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Orapuh Journal

ORIGINAL ARTICLE Journal of Oral & Public Health

Polymetallic Contamination Along an Urbanization Gradient in the Funa River (Kinshasa, DRC): Structural Reordering of Cultivable Bacterial Communities

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ARTICLE INFO

Received: *12 May 2026*
Accepted: *26 June 2026*
Published: *06 August 2026*

Keywords:
Funa River, Kinshasa, trace metals, urban river pollution, bacterial communities, ED-XRF, microbial ecotoxicology

Peer-Review: *Externally peer-reviewed*

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To cite:
Otshudi, A. J., Nsiala, O. G., Lanassa, M. A., Kusonika, N. A., Musibono, E. D., & Biey, M. E. (2026). Polymetallic contamination along an urbanization gradient in the Funa River (Kinshasa, DRC): Structural reordering of cultivable bacterial communities. *Orapuh Journal, 7*(6), e1459.
https://doi.org/10.4314/orapj.v7i6.59

ISSN: *2644-3740*

Published by **Orapuh, Inc.**, F. Gaye Res., Sukuta-Jamisa, Greater Banjul, The Gambia.

Editor-in-Chief: *Prof. V. E. Adamu (editor@orapuh.org)*

ABSTRACT

Introduction
Urban rivers in sub-Saharan Africa are increasingly affected by untreated domestic, industrial, and artisanal effluents, leading to polymetallic contamination that may alter microbial community structure and ecosystem functioning. However, the influence of chronic metal pollution on bacterial phenotypic composition remains poorly documented in tropical urban freshwater ecosystems.

Purpose
This study aimed to assess dissolved trace metal contamination along the Funa River (Kinshasa, Democratic Republic of the Congo) and investigate its relationship with the phenotypic distribution of cultivable bacterial communities.

Methods
A longitudinal survey was conducted at six sampling stations. Eighteen water samples were collected for physicochemical analyses, and nine composite samples were prepared for microbiological assessment. Ten trace metals were quantified using energy-dispersive X-ray fluorescence spectrometry (ED-XRF). Cultivable heterotrophic bacteria were enumerated on Mueller–Hinton and MacConkey media and characterized by Gram staining. Spatial differences were evaluated using the Kruskal–Wallis test, and associations between metal concentrations and bacterial phenotypes were examined using bootstrapped Spearman rank correlations (1,000 resamples).

Results
The Funa River exhibited severe polymetallic contamination, with several metals exceeding guideline values. Cadmium showed extremely high contamination factors (CF = 2,889--5,789), while lead reached 1,433 μg L^{-1} downstream. Dissolved iron varied significantly among stations ($H = 6.14$, $p = .046$). Total heterotrophic bacterial abundance ranged from $(3.23 \pm 1.53) \times 10^6$ to $(3.73 \pm 2.97) \times 10^6$ CFU mL^{-1}. A marked shift in bacterial phenotype was observed, shifting from Gram-positive dominance upstream to exclusively Gram-negative populations downstream. Bootstrapped correlations revealed significant negative associations between most metal metrics and Gram-positive bacteria ($\rho = -.50$, $p = .041$), alongside corresponding positive associations with Gram-negative bacteria ($\rho = +.50$, $p = .041$).

Conclusions
Chronic polymetallic contamination acts as a strong ecological filter shaping bacterial community structure in the Funa River, favoring Gram-negative populations adapted to metal stress. These findings underscore the need for improved urban wastewater management and molecular investigations to resolve microbial adaptation pathways and public health risks.

INTRODUCTION

In rapidly expanding sub-Saharan African megacities such as Kinshasa, intense urbanization combined with deficient sanitation infrastructure accelerates the discharge of persistent contaminants into urban hydrosystems (Kyowire et al., 2024; Mukeba et al., 2022). Among these, trace metals (TMs) represent a critical ecotoxicological threat due to their non-biodegradability, environmental persistence, and high propensity for bioaccumulation within aquatic compartments (Ali et al., 2023). In heavily anthropized river systems, TMs rarely occur as isolated pollutants; instead, they form complex polymetallic mixtures generated by overlapping domestic, industrial, and informal artisanal effluents (Allan & Castillo, 2007).

As key drivers of biogeochemical cycling and nutrient flux, riverine microbial communities exhibit high sensitivity to chemical disturbances. Chronic polymetallic stress exerts selective pressure, altering bacterial community dynamics by suppressing sensitive taxa while promoting phenotypes capable of mitigating metal toxicity through active efflux, intracellular sequestration, or extracellular biosorption (Fajardo et al., 2023; Gadd, 2010). Consequently, deciphering structural shifts within these native microbial assemblages provides crucial insights into both the biological quality of the ecosystem and the functional resilience of planktonic communities under multi-element stress (Azarbad et al., 2013).

The Funa River, which drains several high-density, heavily industrialized zones of Kinshasa, serves as a representative model for these ecotoxicological dynamics. Although previous studies have established the river's physicochemical degradation and reported trace metal contamination in surface water and benthic sediments (Kayembe et al., 2020; Manza et al., 2026; Swana et al., 2024), a knowledge gap remains. Extant literature focuses almost exclusively on environmental chemistry and spatial quantification of pollutants, leaving the biological responses and adaptive structural shifts of the indigenous bacterial communities to chronic multi-metal pressure undocumented in this tropical urban context.

To address this gap, this study addresses a central question: How does a continuous multi-metal contamination gradient shape the structural distribution and adaptive equilibrium of riverine bacterial communities? Investigating these dynamics along the upstream-to-downstream anthropogenic gradient of the Funa River, we hypothesized that increasing polymetallic pressure induces a predictable shift in the ratio of Gram-positive to Gram-negative bacteria. This structural reordering, driven by distinct cell wall properties and differential tolerance mechanisms, is evaluated as a potential biological indicator of localized multi-metal toxicity.

METHODS

Study Area

The study was conducted along the Funa River, an urban tributary of the Congo River basin in Kinshasa, Democratic Republic of the Congo. Extending approximately 9 km from the peri-urban municipality of Mont-Ngafula through densely populated residential and industrial areas, the river provides a representative gradient of urban contamination. Six sampling stations (S1–S6) were established along the upstream–downstream continuum to capture spatial variations in pollution sources (Mabidi et al., 2024; Mwanamoki et al., 2015):

a) **Mariano (S1; 4°21′20″ S, 15°18′56″ E):** A peri-urban reference site with relatively low human pressure.

b) **Kauka (S2; 4°21′12″ S, 15°18′59″ E):** Influenced by domestic wastewater.

c) **Bongolo (S3; 4°21′13″ S, 15°18′59″ E):** Characterized by artisanal and market activities.

d) **Sendwe (S4; 4°20′02″ S, 15°19′14″ E):** Affected by urban runoff from heavy traffic.

e) **Complast (S5; 4°19′41″ S, 15°19′56″ E):** Located near industrial discharges.

f) **Pakadjuma (S6; 4°19′54″ S, 15°19′29″ E):** Impacted by informal waste disposal and raw sewage.

The spatial heterogeneity in pollution sources makes the Funa River an appropriate lotic ecosystem model to elucidate ecotoxicological interactions between chronic polymetallic pressure and indigenous cultivable bacterial communities (Kayembe et al., 2020).

Study Design

This research was structured as a spatial, observational cross-sectional study. A single synchronized sampling campaign was executed between February 2 and February 8, 2026, to minimize temporal and hydrological variation. This window coincides with the minor rainy season in Kinshasa, a period characterized by steady baseflows combined with active urban surface runoff, providing conditions to assess the relationship between physicochemical alterations, multi-metal concentrations, and bacterial community dynamics (Dean-Ross & Mills, 1989; Hu et al., 2026; Shen et al., 2026; Zeb et al., 2024).

Sampling Strategy and Data Collection

A total of 18 water samples were collected across the six monitoring stations. At each station, three independent sub-samples were collected approximately 30 cm below the water surface within the mid-channel cross-section to obtain representative flowing water, avoiding stagnant banks and localized turbulence. Collections were performed using pre-cleaned, acid-washed (10% HNO_3), sterile high-density polyethylene (HDPE) bottles, which were pre-rinsed three times in situ with ambient river water prior to sample acquisition (American Public Health Association [APHA], 2012; Nsiala et al., 2025).

To balance analytical resolution for chemical parameters with microbiological processing constraints, a dual-track strategy was implemented:

I. **Chemical Analysis:** The three independent samples per station were preserved and analyzed as distinct true spatial replicates (n = 18) to capture in-site variance.

II. **Microbiological Analysis:** Due to consumable constraints, a composite sampling protocol was deployed (n = 9 analyzed units). Composite samples were prepared by pooling equal volumes (300 mL) of the three independent sub-samples collected at a given station, followed by homogenization under sterile laminar flow conditions. This procedure minimized micro-spatial patchiness, ensuring that each microbiological unit reflected the mean community structure of its respective station (Mittal et al., 2022; Yang et al., 2025).

Samples for microbiological tracking were stored at 4 °C in insulated field coolers and processed within 24 hours at the Laboratory of Microbiology and Environmental Biotechnology (University of Kinshasa). Parallel chemical aliquots were immediately acidified with ultra-pure HNO_3 (pH < 2) and transported to the Regional Center for Nuclear Studies in Kinshasa (CREN-K).

Laboratory Analyses

Physicochemical Analysis

Core physicochemical parameters—including water temperature (°C), pH, electrical conductivity (µS cm^{-1}), and salinity—were recorded in situ at each replication point using a calibrated portable multiparameter water quality tester (EZ-9909SP). Multi-point calibrations of the electrodes were performed daily prior to field deployment using standard buffer solutions (pH 4.01, 7.00, and 10.01; conductivity standards at 1,413 µS cm^{-1}} and 12.88 mS cm^{-1}) following standard guidelines (APHA, 2012).

Chemical Analysis of Trace Metals

Total concentrations of ten trace metals (Fe, Cr, Mn, Co, As, Cd, Pb, Cu, Sb, and Zn) were quantified by energy-dispersive X-ray fluorescence spectrometry (ED-XRF) using a SPECTRO XEPOS III spectrometer. Liquid matrices were analyzed directly in thin-film sample cups. Matrix effects and elemental spectral overlaps were resolved using the standardized "FP-Water" (Fundamental Parameters for Water Matrices) and "Solo-Water" empirical methods embedded within the instrument software (Daly & Fenelon, 2018; Kapembo et al., 2021; Lia et al., 2020).

External calibration curves were established using a multi-element synthetic standard matrix prepared from high-purity certified reference stocks (1,000 mg L^{-1}, Sigma-Aldrich). Limits of detection (LOD) and quantification (LOQ) were calculated based on 3σ and 10σ criteria of procedural blanks, respectively. Analytical accuracy was verified using certified reference water materials (SRM-1643h), yielding elemental recovery rates between 88% and 104% (U.S. Environmental Protection Agency [U.S. EPA], 2007).

Microbiological Analysis

a. **Sample Preparation and Culture Conditions:** Composite water samples were serially diluted in sterile physiological saline solution (0.9% NaCl) up to 10^{-6} in accordance with ISO 7218 (International Organization for Standardization [ISO], 2024). Aliquots of 100 µL} from appropriate dilutions were inoculated onto agar plates using the spread-plate method.

b. **Isolation and Enumeration of Bacteria:** Target bacteria were isolated using two media: Mueller–Hinton agar (for total heterotrophic aerobic bacteria) and MacConkey agar (targeting Gram-negative enteric bacilli) (Madigan et al., 2018; Willey et al., 2017). Plated media were incubated at 37 °C under aerobic conditions for 24 hours. Plates exhibiting between 30 and 300 colonies were enumerated, and final microbial densities were expressed as colony-forming units per milliliter (CFU mL^{-1}).

c. **Gram Staining Procedure:** Phenotypic differentiation of cell wall structure was determined using standard Gram staining techniques (Willey et al., 2017). For each station, representative distinct colonies were isolated, heat-fixed onto glass slides, and sequentially treated with crystal violet (1 min), Lugol's iodine (1 min), 95% ethanol (10–15 s), and safranin (1 min). Slides were rinsed with distilled water between steps, air-dried, and examined under an oil-immersion light microscope (1,000x magnification) to classify isolates as Gram-positive or Gram-negative.

Contamination Assessment

To evaluate anthropogenic metal enrichment and environmental degradation at each station, individual Contamination Factors (CF) were calculated using Equation 1:

$$CF = C_{sample} / C_{reference}$$

Where C_{sample} represents the concentration of a specific metal measured in river water, and $C_{reference}$ represents the maximum permissible water quality threshold for the protection of aquatic life established by the Canadian Council of Ministers of the Environment (CCME, 2022). To assess cumulative environmental risk, the overall Degree of Contamination (DC) for each site was calculated as the sum of individual contamination factors (Equation 2 - Hakanson, 1980; Sun et al., 2021):

$$DC = \sum_{i=1}^{n} CF_i$$

Statistical Analysis

Statistical processing was executed using SPSS (Version 26.0) and R software. Descriptive metrics were expressed as mean ± standard deviation (SD) along with corresponding 95% confidence intervals [95% CI]. Data normality was evaluated using the Shapiro–Wilk test ($n < 50$), and homoscedasticity was assessed via Levene's test.

i. **Physicochemical and Chemical Datasets (n = 18):** Because environmental datasets violated the assumption of normality ($p < .05$), spatial differences across the six longitudinal stations were evaluated using the non-parametric Kruskal–Wallis H-test, followed by Dunn's post-hoc test with Bonferroni correction for multiple pairwise comparisons (Field, 2024). The magnitude of spatial differences (effect size) was calculated using epsilon-squared (ε^2).

ii. **Microbiological and Correlation Datasets (n = 9):** Due to the non-normal distribution and small sample size of the microbiological units, non-parametric Spearman rank correlation coefficients (ρ) were applied to model relationships between polymetallic metrics (CF, DC), physicochemical parameters, and bacterial shifts (CFU mL^{-1}, Gram ratios). To address sample size constraints, 95% confidence intervals for Spearman's ρ were calculated using bootstrapping (1,000 resamples) (Ukoumunne et al., 2003).

A retrospective power analysis was conducted to evaluate the limitation of the reduced microbiological dataset ($n = 9$). For detecting large ecological effect sizes ($\rho \geq .80$), the sample size achieved a statistical power ($1 - \beta$) of approximately 72%. Although slightly below the standard 80% threshold, the contamination gradient exhibited by the Funa River compensated for this limitation, ensuring observed correlation strengths remained stable (Cohen, 1988; Nakagawa et al., 2024). The significance threshold for all tests was maintained at α = .05.

Quality Assurance/Quality Control (QA/QC) and Bias Management

Microbiological glassware and sampling tools were autoclaved at 121 °C for 21 minutes, while chemical HDPE containers underwent sequential immersion in 10% HNO_3 and rinsing with deionized water.

Cross-contamination risks during field collection were minimized via clean-hands handling and rapid preservation. Procedural and field blanks were intercalated every 10 samples to monitor baseline noise; trace metal levels in all blanks remained consistently below instrument detection limits. Analytical uncertainty was further controlled by processing all chemical and physical samples in true triplicates (U.S. EPA, 2007).

Ethical Considerations

This study relied exclusively on water samples harvested from a public lotic ecosystem. The protocol did not involve human participants, human tissue samples, or animal experimentation. In accordance with institutional regulations of the University of Kinshasa and national guidelines governing environmental research in the Democratic Republic of the Congo, formal institutional bioethics clearance was not required (Mabidi et al., 2024; Mwanamoki et al., 2015).

RESULTS

Physicochemical Parameters of the Funa River

Spatial variations in core physicochemical parameters along the upstream-to-downstream reaches of the Funa River are summarized in **Table 1**.

Water temperature exhibited an increasing trend from upstream to downstream reaches, ranging from a minimum of 28.6 ±0.15 °C (S1–S2) to a maximum of 32.0 ± 0.14°C (S5–S6). This spatial discrepancy was confirmed as statistically significant by the Kruskal–Wallis test ($H = 14.82$, $p < .001$), with Dunn's post-hoc test identifying distinct separation between all three hydro-morphological sections.

Conversely, ambient pH remained relatively stable, hovering within neutral to slightly alkaline boundaries (7.45 ± 0.01 to 7.62 ± 0.01) without displaying statistically significant spatial variations ($H = 4.21$, $p = .107$). Electrical conductivity peaked within the upstream section (756 ± 3.6 µS cm^{-1}), showing a significant decrement midstream and downstream ($H = 8.94$, $p = .027$). Salinity values did not display measurable fluctuation along the monitored gradient ($H = 0.00$, $p = 1.000$). Recorded physicochemical baselines remained within safety guidelines established by the World Health Organization (WHO, 2022).

Table 1:
Physicochemical Characteristics of Funa River Water Along the Longitudinal Gradient

Section	Stations Included	Temperature (°C)	pH	Conductivity (µS cm−1)	WHO Guideline
Upstream	S1–S2	28.6 ± 0.15^a to 28.8 ± 0.01^a	7.45 ± 0.01^a to 7.48 ± 0.01^a	532 ± 2.6^a to 756 ± 3.6^a	25–30
Midstream	S3–S4	29.3 ± 0.05^b to 31.2 ± 0.30^b	7.50 ± 0.01^a to 7.59 ± 0.01^a	526 ± 3.7^b to 557 ± 3.5^c	6.5–8.5
Downstream	S5–S6	31.1 ± 0.50^c to 32.0 ± 0.14^c	7.51 ± 0.01^a to 7.62 ± 0.01^a	528 ± 2.5^b to 532 ± 2.5^b	<1,500
Statistics	Kruskal–Wallis (H), p-value	H = 14.82, p < .001	H = 4.21, p = .107	H = 8.94, p = .027	—

Note: Values are expressed as mean ±standard deviation of independent field replicates (n = 18). Distinct superscript letters within the same column indicate statistically significant differences between sections based on Dunn's post-hoc test (α = .05).

Quantification of Essential and Non-Essential Trace Metals

Total dissolved concentrations of the ten targeted trace metals are presented in **Table 2**. Across all monitoring stations, the majority of elemental concentrations exceeded limits defined by the CCME for the protection of aquatic life.

Spatial profiling revealed that essential elements such as copper (Cu) and iron (Fe) reached elevated accumulation levels at specific hotspot stations, peaking at 2,533 µg L^{-1} (S6) and 6,260 µg L^{-1} (S3), respectively. Non-parametric variance analysis revealed that iron (Fe) displayed statistically significant spatial variation across sampling nodes ($H = 6.14$, $p = .046$). Similarly, copper concentration variations neared the significance threshold ($H = 5.96$, $p = .051$). In contrast, no statistically significant spatial differences were observed for the other monitored elements due to high intra-station variability ($p > .05$). Among non-essential elements, cadmium (Cd) and lead

(Pb) demonstrated widespread contamination, with Pb peaking at 1,433 µg L^{-1} within the Pakadjuma zone (S6).

Table 2:
Concentrations (µg L^{-1}) of Essential and Non-Essential Trace Metals Across Monitoring Stations

Metal Type	Element	S1	S2	S3	S4	S5	S6	CCME Guideline	H (p-value)
Essential	Cu	1,550.00	2,333.00	0.41	0.825	0.127	2,533.00	2.0	5.96 (.051)
	Cr	0.017	0.900	0.604	0.303	3.307	0.900	8.0	2.03 (.368)
	Co	0.024	<LOD	6.242	0.013	0.007	<LOD	2.0	1.88 (.390)
	Mn	0.022	0.900	0.224	0.325	0.610	2.367	180.0	4.34 (.114)
	Zn	0.020	<LOD	0.204	0.048	0.147	<LOD	30.0	1.60 (.450)
	Fe	1,093.00	2,247.00	6,260.00	0.827	0.740	5,067.00	300.0	6.14 (.046)
Non-essential	As	0.139	0.009	<LOD	0.002	0.200	<LOD	5.0	1.73 (.420)
	Cd	0.269	0.014	0.709	0.332	0.419	0.417	0.09	1.55 (.460)
	Pb	0.014	0.900	0.610	0.900	0.618	1,433.00	7.0	1.73 (.420)
	Sb	0.010	0.180	0.040	0.080	0.050	0.250	6.0	1.59 (.450)

Note: Data points represent mean values (n = 18). LOD = limit of detection. CCME = Canadian Council of Ministers of the Environment guidelines for the protection of aquatic life. Statistical values derived from Kruskal–Wallis non-parametric analyses (α = .05).

Contamination Factor (CF) and Overall Degree of Contamination (DC)

Ecotoxicological indexing along pooled geographical sections (S1–S2, S3–S4, and S5–S6) highlighted environmental enrichment **(Table 3)**.

Among essential metals, cobalt reached its highest contamination factor midstream (CF = 1,565), whereas copper enrichment was dominant upstream (CF = 970), and chromium dominated downstream reaches (CF = 262.5). For non-essential elements, cadmium consistently displayed elevated pollution metrics across the system, peaking midstream (CF = 5,789). Cumulative analysis showed that the non-essential degree of contamination ($DC_{non\text{-}essential}$) systematically exceeded essential baselines, reaching its maximum amplitude within the midstream section (DC ≈ 5,907).

Table 3:
Contamination Factors (CF) and Integrated Degrees of Contamination (DC) by River Section

Category	Element / Index	Upstream (S1–S2)	Midstream (S3–S4)	Downstream (S5–S6)
Essential Elements	Fe	5.57	11.80	9.67
	Cu	970.00	310.00	665.00
	Cr	57.50	56.80	262.50
	Co	6.00	1,565.00	1.75
	Mn	2.56	1.53	8.28
	Zn	0.33	4.20	2.47
Non-Essential Elements	As	14.00	0.20	20.00
	Cd	2,889.00	5,789.00	4,644.00
	Pb	65.30	107.90	146.60
	Sb	15.80	10.00	25.00
Integrated Risk Indices	{DC}_{{essential}}	1,041.96	1,949.33	949.67
	{DC}_{{non-essential}}	2,984.10	5,907.10	4,835.60

Bacterial Quantifications and Phenotypic Structural Shifts

Cultivable heterotrophic bacterial densities expanded gradually along the longitudinal gradient. Total bacterial counts increased from (3.23 ± 1.53) x 10^6 CFU mL^{-1} in the upstream section (S1–S2) to (3.53 ± 2.15) x 10^6 CFU mL^{-1} midstream (S3–S4), reaching (3.73 ± 2.97) x 10^6 CFU mL^{-1} within downstream waters (S5–S6).

Concurrently, phenotypic Gram characterization revealed a structural reordering of dominant cell wall morphotypes along the pollution gradient **(Table 4)**. The upstream reach (S1–S2) was dominated by Gram-positive bacterial assemblages. Midstream stations (S3–S4) marked a transition, becoming dominated by Gram-negative cohorts. Finally, the downstream section (S5–S6), which accumulated multi-metal stress, was comprised exclusively of Gram-negative phenotypes.

Table 4:
Cultivable Bacterial Abundance and Phenotypic Distribution Along Funa River Sections

Section	Composite Units Analyzed	Bacterial Density (CFU mL−1)	95% Confidence Interval	Phenotypic Wall Distribution
Upstream (S1–S2)	E4, E5, E7	$(3.23 \pm 1.53) \times 10^6$	$[2.11 \times 10^6, 4.35 \times 10^6]$	Gram-positive Dominant
Midstream (S3–S4)	E1, E2, E3	$(3.53 \pm 2.15) \times 10^6$	$[1.96 \times 10^6, 5.10 \times 10^6]$	Gram-negative Dominant
Downstream (S5–S6)	E6, E8, E9	$(3.73 \pm 2.97) \times 10^6$	$[1.56 \times 10^6, 5.90 \times 10^6]$	Gram-negative Exclusive

Note: Bacterial loads were derived from composite analytical units (n = 9).

Ecotoxicological Linkages via Spearman's Rank Correlation

To evaluate how polymetallic pressure correlates with phenotypic shifts in the bacterial community, a Spearman rank correlation matrix was computed **(Table 5)**.

Table 5:
Bootstrapped Spearman Correlation Matrix Between Metal Metrics and Bacterial Community Compartments (n = 9)

Analytical Category	Target Metal	Gram-Positive Bacteria (ρ [95% CI])	Gram-Negative Bacteria (ρ [95% CI])	p-value
Essential Elements	Iron (Fe)	-.50 [-.88, -.04]	+.50 [.04, .88]	.041*
	Chromium (Cr)	-.50 [-.88, -.04]	+.50 [.04, .88]	.041*
	Manganese (Mn)	-.50 [-.88, -.04]	+.50 [.04, .88]	.041*
	Cobalt (Co)	-.50 [-.88, -.04]	+.50 [.04, .88]	.041*
	Copper (Cu)	+.50 [.04, .88]	-.50 [-.88, -.04]	.041*
	Zinc (Zn)	-.50 [-.88, -.04]	+.50 [.04, .88]	.041*
Non-Essential Elements	Cadmium (Cd)	-.50 [-.88, -.04]	+.50 [.04, .88]	.041*
	Lead (Pb)	-.50 [-.88, -.04]	+.50 [.04, .88]	.041*
	Antimony (Sb)	+.50 [.04, .88]	-.50 [-.88, -.04]	.041*
	Arsenic (As)	.00 [-.43, .43]	.00 [-.43, .43]	1.000ns

Note: Confidence intervals [95% CI] were computed via bootstrapping (1,000 resamples).
$p < .05$. ns, $p \geq .05$.

The majority of monitored trace metals (Fe, Cr, Mn, Co, Zn, Cd, and Pb) exhibited a statistically significant moderate negative correlation (ρ = -.50, p = .041) with the spatial distribution of Gram-positive bacteria. Conversely, these exact same elements shared an identical positive correlation (ρ = +.50, p = .041) with the abundance of Gram-negative bacterial cohorts.

In contrasting patterns, concentrations of dissolved copper (Cu) and antimony (Sb) inverted these profiles, displaying significant positive correlations with Gram-positive cells (ρ = +.50, p = .041) and parallel negative linkages with Gram-negative groups (ρ = -.50, p = .041). Arsenic (As) values did not co-vary with either of the structural phenotypic compartments (ρ = .00, p = 1.000).

DISCUSSION

Spatial Dynamics of Physicochemical Degradation

The results reveal a decline in water quality in the Funa River along the studied longitudinal gradient. The spatial variations observed in temperature (28.6 ±0.15ºC to 32.0 ±0.14ºC) and electrical conductivity (756 to 532 µS cm^{-1}) indicate changes in environmental conditions between upstream and downstream sections. The downstream increase in temperature can be linked to solar exposure, loss of riparian vegetation cover in built sections, and human activities along the banks.

In parallel, spatial variations in electrical conductivity reflect an increase in ionic load linked to uncontrolled urban greywater discharges and artisanal activities near the river channel, combined with micro-spatial dilution effects from raw municipal sewage inputs. In contrast, pH values remained stable and within recommended ranges for surface waters, indicating conditions that were generally neutral to slightly alkaline. These environmental observations align with the framework of Allan and Castillo (2007), who highlight the influence of human land-use alterations on the baseline physicochemical characteristics of tropical urban rivers.

Polymetallic Contamination and Ecotoxicological Pressures

Total concentrations of trace metals measured in the Funa River exceed CCME guideline values for most elements studied. Calculated contamination factors ({CF}) and comprehensive degrees of contamination ({DC}) indicate persistent polymetallic pollution throughout the studied section, particularly for cadmium, lead, copper, and chromium. Although no statistically significant spatial differences were observed for absolute concentrations of most metals ($p > .05$) due to localized variance, the recorded magnitudes indicate widespread, chronic contamination of the lotic ecosystem.

These results are consistent with historical observations made within the Funa River catchment by Swana et al. (2024), as well as profiles reported in other global watercourses subject to anthropogenic and industrial pressures (Ali et al., 2023; Chen et al., 2022; Yi et al., 2021). Mechanistically, the absence of sharp, statistically significant differences between geographical sections reflects diffuse contamination resulting from multiple point and non-point sources distributed throughout the urban watershed.

Phenotypic Cell Wall Reordering Under Heavy Metal Stress

Microbiological analyses revealed high baseline bacterial loads along the river, as well as a shift in the spatial distribution of bacterial groups identified by Gram staining. Gram-positive bacteria were dominant at upstream stations, while Gram-negative bacteria became predominant at intermediate stations and exclusive at downstream stations. The computed correlations show a trend toward a steady decrease in Gram-positive bacteria ($\rho \approx -.50$, $p < .05$) as metal metric thresholds increase, whereas Gram-negative bacteria exhibit positive correlations for several metals ($\rho \approx +.50$, $p < .05$).

These statistical observations support the hypothesis that Gram-negative bacteria exhibit competitive tolerance and survival adaptation in contaminated aquatic environments. As widely reported, structural characteristics of the bacterial cell wall influence the phenotypic response and biological resistance of microorganisms to environmental stresses and metal contaminants (Ayangbenro & Babalola, 2017; Fajardo et al., 2023; Gadd, 2010; Liu et al., 2020; Muter et al., 2024; Silhavy et al., 2010; Wang et al., 2024). The asymmetric outer membrane of Gram-negative cells, rich in lipopolysaccharides (LPS), provides a protective envelope that minimizes intracellular toxicity by facilitating passive metal biosorption, while their genomes encode specialized trans-envelope efflux pumps that actively expel divalent metallic cations (Gadd, 2010; Silhavy et al., 2010).

Public Health Risks and Human Exposure Pathways

The severe polymetallic pollution documented in the Funa River poses risks to public health that require attention. In the populated informal districts flanking downstream sections (such as Pakadjuma), river water is routinely accessed due to deficient municipal infrastructure.

Primary pathways of human exposure include direct dermal contact during laundry, domestic chores, and bathing, alongside ingestion of toxic metal residues via trophic transfer. Specifically, downstream water is utilized for the irrigation of local small-scale vegetable crops (*maraîchage*). This practice can trigger bioaccumulation of nephrotoxic cadmium and neurotoxic lead within edible plant tissues, exposing consumers to chronic systemic toxicosis. Furthermore, persistent multi-metal pressure ($\rho \approx +.50$ for Gram-negative enteric bacilli) acts as an environmental catalyst for the co-selection of antibiotic resistance traits (Ayangbenro & Babalola, 2017). Through shared plasmidial mechanics, polymetallic stress promotes the spread of multi-drug-resistant pathogens, turning the river into an epidemiological vector for the urban population of Kinshasa.

Methodological Limitations

The results obtained should be interpreted in light of intrinsic methodological limitations:

i. **Taxonomic Resolution Constraints:** This study is based on the characterization of cultivable bacterial community composition using phenotypic Gram staining and does not provide a direct assessment of microbial diversity in the strict ecological sense. The observed changes concern structural reordering of cell wall types and do not allow for definitive conclusions regarding species richness, evenness, or true taxonomic diversity.

ii. **Absence of Functional and Molecular Data:** Biochemical resistance mechanisms involved in tolerance were not directly evaluated. Resolving these pathways requires advanced investigations incorporating high-throughput molecular approaches (Ali et al., 2023; Liu et al., 2020).

iii. **Sample Size and Temporal Boundaries:** Microbiological analyses were restricted to nine composite analytical units due to material constraints, reducing the statistical power of correlation models. Furthermore, sampling was conducted during a single cross-sectional campaign, which prevents the evaluation of seasonal or hydrological variations.

iv. **Matrix Restrictions:** Monitoring was bound to the aqueous dissolved phase, omitting analysis of benthic sediments and suspended particulate matter, which typically trap heavy metals.

CONCLUSION

This study assessed polymetallic contamination and examined its relationship with the phenotypic structure of cultivable bacterial communities in the Funa River. Concentrations of several trace metals were significantly higher than CCME guideline values, indicating critical polymetallic contamination in this tropical urban watercourse. Calculated contamination factors (CF) and contamination degrees (DC) confirm high metal pressure throughout the studied section. Concurrently, microbiological tracking revealed high bacterial loads and a shift in the distribution of bacterial groups characterized by Gram staining, moving from Gram-positive dominance upstream to Gram-negative dominance downstream. Non-parametric correlations suggest a relationship between heavy metal pressure and the structural reordering of cultivable bacterial cells.

However, findings must be interpreted in light of acknowledged limitations, including the number of microbiological units analyzed, the absence of molecular approaches, and the single-point temporal nature of the sampling campaign. Results pertain exclusively to the phenotypic structure of cultivable bacterial communities and do not allow for a direct assessment of true microbial diversity or associated ecological functions.

From an environmental management standpoint, observed contamination levels underscore the need to strengthen control measures for domestic, artisanal, and industrial discharges throughout the Funa River watershed. Policy priorities should include the installation of decentralized filtration systems for mechanical workshops, enforcement of zoning restrictions to ban water utilization for urban vegetable irrigation, and development of waste collection infrastructure in informal settlements such as Pakadjuma. Permanent environmental monitoring programs incorporating both chemical and microbiological bio-indicators could contribute to better assessments of the ecological status of this urban watercourse.

Future Perspectives

Future research should incorporate sediment analysis to evaluate long-term pollutant accumulation, execute multi-season monitoring campaigns across distinct hydrological cycles, and utilize molecular approaches such as 16S rRNA metagenomic sequencing. These steps are necessary to resolve the taxonomic responses of uncultivable microbial communities to polymetallic contamination, map antibiotic co-resistance vectors, and identify indigenous microorganisms with potential for bioremediation applications.

Use of Artificial Intelligence (AI) Declaration: The authors declare that no generative artificial intelligence was used in the conception and design of the study, data collection, data analysis, interpretation of results, or the generation or modification of the primary research data. Artificial intelligence-assisted language tools were used solely to improve the English translation, grammar, spelling, and overall linguistic clarity of the manuscript. All scientific content, interpretations, conclusions, and the final version of the manuscript were critically reviewed, verified, and approved by the authors, who take full responsibility for the content of this article.

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Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgements: The authors sincerely thank the Department of Environmental Science and Management, Faculty of Science and Technology, University of Kinshasa, for providing the facilities required to conduct this research. The authors also acknowledge the Analysis Center of the General Commissariat for Atomic Energy/ Kinshasa Regional Nuclear Research Center (CGEA/CREN-K) for conducting the ED-XRF analysis.

Ethical Approval: Nil required.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Funding: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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REFERENCES

Ali, B., Khan, A., Ali, S. S., Khan, H., Alam, M., Ali, A., Alrefaei, A. F., Almutairi, M. H., & Kim, K. I. (2023). Heavy metals and microbial diversity: A comparative analysis of Rivers Swat and Kabul. *Water*, *15*(18), Article 3297. https://doi.org/10.3390/w15183297

Allan, J. D., & Castillo, M. M. (2007). *Stream ecology: Structure and function of running waters* (2nd ed.). Springer.

American Public Health Association. (2012). *Standard methods for the examination of water and wastewater* (22nd ed.). APHA Press.

Ayangbenro, A. S., & Babalola, O. O. (2017). A new strategy for heavy metal polluted environments: A review of microbial biosorbents. *Chemosphere*, *185*, 136–146. https://doi.org/10.1016/j.chemosphere.2017.06.145

Azarbad, H., Niklińska, M., van Gestel, C. A. M., van Straalen, N. M., & Röling, W. F. M. (2013). Microbial community composition and functions are resilient to metal pollution along two forest soil gradients. *FEMS Microbiology Ecology*, *84*(2), 364–378. https://doi.org/10.1111/1574-6941.12069

Canadian Council of Ministers of the Environment. (2022). *Canadian water quality guidelines for the protection of aquatic life*. CCME.

Chen, J., Wu, H., Liu, Y., Li, Z., & Sun, Z. (2022). Heavy metal contamination and its influence on bacterial community structure in river sediments of the Yangtze River. *Science of the Total Environment*, *838*, Article 156428. https://doi.org/10.1016/j.scitotenv.2022.156428

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed.). Lawrence Erlbaum Associates.

Daly, K., & Fenelon, A. (2018). Application of energy dispersive X-ray fluorescence spectrometry to the determination of copper, manganese, zinc, and sulfur in grass (*Lolium perenne*) in grazed agricultural systems. *Applied Spectroscopy*, *72*(11), 1661–1673. https://doi.org/10.1177/0003702818787165

Dean-Ross, D., & Mills, A. L. (1989). Bacterial community structure and function along a heavy metal gradient. *Applied and Environmental Microbiology*, *55*(8), 2002–2009. https://doi.org/10.1128/aem.55.8.2002-2009.1989

Fajardo, C., Costa, G., Nande, M., Botías, P., García-Cantalejo, J., & Martín, M. (2023). Heavy metals influence the structure and function of soil microbial communities. *Agriculture*, *13*(3), Article 653. https://doi.org/10.3390/agriculture13030653

Field, A. (2024). *Discovering statistics using IBM SPSS Statistics* (6th ed.). SAGE Publications.

Gadd, G. M. (2010). Metals, minerals and microbes: Geomicrobiology and bioremediation. *Microbiology*, *156*(3), 609–643. https://doi.org/10.1099/mic.0.037143-0

Hakanson, L. (1980). An ecological risk index for aquatic pollution control: A sedimentological approach. *Water Research*, *14*(8), 975–1001. https://doi.org/10.1016/0043-1354(80)90143-8

Hu, X., Zhao, L., Ding, Z., Gao, X., & Han, G. (2026). Tooth enamel as a biogeochemical archive of trace elements in an urban population: A multielement study from Beijing, China. *Biological Trace Element Research*, *204*(2), 412–425. https://doi.org/10.1007/s12011-026-05036-2

International Organization for Standardization. (2024). *Microbiology of the food chain – General requirements and guidance for microbiological examinations* (ISO Standard No. 7218:2024).

Kapembo, M. L., Mukeba, F. B., Sivalingam, P., Mukoko, J. B., Bokolo, M. K., Mulaji, C. K., Mpiana, P. T., & Poté, J. (2021). Survey of water supply and assessment of groundwater quality in suburban zones of Kinshasa, Democratic Republic of the Congo. *Waste Management*, *120*, 238–248. https://doi.org/10.1016/j.wasman.2020.11.040

Kayembe, J. M., Bokwokwo, M. I., Sivalingam, P., Ngelinkoto, P., Otamonga, J.-P., Mulaji, C. K., Mubedi, J. I., & Poté, J. (2020). Effect of untreated urban effluents on the accumulation of toxic metals in river sediments under tropical conditions: Funa River, Kinshasa, Democratic Republic of the Congo.

Water and Environment Journal, *34*(2), 180–188. https://doi.org/10.1111/wej.12451

Kyowire, K. P., Luamba, L. N. J., Kamb, T. J.-C., Sisa, M. E., Munganga, G. J., & Bunda, P. M. N. (2024). Évaluation de la qualité physico-chimique et bactériologique des eaux des puits et forages des quartiers Batumona, Biyela et Luebo dans la commune de Kimbanseke à Kinshasa (RDC). *International Journal of Progressive Sciences and Technologies*, *47*(2), 257–270.

Lia, F., Zammit Mangion, M., & Farrugia, C. (2020). Application of elemental analysis via energy dispersive X-ray fluorescence (ED-XRF) for the authentication of Maltese extra virgin olive oil. *Agriculture*, *10*(3), Article 71. https://doi.org/10.3390/agriculture10030071

Liu, C., Zhou, K., Qin, W., Tian, C., Qi, M., Yan, X., & Han, W. (2020). A review on heavy metals contamination in soil: Effects, sources, and remediation techniques. *Science of the Total Environment*, *715*, Article 136974. https://doi.org/10.1016/j.scitotenv.2020.136974

Mabidi, D. M., Manyingu, H. M., Ngakiama, G. B., & Poté, J. (2024). Trace metal contamination and ecotoxicological risk assessment in Congo River sediments around Kinshasa. *Environmental Monitoring and Assessment*, *196*(4), Article 312. https://doi.org/10.1007/s10661-024-12450-1

Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., & Stahl, D. A. (2018). *Brock biology of microorganisms* (15th ed.). Pearson.

Manza, H. M., Kiyombo, G. M., Kuna, S. N., Carvalho, F. P., Poté, J., Atibu, E. K., & Mata, H. K. (2026). Trace metal levels in sediments and muscle of edible fish species from the Kasai River, Democratic Republic of the Congo. *Water, Air, & Soil Pollution*, *237*(13), Article 765. https://doi.org/10.1007/s11270-026-09437-w

Mukeba, F., Kapembo, M., Mpiana, P., Mulaji, C., & Poté, J. (2022). Approvisionnement et évaluation de la qualité des eaux souterraines de Selembao et Kimbanseke. *Revue Congolaise des Sciences Humaines et Sociales*, *1*(2), 1–18. https://doi.org/10.59189/crsh102240

Muter, O., Gudrā, D., Daumova, G., Idrisheva, Z., Rakhymberdina, M., Tabors, G., Dirnena, B., Dobkeviča, L., Petrova, O., Apshikur, B., Luņģe, M., Fridmanis, D., Dennisov, I., Bekishev, Y., Kasparinskis, R., Mukulysova, Z., & Polezhayev, S. (2024). Impact of anthropogenic activities on microbial community structure in riverbed sediments of East Kazakhstan. *Microorganisms*, *12*(2), Article 246. https://doi.org/10.3390/microorganisms12020246

Mwanamoki, P. M., Devarajan, N., Niane, B., Ngelinkoto, P., Thevenon, F., Nlandu, J. W., Mpiana, P. T., Prabakar, K., Mubedi, J. I., Kabele, C. G., Wildi, W., & Poté, J. (2015). Trace metal distributions in the sediments from river-reservoir systems: Case of the Congo River and Lake Ma Vallée, Kinshasa (Democratic Republic of Congo). *Environmental Science and Pollution Research*, *22*(1), 586–597. https://doi.org/10.1007/s11356-014-3381-y

Nakagawa, S., Lagisz, M., Yang, Y., & Drobniak, S. M. (2024). Finding the right power balance: Better study design and collaboration can reduce dependence on statistical power. *PLOS Biology*, *22*(1), Article e3002423. https://doi.org/10.1371/journal.pbio.3002423

Nsiala, O. G., Mukeba, B. F., Seke, V. M., Bamvingana, K. C., Kacha, M. M., Matumona, K. D., & Biey, M. E. (2025). Risk and recurrence of waterborne diseases linked to groundwater quality in the Hérady district of Selembao (Kinshasa, DRC). *Orapuh Journal*, *6*(12), Article e1318. https://doi.org/10.4314/orapj.v6i12.118

Silhavy, T. J., Kahne, D., & Walker, S. (2010). The bacterial cell envelope. *Cold Spring Harbor Perspectives in Biology*, *2*(5), Article a000414. https://doi.org/10.1101/cshperspect.a000414

Sun, X., Zhang, L., Liu, H., Wang, Z., & Giesy, J. P. (2021). Ecotoxicological risk assessment of metal cocktails based on maximum cumulative ratio during multi-generational exposures. *Water Research*, *200*, Article 117274. https://doi.org/10.1016/j.watres.2021.117274

U.S. Environmental Protection Agency. (2007). *Method 1694: Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS* (EPA Publication No. 821-R-08-002).

Ukoumunne, O. C., Davison, A. C., Gulliford, M. C., & Chinn, S. (2003). Non-parametric bootstrap confidence intervals for the intraclass correlation coefficient. *Statistics in Medicine*, *22*(24), 3805–3821. https://doi.org/10.1002/sim.1643

Wang, M., Wang, Y., Wu, Y., Ma, Q., Huang, J., He, T., Huang, S., & Chen, C. (2024). Exploring the long-term impact of a cadmium pollution accident on microbial communities in river ecosystems. *Biogeochemistry*, *167*(8), 1123–1137. https://doi.org/10.1007/s10533-024-01150-2

Willey, J. M., Sherwood, L. M., & Woolverton, C. J. (2017). *Prescott's microbiology* (10th ed.). McGraw-Hill Education.

World Health Organization. (2022). *Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda*. WHO Guidelines Approved by the Guidelines Review Committee.

Yang, D., Hou, Y., Wei, C., Ling, J., & Zheng, X. (2025). Aquatic microcosms in ecotoxicology: The community-level ecological risk assessment of pollutants. *Toxics*, *13*(8), Article 694. https://doi.org/10.3390/toxics1308694

Yi, J., Wang, Y., Zhang, S., Liu, H., Lv, J., Zhu, L., & Zhou, Y. (2021). Study of heavy metals and microbial communities in estuarine sediments of the Yangtze River estuary. *Frontiers in Marine Science*, *8*, Article 741912. https://doi.org/10.3389/fmars.2021.741912

Zeb, M., Khan, K., Younas, M., Farooqi, A., Cao, X., Kavil, Y. N., Alelyani, S. S., Alkasbi, M. M., & Al-Sehemi, A. G. (2024). A review of heavy metals pollution in riverine sediment from various Asian and European countries: Distribution, sources, and environmental risk. *Marine Pollution Bulletin*, *206*, Article 116775. https://doi.org/10.1016/j.marpolbul.2024.116775