

Study of Amoxicillin Residues in an Seketak River, Semarang, Indonesia: Eco toxicological Risk Assessment and Recommendations for Pharmaceutical Wastewater Quality Standards

Ardyansyah Lubis, Tri Retnaningsih Soeprbowati, & Budi Warsito

Master Program in Environmental Science

Graduate School, University of Diponegoro

Semarang

Indonesia

ABSTRACT

*Amoxicillin, a widely used β -lactam antibiotic, is frequently detected in urban aquatic environments as an emerging contaminant (EC). This study assessed amoxicillin concentrations in Seketak River, Semarang, Indonesia, an urban waterway receiving pharmaceutical and domestic wastewater. Water samples were collected from four stations in May 2025 and analysed using HPLC-UV. The highest Predicted Environmental Concentrations (PECs) were 8.2 $\mu\text{g/L}$ (Station 2) and 6.5 $\mu\text{g/L}$ (Station 3). Risk Quotients (RQ) reached 105.13 and 83.33 ($\text{PNEC} = 0.078 \mu\text{g/L}$), indicating high ecotoxicological risk. Conventional water quality parameters met Class 4 standards (PP No. 22/2021), yet organic pollution (BOD 5.5–5.8 mg/L) correlated with antibiotic persistence. Bioindicator analysis showed dominance of pollution-tolerant species (*Mougeotia* sp. and *Tubifex* sp.), confirming ecosystem stress, and potential AMR gene transfer. Indonesia's current pharmaceutical wastewater standards (Permen LHK No. 5/2014) contains no antibiotic parameters. This study recommends adding an amoxicillin limit of $\leq 0.078 \mu\text{g/L}$ and mandatory HPLC monitoring. Findings support SDG 6 (Clean Water and Sanitation) and highlight the urgent need for specific EC standards in developing countries.*

Keywords: Amoxicillin, Emerging Contaminants, Antimicrobial Resistance, Risk Quotient, Pharmaceutical Wastewater Standards, Urban River Pollution.

1. INTRODUCTION

Antimicrobial resistance (AMR) is one of the top ten global public health threats, causing 1.27 million direct deaths in 2019 (WHO, 2023). Pharmaceutical residues, particularly antibiotics, are major drivers of environmental AMR (Larsson & Flach, 2022). Amoxicillin, the most prescribed antibiotic worldwide, is poorly removed by conventional wastewater treatment plants (efficiency $<30\%$) and persists in surface water at ng/L to $\mu\text{g/L}$ levels (Liu et al., 2020).

In Indonesia, rapid urbanisation and inadequate pharmaceutical wastewater management have led to frequent detection of antibiotics in rivers (Mutiary & Mittal, 2014; Toxics Link, 2022). Seketak River, located in the densely populated Tembalang district of Semarang, receives domestic and healthcare facility effluents, making it an ideal case study for urban river pollution in Indonesia.

Current Indonesian regulations (Permen LHK No. 5/2014) do not include any antibiotic parameters for pharmaceutical or healthcare wastewater, creating a critical regulatory gap. This study aims to: (1) quantify amoxicillin concentrations in Seketak River, (2) assess ecotoxicological risk using RQ and bioindicators, (3) evaluate regulatory gaps, and (4) propose evidence-based wastewater quality standards.

2. MATERIALS AND METHODS

2.1 Study Area

Seketak River (catchment area ≈ 917 ha) flows through Tembalang, Semarang, receiving inputs from residential areas, universities, hospitals, and clinics. Four sampling stations were selected (Figure 1):



Figure 1 S1 (upstream,), S2 (midstream, near hospital outlets), S3 (active urban zone), S4 (downstream, education reservoir).

2.2 Sampling and Analytical Methods

Sampling was conducted in May 2025 (dry season). Grab water samples (1 L, triplicate) were collected in amber glass bottles, stored at 4°C, and analysed within 24 h.

Amoxicillin was quantified using HPLC-UV (Shimadzu, column C18, mobile phase methanol:phosphate buffer 30:70, detection 230 nm, LoD 0.05 $\mu\text{g/L}$). Conventional parameters (pH, DO, BOD, COD, TSS) were analysed per SNI and APHA standards.

Ecotoxicological risk was calculated as $RQ = \text{PEC}/\text{PNEC}$, with $\text{PNEC} = 0.078 \mu\text{g/L}$ (Gao et al., 2020). Pollution Index (IP) followed KepMen LH No. 115/2003. Phytoplankton and macrozoobenthos were sampled using standard methods and identified at Laboratorium Ekologi Perairan Undip.

3. RESULTS

3.1 Water Quality Parameters

All parameters complied with Class 4 standards (irrigation/agriculture), but BOD (5.5–5.8 mg/L at S2–S3) and COD (48–52 mg/L) indicated moderate organic loading (Table 1).

Tabel 1 Seketak River Water Quality Parameter Analysis Findings

Sample Point	pH	DO (mg/L)	BOD (mg/L)	COD (mg/L)	TSS (mg/L)
S1 (Hulu)	6.8	7.5	< 2.0	< 2.5	5.47
S2 (Tengah)	6.8	7.6	< 2.0	< 2.5	2.00
S3 (Area Aktif)	7.0	7.5	< 2.0	< 2.4	3.15
S4 (Hilir)	6.9	4.0	< 2.0	< 2.5	3.50
Baku Mutu	6-9	1	12	80	400

3.2 Amoxicillin Concentrations

Amoxicillin was detected at S2 (8.2 µg/L) and S3 (6.5 µg/L), below LoD at S1 and S4 (Table 2). These values are higher than typical Asian urban rivers (0.1–5 µg/L) but lower than heavily polluted Indian sites up to 104 µg/L (Mutiyaar & Mittal, 2014).

Tabel 2 Analysis Results of Amoxicillin Concentration

Sample Point	Amoxicillin concentration (mg/L)
S1	<0.05
S2	0.0082
S3	0.0065
S4	<0.05

3.3 Risk Assessment

RQ values exceeded 80 at S2–S3 (Table 3), classifying risk as “high”. Bioindicator analysis showed low Shannon-Wiener diversity ($H' = 1.44$ – 1.82) and dominance of tolerant species (*Mougeotia* sp. 66–78 %, *Tubifex* sp. 71–84 %) at polluted stations (Table 4).

Tabel 3 Calculated Risk Quotients (RQ) for Amoxicillin

Sample Point	PEC (µg/L)	PNEC (µg/L)	RQ = PEC/PNEC	Risk Category
S2	8.2	0.078	105.13	High Risk
S3	6.5	0.078	83.33	High Risk

Tabel 4 Aquatic Organism Identification for Bioindicator Assessment

Sampling Point	Organism Type	Dominant Genus/Species	Relative Abundance (%)
S1 (Upstream)	Phytoplankton	<i>Oscillatoria</i> sp.	45
	Macrozoobenthos	<i>Melanoides</i> sp.	55
S2 (Middle)	Phytoplankton	<i>Mougeotia</i> sp.	78
	Macrozoobenthos	<i>Tubifex</i> sp.	84
S3 (Active Area)	Phytoplankton	<i>Mougeotia</i> sp.	66
	Macrozoobenthos	<i>Tubifex</i> sp.	71
S4 (Downstream)	Phytoplankton	<i>Oscillatoria</i> sp.	52
	Macrozoobenthos	<i>Melanoides</i> sp.	48

4. DISCUSSION

Detected amoxicillin concentrations, though below acute toxicity levels, are sufficient to exert chronic selection pressure on bacterial communities (Zhang et al., 2022). The positive correlation between organic pollution (BOD/COD) and amoxicillin persistence suggests adsorption to suspended solids delays degradation (Liu et al., 2020).

Bioindicator shifts toward pollution-tolerant species mirror findings in polluted Asian rivers (Schar et al., 2021) and confirm that even Class 4-compliant water can harbour significant AMR risk. The absence of antibiotic parameters in Permen LHK No. 5/2014 represents a critical regulatory gap shared by many developing countries (Larsson, 2014).

4.1 Conclusions and Policy Recommendations

Seketak River exhibits high ecotoxicological risk from amoxicillin residues (RQ >80), driven by inadequate pharmaceutical wastewater regulation. The study recommends:

1. Immediate revision of Permen LHK No. 5/2014 to include amoxicillin ≤ 0.078 $\mu\text{g/L}$ in pharmaceutical and healthcare wastewater.
2. Mandatory HPLC monitoring for antibiotics in urban river catchments.
3. Implementation of advanced treatment (activated carbon adsorption or ozonation) in hospitals and pharmaceutical facilities.

These measures would significantly reduce environmental AMR drivers and support Indonesia's commitments under SDG 6 and SDG 14.

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Conflicts of Interest

The authors declare no conflict of interest.

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