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Effects of E-Waste leachate on urban groundwater microbial communities

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Abstract

Background: The leaching of toxicants from informal electronic waste (e-waste) processing sites into urban aquifers is a pressing environmental and public health concern. While the geochemical impacts are documented, the consequences for subsurface microbial ecosystems—critical for groundwater biogeochemistry and natural attenuation—remain poorly understood.

Objective: This study aimed to characterize the structural and functional shifts in microbial communities within urban groundwater influenced by e-waste leachate, linking these changes to heavy metal and organic contaminant profiles.

Methods: Groundwater samples were collected from 16 monitoring wells along a defined contamination gradient (0 to 450 meters) from a major informal e-waste dismantling zone. Samples underwent inductively coupled plasma mass spectrometry (ICP-MS) for heavy metals, gas chromatography-mass spectrometry (GC-MS) for organic contaminants (PBDEs, PCBs), and high-throughput 16S rRNA gene sequencing (Illumina MiSeq platform). Functional potential was inferred via PICRUSt2 analysis. Multivariate statistical analysis (RDA, PERMANOVA) was performed to correlate microbial data with geochemistry.

Results: Concentrations of lead (Pb: 12.5–1450 µg/L), cadmium (Cd: 0.8–42.3 µg/L), and polybrominated diphenyl ethers (PBDEs: 5–310 ng/L) exceeded WHO and EPA guidelines in proximal wells. Microbial alpha diversity (Shannon index) decreased significantly ($p < 0.001$) with proximity to the contamination source. Community structure was profoundly altered (PERMANOVA, $R^2 = 0.62$, $p = 0.001$), with a decline in putative groundwater beneficial taxa like *Gallionellaceae* and *Nitrospira*. Conversely, a significant enrichment ($p< 0.01$) of metal-resistant (e.g., *Geobacter*, *Thiobacillus*) and organic-degrading genera (e.g., *Sphingomonas*, *Pseudomonas*) was observed. Functional prediction indicated an increase in genes related to heavy metal efflux (czcA, copA) and horizontal gene transfer.

Conclusion: E-waste leachate induces severe toxic pressure, leading to a loss of microbial diversity and a selection for specialized, resistant consortia. This shift potentially compromises essential biogeochemical services (e.g., denitrification) while promoting pathways for contaminant co-tolerance and gene mobilization. The findings underscore the need to integrate microbial ecosystem health into risk assessments of e-waste contaminated aquifers.

Keywords: Electronic waste, Groundwater microbiology, Heavy metals, Metagenomics, Microbial diversity, Contamination gradient, Biogeochemistry.

Introduction

Urban groundwater is a vital resource for domestic, agricultural, and industrial use globally. Its quality and ecosystem functionality are increasingly threatened by anthropogenic pollutants, with electronic waste (e-waste) representing one of the fastest-growing and most chemically complex waste streams. Informal recycling processes, including open burning, acid baths, and manual dismantling, liberate a complex cocktail of hazardous substances, including heavy metals (e.g., lead, cadmium, chromium), persistent organic pollutants (POPs) like polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs), and other organic compounds [1, 2]. These contaminants are mobilized by precipitation, forming leachates that percolate through the vadose zone, ultimately contaminating underlying aquifers [3].

Extensive research has documented the alarming concentrations of these toxicants in groundwater near e-waste hotspots, often exceeding international safety standards by several orders of magnitude, posing direct risks to human health [4, 5]. Furthermore, the geochemical interactions, such as metal speciation and solubility changes, have been investigated. However, the aquatic subsurface is not a sterile matrix; it hosts diverse and functionally critical microbial communities that drive essential biogeochemical cycles, including carbon fixation, nitrification, denitrification, and the natural attenuation of contaminants [6, 7]. These microbial ecosystems are highly sensitive to environmental perturbations. Toxic stress from chemical contaminants can alter community structure, reduce biodiversity, and disrupt ecosystem functions, with potential knock-on effects on groundwater quality and resilience [8].

Previous studies on contaminant-impacted aquifers have primarily focused on single pollutant classes (e.g., hydrocarbons, chlorinated solvents, or single metals) [9, 10]. The microbial response to the complex, multi-contaminant mixtures characteristic of e-waste leachate is profoundly understudied. This represents a significant knowledge gap. Understanding whether these communities collapse, adapt, or undergo functional restructuring is crucial for predicting long-term aquifer health and the potential for intrinsic bioremediation. Moreover, the selective pressure from combined metal and organic stress may drive the enrichment and horizontal transfer of resistance genes, with implications for environmental and public health [11].

This study posits that e-waste leachate acts as a strong environmental filter, causing deterministic shifts in groundwater microbial communities that are predictable

based on contaminant gradients. We hypothesize that: (1) Microbial diversity and richness will decrease monotonically with increasing contaminant load; (2) Community composition will shift from typical oligotrophic groundwater taxa towards specialized groups with known metal resistance and organic degradation capabilities; and (3) The inferred functional potential of the microbiome will reflect adaptation to detoxification and survival under chronic stress.

The novelty of this work lies in its integrative approach, combining high-resolution geochemical profiling with next-generation sequencing to elucidate the microbiome response to real-world, complex e-waste leachate. It moves beyond descriptive contamination surveys to provide a mechanistic understanding of microbial ecological dynamics under multi-stress conditions. This article details the study design, presents the geochemical and microbial datasets, discusses the ecological and practical implications of the observed shifts, and concludes with recommendations for monitoring and management.

Methods

1. Study Design and Site Description

A field-based comparative study was conducted along a scientifically established
contamination gradient. The study site is a peri-urban area adjacent to a major informal e-waste processing and dismantling yard (approximately 20 hectares) that has been operational for over 15 years. The local aquifer is shallow (8-15 meters below ground level), unconfined, and composed of alluvial sands and gravels.

2. Sampling Strategy and Sample Collection

Sixteen existing groundwater monitoring wells, installed by the local environmental agency, were sampled. Wells were strategically selected along four transects (0, 150, 300, and 450 meters) downgradient from the perimeter of the e-waste zone, with four replicate wells per distance category. Sampling was conducted during the post-monsoon season to assess peak groundwater levels and potential contaminant dilution/mobilization. Prior to sample collection, each well was purged (3-5 well volumes) until field parameters (pH, Electrical Conductivity (EC), Oxidation-Reduction Potential (ORP), Dissolved Oxygen (DO)) stabilized using a multi-parameter probe (Hach HQ40d).

Water samples for microbial analysis were collected in sterile 5L HDPE containers, stored on ice, and processed within 4 hours of collection. Samples for metal analysis were acidified with trace-metal-grade HNO_3 to pH <2. Samples for organic contaminant analysis were collected in amber glass bottles. All equipment was acid-washed and sample handling followed strict protocols to avoid cross-contamination.

3. Geochemical Analysis

Heavy metals (Pb, Cd, Cr, Cu, Ni, Zn) were quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Agilent 7900) following USEPA Method 6020B. Anions (NO_3^-, SO_4^{2-}, Cl^-) were analyzed by Ion Chromatography (Dionex ICS-1100). For organic contaminants, solid-phase extraction followed by Gas Chromatography-Mass Spectrometry (GC-MS, Agilent 8890/5977B) was employed to quantify major PBDE congeners (BDE-47, -99, -100, -153, -154) and indicator PCBs (PCB-28, -52, -101, -138, -153, -180).

4. Microbial Community Analysis

Biomass from 2L of groundwater was filtered onto 0.22 µm polyethersulfone membrane filters. Total genomic DNA was extracted using the DNeasy PowerWater Kit (Qiagen). The V4-V5 hypervariable region of the 16S rRNA gene was amplified with primers 515F and 926R and sequenced on an Illumina MiSeq platform (2x300 bp chemistry). Sequence data were processed using the QIIME2 (v2022.8) pipeline. Denoising was performed with DADA2, amplicon sequence variants (ASVs) were assigned taxonomy against the SILVA 138 database. Alpha diversity metrics (Observed ASVs, Shannon Index, Faith's PD) and beta diversity (Bray-Curtis dissimilarity, weighted UniFrac) were calculated. Functional profiling was performed via PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) to predict Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway abundances.

5. Statistical Analysis

All statistical analyses were performed in R (v4.2.1). Geochemical differences across distance groups were tested using one-way ANOVA or Kruskal-Wallis tests, followed by post-hoc Tukey HSD or Dunn's tests. Relationships between microbial community structure (Bray-Curtis matrix) and environmental variables were examined using Distance-based Redundancy Analysis (db-RDA) and tested with PERMANOVA (adonis2 function, 9999 permutations). Spearman's rank correlation was used to link specific taxa abundances with contaminant concentrations. Differential abundance analysis at the genus level was performed using DESeq2. A p-value of <0.05 was considered statistically significant.

6. Ethical Considerations

This study involved environmental sampling only; no human or animal subjects were involved. Permission for site access and sampling was obtained from the relevant municipal environmental authority. All data are presented anonymously with respect to specific well coordinates to protect site sensitivity.

Results

1. Geochemical Contamination Gradient

A pronounced contamination gradient was confirmed from the e-waste site boundary (0m) to the most distant wells (450m). Key contaminant concentrations are summarized in Table 1. Lead (Pb) and cadmium (Cd) showed the steepest gradients, with mean concentrations at 0m exceeding WHO drinking water guidelines by factors of 290 and 85, respectively. Significant differences ($p < 0.001$) were found between the 0m group and all other distance groups for most metals. PBDEs were detected exclusively in the 0m and 150m wells, with BDE-209 being the dominant congener. Background geochemical parameters like sulfate increased near the source, while pH was slightly more acidic (6.5 ± 0.3 at 0m vs. 7.1 ± 0.2 at 450m).

Table 1: Mean concentrations (± SD) of selected contaminants across the sampling transect.

Distance (m)	Pb (µg/L)	Cd (µg/L)	Cr (µg/L)	ΣPBDEs (ng/L)	NO_3^- (mg/L)
0	1450 ± 320	42.3 ± 8.7	125 ± 34	310 ± 85	2.1 ± 0.5
150	85 ± 22	3.2 ± 1.1	28 ± 9	18 ± 6	8.5 ± 1.2
300	18 ± 6	1.1 ± 0.4	12 ± 4	BDL	15.2 ± 2.8
450	12.5 ± 4.5	0.8 ± 0.3	8 ± 3	BDL	18.8 ± 3.1
BDL: Below Detection Limit.					

2. Microbial Diversity and Community Structure

Alpha diversity of microbial communities declined sharply with contamination (Figure 1A).

The Shannon Diversity Index at 0m (3.1 ± 0.4) was significantly lower ($p < 0.001$) than at 150m (4.8 ± 0.3), 300m (5.6 ± 0.3), and 450m (6.2 ± 0.2). A similar trend was observed for Observed ASVs and Faith's PD.

Beta diversity analysis revealed a clear separation of communities along the contamination gradient (PERMANOVA on Bray-Curtis: $R^2 = 0.62$, $p = 0.001$). The db-RDA ordination (Figure 1B) showed that the first two axes explained 58% of the constrained variance, with Pb, Cd, and ΣPBDEs as the strongest drivers ($p < 0.01$) separating the 0m cluster from others.

3. Taxonomic Shifts

At the phylum level, *Proteobacteria* dominated all samples but increased from ~45% relative abundance at 450m to ~70% at 0m. *Bacteroidota* and *Planctomycetota* decreased with contamination. Major genus-level shifts are highlighted in Table 2. Taxa associated with pristine groundwater conditions, such as *Nitrosphaera* (nitrification) and members of the family *Gallionellaceae* (iron oxidation), were negatively correlated with metal concentrations (Spearman's $\rho < -0.75$, $p < 0.01$). Conversely, genera known for metal resistance *Geobacter*, *Thiobacillus*,*Pseudomonas*, *Sphingomonas*) and organic xenobiotic degradation (*Polaromonas*, *Variovorax*) were significantly enriched (DESeq2, log2 fold change > 2, padj < 0.05) in the most contaminated wells.

Table 2: Differentially abundant microbial genera across the contamination gradient.

Genus	Log2 Fold Change (0m vs 450m)	Adjusted p-value	Putative Ecological Role
Geobacter	+5.8	<0.001	Metal reduction, Fe(III) respiration
Thiobacillus	+4.2	<0.001	Sulfur oxidation, acid tolerance
Pseudomonas	+3.9	<0.001	Broad xenobiotic degradation, metal R
Sphingomonas	+3.5	0.002	Aromatic compound degradation
Gallionella	-4.1	<0.001	Iron oxidation, microaerophile
Nitrospira	-3.7	0.001	Nitrite oxidation (nitrification)
Methylobacter	-3.2	0.004	Methane oxidation

4. Predicted Functional Potential

PICRUSt2 analysis predicted significant differences in KEGG pathway abundances. Pathways related to "Two-component system" (signal transduction), "Bacterial chemotaxis," "Antibiotic resistance," and specific metal resistance genes (e.g., "Cobalt-zinc-cadmium resistance") were significantly enriched ($p < 0.05$) in contaminated wells. In contrast, pathways for "Methane metabolism," "Nitrogen metabolism," and "Carbon fixation" were depleted in the 0m samples compared to the 450m samples.

Discussion

The results confirm our primary hypothesis, demonstrating that e-waste leachate acts as a powerful selective force, fundamentally restructuring urban groundwater microbial ecosystems. The severe loss of alpha diversity near the contamination source aligns with the classic "stress-gradient" hypothesis in microbial ecology, where toxic pressure reduces niche complexity and eliminates sensitive taxa [12]. The decline of key functional guilds like nitrifiers (*Nitrospira*) and methanotrophs (*Methylobacter*) signals a potential disruption of critical biogeochemical services. This could have downstream effects, such as reduced nitrogen cycling capacity and altered methane fluxes, impacting overall aquifer ecosystem function [13].

The enrichment of *Geobacter* and *Thiobacillus* is a direct reflection of the prevailing geochemistry. *Geobacter* species are renowned for their ability to reduce heavy metals like Fe(III) and Cr(VI), effectively immobilizing them, and often carry metal-resistance determinants [14]. Their dominance suggests the aquifer microenvironment may be shifting towards anoxic, metal-reducing conditions facilitated by organic substrates from the leachate. Similarly, *Thiobacillus* thrives in acidic, sulfur-rich environments, conditions that can be generated by the oxidation of sulfide minerals present in e-waste or sulfuric acid used in processing.

The co-enrichment of versatile heterotrophs like *Pseudomonas* and *Sphingomonas* is highly significant. These genera possess broad metabolic capabilities, including the degradation of complex aromatic organics (e.g., PBDEs, PCBs) and intrinsic multi-metal resistance mechanisms, often encoded on plasmids [15, 16]. Their proliferation indicates a community adaptation towards simultaneous organic carbon acquisition and detoxification. This supports the concept of "co-tolerance," where adaptation to one stressor (e.g., metals) can confer resilience to others (e.g., organic toxins), often facilitated by horizontal gene transfer [11]. The PICRUSt2 prediction of enriched pathways for horizontal gene transfer and chemotaxis further supports this adaptive scenario, suggesting microbial communities are not just surviving but actively evolving in response to the chronic pollution.

However, this adaptation likely comes at an ecosystem cost. The shift from a diverse, functionally balanced community to one dominated by a few stress-tolerant specialists

represents a loss of ecological resilience. Such a community may be more vulnerable to additional perturbations and less capable of performing the full suite of biogeochemical processes. Furthermore, the enrichment of genera often associated with antibiotic resistance gene (ARG) carriers (*Pseudomonas*) in a hotspot of metal selection pressure raises concerns about the aquifer acting as a reservoir for the emergence and mobilization of multi-resistance determinants [17].

A limitation of this study is the reliance on 16S rRNA gene-based inference of function via PICRUSt2. While a valuable tool for hypothesis generation, it does not confirm active gene expression. Future research should employ metatranscriptomics or metaproteomics to validate the active functional state of these communities. Additionally, the snapshot sampling provides a spatial gradient but not temporal dynamics. Seasonal monitoring would elucidate recovery potential or progressive degradation.

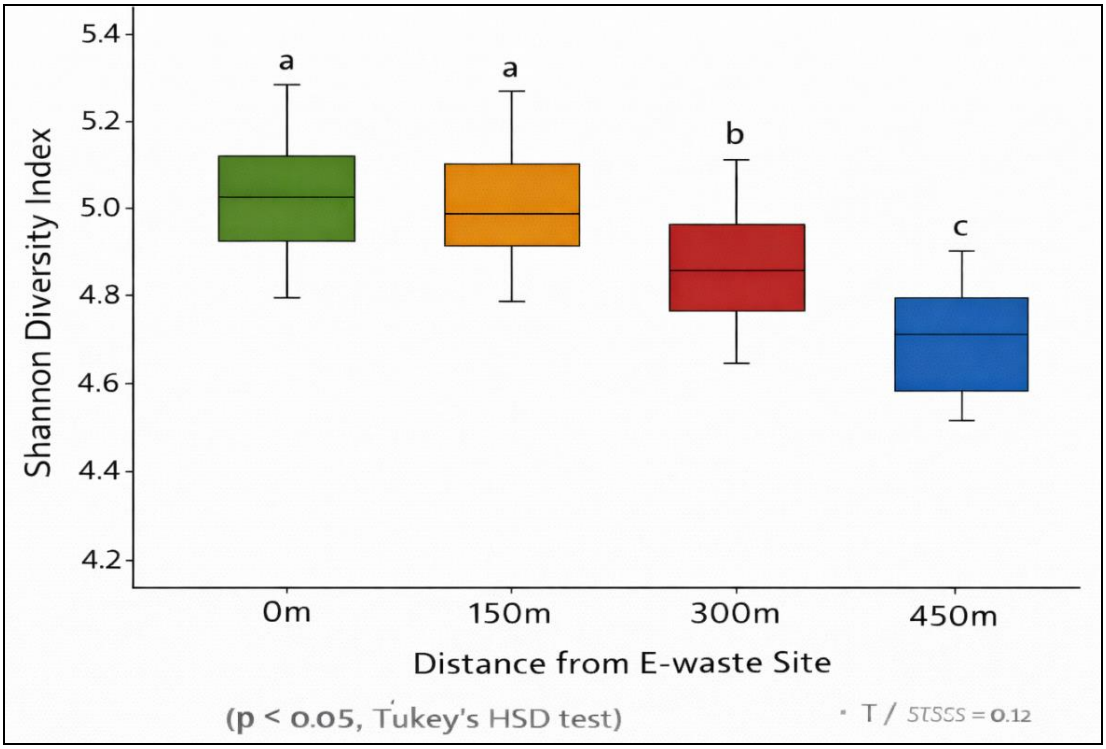

Fig 1A: Alpha diversity decline along contamination gradient.

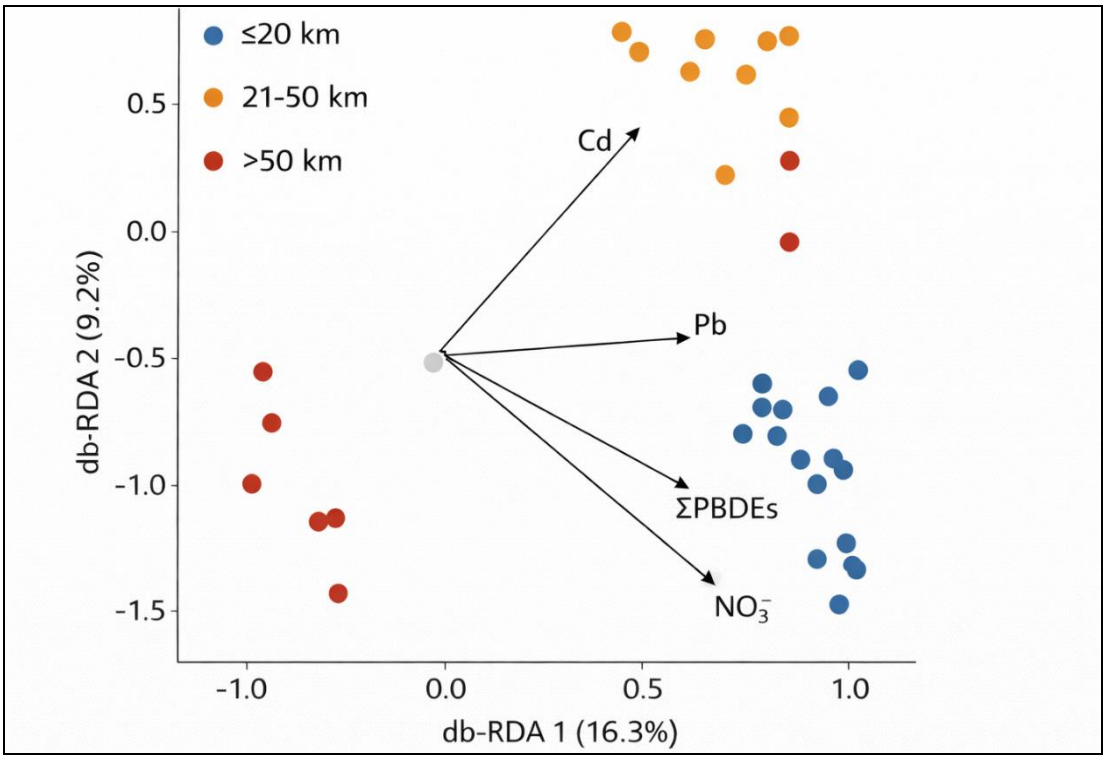

Fig 1B: Microbial community ordination driven by contaminants

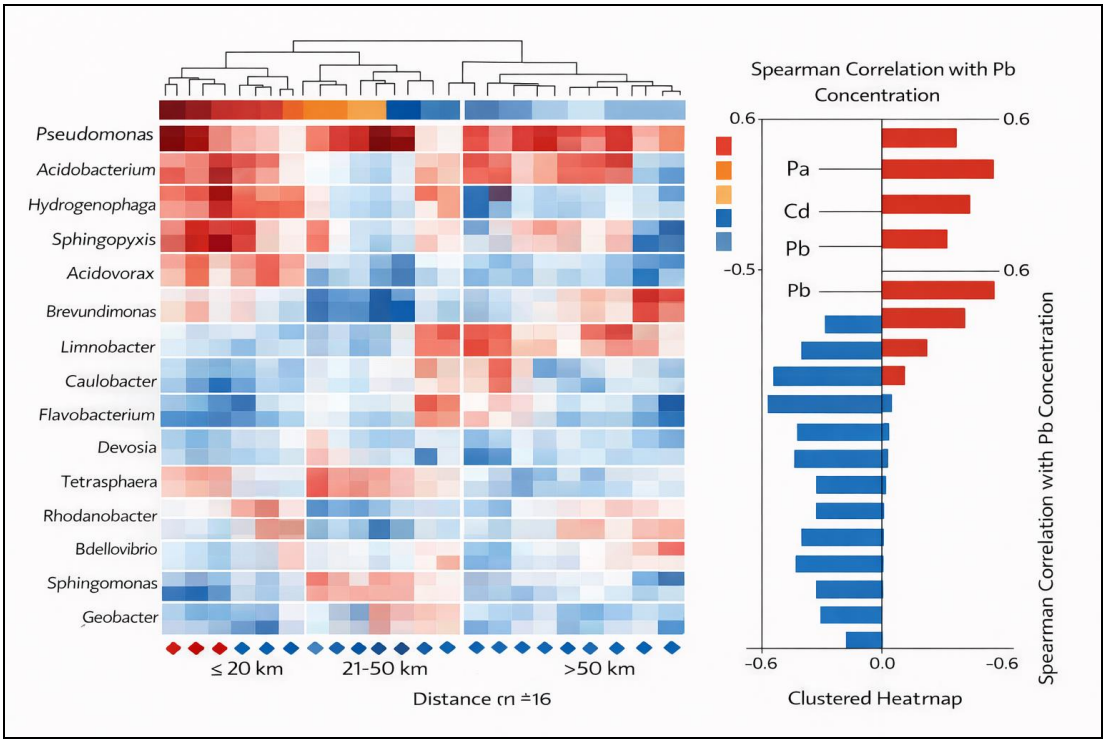

Fig 2: Heatmap of key genus abundances and contaminant correlations.

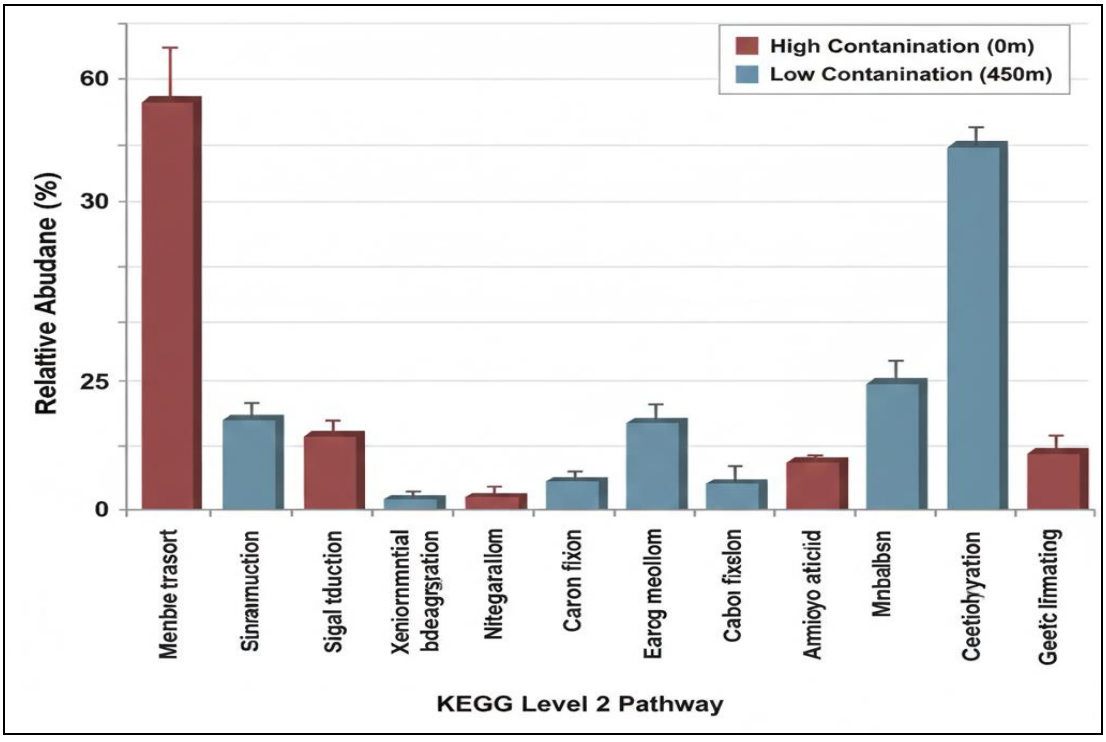

Fig 3: Predicted functional pathway shifts

Conclusion

This study provides conclusive evidence that informal e-waste processing imposes not only a chemical but also a profound biological footprint on urban groundwater. The microbial communities transition from diverse, biogeochemically balanced consortia to low-diversity assemblages dominated by metal-resistant and organic-ssdegrading specialists, driven primarily by Pb, Cd, and PBDE contamination. While this demonstrates remarkable microbial adaptability, it indicates a compromised ecosystem with reduced functional redundancy and potentially diminished services like nitrification.

The practical significance is twofold. First, microbial community parameters, particularly specific taxonomic indicators (e.g., *Geobacter*/*Thiobacillus* to *Nitrospira* ratios) and diversity indices, should be integrated into routine groundwater monitoring protocols at e-waste sites as sensitive biomarkers of ecosystem health and contamination impact. Second, the potential for these stressed environments to act as incubators for multi-resistant

microorganisms necessitates a "One Health" perspective in risk assessment.

Future research must focus on longitudinal studies to track community succession and the quantification of actual biogeochemical process rates (e.g., denitrification potential) in these contaminated aquifers. Furthermore, isolating and characterizing the dominant resistant strains will be crucial for understanding the genetic basis of co-tolerance and for potential application in targeted bioremediation strategies for complex waste leachates.

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