DISTRIBUTION CHARACTERISTICS AND RISK ASSESSMENT OF ANTIBIOTICS AND HEAVY METALS IN THE YUDONG RIVER BASIN

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Abstract. Despite rivers being crucial to human civilization human activities have introduced increasing amounts of pollutants. The combined contamination of river waters by antibiotics and heavy metals has receiving growing attention. However, little is known about the co-occurrence of these contaminants in rivers affected by mining wastewater. By collecting and analyzing water samples from the Yudong River basin, we investigated the distribution patterns of antibiotics and heavy metals, assessed their respective risks, and explored potential relationships between them. Our findings revealed that sulfamethoxazole (143.68 ng/L) had the highest concentration, and the combined risk quotient of all antibiotics ranging from 10.17 to 33.54, which indicated severe antibiotic contamination. None of the heavy metal concentrations exceeded regulatory limits, and the single-factor and Nemero pollution indices suggested the river was non-polluted for heavy metals. Significant spatial variation was observed, with concentrations of oxytetracycline and roxithromycin increasing in the lower reaches, and Zn, As, and Cd concentrations increasing significantly in the middle reaches. A complex correlation between heavy metals and antibiotics was observed across different sections of the river. This study provides novel insights into the co-occurrence of antibiotics and heavy metals in river systems, offering valuable data to inform management strategies.

Keywords: *combined contamination, antibiotics, heavy metals, mining wastewater, Yudong River*

Introduction

Since their discovery, antibiotics have been extensively used to treat infectious diseases in humans and animals and their application have increased in agriculture, animal husbandry, and aquaculture (Deng et al., 2022). However, antibiotics are often not fully absorbed by organisms and are excreted in urine and feces (Gutiérrez et al., 2010). Due to their extensive use and frequent release into the environment, antibiotics are considered “pseudo-persistent” pollutants (Lu et al., 2020). Once introduced into the environment, antibiotics can disrupt carbon and nitrogen cycles and alter microbial community structures, posing a threat to ecosystem stability (Zhou et al., 2022). Similarly, heavy metals are harmful environmental pollutants characterized by persistent toxicity, non-biodegradability, and bioconcentration (Liu et al., 2019). Both antibiotics and heavy metals enter the environment through various pathways, including domestic

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wastewater and animal waste, and exhibit complex environmental behaviors. Moreover, they can interact to form compounds with variable structures and toxicities, which can significantly threaten human health and ecosystem safety (Zhou et al., 2022; Möhler et al., 2017; Zhao et al., 2023).

In recent years, the pollution of aquatic environments by both antibiotics and heavy metals has garnered increasing attention. Activities such as livestock farming, the application of animal manure to land, and the centralized discharge of industrial wastewater have contributed to the combined contamination of surface and groundwater (Dong et al., 2018; Yao et al., 2020). Most existing studies have focused on the presence of either antibiotics or heavy metals alone in water bodies. For instance, research has identified antibiotic contamination in rivers across Chongqing in Southwest China, Kunming in Yunnan, and Kaiyang in Guizhou (Zhang et al., 2021; Zeng et al., 2024; Wang et al., 2020), while other studies have reported heavy metal concentrations in rivers impacted by municipal waste disposal (Sharifi et al., 2016). Only a few studies have investigated the co-occurrence of antibiotics and heavy metals, such as those in the Pearl River and Yitong River basins, where both pollutants have been detected at varying levels (Zhao et al., 2023; Wang et al., 2023a). However, there is limited research on the combined pollution of antibiotics and heavy metals in rivers affected by both mine wastewater and human activities.

The Yudong River, located in Kaili City, Qiandongnan Prefecture, Guizhou Province, has faced severe pollution since the 1980s and 1990s due to uncontrolled coal mining. These activities led to extensive coal mine contamination, causing barren mountains, “rust” deposits, and unchecked acidic mine water that corroded vegetation, polluted soil, surface water, and groundwater, and severely damaged the aquatic ecosystem. Although the coal mines have since been closed and ecological restoration efforts initiated, studies indicate that heavy metal pollution persists in the Yudong River (Liang et al., 2023). In addition, the river is surrounded by residential communities engaged in daily life and agricultural activities, contributing to the discharge of domestic sewage and livestock wastewater, exacerbating water pollution in the basin. While some research has focused on heavy metal contamination in the basin, little is known about the co-occurrence of antibiotics and heavy metals in the Yudong River and their potential interactions.

To address the knowledge gaps in this field, we selected the Yudong River Basin as our study site. The primary objectives of this study were: (i) to analyze the distribution patterns of antibiotics and heavy metals in the river water; (ii) to assess the pollution risks associated with these contaminants; and (iii) to explore the interrelationships between antibiotics and heavy metals in the water bodies of the Yudong River Basin. By thoroughly investigating the distribution, risk levels, and interactions of antibiotics and heavy metals, this study provides critical insights into their coexistence in rivers impacted by mine wastewater and offers foundational data for managing antibiotic and heavy metal pollution in the Yudong River Basin.

Materials and methods

Studying areas and sample collection

The Yudong River, located in Kaili City, Qiandongnan Prefecture, Guizhou Province (*Fig. 1*), is a fourth-order tributary of the Yangtze River, extending 28.5 km with a watershed area of 234 km². It consists of two tributaries: the Baishui River to the north and the Pinglu River to the south. The study area lies in the foothills of the southeastern Guizhou

Plateau, within the transition zone between the Yunnan-Guizhou Plateau and the central hills. The region is characterized by mid-to-low mountainous terrain shaped by erosion and dissolution, with well-developed karst landforms including dissolved hills, depressions, peaks, and funnels. The terrain generally slopes from the southwest to the northeast, with elevations ranging from 600 to 1230 m and relative elevation differences of 50 to 150 m. The area experiences abundant rainfall, supporting rich water resources and dense forests, with a vegetation cover of 53.28%. The Yudong River serves multiple functions, including irrigation, industrial use, drinking water supply, and tourism. Historically, the basin was a major coal mining region, and despite mine closures, over 8 million m^3 of acidic wastewater continue to be discharged annually, severely contaminating both river water and drinking water sources in the basin (Wang et al., 2023b).

As shown in *Figure 1,* sampling was conducted in July 2022 along the Yudong River basin, from upstream to downstream. Sampling points S1-S7 represent upstream locations, S8-S19 midstream, and S20-S23 downstream. GPS was used to geolocate each sampling site, and surface water samples were collected from a depth of 0 to 30 cm using a 1 L plexiglass water sampler. The sampler was rinsed with pure water at each site, followed by thorough washing with river water. Three parallel water samples of equal volume were collected, combined into a single composite sample, and stored in 1 L polyethylene bottles. Samples were sealed, transported to the laboratory under refrigeration, and stored for further analysis.

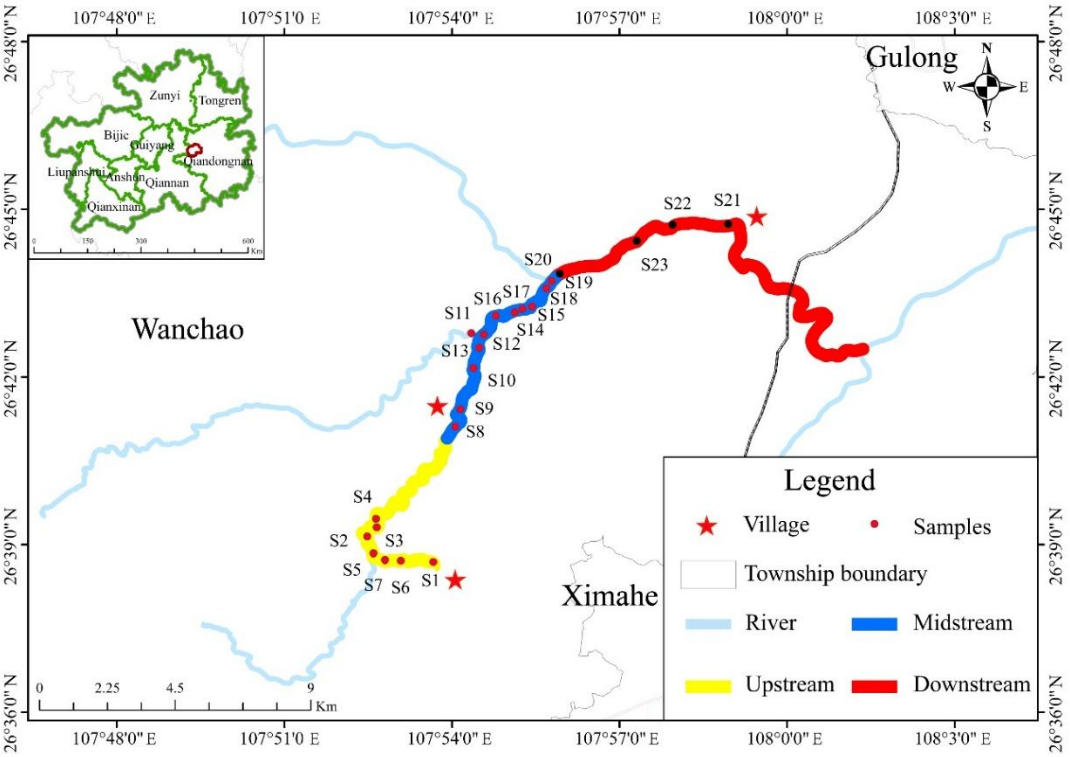

Figure 1. *Overview of the study area and distribution of sampling sites*

Testing for antibiotics

Referring to previous studies (Wang et al., 2020; Wei et al., 2024), water samples were filtered through a 0.45 µm glass fiber membrane to remove suspended particulate matter.

The filtered samples were then adjusted to a pH of 3.0 using 3.0 mol/L sulfuric acid, thoroughly mixed with 0.2 g of $C_{10}H_{14}N_2Na_2O_8$, and supplemented with 100 ng of each standard. The treated water samples were enriched at a flow rate of 10 mL/min by passing them through a 6 mL, 500 mg activated Oasis HLB cartridge (Waters Corporation, Milford, Massachusetts, USA), preconditioned with methanol and ultrapure water. After rinsing the cartridges with 10 mL of ultrapure water to eliminate impurities, they were vacuum dried for 30 min to remove residual moisture. The resulting dried cartridges were eluted with 6 mL of methanol into a brown vial. The solution was gently evaporated to near dryness using a nitrogen blower, then reconstituted in 1 mL of 10% aqueous methanol. After passing through a 0.2 μm needle filter, the solution was transferred to a 1.5 mL brown vial for subsequent assays.

The instrumental analysis of antibiotics was conducted using a high-performance liquid chromatograph tandem mass spectrometer (QTRAP 6500, AB) equipped with a Waters CORTECS C18+ column (1.8 μm, 2.1 × 75 mm) maintained at 40°C. The injection volume was 1 μL, and the flow rate was set to 0.3 mL/min. The analysis utilized electrospray ionization (ESI+) with a spray voltage of 5500 V and an ion source temperature of 450°C. The air curtain gas pressure was maintained at 35 psi, while the atomization gas (GS1) and auxiliary heating gas (GS2) were set at 50 psi. The collision gas (CAD) was maintained at a medium setting.

Detection of heavy metals

The collected water samples were initially filtered through a 0.45 μm cellulose acetate membrane using vacuum pressure to remove suspended particles. The filtrate was then collected in a 30 mL brown glass bottle (Zhao et al., 2023). Subsequently, the concentrations of heavy metals (Cu, Zn, Cr, As, Hg, Cd, and Pb) in the water were determined using inductively coupled plasma emission spectrometry.

Quality control

Stringent laboratory controls were implemented for all experimental procedures. Glassware underwent multiple wash cycles with ultrapure water and was thoroughly dried before use to eliminate residual organic contaminants. Program blanks and method blanks were employed to account for environmental background contamination, while appropriate reagent blanks were prepared for sample testing. National standard samples were utilized for quality control.

Risk evaluation

The risk quotient (RQ) is a widely used approach for assessing the ecological risk of antibiotics in aquatic environments. In this study, the ecological risk of antibiotics in the Yudong River Basin was estimated using the Risk Quotient Method described in the European Technical Guidance Document on Risk Evaluation (Papageorgiou et al., 2019). This assessment was calculated using the following formula:

$$RQ = \frac{MEC}{PNEC} \qquad \text{(Eq.1)}$$

where MEC represents the measured concentration of antibiotics in the environment (ng/L), while PNEC refers to the predicted no-effect concentration (ng/L). PNEC is calculated as the ratio of chronic toxicity data (EC50) or acute toxicity data (LC50) to the assessment factor (AF). The PNECs utilized in this study are Oxytetracycline of 4930 ng/L, Sulfamethoxazole of 7.5 ng/L, Norfloxacin of 113 ng/L, Tetracycline of 115 ng/L and Roxithromycin of 100 ng/L. When RQ is less than 0.01, it indicates no potential ecological risk; an RQ between 0.01 and 0.1 suggests low risk; an RQ between 0.1 and 1 denotes medium risk; and an RQ greater than 1 indicates high risk (Wei et al., 2024; Han et al., 2020).

The presence of multiple antibiotics in aquatic environments has been shown to result in synergistic effects, leading to an enhanced toxicological impact (Cleuvers, 2003). Accordingly, the joint risk quotient (RQmix) was employed to assess the collective ecological risk of antibiotics to aquatic ecosystems (Gong et al., 2022). The calculation is as follows:

$$RQ_{\mathrm{mix}} = \sum RQ \tag{Eq.2}$$

where RQ is the ecological risk quotient for a single antibiotic; RQmix is evaluated at the same level as RQ.

In this study, the pollution risk assessment of heavy metals in watersheds was conducted using both the single-factor pollution index method and the Nemero Comprehensive Pollution Index Method, which are widely employed internationally and domestically. The following formulas were applied.

The Single Factor Pollution Index Method (Chen et al., 2023):

$$P_i = \frac{C_i}{S_i} \tag{Eq.3}$$

The Nemiro Comprehensive Pollution Index Method (Chen et al., 2017):

$$P_n = \sqrt{\frac{MAX\left(P_i\right)^2 + AVE\left(P_i\right)^2}{2}} \tag{Eq.4}$$

where Pi is the single-factor pollution index of the ith heavy metal in the water body, Ci denotes the measured concentration of the ith heavy metal, and Si represents the evaluation standard for that metal. The water quality standard limit for Class III water, as defined in the Environmental Quality Standard for Surface Waters (GB3838-2002). Additionally, Pn is the combined pollution index of heavy metals in the watershed. The Pi values can be categorized into four levels: Pi ≤ 1, 1 < Pi ≤ 1, 1 < Pi ≤ 2, 2 < Pi ≤ 3, and Pi > 3, corresponding to non-pollution, low pollution, medium pollution, and high pollution, respectively. The Pn values can also be classified into four categories: Pn ≤ 0.7, 0.7 < Pn ≤ 1, 1 < Pn ≤ 2, 2 < Pn ≤ 3, and Pn > 3, indicating non-pollution, low pollution, medium pollution, high pollution, and very high pollution, respectively (Shetaia et al., 2023).

Risk evaluation

Data was initially processed using Microsoft Excel (Office 2003, Redmond, WA, USA). Graphical representations and statistical analyses were performed with Origin

(Gamma Design Software, Origin 6.1, LLC, Plainwell, MI, USA). A one-way analysis of variance (ANOVA) was conducted, and the Least Significant Difference (LSD) method was applied for multiple comparisons. Pearson correlation analysis was utilized to assess relationships between variables.

Results and discussion

Concentrations of antibiotics and heavy metals in the water of the Yudong River

Surface water from the Yudong River was found to contain five distinct types of antibiotics: oxytetracycline (OTC), sulfamethoxazole (SMX), norfloxacin (NOR), tetracycline (TC), and roxithromycin (ROX). All detected antibiotics were presented at concentrations within the ng/L range, as illustrated in *Figure 2*. The concentrations of these antibiotics in surface water were ranked as follows: SMX (143.68 ng/L) > TC (133.00 ng/L) > ROX (98.09 ng/L) > NOR (69.93 ng/L) > OTC (59.00 ng/L). Sulfamethoxazole exhibited the highest concentration, ranging from 56.24 to 227.76 ng/L, followed by tetracycline, which ranged from 0.03 to 252.76 ng/L. In contrast, OTC had the lowest concentration, with a range of 0.05 to 104.69 ng/L. Compared to antibiotic concentrations in river water from other regions, the Yudong River displays notably higher levels, particularly for SMX and TC (*Table 1*). Specifically, the concentration of SMX in Yudong River water was found to be 139.50 times greater than that in Shandong Province’s surface water bodies (1.30 ng/L) (Hanna et al., 2018), and 1.28 times higher than that in the urban river water of Guiyang (112 ng/L) (Wang et al., 2018). This elevated level of SMX, compared to the other antibiotics, can be attributed to its status as one of the most frequently prescribed sulfonamides in both human and veterinary medicine, as well as its prevalent use in the region. Furthermore, sulfamethoxazole’s resistance to removal processes may contribute to its heightened concentration in the river water (Yang et al., 2020; Zhao et al., 2024; Li et al., 2019). The observed discrepancies in antibiotic concentrations across different regions may reflect variations in local antibiotic usage patterns (Yang et al., 2020).

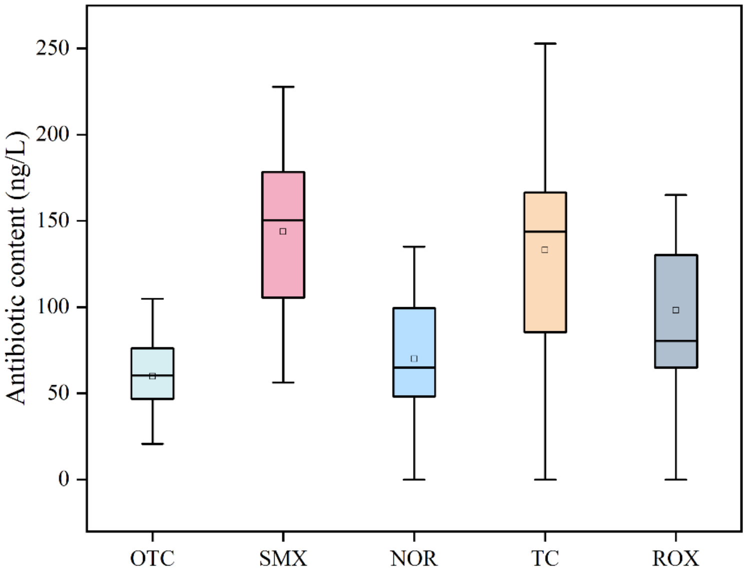

Figure 2. *Characteristics of antibiotic concentrations in the water*

Table 1. *Characterization of antibiotic concentrations in rivers in different regions (ng/L)*

OTC (ng/L)	SMX (ng/L)	NOR (ng/L)	TC (ng/L)	ROX (ng/L)	Areas	References
-	36.59	-	-	1.14	Beijing	Zhao et al., 2024
-	-	0.51	0.42	-	Chengdu	Han et al., 2023
-	-	-	-	9.73	Beijing	Wu et al., 2024
34.5	112	30.0	18.1	102	Guiyang	Wang et al., 2018
2.66	-	-	2.95	-	Bohai Rim Basin	Zhao et al., 2022
17.77	41.92	-	16.45	-	Guangzhou	Tang et al., 2019
10.42	-	6.70	3.55	23.91	Kaiyang	Zhang et al., 2021
4.98	6.76	19.32	8.38	-	Chongqing	Wang et al., 2020
-	1.30	4.36	-	-	Shandong	Hanna et al., 2018
59.71	143.68	69.93	133.00	98.09	Yudong River	This study

The concentrations of heavy metals in Yudong River water were presented in *Table 2*. The measured concentrations of Cu, Zn, Cr, As, Hg, Cd, and Pb were 2.04, 61.80, 6.56, 0.65, 0.01, 0.21, and 5.02 μg/L, respectively, with the following order: Zn > Cr > Pb > Cu > As > Cd > Hg. Prior studies indicate that Yudong River water quality conforms to the Class III water standard established by the Environmental Quality Standard for Surface Water (GB3838-2002) (Liang, 2019). Compared to this standard, none of the seven heavy metals detected exceeded permissible limits. The coefficient of variation (CV), a statistical measure of data dispersion, revealed the following order: As > Cd > Hg > Zn > Cu > Cr > Pb. The CVs for As and Cd were 120.45% and 88.26%, respectively, indicating significant variability and highlighting marked spatial differences in As and Cd concentrations within the Yudong River Basin. In comparison to heavy metal concentrations in other river systems, Cu, Zn, As, and Cd levels in the Yudong River were generally lower, whereas concentrations of Cr and Pb were comparatively higher (*Table 3*). The observed discrepancies in heavy metal concentrations across different regions may stem from various factors, including differing heavy metal sources and land use practices.

Table 2. *Overall characteristics of heavy metal concentrations in the water of Yu Dong River*

	Cu (μg/L)	Zn (μg/L)	Cr (μg/L)	As (μg/L)	Hg (μg/L)	Cd (μg/L)	Pb (μg/L)
Max	3.70	118.59	10.19	3.70	0.02	0.68	7.45
Min	0.71	31.64	0.23	0.14	0.002	0.04	1.51
Mean	2.04	61.80	6.56	0.65	0.01	0.21	5.02
SD	0.75	25.27	2.32	0.79	0.005	0.19	1.64
CV (%)	36.56	40.88	35.39	120.45	64.57	88.26	32.60
GB3838-2022	1000	1000	50	50	0.1	5	50

Spatial variation of antibiotic and heavy metal concentrations in water

From the upper reaches to the lower reaches, the concentrations of OTC and ROX exhibited an increasing trend, while SMX concentrations showed a decreasing trend. NOR concentrations initially increased before declining, and TC concentrations first decreased and then increased, ultimately reflecting an overall decline (*Fig. 3*). OTC and ROX are extensively used in animal husbandry and aquaculture (Zhang et al., 2019; Antos et al., 2024). As the Yudong River flows downstream, population density rises, accompanied by an increase in livestock and poultry farming. Consequently, concentrations of antibiotics, such as OTC and ROX, increase due to factors such as healthcare practices and the discharge of farm wastewater. The observed decline in SMX concentration may be attributed to the self-purification of the water body. Furthermore, the occurrence of antibiotics in aquatic environments is influenced by various factors, including rainfall, temperature, photolysis, microbial metabolism, and human activities. Variations in these factors can lead to complex changes in the distribution of antibiotics (Ding et al., 2021).

Table 3. *Characteristics of heavy metal concentrations in rivers in different regions (µg/L)*

Cu	**Zn**	**Cr**	**As**	**Hg**	**Cd**	**Pb**	**Areas**	**Reference**
32.34	59.79	14.60	-	-	10.72	34.42	Brazil	Da Silva et al., 2017
121.75	89.38	0.48	-	-	5.08	1.34	Chaohu Lake Basin	He et al., 2021
4.25	8.03	0.89	1.11	-	0.11	1.17	Ganjiang River Basin	Jiang et al., 2017
78.53	30.75	6.00	24.22	1.83	1.03	2.37	Zhijin county, Guizhou	Chen et al., 2017
-	1.29	8.30	1.45	-	-	0.013	Akcay River	Leventeli et al., 2021
6.66	33.29	6.00	4.92	-	-	6.29	Caroni River	Banerjee et al., 2019
2.00	110	-	-	-	6.00	1.00	Yudong River	Liang et al., 2023
2.04	61.80	6.56	0.65	0.01	0.21	5.02	Yudong River	This study

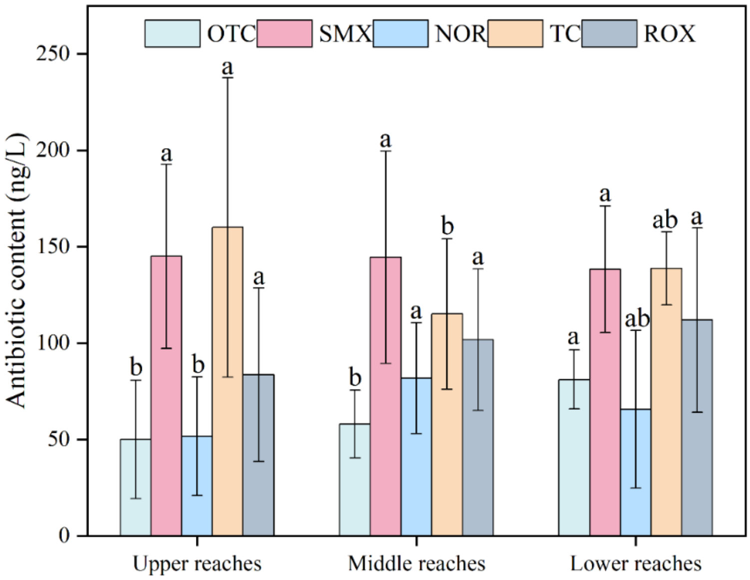

Figure 3. *Differences of same antibiotic concentrations in water in upper, middle and lower reaches. Different lower-case letters between indicate significant differences at a significant level of* $p < 0.05$

The concentration of heavy metals in the water body exhibited complex variations. In the middle reaches of the Yudong River, the concentrations of Zn, As, and Cd increased significantly (*Fig. 4A*, *B*), exceeding those observed in both the upper and lower reaches. This increase may be attributed to the discharge of mine wastewater in the middle reaches, which elevates the concentration of certain heavy metals. The rise in Cr concentration is not solely linked to mining activities; it may also be influenced by various human production and domestic activities. Conversely, the decrease in Cu and Pb concentrations could result from the river's self-purification processes, as well as ecological restoration efforts implemented in recent years.

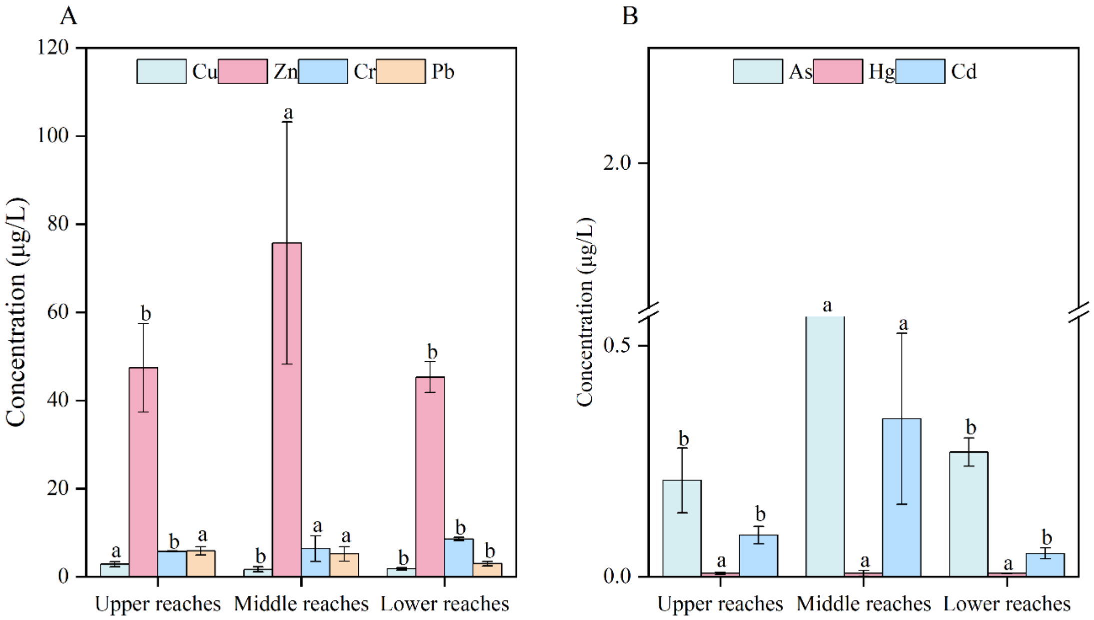

Figure 4. *Distribution characteristics of heavy metal concentrations in the upper, middle and lower water reaches in Yudong rive*

Spatial variation of antibiotic and heavy metal concentrations in water

To deepen our understanding of the potential harm caused by antibiotic and heavy metal pollution in aquatic environments, we conducted a risk assessment of both contaminants in the Yudong River. The results were presented in *Tables 4* and *5*. For the seven heavy metals Cu, Zn, Cr, As, Hg, Cd, and Pb in the Yudong River, we calculated the single-factor pollution index and the Nemero comprehensive pollution index. The findings indicated (*Table 4*) that the single-factor pollution index for all heavy metals was substantially below 1, categorizing the water as clean. Notably, while the Nemero comprehensive pollution index suggested that the Yudong River was not at risk of heavy metal pollution, the lower reaches exhibited the highest index values, indicating potential risks of heavy metal contamination. Additionally, a study suggested that the water in the lower reaches of the Yudong River may be at significant risk of pollution (Liang, 2019). This area is characterized by a high population density and the discharge of mine wastewater, both of which contribute to the risk of heavy metal pollution.

The ecological risk evaluation based on the risk quotient (RQ) for the single and combined toxicity of five antibiotics is summarized in *Table 5*. Regarding the single toxicity of antibiotics, the RQ values for oxytetracycline (OTC) ranged from 0 to 0.02, indicating a low ecological risk. Among the sampling points, 13.04% displayed no risk, primarily concentrated in the upper reaches of the river. In contrast, the RQ values for NOR and ROX ranged from 0 to 1.19 and 0 to 1.65, respectively, reflecting a medium ecological risk (NOR: 82.61%; ROX: 52.17%), with certain sampling points showing a high ecological risk (NOR: 13.04%; ROX: 43.48%). The RQ value for TC was 0 to 2.20, with 30.43% of sampling points indicating moderate ecological risk and 65.22% indicating high ecological risk. The RQ value for SMX ranged from 7.50 to 30.37, substantially exceeding those of the other four antibiotics and indicating the highest ecological risk. The RQmix was calculated at 10.17–33.54, all significantly greater than 1, suggesting a substantial ecological risk associated with antibiotic pollution in the Yudong River. The highest RQmix value was observed in the middle reaches of the river, likely due to a greater number of antibiotic sources in this region. Moreover, most current studies utilize ecological risk assessments based on risk quotients. The values for PNECs used in calculating these quotients are often derived from a synthesis of published literature or researchers' own calculations (Wang et al., 2020; Chen et al., 2023), resulting in a lack of standardized PNECs. This inconsistency poses significant challenges to ecological risk research on antibiotics and introduces uncertainty into the outcomes of these assessments. Thus, the assessment methods and related standards for evaluating the ecological risk of antibiotics require further refinement.

The interrelationship between antibiotic and heavy metal pollution in water

We conducted a correlation analysis to investigate the relationship between antibiotic and heavy metal concentrations in the water bodies of the Yudong River basin. The results indicated that, with the exception of norfloxacin, the four antibiotics generally exhibited negative correlations with heavy metals (*Fig. 5a*). However, these relationships displayed complex variations across different river reaches. In the upper reaches, Cu and Ni showed positive correlations with antibiotics (OTC, NOR, TC, and ROX), with Cu demonstrating a highly significant positive correlation with NOR ($p < 0.01$) and Ni showing a significant positive correlation with TC ($p < 0.05$). Conversely, As was generally significantly ($p < 0.05$) or highly significantly ($p < 0.01$) negatively correlated with the remaining four antibiotics, except SMX. Hg exhibited a significant negative correlation with ROX, while Cd was significantly negatively correlated with both SMX and OTC. In the middle reach, As and Cd showed significant and highly significant positive correlations with TC, Hg was positively correlated with NOR, and Cu demonstrated significant negative correlations with both OTC and SMX. Cr exhibited a highly significant negative correlation with SMX, and Pb showed significant negative correlations with OTC and highly significant negative correlations with SMX. In the lower reach, Cd was significantly positively correlated with SMX, and Ni was significantly positively correlated with both OTC and ROX. In this study, the upstream was closer to the pollution source, such as the discharge points of mining areas and intensive breeding farms. The area was of the intensive human activities and high input concentration. Specific heavy metals (such as Cu, Ni) showed significant local positive correlations with antibiotics, mainly because pollutants had the high homology, and they mainly came from the same mine wastewater or livestock manure (Li et al., 2024). The midstream was a critical section for the mixing, migration, and transformation of pollutants, which received input from upstream water and dispersed sources in the region (such as new mine wastewater and agricultural runoff), leading to complex or unstable correlations between pollutants (McArthur and Tuckfield, 2000). Firstly, the mixture of multiple sources can dilute or mask specific upstream pollution fingerprints, leading to a weakening of the original significant correlation (Li et al., 2022). Additionally, a new and stronger positively correlated hotspot may be formed at specific convergence points (such as the main tributary confluence or downstream of large sewage outlets). The downstream water had a large volume and strong dilution effect. The slowing down of water flow velocity promoted the sedimentation of pollutants, and the absolute concentration of most pollutants will decrease due to dilution and sedimentation. However, the differences in environmental behavior between heavy metals and antibiotics can further weaken their correlation or even lead to separation. For example, heavy metals were prone to adsorb onto particulate matter and deposit into sediment, while some antibiotics may be more soluble in water or undergo photolysis or biodegradation (Wu et al., 2022). Therefore, in the aqueous phase, the heavy metals and antibiotic may not exhibit the significant spatial co-occurrence. An early study even found a negative correlation between metal concentration scores and aminoglycoside antibiotic resistance scores in downstream sediments (Toothman et al., 2009), suggesting that in complex and long-term polluted environments, the adaptation mechanisms of organisms to various stresses may be more complex, which is consistent with the weakened correlation between heavy metals and antibiotics downstream in this study.

To elucidate the relationship between antibiotics and heavy metals, we analyzed the correlation between the antibiotic risk quotient (RQ) and the heavy metal pollution index (Pi, Pn). The correlation analysis revealed that the antibiotic risk quotient was predominantly negatively correlated with the heavy metal pollution index (*Fig. 6*). For instance, in the upper reach, the pollution index for Pi-As was significantly or highly significantly negatively correlated with the RQ of OTC, NOR, TC, and ROX. Notably, across all reaches, the pollution index for Pi-Cu was significantly and positively correlated with the RQ for TC; specifically, in the upstream region, Pi-Cu was highly significantly positively correlated with the RQ for NOR. In the middle reach, Pi-As and Pi-Cd were significantly and highly significantly positively correlated with the RQ for TC, while the pollution index for Pi-Hg showed a significant positive correlation with the RQ for NOR. In the downstream region, Pi-Cd was significantly positively correlated with the RQ for SMX. These results mirror the observed correlations between heavy metals and antibiotics, suggesting that the risks associated with antibiotics and heavy metals are influenced by their respective concentrations. The significant or highly significant positive correlations between the antibiotic risk quotient and the heavy metal contamination index highlight the potential for joint contamination resulting from the interactions between specific antibiotics and heavy metals. Previous studies have indicated that antibiotics and heavy metals can form complexes that exert toxic effects on aquatic organisms (Wang et al., 2022). Additionally, it is crucial to further investigate the potential hazards associated with antibiotic-heavy metal interactions to mitigate related pollution risks.

Table 4. *Evaluation of heavy metal pollution of water in the Yudong River*

		P_i	Cu	Zn	Cr	As	Hg	Cd	Pb	P_n
Total		Max	0.0037	0.1186	0.2037	0.0739	0.2400	0.1370	0.1489	0.1489
		Mean	0.0020	0.0618	0.1312	0.0131	0.0738	0.0429	0.1004	0.1004
		Min	0.0007	0.0316	0.0047	0.0027	0.0182	0.0078	0.0303	0.0303
Different reach	Upper reach	Max	0.0037	0.0595	0.1180	0.0070	0.1138	0.0235	0.1442	0.1096
		Mean	0.0028	0.0474	0.1145	0.0042	0.0776	0.0180	0.1175	0.0963
		Min	0.0019	0.0329	0.1106	0.0027	0.0302	0.0113	0.0875	0.0862
	Middle reach	Max	0.0028	0.1186	0.2037	0.0739	0.2400	0.1370	0.1489	0.1842
		Mean	0.0017	0.0757	0.1276	0.0208	0.0722	0.0684	0.1039	0.1159
		Min	0.0007	0.0316	0.0047	0.0027	0.0182	0.0133	0.0303	0.0472
	Lower reach	Max	0.0021	0.0496	0.1816	0.0062	0.0796	0.0134	0.0734	0.1340
		Mean	0.0018	0.0453	0.1713	0.0054	0.0722	0.0101	0.0597	0.1266
		Min	0.0013	0.0408	0.1610	0.0045	0.0658	0.0078	0.0465	0.1200

Table 5. *Ecological risk assessment of antibiotics in the Yudong River*

	RQ	OTC	SMX	NOR	TC	ROX	RQ$_{mix}$
Upper reach	S1	0.00	19.34	0.00	0.00	0.00	19.34
	S2	0.01	27.71	0.20	1.25	0.80	29.97
	S3	0.01	8.92	0.53	2.15	0.93	12.54
	S4	0.00	23.12	0.88	1.45	1.29	26.74
	S5	0.01	21.35	0.42	1.45	0.76	23.98
	S6	0.01	11.16	0.49	2.20	1.49	15.34
	S7	0.02	23.78	0.70	1.25	0.58	26.34
Middle reach	S8	0.01	30.37	0.79	0.72	1.65	33.54
	S9	0.02	22.76	0.66	1.52	0.71	25.67
	S10	0.01	13.31	1.04	0.81	0.76	15.93
	S11	0.01	18.83	0.51	0.61	1.30	21.26
	S12	0.01	8.89	0.27	1.42	0.73	11.32
	S13	0.02	14.06	0.68	0.72	1.19	16.67
	S14	0.01	21.78	0.60	1.10	0.63	24.12
	S15	0.02	27.77	0.97	1.42	1.28	31.47
	S16	0.02	20.06	0.47	0.80	1.08	22.42
	S17	0.01	16.18	1.17	0.74	0.65	18.75
	S18	0.00	7.50	0.57	1.46	0.63	10.17
	S19	0.01	29.91	0.96	0.71	1.61	33.20
Lower reach	S20	0.01	24.58	0.45	1.45	0.52	27.01
	S21	0.01	14.53	0.25	1.25	0.79	16.84
	S22	0.02	14.09	1.19	1.00	1.56	17.86
	S23	0.02	20.62	0.43	1.14	1.62	23.83

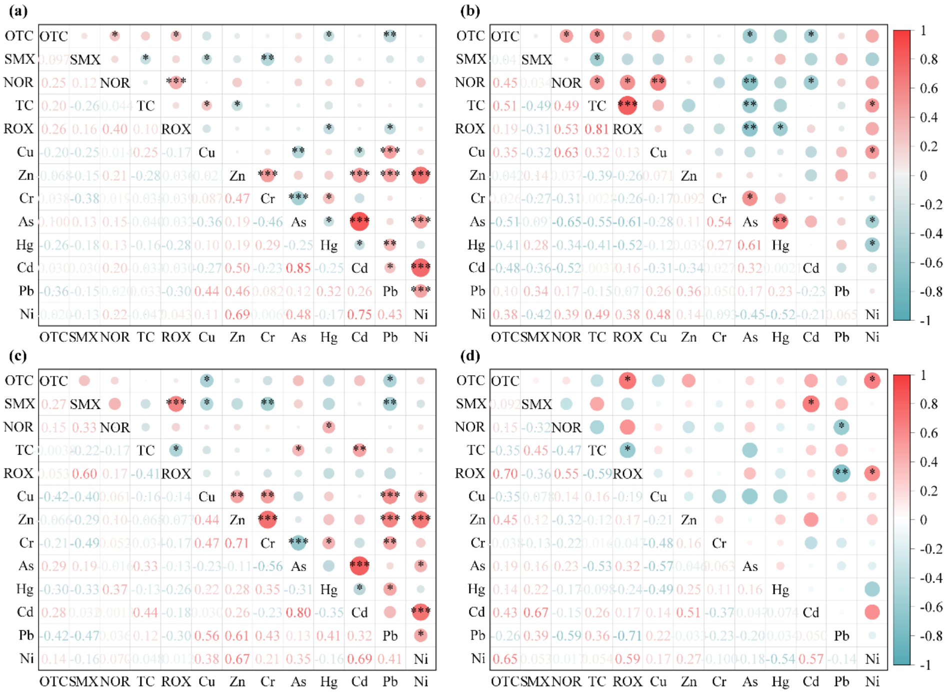

Figure 5. *Relationship between antibiotic and heavy metal concentrations in water. Whole watershed (a); upper reach (b); middle reach (c); lower reach (d). oxytetracycline, OTC; sulfamethoxazole, SMZ; norfloxacin, NOR; tetracycline, TC; and roxithromycin, ROX. *, **, *** denote p-values less than 0.05, 0.01, and 0.001, respectively*

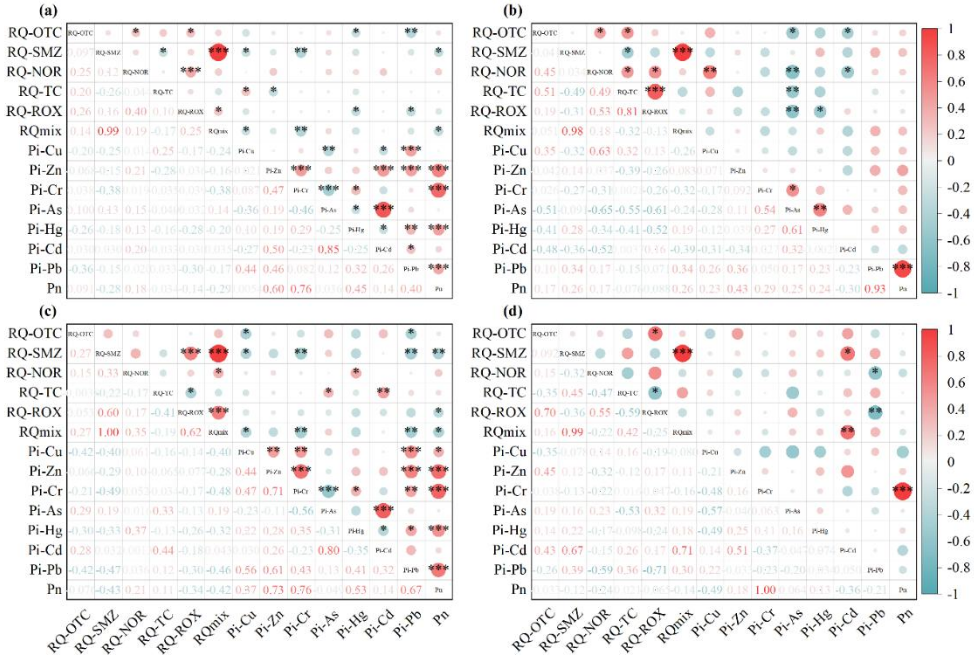

Figure 6. *Relationship between antibiotics and risk of heavy metal pollution in water. Whole watershed (a); upper reach (b); middle reach (c); lower reach (d). RQ-(OTC, SMX, NOR, TC, ROX), denotes the risk quotient of different types of antibiotics; RQ$_{mix}$, the joint risk quotient of different types of antibiotics; Pi-(Cu, Zn, Cr, As, Hg, Cd, Pb), denotes the different heavy metals' single-factor pollution index; Pn, Nemero combined pollution index. *, **, *** denote p-values less than 0.05, 0.01, and 0.001, respectively*

Conclusion

In this study, we investigated the distribution characteristics of antibiotics and heavy metals in the water body of the Yudong River Basin in Qiandongnan, Guizhou Province. We assessed the environmental impacts of these pollutants and explored their interrelationships. The antibiotics with the highest concentrations were sulfamethoxazole (56.24–227.76 ng/L), followed by tetracycline (0.03–252.76 ng/L). The concentrations of seven heavy metals, including Cu and Cr, did not exceed acceptable levels. Notably, the concentrations of oxytetracycline and roxithromycin exhibited an increasing trend from upstream to downstream, while SMX concentrations showed a decreasing trend. Zn, As, and Cd concentrations peaked in the middle reach, whereas Cr concentrations increased and those of Cu and Pb decreased. The heavy metal pollution indices (Pi, Pn) indicated that the risk of heavy metal pollution in the Yudong River was at a clean level. In contrast, the antibiotic risk quotient and joint risk quotient suggested a medium-high risk level for antibiotic pollution in the river. Both antibiotics and heavy metals may share common sources, and the potential for joint pollution resulting from their interactions requires urgent attention. This study provides foundational data and theoretical support for the prevention, control, and management of antibiotic and heavy metal pollution in the Yudong River Basin.

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