disasters (CIC-FEMD), Nanjing University of Information Science and Technology, Nanjing 210044, China; 202283090021@nuist.edu.cn (S.L.); bapaul@nuist.edu.cn (R.T.M.); 202283090016@nuist.edu.cn (Q.Z.); 202183090023@nuist.edu.cn (T.Z.)

² Centre for Borneo Regionalism and Conservation, University of Technology Sarawak, No. 1 Jalan University, Sibul 96000, Malaysia; armstrong@uts.edu.my

³ School of Geography and Environmental Science, University of Reading, Reading RG6 7BE, UK

⁴ LOMC Laboratory, Civil Engineering Department, Université Le Havre Normandie, Normandie Université, UMR 6294 CNRS, 53 Rue De Prony, Cedex 76058 Le Havre, France

* Correspondence: a.rajasekar@reading.ac.uk (A.R.); tariq.ouahbi@univ-lehavre.fr (T.O.)

Abstract: The interaction between antibiotics and antibiotic resistance genes (ARGs) in freshwater ecosystems has become a critical environmental concern. This study investigates seasonal variations of sulfonamide and tetracycline antibiotics and their relationship with ARGs in three urban reservoirs in Nanjing, China: Pingshan Forest Park, Shanhua Lake Wetland Park, and Zhaoqiao Reservoir. Sampling was conducted in May and September 2023 to assess water quality, antibiotic concentrations, and ARG abundance. A total of 30 water samples were analyzed in regard to their physicochemical parameters, heavy metals, and antibiotics. A quantitative PCR assay was used to measure the ARG abundance relative to the 16S rRNA gene. Sulfonamide concentrations ranged from 30 to 120 ng/L, while the concentrations of tetracyclines were 50–160 ng/L. Notably, sulfamethazine decreased significantly in two reservoirs (Shanhua and Zhaoqiao, $p < 0.05$), while other antibiotics showed minimal variation, indicating persistent contamination from agricultural runoff and wastewater discharge. ARG abundance was lower in May than in September, with sulfonamide resistance genes being lower cumulatively than tetracycline resistance genes. Strong correlations ($r > 0.7$) were observed between ARGs and parameters like dissolved oxygen and pH. High antibiotic levels were observed in areas without nearby hospitals or pharmaceutical companies, implicating agriculture as a major pollution source. By analyzing sulfonamide and tetracycline antibiotics and their resistance genes across three eutrophic reservoirs in Nanjing, China, we highlight critical environmental drivers of ARG proliferation and propose targeted mitigation strategies.

Keywords: antibiotic resistance genes; antibiotic residues; sulfonamide; tetracycline; freshwater reservoirs; water quality; seasonal variation; environmental factors



Academic Editors: Tilemachos K. Koliopoulos and Gregorio García

Received: 12 February 2025

Revised: 12 March 2025

Accepted: 13 March 2025

Published: 18 March 2025

Citation: Li, S.; Murava, R.T.; Zhang, Q.; Zhou, T.; Omoregie, A.I.; Rajasekar, A.; Ouahbi, T. Linking Antibiotic Residues and Antibiotic Resistance Genes to Water Quality Parameters in Urban Reservoirs: A Seasonal Perspective. *Environments* **2025**, *12*, 96. <https://doi.org/10.3390/environments12030096>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The widespread use of antibiotics, especially in healthcare, animal husbandry, and aquaculture, has resulted in antibiotic resistance genes (ARGs) becoming a novel contaminant that seriously threatens both human health and aquatic ecosystems [1–3]. Antibiotics

have significantly improved human and animal health by reducing mortality from pathogen infections [4]. Sulfonamides and tetracyclines are among the most widely used antibiotics globally, with significant applications in human medicine, livestock farming, and aquaculture [5,6]. These antibiotics enter aquatic ecosystems primarily through agricultural runoff, manure application, and untreated or partially treated wastewater discharge [7,8]. However, the misuse and overuse of antibiotics have accelerated the development of antibiotic-resistant bacteria (ARB) [3]. These resistant bacteria exacerbate the problem by transferring resistance genes to other environmental microorganisms through horizontal gene transfer [9].

ARGs are now recognized as emerging environmental contaminants due to their persistence, mobility, and ability to spread across ecosystems through horizontal gene transfer (HGT) [10,11]. The World Health Organization (WHO) has classified antibiotic resistance as one of the most pressing global health challenges of the 21st century, emphasizing the urgent need for comprehensive research and mitigation strategies [6]. ARGs are prevalent in water bodies, and their spread is particularly noticeable in ecosystems affected by human activities [12,13]. Even at low concentrations (2–10 ng/L), the persistence of antibiotics in the environment can cause long-term toxic effects on aquatic organisms, while fostering microbial resistance [7,14]. Both ARGs and ARB are highly toxic and difficult to remove using existing water treatment technologies, leading to their accumulation in water bodies and the food chain, where they may ultimately threaten human health through direct contact or ingestion [10–13]. Given their potential threat to public health and the environmental challenges posed by ARGs, the World Health Organization has classified induced antibiotic resistance as one of the most pressing public health issues of the 21st century.

In recent years, water bodies, such as reservoirs, lakes, and rivers, across China have increasingly faced pollution problems due to ARGs and water eutrophication, especially in the Yangtze and Yellow River basins [12,15]. Recent studies have highlighted the role of ARGs in facilitating the spread of multidrug-resistant pathogens, further exacerbating the global burden of antibiotic resistance [16–18]. Globally, ARG proliferation in aquatic environments has been linked to anthropogenic activities, such as urbanization, industrial discharge, and agricultural runoff. However, the issue is exacerbated in China due to rapid urbanization, intensive agricultural practices, and insufficient wastewater treatment infrastructure. For instance, studies have shown higher concentrations of sulfonamides and tetracyclines in Chinese water bodies compared to regions like Europe or North America, where stricter regulations on antibiotic use and wastewater management are in place [19,20]. Additionally, the prevalence of eutrophication in Chinese lakes and reservoirs further amplifies ARG proliferation, creating unique challenges for water quality management. The proliferation of ARGs in water bodies has become more severe, driven by human activities such as agriculture, urban wastewater discharge, and aquaculture [21]. Antibiotics enter the environment primarily through discharge from sewage treatment plants, agricultural runoff, and medical wastewater [22]. They accumulate in relatively closed reservoirs and lakes, forming a “reservoir” of ARGs, severely threatening water quality and the ecological environment [23]. The long-term closure and extended water residence times in lakes make these bodies particularly high-risk areas for ARGs.

Eutrophication is a well-known environmental issue affecting freshwater systems globally, driven by excessive nutrient inputs from agricultural runoff, sewage discharge, and industrial activities. It remains a persistent challenge, particularly in rapidly developing regions like China, where nutrient loading exceeds critical thresholds [24,25]. Studies have shown that the abundance of ARGs in many reservoirs correlates closely with water quality indicators, such as nutrient levels, pH, and chemical oxygen demand (COD) [3,26]. For example, ref. [3,15,27] demonstrated that nutrient enrichment in aquatic systems creates

favorable conditions for the proliferation of ARG-carrying bacteria. Eutrophic water bodies typically contain high concentrations of nutrients like total nitrogen, total phosphorus, and chlorophyll-a, creating favorable conditions for microbial communities [28]. Rapid algal blooms reduce dissolved oxygen and create an environment conducive to bacterial growth, facilitating the spread of ARGs in water bodies through horizontal gene transfer [29]. Antibiotic residues in the water column alter microorganisms' metabolic activities, increasing the resistance gene transfer frequency [30].

The long-term use of certain antibiotics, such as sulfonamides and tetracyclines, has significantly increased the abundance of their associated resistance genes in reservoirs through plasmid transfers [31]. These changes affect aquatic organisms' health and potentially threaten human health through the food chain or direct contact [32]. Heavy metals, such as chromium (Cr), copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn), have also been identified as key contributors to the proliferation of ARGs in aquatic environments [33]. These metals exert selective pressure on microbial communities, promoting the co-selection of metal tolerance and antibiotic resistance genes, which often reside on identical plasmids or integrons [34]. For example, Cr(VI) has been shown to induce oxidative stress, triggering DNA damage and enhancing the transfer of resistance genes via integrase activity [35]. Similarly, Cu and Zn generate reactive oxygen species, prompting bacteria to upregulate efflux pumps that expel metals and antibiotics, thereby facilitating bacterial survival under metal stress [34]. Understanding the dynamic behavior of ARGs in aquatic ecosystems can help identify high-risk areas and develop targeted management strategies. This research could provide scientific insights for future pollution prevention policies and enhance wastewater treatment technologies. Furthermore, investigating the environmental behavior of ARGs can inform stricter antibiotic use management, reducing the risk of spreading drug-resistant genes and mitigating the long-term threat of antibiotic resistance to public health. Given the backdrop of rapid population growth, urbanization in China, and the escalating issue of water pollution, the environmental impact of antibiotic use and discharge has garnered global attention [36].

While previous studies have examined antibiotics and antibiotic resistance genes (ARGs) in Chinese freshwater systems, most have focused on large rivers or heavily polluted urban freshwater lakes like the Yangtze River, Taihu Lake, and Chaohu Lake [37,38]. Few have explored the seasonal dynamics of ARGs in urban reservoirs, which are critical freshwater sources that are vulnerable to eutrophication and anthropogenic pressures. This study addresses this gap by analyzing ARGs, water quality parameters, and heavy metals in three Nanjing reservoirs: Pingshan Forest Park, Shanhu Lake Wetland Park, and Zhaoqiao Reservoir. These reservoirs were chosen for their varying eutrophication levels, their role as freshwater sources, and their exposure to agricultural runoff and wastewater discharge. The objectives were to assess ARG pollution and examine the relationships between ARGs and environmental variables, particularly under eutrophic conditions. This research elucidates ARG proliferation mechanisms and their interactions with water quality parameters by studying tetracycline genes (*tetA*, *tetX*, *tetO*, *tetC*, *tetE*, *tetD*), *int1*, and sulfonamide resistance genes (*sul1*, *sul2*). The findings contribute to understanding ARG dynamics in freshwater ecosystems, offering valuable data for regional and global water management strategies. This study is crucial for protecting China's water resources and advancing global antibiotic resistance research [20].

2. Materials and Methods

2.1. Study Area

This study was conducted in three reservoirs located in Nanjing, China: Pingshan Forest Park, Shanhu Lake Wetland Park, and Zhaoqiao Reservoir. These reservoirs were

selected due to their diverse degrees of eutrophication, their role as significant freshwater sources, and their vulnerability to anthropogenic pressures, including agricultural runoff and urban wastewater discharge. The reservoirs exhibit varying levels of water quality degradation, making them ideal for investigating the relationship between water quality parameters and the abundance of antibiotic resistance genes (ARGs). Pingshan Forest Park is characterized by low anthropogenic influence, while Shanhu Lake Wetland Park and Zhaoqiao Reservoir are more affected by agricultural activities and moderate anthropogenic activities. A map of the study area, including the sampling locations, is provided in Figure S1.

2.2. Water Sampling

Surface water samples were collected from the three reservoirs in May and September 2023. Five sampling points were selected at each reservoir to ensure spatial variability across the water body. These points were strategically distributed to represent different zones, including nearshore areas that were influenced by anthropogenic activities (e.g., agricultural runoff and urban wastewater discharge) and open-water zones that were less affected by direct inputs (Figure 1). While a regular sampling grid was not employed, the selection of the sampling points aimed to capture the spatial heterogeneity within each reservoir. Each sampling point was sampled in triplicate to account for variability, resulting in 30 water samples (5 samples $\times$ 3 reservoirs $\times$ 2 sampling times). A map of the sampling locations is provided in Figure 1. The GPS locations are shown in Table S1. Water samples were collected from the surface layer (approximately 0.5 m below the water surface), using sterile high-density polyethylene (HDPE) bottles. At each sampling point, 1500 mL of water was collected in triplicate, resulting in a total volume of 4500 mL per sampling point. The samples were stored in ice during transportation to the laboratory for further analysis. In regard to the antibiotic concentration determination, the water samples were collected in brown bottles. Research has shown that tetracyclines and sulfonamides are sensitive to light, which can lead to photodegradation, so we used brown bottles to preserve the integrity of the samples during transportation and storage [39].

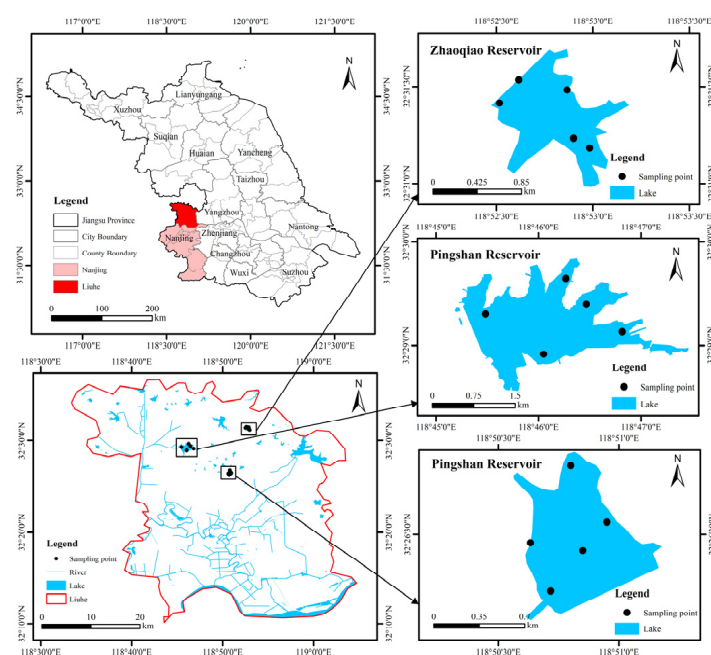


Figure 1. The locations of the three reservoirs.

2.3. Water Quality Parameters

We conducted a comprehensive assessment of the eutrophication level of the water bodies in three reservoirs (Pingshan Reservoir, Shanhu Lake, and Zhaoqiao Reservoir), based on the eutrophication evaluation index for lakes (reservoirs), published by the Ministry of Ecology and Environment of the People's Republic of China. The following water quality parameters were measured in regard to each water sample: pH, temperature, electrical conductivity (EC), total dissolved solids (TDS), biochemical oxygen demand (BOD₅), dissolved oxygen (DO), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), and chlorophyll-a (Chl-a). The temperature, pH, DO, and conductivity were measured using portable meters (HACH, Loveland, CO, USA). The Chl-a, TN, and TP concentrations were determined by spectrophotometric analysis, using a UV-Vis spectrophotometer (Thermo Scientific Genesys 10S, Waltham, MA, USA). The instrument was calibrated using blank solutions and standard calibration curves to ensure a good level of accuracy was achieved. Standard methods for TN, TP, Chl-a, BOD₅, and COD determination were followed, as outlined in the Standard Methods for the Examination of Water and Wastewater [40]. COD was measured using the closed reflux colorimetric method, while BOD₅ was determined using the 5-day incubation method at 20 °C in a controlled environment. The instrument was calibrated daily using blank solutions and standard calibration curves to ensure a good level of accuracy was achieved.

2.4. Heavy Metal Analysis

Heavy metals, including arsenic (As), cadmium (Cd), lead (Pb), and zinc (Zn), were measured in the water samples, using atomic absorption spectrophotometry (AAS) (ZEEnit 700P, Analytik Jena GmbH + Co. KG, Jena, Germany). The water samples were first acidified with nitric acid to a pH < 2 and then digested using a microwave digestion system (CEM, Matthews, NC, USA). For each metal, calibration curves were generated using standard solutions (Sigma-Aldrich, St. Louis, MO, USA), and the concentrations of the metals were determined in triplicate for each sample. The protocol and standards used are mentioned in our previous paper [41].

2.5. Antibiotic Residues

Antibiotic residues in the water samples were analyzed to determine the presence and concentration of commonly used antibiotics. The procedure was extracted from previous literature on sulfonamide and tetracycline antibiotics [39,42]. The antibiotics selected for analysis included tetracycline, sulfonamide, and their derivatives, which are frequently detected in aquatic environments. The antibiotics were extracted from the water samples using solid-phase extraction (SPE), with an Oasis HLB cartridge (Waters, Milford, MA, USA). A total of 500 mL of water was filtered and passed through the SPE cartridge, preconditioned with methanol and water. After the cartridge was washed with water, the antibiotics were eluted with methanol, and the eluate was concentrated using a rotary evaporator. Matrix-matched calibration standards were prepared by spiking the blank water samples with known concentrations of antibiotic standards, ensuring accurate quantification in the presence of potential interferences. Additionally, surrogate compounds (e.g., isotopically labeled internal standards such as sulfamethoxazole-d4 and tetracycline-d6) were used to monitor the recovery rates and correct for matrix-induced variations in the extraction efficiency. The average recovery rates for the target antibiotics ranged from 85 to 110%, indicating high reliability of the method. Furthermore, procedural blanks and spiked samples were analyzed alongside the environmental samples to verify the absence of contamination and ensure data accuracy. The concentration of the antibiotics was determined using high-performance liquid chromatography (HPLC), coupled with a

UV detector (Agilent 1260, Santa Clara, CA, USA), at 280 nm for tetracycline and 265 nm for sulfonamide antibiotics. The quantification was based on calibration curves generated using standard antibiotic solutions (Sigma-Aldrich, St. Louis, MO, USA).

2.6. ARG Detection

The detection of ARGs was carried out using quantitative PCR (qPCR) analysis. Specific primers for tetracycline resistance genes (*tetA*, *tetX*, *tetO*, *tetC*, *tetE*, *tetD*), sulfonamide resistance genes (*sul1*, *sul2*), and *intl1* were selected based on previous studies and primer sequences in the literature [43,44]. The 16S rRNA gene was quantified from the extracted DNA to assess the bacterial community. The 16S rRNA gene was selected as a universal marker for microbial diversity, enabling an estimate of the total bacterial abundance. For qPCR amplification, the primers used for the 16S rRNA gene were adapted from those included in previously published studies [45]. The qPCR reactions for the 16S rRNA gene were performed under the same conditions as those for ARGs, with primers specific to bacterial 16S rRNA sequences. The DNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher, Waltham, MA USA) and stored at -20°C , until further analysis. For the qPCR amplification, reactions were carried out in 20 μL volumes, containing 10 μL of 2 $\times$ SYBR Green PCR Master Mix (Thermo Fisher, Waltham, MA, USA), 1 μL of each primer (10 μM), and 2 μL of template DNA (100 ng/ μL). The PCR conditions were as follows: initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at $55\text{--}60^{\circ}\text{C}$ (depending on the specific primer pair) for 30 s, and extension at 72°C for 30 s. Melting curve analysis was performed at the end of each qPCR cycle to verify the specificity of the amplification.

2.7. Risk Quotient Calculation

The risk quotient (RQ) assesses the ecological risk posed by antibiotic residues in water samples. The RQ is calculated based on the measured environmental concentrations (MECs) and their respective predicted no-effect concentrations (PNECs). The RQ is a widely used tool in regard to environmental risk assessments, providing a quantitative basis for evaluating the potential harm of contaminants in aquatic ecosystems [46]. The formula/equation for calculating the RQ is as follows:

$$\text{RQ} = \text{MEC} / \text{PNEC}$$

where MEC represents the measured environmental concentration of the antibiotic (ng/L), which reflects the actual levels of contamination detected in water samples. PNEC denotes the predicted no-effect concentration (ng/L), derived from available ecotoxicity data, representing the threshold concentration below which adverse effects on aquatic organisms are unlikely to occur [3,30].

The European Commission's Technical Guidance Document [47] outlines two primary methods for determining the PNEC, namely the assessment factor approach and the species sensitivity distribution method, with the choice depending on data availability. According to [32,34], the PNEC can be calculated using the following formula:

$$\text{PNEC} = \text{LC}_{50}, \text{EC}_{50} \text{ or } \text{NOEC} / \text{AF}$$

In this equation:

LC_{50} refers to the concentration that causes a 50% mortality rate in test organisms. EC_{50} represents the concentration at which 50% of the maximum effect is observed. NOEC denotes the highest concentration at which no adverse effects are detected.

AF is the assessment factor, which varies based on the type of toxicity data available. For acute or short-term toxicity data (LC_{50} or EC_{50}), an AF of 1000 is typically applied. For chronic or long-term toxicity data (NOEC), the AF ranges from 10 to 100, depending on the trophic level of the organisms being assessed, namely primary producers (e.g., algae), secondary consumers (e.g., invertebrates), or tertiary consumers (e.g., fish) [48].

The RQ value serves as a key metric for categorizing the ecological risk posed by antibiotics in aquatic environments. Based on the RQ value, the risk levels are classified as follows: high risk ($RQ > 1$), medium risk ($0.1 \leq RQ \leq 1$), low risk ($0.01 \leq RQ < 0.1$), and very low risk ($RQ < 0.01$) [23,49]. In this study, the ecological risk assessment focused on two groups of aquatic organisms: algae and fish.

Integrating chemical indexes, such as the MEC and PNEC, is critical for understanding the persistence, bioavailability, and ecological impact of antibiotics in aquatic systems [8]. These parameters enable high-risk compounds to be prioritized and help to guide targeted mitigation strategies and regulatory frameworks to reduce pharmaceutical pollution. For instance, studies have shown that antibiotics, like tetracyclines and sulfonamides, exhibit varying persistence and toxicity depending on the environmental conditions and microbial community dynamics [6,30,50]. This study's PNEC values for tetracycline and sulfonamide antibiotics were sourced from the existing literature, ensuring robustness in regard to the risk assessment process. Antibiotic residues in aquatic environments contribute significantly to the global challenge of antimicrobial resistance (AMR), disrupt microbial communities, and harm non-target organisms [51]. Chronic exposure to sub-lethal concentrations of antibiotics can alter the structure and function of aquatic ecosystems, leading to cascading effects on biodiversity and ecosystem services [52]. Furthermore, the bioaccumulation of antibiotics in aquatic food webs poses additional risks to species at higher trophic levels, including humans, through dietary exposure [53].

The RQ framework provides a standardized and scientifically sound approach to quantifying these risks by integrating chemical indexes, like the MEC and PNEC, into the RQ calculation. Researchers can identify priority pollutants and develop evidence-based interventions to mitigate their environmental impact.

2.8. Data Analysis

An analysis of variance (ANOVA) was performed for the physicochemical and antibiotic concentrations to identify significance among seasonal variations. We have conducted formal tests for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) to ensure the assumptions of the ANOVA were met. The results confirmed that our data satisfied these assumptions, justifying the use of an ANOVA for our analysis. The relationship between ARG abundance, water quality parameters, heavy metals, and antibiotic concentrations was assessed using Spearman's correlation coefficients (r) and principal component analysis (PCA). The Bonferroni correction was performed to adjust the p -values for multiple comparisons. Statistical significance was set at $p < 0.05$ at a 95% CI. All the statistical analyses, including the creation of graphs and plots, were performed using OriginPro 2024b software.

3. Results and Discussion

3.1. Seasonal Variations and Relationships in Regard to Water Quality Parameters and Heavy Metals Among Three Reservoirs

As shown in Table S2, the results showed that the COD in the three reservoirs was significantly higher in May than in September, the total nitrogen concentration was higher in September than in May, and all of them were in the range of 2.41–3.37 mg/L, which far exceeded the national standard, indicating that the water bodies were seriously pol-

luted [54]. The total phosphorus concentrations showed some differences, except for the Pingshan Reservoir; the other two reservoirs had higher total phosphorus concentrations in September than in May, but the data did not exceed the national standard. The BOD₅ values were significantly higher in May than in September, with a range of 7.59 to 8.35 in May, compared with 6.75 to 7.01 in September. The Chl-a concentrations in the reservoirs, except for Pingshan Reservoir, had higher values in May than in September, but the values were still significantly higher than the national standard, further verifying the higher degree of eutrophication in the water bodies. The DO values ranged from 6.64 to 8.47 in September, which were significantly higher than those of 5.34 to 6.46 in May, indicating that the oxygen status of the water body was relatively better in September. However, compared to Lake Taihu, the DO concentrations were significantly lower in September, while the Chl-a values were much higher [29]. Compared with Daihai Lake, Daihai Lake had higher total nitrogen (TN) and total phosphorus (TP) concentrations (TN: 1.13 mg/L, TP: 0.0358 mg/L) and lower DO values than the reservoirs in this study, further indicating that these reservoirs are more seriously polluted [55]. Compared to Chaohu Lake, the DO was lower in all three reservoirs, while the total nitrogen and phosphorus concentrations were significantly higher, and the Chl-a values were lower [56,57]. Spearman's correlation analysis revealed significant relationships between the physicochemical parameters and heavy metals across all three reservoirs (Figure 2). For instance, strong positive correlations were observed between TP and Chl-a in multiple reservoirs, such as May–Pingshan, Sep–Pingshan, and May–Shanhu. These findings reinforce the critical role of phosphorus in promoting algal blooms, a key driver of eutrophication. This relationship is likely due to phosphorus acting as a limiting nutrient in freshwater systems, where its excess stimulates phytoplankton growth, leading to elevated Chl-a levels. As the concentration of TP increases, it usually promotes the growth of algae, which in turn increases the Chl-a level, leading to a positive correlation between TP and Chl-a [58]. The PCA results further support this, by showing that TP and Chl-a often load strongly on the same principal components, particularly in May–Shanhu and Sep–Shanhu reservoirs. For instance, in the Sep–Shanhu Reservoir, Chl-a had the highest loading on PC1, while TP also showed a notable contribution, underscoring their linked influence on water quality. Similarly, temperature strongly correlated with total nitrogen in the May–Zhaoqiao Reservoir, suggesting that higher temperatures enhance nitrogen enrichment through increased microbial activity and nutrient cycling. Another notable finding was the significant positive correlation between pH and certain heavy metals, such as Cd and Zn, in the May–Shanhu Reservoir. This indicates that pH could potentially play a crucial role in metal speciation and solubility. At higher pH levels, metals like Cd and Zn may become less soluble and precipitate out of the solution, reducing their bioavailability. Conversely, these metals can dissolve more readily at lower pH levels, increasing their mobility and potential toxicity. The PCA findings corroborate this relationship, as pH showed varying contributions across different principal components, reflecting its complex interactions with other parameters. For example, in the May–Shanhu Reservoir, pH loaded moderately on PC1, while Cd and Zn also had notable loadings, suggesting their interdependence. In the Sep–Zhaoqiao Reservoir, EC exhibited a strong positive correlation with Zn, suggesting that salinity-driven mobilization contributes to Zn contamination. This highlights the influence of ionic strength on metal behavior in aquatic systems. The ANOVA analysis showed that TN, TP, DO, COD, BOD, Chl-a, and other indicators for assessing the eutrophication of the water bodies in the three reservoirs were significantly seasonally correlated, indicating that the eutrophication level of the water bodies in the reservoirs changed significantly between May and September, with eutrophication being significantly higher in May than in September. The PCA results further emphasized this seasonal variation, as the loadings of parameters like TN, TP, and Chl-a on PC1 were

generally higher in May, reflecting their greater contribution to water quality variability during this period. For instance, in the May–Zhaoqiao Reservoir, TN and TP strongly contributed to PC1, consistent with the observed seasonal trends.

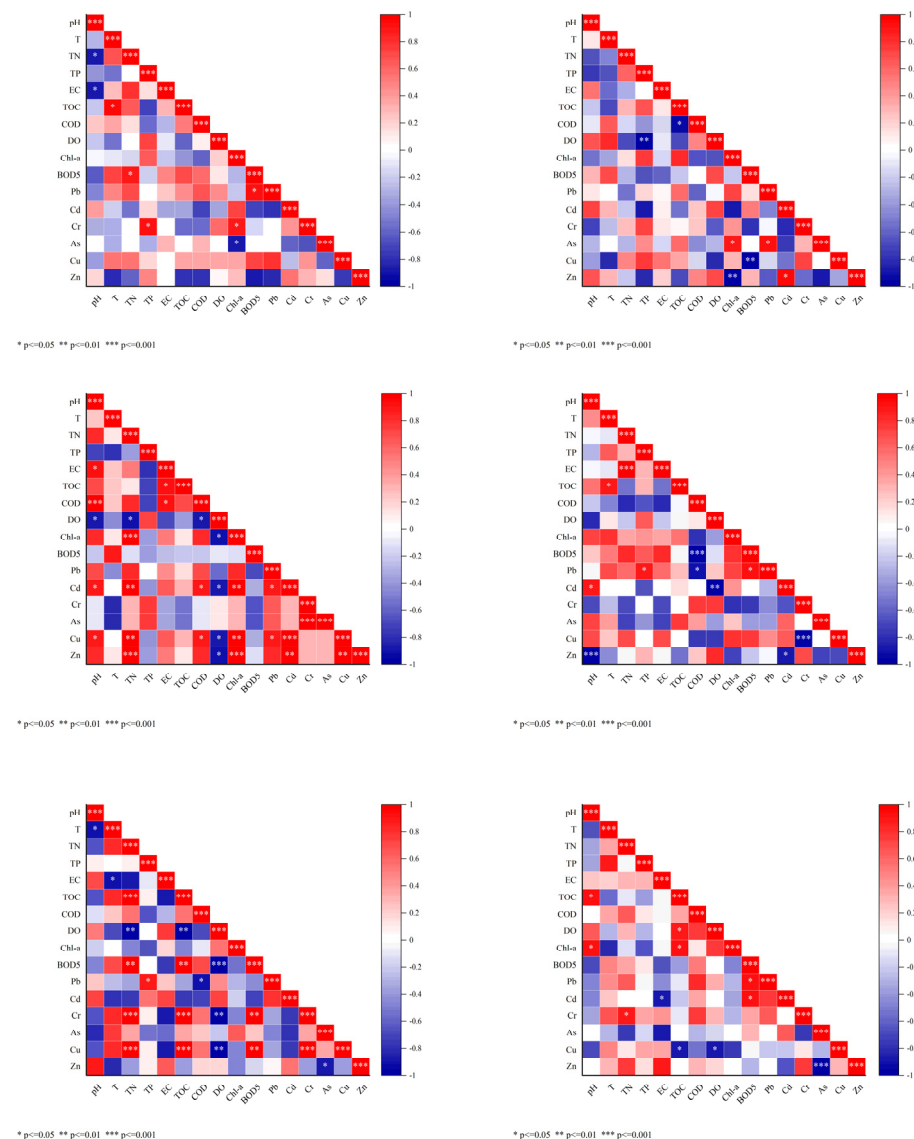


Figure 2. Relationships between physicochemical parameters and heavy metals using Spearman’s correlation analysis in three reservoirs (red means positively related to each other, blue means negatively related to each other, * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$): (**top row**) May–Pingshan, Sep–Pingshan; (**middle row**) May–Shanhu, Sep–Shanhu; (**bottom row**) May–Zhaoqiao, Sep–Zhaoqiao.

The areas in which these reservoirs are located are mainly characterized by natural landscapes, and no significant sources of industrial pollution have been identified. However, agricultural activities and domestic sewage discharge may harm water quality, especially during the peak tourist season. Meteorological conditions may be one of the most important factors in regard to water quality changes. The average temperature in Nanjing from May to September ranges from 19 to 26 °C, which provides a better environment for algae growth [59]. Precipitation in May is more concentrated than in September and is often accompanied by heavy precipitation events. These precipitation events may accelerate the entry of pollutants from agricultural runoff into the water column, resulting in higher pollutant concentrations, which may increase the degree of eutrophication in

the water column. Previous studies have also observed that a greater wet season (high precipitation) increases algal growth [59,60]. This effect was also reflected in the decrease in DO concentrations in the May water samples, which were relatively high in September. A significant negative correlation ($p < 0.001$) between BOD and DO indicates that the BOD of the water body increased significantly with the decrease in DO (Figures 2 and 3). The PCA findings supported this relationship, as BOD₅ and DO were often loaded on opposing principal components, indicating their inverse relationship. For example, in the May–Pingshan Reservoir, BOD₅ had a high positive loading on PC1, while DO showed a moderate negative loading, consistent with the observed seasonal patterns. In addition, nutrients (nitrogen and phosphorus) enter the lake through wet and dry atmospheric deposition, which converts them into finer particles and then dissolves them, resulting in high levels of total nitrogen and total phosphorus, which promotes algal overgrowth and blooms [58,61]. Despite precipitation’s diluting effect, pollutant concentrations flowing into the water body increase faster than the dilution capacity, resulting in significantly higher conductivity in May than in September.

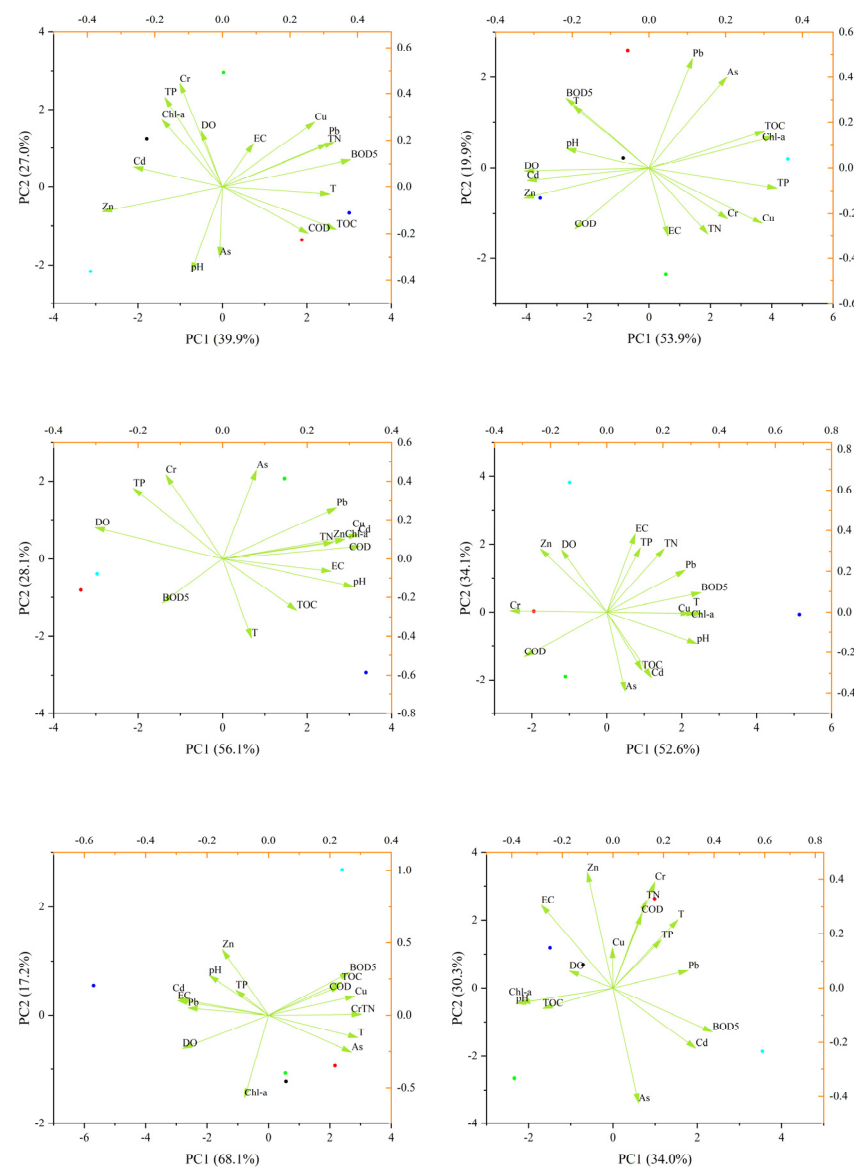


Figure 3. Principal component analysis of the quantitative correlation between physicochemical parameters and heavy metals in three reservoirs: (top row) May–Pingshan, Sep–Pingshan; (middle row) May–Shanhu, Sep–Shanhu; (bottom row) May–Zhaoqiao, Sep–Zhaoqiao.

Temperature differences likewise had a significant impact on water quality. The average temperatures in September were approximately 5 °C higher than in May, and this difference promoted the decomposition of organic matter in the water column, so the TOC concentrations were typically higher in September than in May, except in Mountain Lake Reservoir. The existing literature on freshwater bodies has observed higher amounts of TOC during summer compared to other seasons [62,63]. The PCA results highlighted TOC's role in explaining variance, particularly in September, as seen in the Sep–Shanhu Reservoir, where TOC had moderate loading on PC1, reflecting its increased importance during warmer months. For other water quality indicators, the pH of the reservoirs was maintained in the neutral range (7–8), and the concentrations of heavy metals were below the national standards, except for Cr, which did not show significant temporal differences, and, except for As, the concentrations of other heavy metals did not change significantly between the different periods (Table S2). These results indicate that the eutrophication trend in terms of the water body is generally stable, but the water quality is affected by precipitation, agricultural runoff, and temperature during different seasons, which show seasonal fluctuations. There was a significant positive correlation between As and Cr and a significant positive correlation between As and Zn (Figures 2 and 3). At the same time, a positive correlation was also found between Cr and Pb [57,58]. The PCA findings confirmed these relationships: As, Cr, and Zn were often loaded together on the same principal components, particularly in the Sep–Zhaoqiao Reservoir, where As and Zn had notable contributions to PC1 and PC2, respectively. The significant positive correlation between chemical oxygen demand (COD) and Cr in municipal wastewater further proves that the high level of eutrophication in these three reservoirs may be due to the excessive use of nitrogen and phosphate fertilizers on nearby farmland, metal insecticides or herbicides as part of agriculture, and domestic wastewater [57,58]. The presence of these pollutants, particularly heavy metals, can contribute to the enrichment of nutrients, enhancing eutrophication by promoting algae blooms and depleting dissolved oxygen levels [64].

In regard to Zhaoqiao Reservoir, the correlation analysis showed that Cu, Pb, and Zn concentrations were strongly influenced by temperature, with the pollution levels of these heavy metals intensifying at higher temperatures (Figures 2 and 3). These findings may be attributed to higher temperatures promoting the accumulation of anthropogenic pollutants in the polluted areas and accelerating the transport of heavy metals in the form of particles in the water column, thus exacerbating water quality pollution [65]. The PCA results supported this observation, as Cu, Pb, and Zn often loaded strongly on PC1 in September, particularly in the Sep–Zhaoqiao Reservoir, where Pb and Cu had high loadings, respectively, suggesting their increased influence during warmer months. Previous studies have demonstrated that elevated temperatures can increase the solubility and mobility of heavy metals, making them more bioavailable and more easily transported through aquatic systems [66–68]. These studies have shown that warmer temperatures accelerate the dissolution of metal-bound particles, further elevating metal concentrations and contributing to the deterioration of water quality.

3.2. Role of Water Quality Parameters and Heavy Metals in ARG Proliferation

In water quality studies, factors associated with the eutrophication of water bodies, such as TN, TP, DO, and Chl-a, have complex and variable effects on antibiotic resistance genes (ARGs). Studies have shown that environmental factors, such as water temperature, pH, nitrogen and phosphorus concentrations, and anthropogenic activities (e.g., aquaculture, livestock and poultry fecal discharge, and municipal wastewater discharge) are the main factors influencing the distribution of ARGs in aquatic environments [39].

According to other related studies, DO is negatively correlated with the abundance of ARGs (Figures 4 and 5). We believe this could be because nutrients are released from sediments in low DO conditions, leading to higher concentrations of TN, TP, and COD, which provide favorable conditions for the proliferation of ARGs [3,30]. Lower DO levels may cause nutrients to be released into the water, potentially promoting the spread of antibiotic resistance genes; the energy demands placed on the bacteria carrying these genes in low-oxygen environments could reduce their prevalence. Further research is needed to better understand how these opposing factors interact. Our results showed that the eutrophication of water bodies was higher in May than in September, but the abundance of ARG pollution was higher in September than in May. This may be related to higher bottom water temperatures and more favorable water-mixing conditions in September [3,30]. In this case, low concentrations of ammonia and phosphate instead favored the proliferation of ARGs, while higher concentrations of nitrogen and phosphorus may have inhibited their proliferation [12]. Thus, high concentrations of nitrogen and phosphorus in May resulted in low concentrations of ARGs. The PCA analysis supports this observation, showing that parameters like TN and TP had weaker correlations with ARGs in May compared to September, where lower nutrient levels coincided with a higher ARG abundance. For instance, in Pingshan Reservoir, TN and TP were negatively correlated with *sul1* and *tetX* in September, suggesting that reduced nutrient levels may favor ARG proliferation. Similarly, in Shanhu Lake, the PCA analysis revealed stronger negative correlations between TP and ARGs in May, aligning with the observed seasonal trends. Environmental parameters, such as temperature and pH, affected the efficiency of plasmid transfer, and pH was negatively correlated with the abundance of *sul1* and *sul2*, a result consistent with the study in Lake Xuanwu, as well as weakly correlated with other antibiotics, which was in line with the results of the study on Yitong River [3,30,69]. Organic matter also plays a critical role in shaping ARG concentrations across seasons. Elevated levels of organic matter derived from agricultural runoff, decaying vegetation, or wastewater inputs provide a rich carbon source for microbial communities, promoting bacterial growth and horizontal gene transfer (HGT) [36]. In May, increased rainfall may transport organic matter into water bodies, enhancing microbial activity and ARG proliferation. Conversely, in September, higher temperatures may accelerate the decomposition of organic matter, further fueling microbial metabolism and ARG dissemination. The PCA findings highlight the importance of organic matter and heavy metals in regard to ARG dynamics. For example, in Zhaoqiao Reservoir, Zn showed strong positive correlations with ARGs in May and September, emphasizing its role in promoting ARG proliferation. Similarly, in Shanhu Lake, heavy metals like Cr and As exhibited significant correlations with ARGs, particularly in September, when microbial activity was higher. These findings underscore the need to monitor heavy metal pollution as a contributing factor to ARG dissemination. The interplay between organic matter, microbial activity, and environmental conditions underscores the complexity of ARG dynamics in aquatic ecosystems [70]. Pingshan Reservoir exhibited higher ARG contamination due to its proximity to agricultural areas and urban runoff. Shanhu Lake showed elevated sulfonamide gene concentrations, linked to nearby agricultural activities. Zhaoqiao Reservoir had lower ARG levels, due to its remote location and limited anthropogenic influence. Our study's observed trends in regard to ARG proliferation align with the findings from eutrophic lakes in Brazil and North America, highlighting the global relevance of these dynamics [71,72]. For instance, studies on Lake Erie in North America and Bolonha in Brazil have reported elevated ARG levels during times when there is increased microbial activity and nutrient enrichment, similar to our observations in September. These regional variations underscore the importance of location-specific monitoring and management strategies to address ARG pollution effectively.

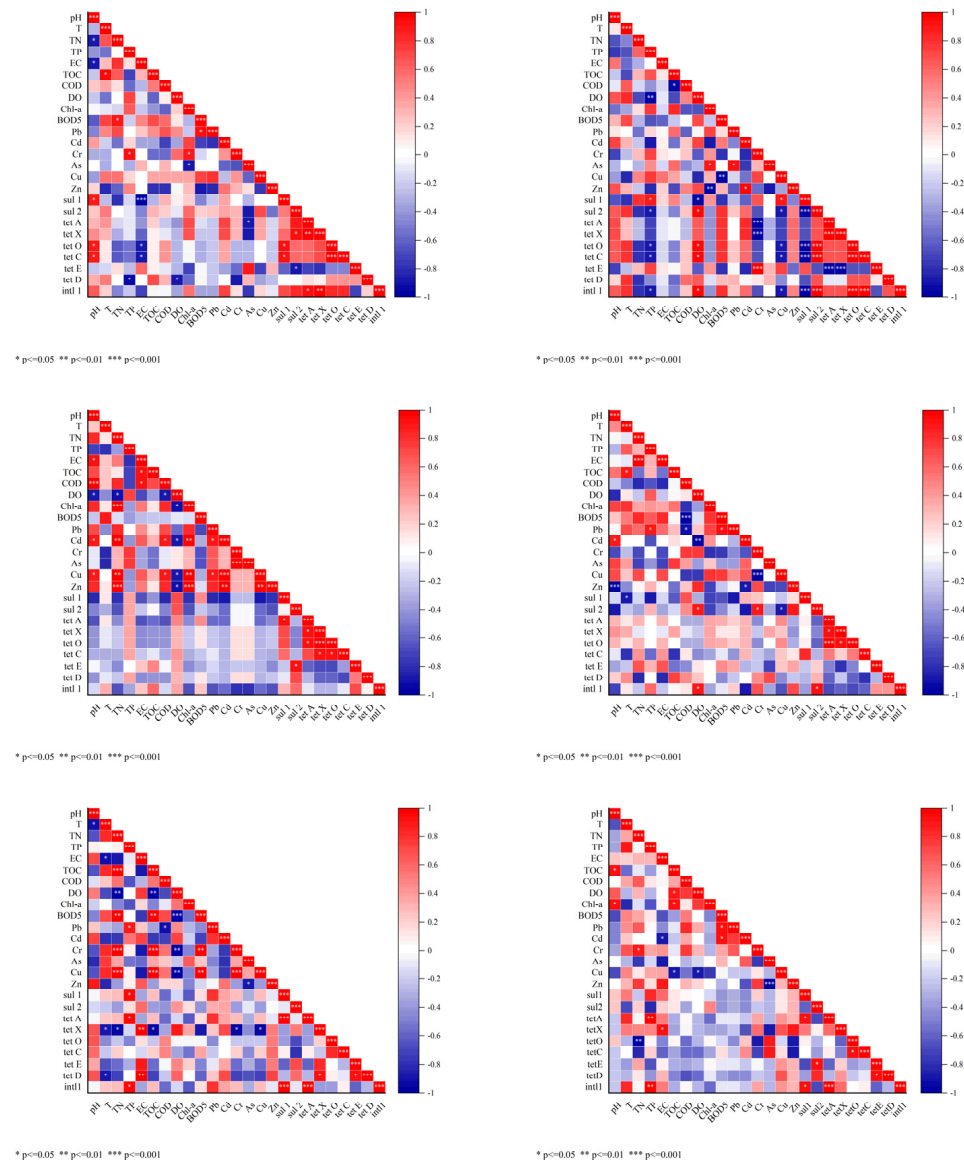


Figure 4. Relationships between physicochemical parameters and antibiotic resistance genes using Spearman's correlation analysis in three reservoirs (red means positively related to each other, blue means negatively related to each other, * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$): (top row) May–Pingshan, Sep–Pingshan; (middle row) May–Shanhu, Sep–Shanhu; (bottom row) May–Zhaoqiao, Sep–Zhaoqiao.

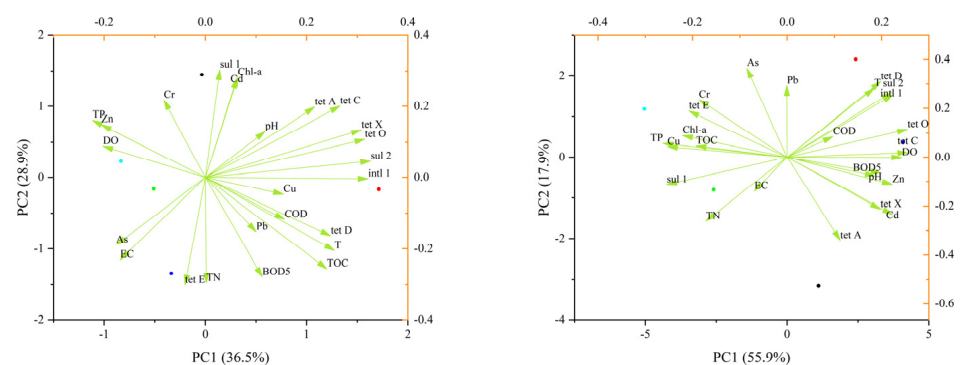


Figure 5. Cont.

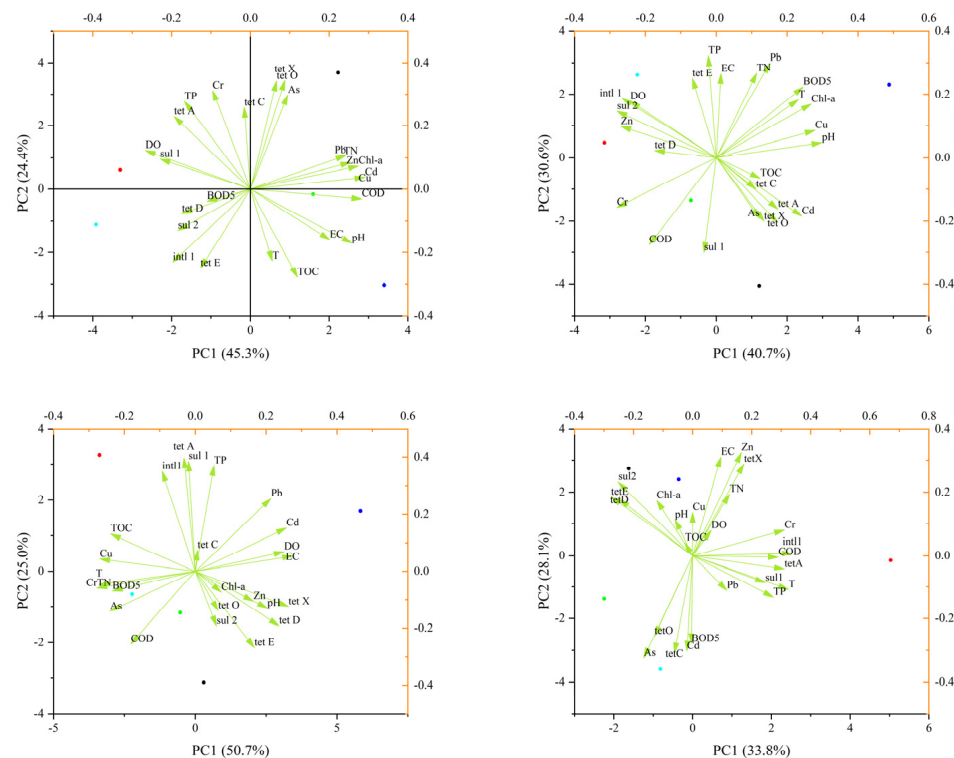


Figure 5. Principal component analysis of the quantitative correlation between ARGs and physicochemical parameters in three reservoirs: (**top row**) May–Pingshan, Sep–Pingshan; (**middle row**) May–Shanhu, Sep–Shanhu; (**bottom row**) May–Zhaoqiao, Sep–Zhaoqiao.

The Chl-a concentration was negatively or weakly correlated with antibiotics, contrary to the general rule that a higher Chl-a concentration means poorer water quality and a higher ARG concentration [2,13]. This suggests that although nutrients significantly influence antibiotics in the water environment, the overgrowth of algae during eutrophication may inhibit the spread of ARGs [30]. Chl-a was significantly negatively correlated with *sul* genes (Figures 4 and 5), which is in agreement with the results of the study on the Yangtze River estuary. This may be related to the longer water residence time and eutrophication status, reflecting the competitive inhibitory effect of algal growth in eutrophic environments, weakening the diffusion ability of ARG-carrying bacteria in the microbial community [73]. Overall, the correlation between water quality parameters and resistance genes was weak, indicating that the water quality parameters had little effect on resistance genes in the environment of the three reservoirs, which is the same finding as the results obtained for the Yitong River [30]. The negative correlation between Chl-a and ARGs suggests that algal blooms may exert competitive inhibitory effects on ARG-carrying bacteria. During periods of high eutrophication, rapid algal growth can alter the microbial community structure, potentially favoring non-ARG-carrying species. This phenomenon aligns with findings from earlier studies, which have shown that eutrophic conditions can suppress the spread of ARGs by altering bacterial community dynamics [24]. The PCA analysis reinforces this conclusion, showing that Chl-a had weak or negative correlations with ARGs across all the reservoirs. For example, in Pingshan Reservoir, Chl-a was negatively correlated with *sul2* and *tetX* in May, while Shanhu Lake showed a weak negative correlation with *sul1* in September. These findings suggest that algal blooms may be protective by reducing ARG spread, particularly during periods of high eutrophication. Metals and antibiotics accelerate the spread of antibiotic-resistant bacteria (ARB)-carrying antibiotic-resistance genes (ARGs) by exerting selective pressure on bacteria, which in turn exacerbates the spread of ARGs in the ecosystem [15,70]. Cr, Cu, Cd, Pb, and Zn released

from industrial emissions, agricultural activities, and transportation pollution enter the environment through rainwater runoff, atmospheric deposition, and other pathways [65]. These heavy metals showed different correlations with ARGs. Cd was positively correlated with *tetX* and *tetA*, but negatively correlated with *sul2* (Figures 4 and 5). Cd induces oxidative stress in bacterial cells, activating efflux pumps like *tetA* and *tetX*, which expel tetracyclines, promoting bacterial survival and increasing ARG prevalence. In contrast, Cd inhibits sulfur metabolism in sulfate-reducing bacteria, potentially reducing *sul2* gene abundance. Additionally, Cd interferes with biofilm formation, further hindering the proliferation of sulfonamide-resistant bacteria [17]. Cr was significantly positively correlated with *sul1* and *tetE*; Cu and Zn were positively correlated with *tetX* and *tetD*; and Pb was positively correlated with *sul1*, *tetA*, and *tetC*, but possibly negatively correlated with *tetE* (Figures 4 and 5). Cr, particularly Cr(VI), induces oxidative stress, triggering DNA damage and promoting the transfer of resistance genes like *sul1* and *tetE* via integrase activity, enhancing gene spread in bacterial communities exposed to chronic Cr stress [1,12]. Cu and Zn generate reactive oxygen species, prompting bacteria to upregulate efflux pumps like *tetX* and *tetD* to expel metals and antibiotics, facilitating bacterial survival under metal stress [7]. Metal tolerance genes and antibiotic resistance genes often reside on the same plasmids or integrons, promoting co-selection and increasing the likelihood of resistance in contaminated environments [73]. Pb causes oxidative damage and can select bacteria that tolerate stress, co-selecting *sul1*, *tetA*, and *tetC* resistance genes due to their association with mobile genetic elements [12,17]. The negative correlation between Pb and *tetE* may arise from specific microbial community dynamics or interference with conjugation mechanisms, shifting the resistance profile toward other mechanisms [7]. Thus, Pb could select alternative resistance strategies, reducing the prominence of *tetE*. These heavy metals alter the microbial community structure, and heavy metals with substantial toxicity and antioxidant properties, such as Cr, trigger microbial stress responses, selectively promote the proliferation of drug-resistant bacteria, enhance the adaptability of bacteria-harboring resistance genes, and promote the spread of resistance genes [1,7,28,74,75].

3.3. Antibiotic Resistance Gene (ARG) Dynamics Across Seasons and Their Relationship with Antibiotics

According to the data analysis in Table 1, the degree of antibiotic resistance gene (ARG) contamination in Pingshan Forest Park, Shanhu Lake Wetland Park, and Zhaoqiao Reservoir differed, reflecting the environmental characteristics of each area and the influence of human activities on ARGs.

Table 1. Abundance of ARGs (copies/16S rRNA) (mean $\pm$ S.D) found in three reservoirs in May and September.

	May– Pingshan	Sep– Pingshan	May– Shanhu	Sep– Shanhu	May– Zhaoqiao	Sep– Zhaoqiao
Sulfonamides	0.16 $\pm$ 0.14	0.31 $\pm$ 0.08	0.6 $\pm$ 0.18	0.73 $\pm$ 0.16	0.13 $\pm$ 0.02	0.34 $\pm$ 0.08
Tetracyclines	1.91 $\pm$ 0.68	2.5 $\pm$ 0.92	0.42 $\pm$ 0.18	1.22 $\pm$ 0.15	0.52 $\pm$ 0.16	0.52 $\pm$ 0.16

First, the highest concentration of Σ ARG was found in Pingshan Reservoir, especially the concentration of tetracycline, which was significantly higher than that of Shanhu and Zhaoqiao, indicating that antibiotic resistance gene contamination was more serious in this area. In contrast, we observed that Zhaoqiao Reservoir's ARG concentration was cumulatively lower when compared with other reservoirs, especially in different seasons. The concentrations of sulfonamide resistance genes and tetracycline resistance genes in September were significantly higher than those in May, indicating that seasonal variations

significantly impacted the ARG concentration, which might be related to the heavy rainfall. In Nanjing, the precipitation was more concentrated in May than in September, and the degree of eutrophication in the water bodies was higher in May than in September. On the one hand, the heavy rainfall delivered new ARGs into the aquatic environment through surface runoff, and, on the other hand, the heavy rainfall significantly diluted the concentration of ARGs in the water bodies [2,13,76,77]. Here, the concentration in September may be higher than that in May because surface runoff in May brings new ARG pollution, which has less of an impact than the effect of substantial rainfall in diluting the ARG concentration in the water body. The higher abundance of ARGs in September may be attributed to several factors. First, elevated temperatures in September can enhance microbial activity and facilitate horizontal gene transfer (HGT), a key mechanism for ARG dissemination. Second, reduced rainfall during this period likely decreases the dilution of ARGs, resulting in higher concentrations compared to May. Additionally, seasonal agricultural activities, such as fertilizer application, may introduce nutrients that promote microbial growth and ARG proliferation.

Further analysis showed that the tetracycline genes in Shanhu showed the most significant difference, while those in Zhaoqiao Reservoir remained almost the same. However, Zhaoqiao showed the most significant difference in regard to sulfonamide resistance genes. The concentration of sulfonamide resistance genes in Shanhu was significantly higher than that in the other reservoirs, which might be related to environmental factors or pollutants in the area. Although the levels of ARGs in these reservoirs were lower than those usually found in polluted environments, they were slightly higher than those found in Shaanxi Province, China's Heihe Reservoir, Guangzhou City's Xikeng Reservoir, and Chaohu Lake [3,30]. This suggests that some anthropogenic activities may have affected the source reservoirs. However, the ARG loads are still lighter compared to more heavily polluted environments and are lower overall than those found in urban rivers that are more heavily affected by anthropogenic activities, such as the Minxin River in Shijiazhuang [78,79]. The higher abundance of *sul* genes is closely related to the greater persistence of sulfonamide antibiotics and their long history of application, as reported by multiple studies [19,50,80]. Moreover, *sul1* genes were the most abundant among the sulfonamide resistance genes [32,78,79]. In contrast, the *sul2* gene was less abundant, possibly related to its presence in restricted non-conjugative plasmids, thus affecting its spread and distribution [70]. Based on the correlation between antibiotics and resistance genes, *sul* was significantly positively correlated with *intl1* (Figures S1 and S2), probably because the resistance genes of sulfonamide antibiotics were easily captured by *intl1*, and *sul* genes were mainly carried by integrons (*intl1*) [70,78,79]. The PCA findings also revealed that *sul1* was consistently correlated with *intl1* across all the reservoirs, underscoring the role of mobile genetic elements in spreading sulfonamide resistance genes. Notably, in Zhaoqiao Reservoir, *sul1* showed a strong positive correlation with *intl1* in September, emphasizing its importance in shaping ARG profiles during this season. This conclusion is further supported by the fact that higher abundances of *sul1* and *sul2* were also detected in studies on the Minxin River in Shijiazhuang and the Liao River in northeast China [78,79]. The current study suggests that mobile integrons play an important role in spreading antibiotic resistance genes (ARGs), especially in regard to environmental pollution, where they promote the level and spread of ARGs [23].

In the tetracycline resistance genes (*tet* genes) analysis, *tetC* was generally the most abundant in the water column, while *tetA* and *tetX* were detected at a lower frequency. Moreover, *tetO* and *tetC* had a higher abundance, which may be closely related to agricultural activities, such as soil fertilization [80,81]. In particular, the abundance of tetracycline and sulfonamide genes (e.g., *tetM*, *tetO*, *sul1*, *sul2*) was higher in soils with manure ap-

plication, suggesting that agricultural activities are one of the most important sources of tetracycline resistance genes [82]. The use of tetracycline antibiotics and the spread of resistance genes are closely related to environmental selection pressure [70]. In this environment, plasmids act as important carriers of antibiotic resistance genes (ARGs) and facilitate the spread of resistance genes [12]. The integron *int1*, a marker of anthropogenic contamination-induced selective pressure on ARGs, can facilitate the extensive spread of antibiotic resistance through horizontal gene transfer [78,79]. Higher *int1* abundance revealed that horizontal gene transfer may occur in multiple environmental media, further promoting the spread of antibiotic resistance genes in different ecosystems [32,70].

In the specific reservoir studies, *sul2* was significantly and positively correlated with the concentration of SMX (Figures S1 and S2), similar to the findings in Honghu Lake. The PCA findings show that *sul2* and SMX are positively correlated across all the reservoirs, particularly in Zhaoqiao Reservoir (e.g., May–Zhaoqiao). This suggests that higher SMX concentrations may drive the prevalence of *sul2*, aligning with the findings in Honghu Lake. In Shanhu Lake, *tetC* was positively correlated with the concentration of OTC (Figures S1 and S2), similar to the findings in Honghu Lake [23]. It is crucial that SMZ was significantly negatively correlated with STZ in our three reservoirs, indicating significant non-homology between the two, contrary to the results from the Yitong River [30]. In addition, the negative correlation between *sul1* and *sul2* (Figures S1 and S2), which was different from most other studies, might be related to the different distribution modes of the mobile genetic elements, suggesting that mobile genetic elements, such as integrons, play a crucial role in the dissemination of ARGs [21,70]. The negative correlation could arise from how *sul1* and *sul2* are associated with MGEs, selective pressures, and the dynamics of horizontal gene transfer within microbial communities. In Pingshan Reservoir, *sul1*, *sul2*, *tetE*, *tetX*, *tetO*, *tetC*, and *int1* were positively correlated with each other (Figures S1 and S2), suggesting potential co-selection mechanisms, but does not necessarily imply shared contamination sources [21]. Similar findings were observed in the PCA analysis, thus further emphasizing the potential possibility of a co-selection mechanism (Figure S2). These results indicate significant correlations among most antibiotics, reflecting their interactions and co-transmission mechanisms in water bodies. They provide an important reference for the control and management of sulfonamide antibiotic pollution in urban rivers [50].

3.4. Seasonal Variation in the Antibiotics Among the Reservoirs

Figure 6 illustrates the seasonal variations in sulfonamide and tetracycline antibiotic concentrations observed in the Pingshan, Shanhu, and Zhaoqiao reservoirs. The analysis revealed significant changes in sulfamethazine in two reservoirs (Shanhu and Zhaoqiao). In contrast, no significant differences were observed for other antibiotics (Table S3).

The sulfamethazine concentrations decreased significantly in Shanhu (from 2.15 ng/L in May to 1.23 ng/L in September, $p = 0.01$) and Zhaoqiao (from 1.13 ng/L in May to 0.71 ng/L in September, $p = 0.03$). These reductions suggest that sulfamethazine contamination is highly sensitive to seasonal factors, likely influenced by agricultural runoff patterns. Sulfamethazine is commonly used in veterinary practices, and its decline during the drier months of September may reflect reduced application or leaching into water bodies during this period [83]. For instance, tetracycline levels in Zhaoqiao were consistently high (12.45 ± 3.48 ng/L in May vs. 12.45 ± 2.98 ng/L in September), suggesting persistent contamination from continuous sources, such as livestock farming or wastewater discharge [5]. Similarly, sulfonamides like sulfadiazine and sulfamethoxazole exhibited minimal seasonal variability, indicating their persistence in the environment and resistance to degradation [84].

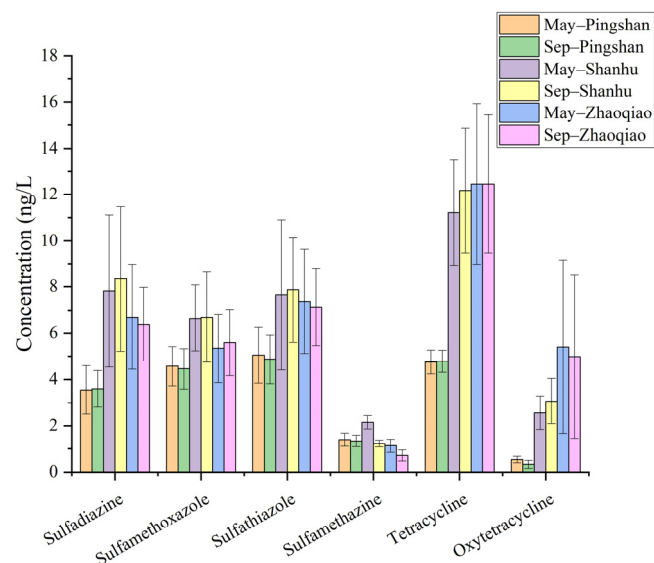


Figure 6. Seasonal variations in sulfonamide and tetracycline antibiotic concentrations across three reservoirs.

The observed variations highlight the complex interplay between antibiotic usage patterns, environmental conditions, and contamination sources. The significant reduction in sulfamethazine during September underscores its sensitivity to seasonal agricultural activities, making it a potential target for mitigation strategies during peak usage [3]. The seasonal variation in sulfamethazine concentrations may be linked to its widespread use in agriculture and aquaculture practices. Globally, sulfamethazine is commonly used in veterinary medicine to treat bacterial infections in livestock, as well as in aquaculture and crop farming to prevent disease outbreaks [85,86]. Manure or organic fertilizers containing antibiotic residues peak during planting in many regions, often coinciding with late spring or early summer. These practices can lead to higher runoff of sulfamethazine into water bodies during increased precipitation, such as in May in the areas in our study. In contrast, the persistence of other antibiotics, such as tetracycline and sulfonamides, suggests ongoing inputs from consistent sources, emphasizing the need for long-term management practices to address these contaminants. Tetracyclines, in particular, are widely used in livestock farming and aquaculture, and their elevated concentrations in Zhaoqiao indicate localized contamination from these activities [4]. The lack of significant seasonal changes for most antibiotics also highlights their environmental stability and resistance to degradation, posing risks for the development of antibiotic-resistant bacteria (ARB) and resistance genes (ARGs) [23]. These findings align with previous studies showing that antibiotics like tetracycline and sulfonamides can persist in aquatic environments due to their chemical stability and continuous release from anthropogenic sources [49]. The reservoir-specific results further emphasize the importance of location-specific monitoring programs. For example, Shanhu exhibited higher sulfonamide concentrations, likely due to its proximity to agricultural areas, while Zhaoqiao's elevated tetracycline levels suggest contamination from livestock farming or aquaculture. These insights can inform targeted interventions, such as reducing antibiotic use during critical periods or improving waste management practices, to mitigate environmental and public health risks associated with antibiotic pollution.

Figure 7 illustrates the risk quotients (RQs) for the target antibiotics in the surface water of the three reservoirs during May and September. The results show that for SMX and TC, Shanhu and Zhaoqiao reservoirs present moderate acute ecological risk in river water, while the remaining antibiotics exhibit low acute risk levels. Antibiotic concentrations in the target water bodies have not yet reached a high-risk level. Therefore, pollution control

and management must be further strengthened to reduce the ecological risk and ensure the RQ does not exceed the guideline values [30].

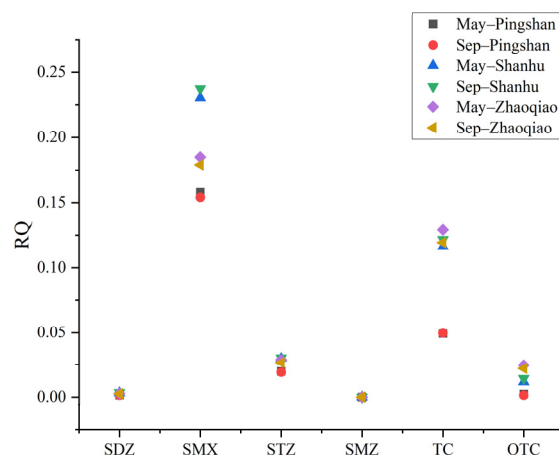


Figure 7. The mean RQ of the target antibiotics in surface water.

Although no large-scale factories or hospitals are located around these reservoirs, the antibiotic resistance gene (ARG) contamination in these two reservoirs remains relatively severe. It may be closely related to the surrounding area's small-scale residential and agricultural activities. In particular, antibiotics and their residues enter water bodies through agricultural wastewater and surface runoff, one of the primary sources of ARG pollution [12]. Agricultural activities, especially applying manure and organic waste containing antibiotics, significantly increased the concentration of resistance genes in water bodies [87]. In addition, the three reservoirs we studied are located along the middle and lower reaches of the Yangtze River, and the connectivity of the regional water network makes it easy for pollutants to spread between different water bodies [17]. The exchange and flow between water bodies exacerbated the spread of contamination, further expanding the scope of contamination [69]. In contrast, Zhaoqiao Reservoir is located in a more remote area, surrounded by a lower population density, a relatively better ecological environment, and is less affected by human activities. Hence, the concentration of ARGs in its water bodies is lower. There are no significant agricultural or industrial pollution sources around Zhaoqiao Reservoir, and the use of antibiotics is low, which results in a lower level of ARG pollution in this reservoir, indicating that differences in geographic location and population density have a significant effect on the extent of ARG pollution [78,79].

3.5. Correlation Between Antibiotics, Heavy Metals, and Water Quality Parameters

Figure 8 presents the Spearman correlation analysis of the antibiotics and physicochemical parameters across the three reservoirs during May and September. The analysis revealed intricate relationships that highlight the complex interactions within aquatic ecosystems. In the Pingshan Reservoir during May, SDZ exhibited a consistent correlation with several physicochemical parameters, particularly showing a moderate negative correlation with pH and a positive correlation with temperature, similar to the findings reported by [30]. Figure 9 displays the principal component analysis (PCA) results, which quantify the correlations between antibiotics and physicochemical parameters across the three reservoirs. The PCA results for the May-Pingshan Reservoir confirm this, showing SDZ loading significantly on PC1 and PC2, which are associated with temperature and pH changes. These patterns persisted in September, where SDZ maintained significant correlations with TOC and COD, suggesting that the presence of the antibiotics might be linked to organic pollution levels, as noted in freshwater lake and river studies [50,88]. The relationship between SMX and conventional water quality parameters demonstrated even

stronger connections, particularly in the Shanhui Reservoir during May. SMX showed a strong positive correlation with TN, while maintaining significant relationships with heavy metals like Cu and Zn, supporting the observations in similar studies [19].

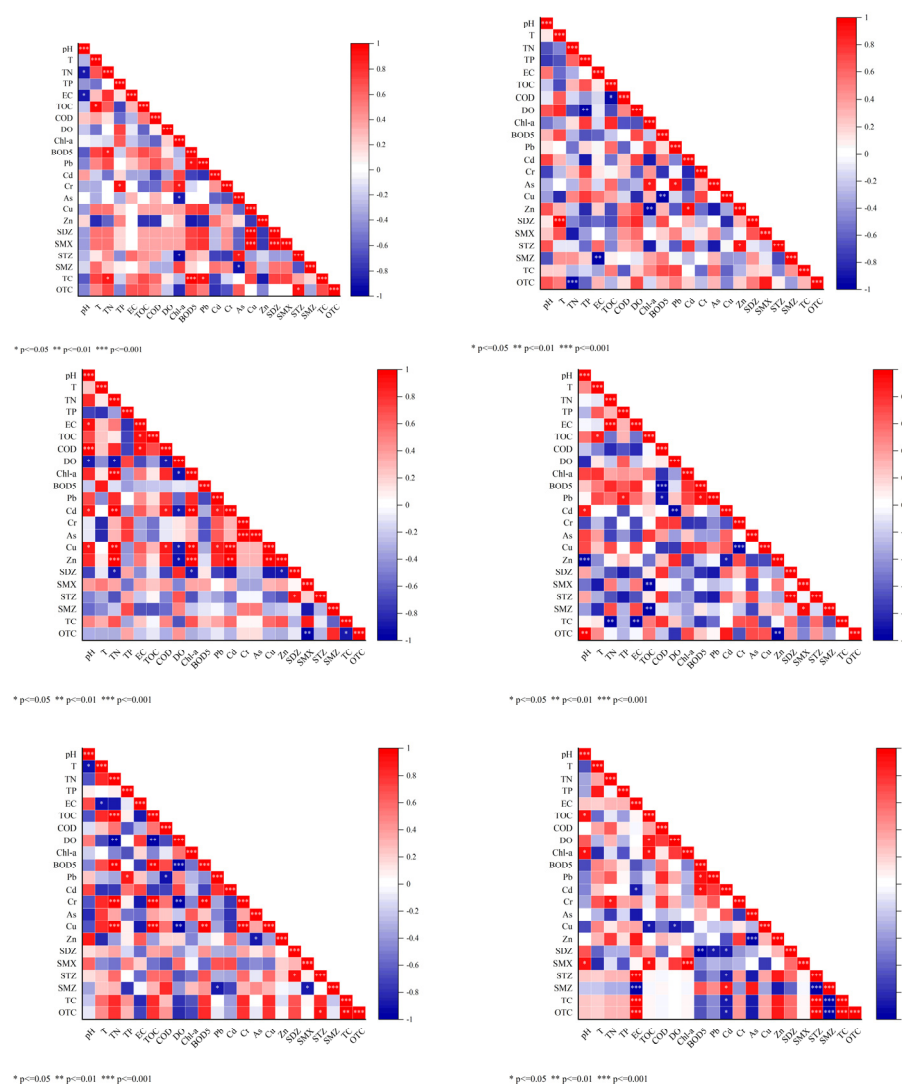


Figure 8. Relationships between antibiotics and physicochemical parameters using Spearman's correlation analysis in three reservoirs: (**top row**) May–Pingshan, Sep–Pingshan; (**middle row**) May–Shanhui, Sep–Shanhui; (**bottom row**) May–Zhaoqiao, Sep–Zhaoqiao.

In the Zhaoqiao Reservoir, the correlation patterns between the antibiotics and other parameters revealed particularly noteworthy seasonal variations. During May, SMX demonstrated a strong negative correlation with DO, while showing a significant positive relationship with the biological oxygen demand, BOD, aligning with other global studies indicating that antibiotic concentrations often increase in conditions involving higher organic loads [53]. The PCA results for the May–Zhaoqiao Reservoir emphasized this, showing SMX loading heavily on PC2 and PC4, which were linked to biological oxygen demand and dissolved oxygen levels. These relationships shifted dramatically in September, wherein SMX exhibited stronger correlations with heavy metals, particularly showing a moderate positive correlation with cadmium, Cd, and Cr. The consistent correlation patterns observed between the antibiotics and traditional water quality parameters suggest that these compounds might serve as indicators of broader anthropogenic impacts, which resonates with emerging research from rapidly developing regions documented by [14,27,51]. Furthermore, the strong correlations observed between STZ and various

water quality parameters across all the studied reservoirs indicate that antibiotic contamination might be more deeply integrated into the overall water quality profile than previously understood, supporting the hypothesis proposed by [85].

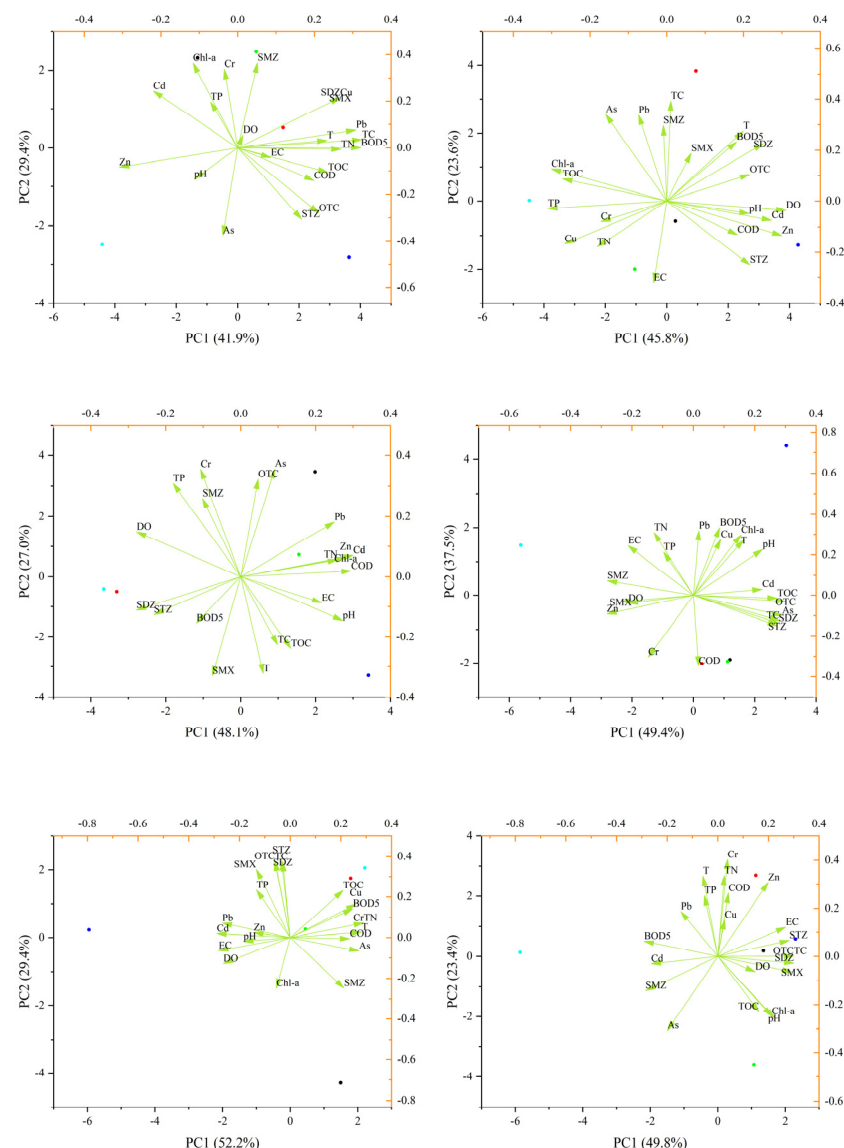


Figure 9. Principal component analysis of the quantitative correlation between antibiotics and physicochemical parameters in three reservoirs: (**top row**) May–Pingshan, Sep–Pingshan; (**middle row**) May–Shanhu, Sep–Shanhu; (**bottom row**) May–Zhaoqiao, Sep–Zhaoqiao.

The temporal variations observed between the May and September samplings highlight the dynamic nature of antibiotic interactions within aquatic environments. In the Pingshan Reservoir, the shift from moderate correlations between SMX and Pb in May to stronger relationships with heavy metals like Cu in September reflects seasonal changes documented in other studies [88]. These fluctuations likely result from the combined effects of temperature variations, biological activity cycles, and human activities within the watershed. The consistently strong correlations between antibiotics and nutrients across all the sites confirm established ecological relationships, but reveal site-specific nuances that could inform targeted management strategies. For example, the exceptionally high correlation between SMX and TN in Zhaoqiao Reservoir during May suggests this location might be particularly vulnerable to combined pollution events, supporting the vulnerability assessment framework proposed by [39]. Compared to global datasets, our findings show

similarities and distinct differences that could be attributed to regional characteristics. The observed relationships between antibiotics and organic pollutants, particularly the consistent correlation patterns between SDZ and various heavy metals, differ from those reported in other studies [89,90], potentially indicating different contamination pathways or environmental behaviors.

The observed relationships between antibiotics and other water quality parameters across the different reservoirs reveal expected and unexpected patterns warranting detailed examination. Across all the studied reservoirs, SDZ showed significant correlations with multiple parameters, particularly TN, TP, and EC, similar to other global studies indicating that antibiotic presence is a crucial indicator of overall water quality and ecosystem health [50,90]. Notably, the strong negative correlation between SDZ and pH in the May–Pingshan Reservoir mirrors observations from similar freshwater systems worldwide, suggesting that increased nutrient loads typically lead to decreased pH levels through various biochemical processes. However, the positive correlation between SDZ and EC observed in the Shanhu Reservoir during September presents an interesting contrast to many international studies, where such a strong relationship is less commonly reported. This discrepancy might be attributed to local geological characteristics or specific seasonal variations unique to this region, as suggested by [91]. Temperature emerged as another critical factor influencing antibiotic dynamics, showing complex interactions with BOD and heavy metals like Cd and Cr. The PCA findings highlighted temperature's role on various principal components, particularly those associated with BOD and heavy metals. The negative correlation between temperature and DO across most reservoirs confirms well-documented global patterns, yet the strength of these relationships varied significantly between sampling periods and locations. The integration of PCA findings with correlation analyses underscores the complex interplay between antibiotics, heavy metals, and water quality parameters, revealing trends and site-specific dynamics that reflect regional environmental conditions and anthropogenic pressures. These insights emphasize the need for site-specific management strategies to address antibiotic contamination and its implications for aquatic ecosystem health.

4. Future Recommendations

First, expanding monitoring efforts is essential to capture long-term trends and seasonal variations in ARG abundance. This includes increasing sample sizes, incorporating additional reservoirs across diverse geographical regions, and utilizing advanced analytical techniques, such as metagenomic sequencing and high-throughput qPCR, to comprehensively profile ARGs, mobile genetic elements (MGEs), and microbial communities. Investigating anthropogenic sources, such as agricultural runoff, wastewater discharge, aquaculture, and urban activities, is also critical. Source tracking studies can help identify and quantify contributions from organic fertilizers, manure, sewage sludge, potential ARGs, and heavy metals sources. Additionally, developing mitigation strategies through stricter regulations on antibiotic use in agriculture, aquaculture, and healthcare can reduce environmental inputs of antibiotics and ARGs. Investigating the role of algal blooms and microbial community dynamics in modulating ARG proliferation can inform strategies to mitigate their impacts. Exploring the impacts of climate change-induced factors, such as rising temperatures, altered precipitation patterns, and extreme weather events, on ARG dynamics is also necessary to understand their combined effects with anthropogenic stressors. Finally, targeting high-risk reservoirs, those with significant eutrophication, heavy metal contamination, or proximity to intensive agricultural and urban areas, can guide targeted interventions and remediation efforts. Developing location-specific management

plans based on each reservoir's unique characteristics and vulnerabilities will ensure that solutions are effective and sustainable.

5. Conclusions

This study highlights the presence and dynamics of antibiotic resistance genes (ARGs) in urban reservoirs, emphasizing their role as potential hotspots for ARG proliferation. The findings underscore the importance of integrating water quality parameters, heavy metal contamination, and seasonal variations into future assessments of ARG contamination. While this study provides valuable insights into ARGs' spatial and temporal patterns, further research is needed to understand the mechanisms driving ARG proliferation in aquatic environments.

Future investigations should prioritize expanding the sample size and incorporating longitudinal monitoring across multiple seasons to capture dynamic ARG abundance and distribution changes. Additionally, exploring the interactions between ARGs, heavy metals, and microbial communities will shed light on co-selection mechanisms that may exacerbate resistance gene spread. The observed correlations between water quality indicators, such as total nitrogen (TN), total phosphorus (TP), and chlorophyll-a (Chl-a), and ARG abundance suggest that nutrient management strategies could play a critical role in mitigating ARG proliferation in eutrophic reservoirs.

Further research should also examine the contribution of urban reservoirs to the broader dissemination of ARGs in aquatic ecosystems and their potential impact on public health. Investigating the role of anthropogenic pressures, such as agricultural runoff and wastewater discharge, in shaping ARG profiles will provide actionable insights for policymakers. Advanced molecular techniques, including metagenomic analysis, could help identify specific microbial taxa associated with ARGs and elucidate horizontal gene transfer pathways.

Ultimately, this study underscores the need for integrated water quality management strategies that consider environmental and anthropogenic factors. By addressing these knowledge gaps, future research can inform targeted interventions to reduce ARG contamination and mitigate antibiotic resistance's ecological and public health risks in freshwater systems.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/environments12030096/s1>, Table S1: Sampling locations from the three reservoirs; Table S2: Spatial and seasonal correlation of heavy metals, physicochemical parameters, and antibiotics in three reservoirs (NS means $p > 0.05$, * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$, **** means $p < 0.0001$); Table S3: Seasonal concentration of antibiotics observed among three reservoirs; Figure S1: Relationships between antibiotics and antibiotic resistance genes using Spearman's correlation analysis in three reservoirs. (a: May–Pingshan, b: Sep–Pingshan, c: May–Shanhu, d: Sep–Shanhu, e: May–Zhaoqiao, f: Sep–Zhaoqiao); Figure S2: Principal component analysis of the quantitative correlation between antibiotics and ARGs in three reservoirs. (a: May–Pingshan, b: Sep–Pingshan, c: May–Shanhu, d: Sep–Shanhu, e: May–Zhaoqiao, f: Sep–Zhaoqiao).

Author Contributions: Conceptualization, A.R. and R.T.M.; methodology, S.L., Q.Z. and T.Z.; software, A.I.O.; validation, A.I.O., S.L. and T.Z.; formal analysis, A.I.O. and R.T.M.; investigation, A.R. and S.L.; resources, T.O.; data curation, Q.Z.; writing—original draft preparation, A.R. and S.L.; writing—review and editing, T.O. and A.I.O.; visualization, R.T.M.; supervision, A.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data requests can be made to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Okoye, C.O.; Nyaruaba, R.; Ita, R.E.; Okon, S.U.; Addey, C.I.; Ebido, C.C.; Opabunmi, A.O.; Okeke, E.S.; Chukwudozie, K.I. Antibiotic resistance in the aquatic environment: Analytical techniques and interactive impact of emerging contaminants. *Environ. Toxicol. Pharmacol.* **2022**, *96*, 103995. [\[CrossRef\]](#) [\[PubMed\]](#)
- Wang, S.; Nie, W.; Gu, Q.; Wang, X.; Yang, D.; Li, H.; Wang, P.; Liao, W.; Huang, J.; Yuan, Q.; et al. Spread of antibiotic resistance genes in drinking water reservoirs: Insights from a deep metagenomic study using a curated database. *Water Res.* **2024**, *256*, 121572. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zhang, Y.; Liu, L.; Liu, Y.; Chen, L.; Wang, J.; Li, Y.; Wang, K.; Wang, W. Deciphering the natural and anthropogenic drivers on the fate and risk of antibiotics and antibiotic resistance genes (ARGs) in a typical river-estuary system, China. *J. Hazard. Mater.* **2024**, *480*, 136006. [\[CrossRef\]](#) [\[PubMed\]](#)
- Cherian, T.; Ragavendran, C.; Vijayan, S.; Kurien, S.; Peijnenburg, W.J.G.M. A review on the fate, human health and environmental impacts, as well as regulation of antibiotics used in aquaculture. *Environ. Adv.* **2023**, *13*, 100411. [\[CrossRef\]](#)
- Dong, J.; Yan, D.; Mo, K.; Chen, Q.; Zhang, J.; Chen, Y.; Wang, Z. Antibiotics along an alpine river and in the receiving lake with a catchment dominated by grazing husbandry. *J. Environ. Sci.* **2022**, *115*, 374–382. [\[CrossRef\]](#)
- Xu, L.; Zhang, H.; Xiong, P.; Zhu, Q.; Liao, C.; Jiang, G. Occurrence, fate, and risk assessment of typical tetracycline antibiotics in the aquatic environment: A review. *Sci. Total Environ.* **2021**, *753*, 141975. [\[CrossRef\]](#)
- Barathe, P.; Kaur, K.; Reddy, S.; Shriram, V.; Kumar, V. Antibiotic pollution and associated antimicrobial resistance in the environment. *J. Hazard. Mater. Lett.* **2024**, *5*, 100105. [\[CrossRef\]](#)
- Jiang, S.; Shi, B.; Zhu, D.; Cheng, X.; Zhou, Z.; Xie, J.; Chen, Z.; Sun, L.; Zhang, Y.; Xie, Y.; et al. Cross-contamination and ecological risk assessment of antibiotics between rivers and surrounding open aquaculture ponds. *Environ. Pollut.* **2024**, *344*, 123404. [\[CrossRef\]](#)
- Ahmed, S.K.; Hussein, S.; Qurbani, K.; Ibrahim, R.H.; Fareeq, A.; Mahmood, K.A.; Mohamed, M.G. Antimicrobial resistance: Impacts, challenges, and future prospects. *J. Med. Surg. Public Health* **2024**, *2*, 100081. [\[CrossRef\]](#)
- Huang, H.; Zeng, S.; Dong, X.; Li, D.; Zhang, Y.; He, M.; Du, P. Diverse and abundant antibiotics and antibiotic resistance genes in an urban water system. *J. Environ. Manag.* **2019**, *231*, 494–503. [\[CrossRef\]](#)
- Wang, H.; Hou, L.; Liu, Y.; Liu, K.; Zhang, L.; Huang, F.; Wang, L.; Rashid, A.; Hu, A.; Yu, C. Horizontal and vertical gene transfer drive sediment antibiotic resistome in an urban lagoon system. *J. Environ. Sci.* **2021**, *102*, 11–23. [\[CrossRef\]](#)
- Wang, S.; Fang, L.; Sun, X.; Lu, W. Occurrence and distribution of antibiotic resistance genes in urban rivers with black-odor water of Harbin, China. *Environ. Res.* **2024**, *259*, 119497. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zhang, K.; Xin, R.; Zhao, Z.; Ma, Y.; Zhang, Y.; Niu, Z. Antibiotic Resistance Genes in drinking water of China: Occurrence, distribution and influencing factors. *Ecotoxicol. Environ. Saf.* **2020**, *188*, 109837. [\[CrossRef\]](#) [\[PubMed\]](#)
- Li, Y.; Wang, J.; Lin, C.; Lian, M.; He, M.; Liu, X.; Ouyang, W. Occurrence, removal efficiency, and emission of antibiotics in the sewage treatment plants of a low-urbanized basin in China and their impact on the receiving water. *Sci. Total Environ.* **2024**, *921*, 171134. [\[CrossRef\]](#)
- Li, S.; Ondon, B.S.; Ho, S.-H.; Zhou, Q.; Li, F. Drinking water sources as hotspots of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs): Occurrence, spread, and mitigation strategies. *J. Water Process Eng.* **2023**, *53*, 103907. [\[CrossRef\]](#)
- Motsinger-Reif, A.A.; Reif, D.M.; Akhtari, F.S.; House, J.S.; Campbell, C.R.; Messier, K.P.; Fargo, D.C.; Bowen, T.A.; Nadadur, S.S.; Schmitt, C.P.; et al. Gene-environment interactions within a precision environmental health framework. *Cell Genom.* **2024**, *4*, 100591. [\[CrossRef\]](#)
- Li, T.; Tao, S.; Ma, M.; Liu, S.; Shen, M.; Zhang, H. Is the application of organic fertilizers becoming an undeniable source of microplastics and resistance genes in agricultural systems? *Sci. Total Environ.* **2024**, *912*, 169571. [\[CrossRef\]](#)
- Ji, W.; Ma, J.; Zheng, Z.; Al-Herrawy, A.Z.; Xie, B.; Wu, D. Algae blooms with resistance in fresh water: Potential interplay between Microcystis and antibiotic resistance genes. *Sci. Total Environ.* **2024**, *940*, 173528. [\[CrossRef\]](#)
- Yang, Y.; Song, W.; Lin, H.; Wang, W.; Du, L.; Xing, W. Antibiotics and antibiotic resistance genes in global lakes: A review and meta-analysis. *Environ. Int.* **2018**, *116*, 60–73. [\[CrossRef\]](#)
- Zheng, D.; Yin, G.; Liu, M.; Chen, C.; Jiang, Y.; Hou, L.; Zheng, Y. A systematic review of antibiotics and antibiotic resistance genes in estuarine and coastal environments. *Sci. Total Environ.* **2021**, *777*, 146009. [\[CrossRef\]](#)
- Ohore, O.E.; Addo, F.G.; Han, N.; Li, X.; Zhang, S. Profiles of ARGs and their relationships with antibiotics, metals and environmental parameters in vertical sediment layers of three lakes in China. *J. Environ. Manag.* **2020**, *225*, 109583. [\[CrossRef\]](#) [\[PubMed\]](#)
- Chu, K.; Liu, Y.; Hua, Z.; Lu, Y.; Ye, F. Spatio-temporal distribution and dynamics of antibiotic resistance genes in a water-diversion lake, China. *J. Environ. Manag.* **2023**, *348*, 119232. [\[CrossRef\]](#) [\[PubMed\]](#)
- Luo, Y.; Liu, C.; Wang, Y.; Yang, Y.; Mishra, S. Occurrence, distribution and their correlation with different parameters of antibiotics and antibiotic resistance genes in lakes of China: A review. *Mar. Pollut. Bull.* **2023**, *193*, 115189. [\[CrossRef\]](#)

24. Zhang, Q.; Zhang, Z.; Lu, T.; Peijnenburg, W.; Gillings, M.; Yang, X.; Chen, J.; Penuelas, J.; Zhu, Y.G.; Zhou, N.Y.; et al. Cyanobacterial blooms contribute to the diversity of antibiotic-resistance genes in aquatic ecosystems. *Commun. Biol.* **2020**, *3*, 737. [\[CrossRef\]](#)
25. Li, X.D.; Chen, Y.H.; Liu, C.; Hong, J.; Deng, H.; Yu, D.J. Eutrophication and Related Antibiotic Resistance of Enterococci in the Minjiang River, China. *Microb. Ecol.* **2020**, *80*, 1–13. [\[CrossRef\]](#)
26. Zhang, Y.; Wang, M.; Zhou, X.; Cheng, W.; Ren, J.; Wan, T.; Liu, X. Transmission mechanism of antibiotic resistance genes and their differences between water and sediment in the Weihe River Basin. *Environ. Res.* **2024**, *252*, 119057. [\[CrossRef\]](#)
27. Flach, K.A.; Bones, U.A.; Wolff, D.B.; de Oliveira Silveira, A.; da Rosa, G.M.; Carissimi, E.; Silvestri, S. Antibiotic resistant bacteria and genes (ARB and ARG) in water and sewage treatment units: A review. *Environ. Nanotechnol. Monit. Manag.* **2024**, *21*, 100941. [\[CrossRef\]](#)
28. Wang, M.; Masoudi, A.; Wang, C.; Wu, C.; Zhang, Z.; Zhao, X.; Liu, Y.; Yu, Z.; Liu, J. Impacts of net cages on pollutant accumulation and its consequence on antibiotic resistance genes (ARGs) dissemination in freshwater ecosystems: Insights for sustainable urban water management. *Environ. Int.* **2024**, *183*, 108357. [\[CrossRef\]](#)
29. Du, C.; Li, G.; Xia, R.; Li, C.; Zhu, Q.; Li, X.; Li, J.; Zhao, C.; Tian, Z.; Zhang, L. New insights into cyanobacterial blooms and the response of associated microbial communities in freshwater ecosystems. *Environ. Pollut.* **2022**, *309*, 119781. [\[CrossRef\]](#)
30. Zhao, K.; Li, C.; Wang, Q.; Lu, H. Distribution of Sulfonamide Antibiotics and Resistance Genes and Their Correlation with Water Quality in Urban Rivers (Changchun City, China) in Autumn and Winter. *Sustainability* **2022**, *14*, 7301. [\[CrossRef\]](#)
31. Venkatesan, M.; Fruci, M.; Verellen, L.A.; Skarina, T.; Mesa, N.; Flick, R.; Pham, C.; Mahadevan, R.; Stogios, P.J.; Savchenko, A. Molecular mechanism of plasmid-borne resistance to sulfonamide antibiotics. *Nat. Commun.* **2023**, *14*, 4031. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Guo, X.; Song, R.; Lu, S.; Liu, X.; Chen, J.; Wan, Z.; Bi, B. Multi-Media Occurrence of Antibiotics and Antibiotic Resistance Genes in East Dongting Lake. *Front. Environ. Sci.* **2022**, *10*, 866332. [\[CrossRef\]](#)
33. Zhang, Y.; Wang, H.; Hu, M.; Cai, R.; Miao, Y.; Zhu, X. Heavy metals potentially drive co-selection of antibiotic resistance genes by shifting soil bacterial communities in paddy soils along the middle and lower Yangtze River. *Pedosphere* **2024**, *34*, 606–619. [\[CrossRef\]](#)
34. Wu, T.; Zhang, Y.; Wang, B.; Chen, C.; Cheng, Z.; Li, Y.; Wang, B.; Li, J. Antibiotic resistance genes in Chishui River, a tributary of the Yangtze River, China: Occurrence, seasonal variation and its relationships with antibiotics, heavy metals and microbial communities. *Sci. Total Environ.* **2022**, *846*, 157472. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Zhang, W.; Suyamud, B.; Lohwacharin, J.; Yang, Y. Large-scale pattern of resistance genes and bacterial community in the tap water along the middle and low reaches of the Yangtze River. *Ecotoxicol. Environ. Saf.* **2021**, *208*, 111517. [\[CrossRef\]](#)
36. Igere, B.E.; Onohuean, H.; Nwodo, U.U. Modern knowledge-scape possess petite influence on the factual persistence of resistance determinants (ARGs/MGEs): A map and assessment of discharged wastewater and water bodies. *Heliyon* **2022**, *8*, e12253. [\[CrossRef\]](#)
37. Zhang, G.; Lu, S.; Wang, Y.; Liu, X.; Liu, Y.; Xu, J.; Zhang, T.; Wang, Z.; Yang, Y. Occurrence of antibiotics and antibiotic resistance genes and their correlations in lower Yangtze River, China. *Environ. Pollut.* **2020**, *257*, 113365. [\[CrossRef\]](#)
38. Zhang, J.; Chen, J.; Wang, C.; Wang, P.; Gao, H.; Feng, B.; Fu, J. Vertical variation of antibiotic resistance genes and their interaction with environmental nutrients in sediments of Taihu lake. *J. Environ. Manag.* **2024**, *370*, 122661. [\[CrossRef\]](#)
39. Zainab, S.M.; Junaid, M.; Xu, N.; Malik, R.N. Antibiotics and antibiotic resistant genes (ARGs) in groundwater: A global review on dissemination, sources, interactions, environmental and human health risks. *Water Res.* **2020**, *187*, 116455. [\[CrossRef\]](#)
40. APHA. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 2017.
41. Rajasekar, A.; Murava, R.T.; Norgbey, E.; Zhu, X. Spatial Distribution, Risk Index, and Correlation of Heavy Metals in the Chuhe River (Yangtze Tributary): Preliminary Research Analysis of Surface Water and Sediment Contamination. *Appl. Sci.* **2024**, *14*, 904. [\[CrossRef\]](#)
42. Lu, S.; Lin, C.; Lei, K.; Wang, B.; Xin, M.; Gu, X.; Cao, Y.; Liu, X.; Ouyang, W.; He, M. Occurrence, spatiotemporal variation, and ecological risk of antibiotics in the water of the semi-enclosed urbanized Jiaozhou Bay in eastern China. *Water Res.* **2020**, *184*, 116187. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Aminov, R.I.; Garrigues-Jeanjean, N.; Mackie, R.I. Molecular Ecology of Tetracycline Resistance: Development and Validation of Primers for Detection of Tetracycline Resistance Genes Encoding Ribosomal Protection Proteins. *Appl. Environ. Microbiol.* **2001**, *67*, 22–32. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Gao, P.; Mao, D.; Luo, Y.; Wang, L.; Xu, B.; Xu, L. Occurrence of sulfonamide and tetracycline-resistant bacteria and resistance genes in aquaculture environment. *Water Res.* **2012**, *46*, 2355–2364. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Frank, J.A.; Reich, C.I.; Sharma, S.; Weisbaum, J.S.; Wilson, B.A.; Olsen, G.J. Critical Evaluation of Two Primers Commonly Used for Amplification of Bacterial 16S rRNA Genes. *Appl. Environ. Microbiol.* **2008**, *74*, 2461–2470. [\[CrossRef\]](#)

46. Wang, Z.; Han, M.; Li, E.; Liu, X.; Wei, H.; Yang, C.; Lu, S.; Ning, K. Distribution of antibiotic resistance genes in an agriculturally disturbed lake in China: Their links with microbial communities, antibiotics, and water quality. *J. Hazard. Mater.* **2020**, *393*, 122426. [\[CrossRef\]](#)
47. European Commission. AC01769567, A. Technical Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances; Office for Official Publications of the European Communities: Luxembourg, 1996.
48. Jiang, Y.; Li, M.; Guo, C.; An, D.; Xu, J.; Zhang, Y.; Xi, B. Distribution and ecological risk of antibiotics in a typical effluent-receiving river (Wangyang River) in north China. *Chemosphere* **2014**, *112*, 267–274. [\[CrossRef\]](#)
49. Zhang, Y.; Wang, M.; Cheng, W.; Huang, C.; Ren, J.; Wan, T.; Gao, K. Effects of water environmental factors and antibiotics on bacterial community in urban landscape lakes. *Aquat. Toxicol.* **2023**, *265*, 106740. [\[CrossRef\]](#)
50. Duan, W.; Cui, H.; Jia, X.; Huang, X. Occurrence and ecotoxicity of sulfonamides in the aquatic environment: A review. *Sci. Total Environ.* **2022**, *820*, 153178. [\[CrossRef\]](#)
51. Okeke, E.S.; Nwankwo, C.E.I.; Ezeorba, T.P.C.; Ogugofor, M.O.; Nwuche, C.O. Antibiotic residues and antibiotic resistance genes in African water systems: Implications for safe drinking water, aquatic ecosystems, and Sustainable Development Goals. *J. Water Process Eng.* **2025**, *69*, 106636. [\[CrossRef\]](#)
52. Ripanda, A.; Rwiza, M.J.; Nyanza, E.C.; Hossein, M.; Alfred, M.S.; Mahmoud, A.E.D.; Murthy, H.C.A.; Bakari, R.; Vuai, S.A.H.; Machunda, R.L. Ecological Consequences of Antibiotics Pollution in Sub-Saharan Africa: Understanding Sources, Pathways, and Potential Implications. *Emerg. Contam.* **2025**, *11*, 100475. [\[CrossRef\]](#)
53. Hu, T.; Zhang, J.; Xu, X.; Wang, X.; Yang, C.; Song, C.; Wang, S.; Zhao, S. Bioaccumulation and trophic transfer of antibiotics in the aquatic and terrestrial food webs of the Yellow River Delta. *Chemosphere* **2023**, *323*, 138211. [\[CrossRef\]](#) [\[PubMed\]](#)
54. SEPA. *Environmental Quality Standard for Surface Water*; Ministry of Environmental Protection: Beijing, China, 2002.
55. Wu, R.; Liu, Y.; Zhang, S.; Shi, X.; Zhao, S.; Lu, J.; Kang, X.; Wang, S.; Wu, Y.; Arvola, L. Characterization of nitrogen and phosphorus at the ice-water-sediment interface and the effect of their migration on overlying water quality in Daihai Lake (China) during the freezing period. *Sci. Total Environ.* **2023**, *893*, 164863. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Yang, C.; Yang, P.; Geng, J.; Yin, H.; Chen, K. Sediment internal nutrient loading in the most polluted area of a shallow eutrophic lake (Lake Chaohu, China) and its contribution to lake eutrophication. *Environ. Pollut.* **2020**, *262*, 114292. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Zhao, J.; Hou, S.; Zhang, H.; Sun, S.; Guo, C.; Zhang, X.; Song, G.; Xu, J. Spatiotemporal variations and priority ranking of emerging contaminants in nanwan reservoir: A case study from the agricultural region in huaihe river basin in China. *J. Environ. Manag.* **2024**, *368*, 122195. [\[CrossRef\]](#)
58. Lin, S.-S.; Shen, S.-L.; Zhou, A.; Lyu, H.-M. Assessment and management of lake eutrophication: A case study in Lake Erhai, China. *Sci. Total Environ.* **2021**, *751*, 141618. [\[CrossRef\]](#)
59. Chen, L.; Shi, Y.; Wang, S.; Sun, M.; Wang, M.; Ren, X.; Gao, Z.; Zhou, Y.; Zhang, J.; Zhuang, W.; et al. Temperature and phosphorus: The main environmental factors affecting the seasonal variation of soil bacterial diversity in Nansi Lake Wetland. *Front. Microbiol.* **2023**, *14*, 1169444. [\[CrossRef\]](#)
60. Chen, Q.; Liu, Y.; Zhang, M.; Lin, K.; Wang, Z.; Liu, L. Seasonal responses of microbial communities to water quality variations and interaction of eutrophication risk in Gehu Lake. *Sci. Total Environ.* **2024**, *955*, 177199. [\[CrossRef\]](#)
61. Xia, K.; Wu, T.; Li, X.; Wang, S.; Tang, H.; Zu, Y.; Yang, Y. A novel method for assessing water quality status using MODIS images: A case study of large lakes and reservoirs in China. *J. Hydrol.* **2024**, *638*, 131545. [\[CrossRef\]](#)
62. Jiang, Y.; He, K.; Li, Y.; Qin, M.; Cui, Z.; Zhang, Y.; Yao, Y.; Chen, X.; Deng, M.; Gray, A.; et al. Driving Factors of Total Organic Carbon in Danjiangkou Reservoir Using Generalized Additive Model. *Water* **2022**, *14*, 891. [\[CrossRef\]](#)
63. Lee, K.-H.; Kam, J. Spatiotemporal patterns of water volume and total organic carbon concentration of agricultural reservoirs over South Korea. *Water Res.* **2024**, *256*, 121610. [\[CrossRef\]](#)
64. Ahmad, N.B.; Jaafaru, M.S.; Isa, Z.; Abdulhamid, Y.; Kakudi, R.A.; Ugya, A.Y.; Meguellati, K. High pollution loads engineer oxygen dynamics, ecological niches, and pathogenicity shifts in freshwater environments. *J. Hazard. Mater. Adv.* **2024**, *14*, 100425. [\[CrossRef\]](#)
65. Wang, L.; Liu, D.; Sun, Y.; Zhang, Y.; Chen, W.; Yuan, Y.; Hu, S.; Li, S. Machine learning-based analysis of heavy metal contamination in Chinese lake basin sediments: Assessing influencing factors and policy implications. *Ecotoxicol. Environ. Saf.* **2024**, *283*, 116815. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Tshisikhawe, C.S.; Ngole-Jeme, V.M. Temperature induced changes on heavy metal geochemical partitioning and mobility in contaminated and uncontaminated soils. *Environ. Pollut. Bioavailab.* **2024**, *36*, 2317747. [\[CrossRef\]](#)
67. Liu, L.; Ouyang, W.; Wang, Y.; Tysklind, M.; Hao, f.; Liu, H.; Hao, X.; Xu, Y.; Lin, C.; Su, L. Heavy metal accumulation, geochemical fractions, and loadings in two agricultural watersheds with distinct climate conditions. *J. Hazard. Mater.* **2020**, *389*, 122125. [\[CrossRef\]](#)

68. Xiao, W.; Zhang, Y.; Chen, X.; Sha, A.; Xiong, Z.; Luo, Y.; Peng, L.; Zou, L.; Zhao, C.; Li, Q. The Easily Overlooked Effect of Global Warming: Diffusion of Heavy Metals. *Toxics* **2024**, *12*, 400. [\[CrossRef\]](#)
69. Rajasekar, A.; Qiu, M.; Wang, B.; Murava, R.T.; Norgbey, E. Relationship between water quality, heavy metals and antibiotic resistance genes among three freshwater lakes. *Environ. Monit. Assess.* **2022**, *194*, 64. [\[CrossRef\]](#)
70. Shi, X.; Xia, Y.; Wei, W.; Ni, B.-J. Accelerated spread of antibiotic resistance genes (ARGs) induced by non-antibiotic conditions: Roles and mechanisms. *Water Res.* **2022**, *224*, 119060. [\[CrossRef\]](#)
71. Alves, J.; Dias, L.; Mateus, J.; Marques, J.; Graças, D.; Ramos, R.; Seldin, L.; Henriques, I.; Silva, A.; Folador, A. Resistome in Lake Bolonha, Brazilian Amazon: Identification of Genes Related to Resistance to Broad-Spectrum Antibiotics. *Front. Microbiol.* **2020**, *11*, 67. [\[CrossRef\]](#)
72. Lee, J.; Lee, S.; Hu, C.; Marion, J.W. Beyond cyanotoxins: Increased *Legionella*, antibiotic resistance genes in western Lake Erie water and disinfection-byproducts in their finished water. *Front. Microbiol.* **2023**, *14*, 1233327. [\[CrossRef\]](#)
73. Huang, Y.; Fu, M.; Chen, G.; Zhang, J.; Xu, P.; Pan, L.; Zhang, X.; Chen, X. Reducing the water residence time is inadequate to limit the algal proliferation in eutrophic lakes. *J. Environ. Manag.* **2023**, *330*, 117177. [\[CrossRef\]](#)
74. Komijani, M.; Shamabadi, N.S.; Shahin, K.; Eghbalpour, F.; Tahsili, M.R.; Bahram, M. Heavy metal pollution promotes antibiotic resistance potential in the aquatic environment. *Environ. Pollut.* **2021**, *274*, 116569. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Li, Z.-T.; Song, X.; Yuan, S.; Zhao, H.-P. Unveiling the inhibitory mechanisms of chromium exposure on microbial reductive dechlorination: Kinetics and microbial responses. *Water Res.* **2024**, *253*, 121328. [\[CrossRef\]](#) [\[PubMed\]](#)
76. Hu, Y.; Jiang, L.; Sun, X.; Wu, J.; Ma, L.; Zhou, Y.; Lin, K.; Luo, Y.; Cui, C. Risk assessment of antibiotic resistance genes in the drinking water system. *Sci. Total Environ.* **2021**, *800*, 149650. [\[CrossRef\]](#)
77. Yu, W.; Xu, Y.; Wang, Y.; Sui, Q.; Xin, Y.; Wang, H.; Zhang, J.; Zhong, H.; Wei, Y. An extensive assessment of seasonal rainfall on intracellular and extracellular antibiotic resistance genes in Urban River systems. *J. Hazard. Mater.* **2023**, *455*, 131561. [\[CrossRef\]](#)
78. Zhang, R.-D.; Gao, F.-Z.; Shi, Y.-J.; Zhao, J.-L.; Liu, Y.-S.; He, L.-Y.; Ying, G.-G. Metagenomic investigation of antibiotic resistance genes and resistant bacteria contamination in pharmaceutical plant sites in China. *Environ. Pollut.* **2024**, *357*, 124482. [\[CrossRef\]](#)
79. Yin, F.; Hao, W.; Zhang, H.; Miao, J.; Shi, H. Pollution status and spatial distribution of antibiotic resistance genes in urban surface water and surrounding soil media. *J. Environ. Chem. Eng.* **2024**, *12*, 113856. [\[CrossRef\]](#)
80. Szekeres, E.; Chiriac, C.M.; Baricz, A.; Szoke-Nagy, T.; Lung, I.; Soran, M.L.; Rudi, K.; Dragos, N.; Coman, C. Investigating antibiotics, antibiotic resistance genes, and microbial contaminants in groundwater in relation to the proximity of urban areas. *Environ. Pollut.* **2018**, *236*, 734–744. [\[CrossRef\]](#)
81. Hao, H.; Shi, D.-y.; Yang, D.; Yang, Z.-w.; Qiu, Z.-g.; Liu, W.-l.; Shen, Z.-q.; Yin, J.; Wang, H.-r.; Li, J.-w.; et al. Profiling of intracellular and extracellular antibiotic resistance genes in tap water. *J. Hazard. Mater.* **2019**, *365*, 340–345. [\[CrossRef\]](#)
82. Lu, L.; Liu, J.; Li, Z.; Zou, X.; Guo, J.; Liu, Z.; Yang, J.; Zhou, Y. Antibiotic resistance gene abundances associated with heavy metals and antibiotics in the sediments of Changshou Lake in the three Gorges Reservoir area, China. *Ecol. Indic.* **2020**, *113*, 106275. [\[CrossRef\]](#)
83. Qi, M.; He, P.; Hu, H.; Zhang, T.; Li, T.; Zhang, X.; Qin, Y.; Zhu, Y.; Guo, Y. An Automated Solid-Phase Extraction–UPLC–MS/MS Method for Simultaneous Determination of Sulfonamide Antimicrobials in Environmental Water. *Molecules* **2023**, *28*, 4694. [\[CrossRef\]](#)
84. Chen, H.; Zheng, W.; Shen, X.; Zhang, F.; Zhou, X.; Shen, J.; Lu, M. Occurrence, Distribution, and Ecological Risk Assessment of Antibiotics in Different Environmental Media in Anqing, Anhui Province, China. *Int. J. Environ. Res. Public Health* **2021**, *18*, 8112. [\[CrossRef\]](#) [\[PubMed\]](#)
85. Maghsodian, Z.; Sanati, A.M.; Mashifana, T.; Sillanpää, M.; Feng, S.; Nhat, T.; Ramavandi, B. Occurrence and Distribution of Antibiotics in the Water, Sediment, and Biota of Freshwater and Marine Environments: A Review. *Antibiotics* **2022**, *11*, 1461. [\[CrossRef\]](#)
86. Salma, U.; Nishimura, Y.; Tokumura, M.; Hossain, A.; Watanabe, K.; Noro, K.; Raknuzzaman, M.; Amagai, T.; Makino, M. Occurrence, seasonal variation, and environmental risk of multiclass antibiotics in the urban surface water of the Buriganga River, Bangladesh. *Chemosphere* **2025**, *370*, 143956. [\[CrossRef\]](#)
87. Fernandes, J.M.O.; Laht, M.; Karkman, A.; Voolaid, V.; Ritz, C.; Tenson, T.; Virta, M.; Kisand, V. Abundances of Tetracycline, Sulphonamide and Beta-Lactam Antibiotic Resistance Genes in Conventional Wastewater Treatment Plants (WWTPs) with Different Waste Load. *PLoS ONE* **2014**, *9*, e103705. [\[CrossRef\]](#)
88. Tong, L.; Qin, L.; Guan, C.; Wilson, M.E.; Li, X.; Cheng, D.; Ma, J.; Liu, H.; Gong, F. Antibiotic resistance gene profiling in response to antibiotic usage and environmental factors in the surface water and groundwater of Honghu Lake, China. *Environ. Sci. Pollut. Res.* **2020**, *27*, 31995–32005. [\[CrossRef\]](#)
89. Han, Q.F.; Zhao, S.; Zhang, X.R.; Wang, X.L.; Song, C.; Wang, S.G. Distribution, combined pollution and risk assessment of antibiotics in typical marine aquaculture farms surrounding the Yellow Sea, North China. *Environ. Int.* **2020**, *138*, 105551. [\[CrossRef\]](#)

90. Li, Q.; Na, G.; Zhang, L.; Lu, Z.; Gao, H.; Li, R.; Jin, S. Effects of corresponding and non-corresponding contaminants on the fate of sulfonamide and quinolone resistance genes in the Laizhou Bay, China. *Mar. Pollut. Bull.* **2018**, *128*, 475–482. [[CrossRef](#)]
91. Guo, X.; Feng, C.; Gu, E.; Tian, C.; Shen, Z. Spatial distribution, source apportionment and risk assessment of antibiotics in the surface water and sediments of the Yangtze Estuary. *Sci. Total Environ.* **2019**, *671*, 548–557. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.