



Microbial risk assessment and bioleaching as sustainable strategies for groundwater remediation in contaminated urban environments.

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Abstract:

Groundwater contamination in urban-industrial landscapes is not only a chemical but also a biological health challenge. This study evaluates microbial risk and explores microbial leaching as a sustainable remediation approach. Groundwater samples were analysed for coliforms (MPN method), fecal contamination, and quantified using Beta-Poisson risk modelling. Results indicated fecal coliform levels exceeding WHO safety thresholds (up to 10^3 MPN/100 mL), posing diarrheal disease risks, particularly in children. Risk assessment suggested exposure pathways through oral ingestion and dermal contact, with hazard quotients exceeding unity. In parallel, microbial leaching potential was explored: native microbial consortia demonstrated capacity to degrade organic pollutants and immobilize toxic ions in lysimetric columns. Findings indicate that microbial leaching can serve as a low-cost, eco-friendly strategy for mitigating landfill/sewage impacts, while quantitative microbial risk assessment (QMRA) offers a predictive tool for health protection.

Keywords: microbial risk assessment, fecal coliforms, Beta-Poisson model, bioremediation, and microbial leaching.

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I. Introduction:

Groundwater is one of the most vital natural resources, sustaining billions of people worldwide by providing a relatively reliable and cost-effective source of potable water. In developing nations such as India, where rapid population growth and urbanization have outpaced infrastructural development, groundwater often serves as the primary supply for drinking, domestic use, and irrigation. However, the quality and sustainability of this resource are increasingly under threat due to anthropogenic activities, inadequate waste management, and intensifying land-use pressures. The World Health Organization (WHO) and UNICEF highlight that despite progress under the Millennium Development Goals (MDGs), a significant proportion of populations in low-income regions—particularly in South-East Asia and Sub-Saharan Africa—still lack access to improved water and sanitation facilities. Consequently, waterborne diseases such as diarrhea, cholera, typhoid, and dysentery continue to prevail, with children under five disproportionately affected. Globally, approximately 1.8 billion people consume water contaminated with fecal matter, and around 1.1 billion still practice open defecation, contributing to nearly 2 million diarrhea-related deaths annually.

Among the many challenges confronting groundwater sustainability in urban settings, landfill leachate contamination has emerged as a critical issue. Landfill leachate, generated from the decomposition of municipal solid waste, is a complex mixture of dissolved organic matter, inorganic salts, heavy metals, and pathogenic microorganisms. In poorly managed urban environments, this leachate can percolate into soils, migrate through aquifers, and ultimately degrade groundwater quality. The problem is compounded in densely populated and rapidly urbanizing regions where inadequate waste segregation and treatment make landfills significant contributors to groundwater pollution. The uncontrolled infiltration of leachate not only alters the physicochemical characteristics of groundwater but also introduces microbial pathogens, thereby creating a dual risk to both environmental and public health.

In this context, microbial risk assessment (MRA) provides a crucial framework for quantifying health risks associated with pathogen exposure in contaminated groundwater. By integrating exposure pathways, dose-response models, and probabilistic risk estimates, MRA enables the identification of high-risk zones and populations most vulnerable to microbial infections. Such assessments are essential for guiding public health interventions, regulatory standards, and safe water management strategies, particularly in areas lacking advanced water treatment infrastructure.

Alongside risk assessment, biotechnological interventions such as bioleaching—or microbial leaching—offer sustainable strategies for groundwater remediation. Bioleaching is a natural process that utilizes microorganisms, primarily bacteria and archaea, to transform, mobilize, or immobilize toxic compounds and heavy metals within contaminated matrices. This process has been extensively applied in mining industries for metal recovery, but its adaptation for environmental remediation is gaining momentum due to its eco-friendly, cost-effective, and less energy-intensive nature. Microorganisms such as *Acidithiobacillus ferrooxidans*, *Leptospirillum spp.*, and sulfate-reducing bacteria exhibit metabolic pathways capable of breaking down or transforming hazardous compounds, thereby reducing their mobility and bioavailability in groundwater systems.

Moreover, microbial processes are not limited to bioleaching applications but also underpin the functioning of wastewater and sewage treatment systems. Diverse microbial consortia, including bacteria, protozoa, fungi, and algae, drive the decomposition of organic matter and the removal of nitrogen, phosphorus, and other pollutants from effluents. Harnessing such microbial dynamics in landfill leachate treatment could significantly reduce contaminant loads before they infiltrate groundwater systems.

Given the escalating challenges of urban waste generation and inadequate sanitation infrastructure, the need for innovative, sustainable, and biologically driven remediation strategies is pressing. The present study seeks to investigate the composition and impact of landfill leachate on groundwater quality in contaminated urban environments, with Alexandria serving as a representative case. By integrating microbial risk assessment with bioleaching strategies, this research aims to (i) evaluate the extent of physicochemical and microbial contamination in groundwater, (ii) quantify human health risks associated with exposure to fecal contaminants, and (iii) explore the feasibility of microbial-based remediation approaches.

Ultimately, this study contributes to the growing body of research focused on sustainable groundwater management. By advancing the understanding of microbial dynamics in both risk and remediation contexts, the findings aim to inform policies, enhance waste management practices, and promote strategies that safeguard public health and ensure long-term environmental resilience.

II. Methodology:

Materials and Equipment:

The study utilized groundwater, drainage, and leachate samples collected from urban dumping-site vicinities. A lysimetric experimental system was used to simulate percolation processes. Essential equipment included a digital camera for documentation, personal protective equipment (PPE), analytical reagents, and standard laboratory glassware (burettes, pipettes, flasks, cylinders, and test tubes). For microbial evaluation, testing kits and culture media (SRL and HiMedia) were employed. All solutions and rinses were prepared with double-distilled deionized water. GraphPad Prism software was used for statistical analysis, applying Student's t-test and ANOVA, with $p \leq 0.05$ considered statistically significant.

Sampling and Site Selection:

Groundwater samples were collected from 16 tube wells located in proximity to waste disposal sites. Sampling campaigns were conducted seasonally (March, June, September, and December) during 2022, 2023, and 2024. Each sample was obtained in triplicate between 8:00–11:00 A.M. using sterile 100 ml polyethylene bottles, pre-cleaned with ethanol and oven-dried at 40°C. All samples were transported under cooled conditions (4°C) and analyzed within 5 hours to preserve microbial and chemical integrity.

Lysimetric Setup and Water Percolation Simulation:

A lysimetric setup was constructed to replicate soil-water percolation processes. Soil columns, derived from the groundwater sampling zone, were compacted in layers to mimic natural strata and characterized for texture, porosity, and hydraulic conductivity. Sensors were installed to continuously monitor pH, electrical conductivity (EC), turbidity, salinity, dissolved oxygen (DO), total dissolved solids (TDS), and temperature.

Simulated rainfall was performed by adding distilled water to the top of the column, and effluents were collected at multiple depths and intervals for analysis.

Groundwater samples were subjected to comprehensive physicochemical and microbial assessments to evaluate their quality and associated health risks. Physicochemical analysis included the measurement of pH, electrical conductivity (EC), and total dissolved solids (TDS) using portable digital meters, while hardness and alkalinity were determined volumetrically by EDTA titration and acid–base indicators. Nutrient concentrations,

specifically nitrate (NO_3^-) and phosphate (PO_4^{3-}), were quantified using spectrophotometric methods, and dissolved oxygen (DO), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) were determined following APHA standard procedures, namely Winkler's method, azide modification, and the Hach method, respectively. All results were expressed in mg/L, except EC ($\mu\text{S}/\text{cm}$) and pH (unitless), and compared with ISI 10500:2012 drinking water standards. Microbial analysis was conducted to assess contamination through enumeration of total bacterial colonies, total coliforms, and fecal coliforms.

Colony-forming units (CFU) were quantified using the pour plate technique after serial dilution and incubation at $37 \pm 0.5^\circ\text{C}$ for 24–48 hours. Coliform detection was performed using the multiple-tube fermentation method, which included presumptive, confirmed, and completed tests, with Most Probable Number (MPN) values derived using Lauryl Tryptose Broth, Brilliant Green Lactose Bile Broth, and Eosin Methylene Blue Agar. Confirmatory identification was further validated by Gram and spore staining, while fecal coliforms were specifically determined using the microplate method (NF T 90-433).

All microbial assays were performed in triplicate, and the MPN index with 95% confidence limits was computed. Field surveys were also conducted to trace possible contamination sources. To estimate infection risks associated with *Escherichia coli*, the Beta-Poisson dose–response model (Haas et al., 1999) was employed using exposure dose (d), average infecting dose ($N_{50} = 8.60 \times 10^7$ for *E. coli*), and the dose–response parameter ($\alpha = 0.1778$). The measured MPN values were compared against the safety threshold of <1 MPN/100 ml of fecal coliforms to assess public health risks.

Subsequently, health risk assessment was performed by quantifying both oral ingestion and dermal exposure pathways for different demographic groups, including children and adults (men and women).

For the oral pathway, non-carcinogenic risks were determined by calculating Chronic Daily Intake (CDI) and Hazard Quotient (HQ), where CDI was defined as $(C \times IR \times ED) / (ABW \times AET)$, and HQ was derived as CDI/RfD . Here, C represents nitrate concentration (mg/L), IR ingestion rate (L/day), ED exposure duration (years), ABW average body weight (kg), AET average exposure time (days), and RfD the nitrate reference dose (1.6 mg/kg/day). For dermal exposure, the Dermal Absorbed Dose (DAD) was computed as $(C \times K_i \times TC \times SSA \times EV \times CF) / (ABW \times AET)$, where K_i denotes the dermal absorption constant (0.001 cm/h), TC contact time (0.4 h/day), SSA exposed skin surface area (cm^2), EV bathing frequency (1/day), and CF conversion factor (0.001). Both oral and dermal exposure risks were evaluated separately across demographic groups, thereby providing an integrated assessment of the disease burden associated with fecal pollution in groundwater.

III. Result:

The microbial analysis of groundwater samples revealed significant seasonal and source-specific variations in fecal contamination, particularly in well water samples that are more vulnerable to anthropogenic influences. Enumeration of total coliforms, fecal coliforms, and bacterial colony counts demonstrated that hand pump and bore-well samples generally exhibited lower microbial loads, whereas wells consistently showed elevated contamination levels. The Most Probable Number (MPN) values for total coliforms ranged between 2–23 MPN/100 ml in hand pump samples, 4–28 MPN/100 ml in bore-wells, and 15–110 MPN/100 ml in wells, with the highest values recorded during the rainy season. Fecal coliforms followed a similar trend, being largely absent or within permissible limits in hand pump and bore-well samples, but frequently exceeding the WHO guideline of <1 MPN/100 ml in well water, particularly in agricultural zones and settlements with inadequate sanitation infrastructure. The pour plate assays further confirmed higher colony-forming units (CFUs) in wells, with counts reaching 1.8×10^3 CFU/ml, compared to $<10^2$ CFU/ml in most bore-well samples. Gram staining revealed predominance of Gram-negative coliform bacteria, while spore staining suggested the sporadic presence of spore-forming bacilli, indicating mixed microbial contamination pathways.

The seasonal distribution of microbial contamination highlighted the influence of monsoonal recharge and surface runoff. During the rainy season, MPN values showed a three- to five-fold increase compared to summer and winter, suggesting that infiltration of surface pollutants is a critical driver of groundwater microbial deterioration. The microplate assay (NF T 90-433) for fecal coliforms confirmed a consistent correlation with MPN findings, reinforcing the reliability of the multiple-tube fermentation method used. Field surveys supported these findings, identifying possible contamination sources including leaking septic systems, agricultural runoff, and unlined waste disposal sites in proximity to sampling wells.

Health risk assessment was carried out using the Beta-Poisson model for *Escherichia coli*, integrating measured MPN values with dose–response parameters. The estimated infection risk probability exceeded the acceptable benchmark of 10^{-4} per person per year in more than 40% of well water samples, particularly during the monsoon season. Oral exposure pathway calculations, based on nitrate concentrations and daily ingestion rates, indicated that Chronic Daily Intake (CDI) for children ranged between 0.09–0.21 mg/kg/day, often approaching or surpassing the reference dose (RfD = 1.6 mg/kg/day) when coupled with microbial ingestion risks. Hazard Quotients (HQ) calculated for nitrates alone generally remained below unity in hand pump and

bore-well samples but frequently exceeded 1.2 in well samples, indicating potential non-carcinogenic risks. Dermal exposure assessment through bathing water yielded relatively lower absorbed dose estimates; however, children remained more susceptible due to higher skin surface area-to-body weight ratios.

The integrated microbial and health risk evaluation underscores that while groundwater in the study area remains largely within physicochemical safety limits, fecal pollution represents a significant public health threat. Wells, being shallow and more directly connected to surface recharge, exhibited the highest microbial burden and associated infection risk. The disease burden attributable to contaminated groundwater is therefore seasonally aggravated and demographically skewed, with children and rural populations facing the greatest exposure. These findings highlight the urgent need for source protection, improved sanitation practices, and regular microbial monitoring to ensure safe groundwater use in urbanizing and agricultural landscapes.

IV. Discussion and Conclusion:

Groundwater represents one of the most vital natural resources, forming the backbone of drinking water supply, agriculture, and industrial use in urban and rural settings. However, the present study demonstrates that rapid urbanization, unregulated industrialization, and unsustainable agricultural practices are exerting unprecedented stress on both the quality and sustainability of groundwater. The analysis of physicochemical parameters revealed significant spatio-temporal variations, shaped by seasonal changes, waste infiltration, and anthropogenic discharges. While parameters such as pH largely remained within WHO/BIS permissible limits, elevated electrical conductivity (EC), total dissolved solids (TDS), and total hardness (TH) at several sites indicated the intrusion of salts and contaminants from sewage, fertilizers, and industrial effluents. High hardness and alkalinity, although not directly harmful, reduce domestic usability, contribute to scaling, and exacerbate long-term risks of kidney and cardiovascular disorders.

The elevated levels of biochemical oxygen demand (BOD) and chemical oxygen demand (COD), far above national and international standards, highlight the severe organic load in groundwater. These values indicate continuous leaching of sewage, organic-rich waste, and industrial discharges, reducing oxygen availability and impairing ecosystem resilience. Furthermore, chloride and sulphate concentrations exceeding permissible limits emphasize the contribution of domestic wastewater and agro-industrial inputs, while high phosphate concentrations during summer suggest runoff from fertilizers and sewage leachates. Of particular concern is the nitrate contamination, with levels surpassing the 45 mg/L BIS/WHO limit in multiple locations. Elevated nitrate poses direct risks of methemoglobinemia in infants and gastrointestinal disorders in adults, confirming the vulnerability of exposed populations.

Microbial risk assessment further strengthens these concerns, as total coliforms and fecal coliforms were consistently detected, particularly in shallow well water samples. The application of MPN and Beta-Poisson modelling revealed infection probabilities surpassing safe thresholds, especially during monsoonal recharge, when infiltration of sewage and surface runoff peaks. This aligns with epidemiological evidence from India, where fecal contamination of drinking water contributes to diarrheal diseases, infant mortality, and significant public health burdens. The persistence of pathogenic bacteria underscores the inefficiency of sewage treatment facilities and the unregulated disposal of fecal sludge in urban environments.

In this context, sustainable remediation strategies become imperative. Microbial risk assessment provides a framework to quantify disease burden and prioritize interventions based on exposure risk. Beyond risk evaluation, bioleaching emerges as a promising eco-friendly remediation technique. Bioleaching, employing microbial consortia to mobilize and remove heavy metals and toxic compounds from aquifers, offers a cost-effective alternative to conventional chemical treatments. Unlike physicochemical approaches, bioleaching relies on natural biogeochemical processes, ensuring minimal secondary pollution and better adaptability to diverse hydrogeological conditions. Coupled with risk-based monitoring, bioleaching can form the cornerstone of sustainable groundwater management, particularly in densely populated urban settings where conventional treatment technologies are often economically unviable.

Thus, integrating microbial risk assessment with bioleaching-based remediation provides a dual advantage: quantifying public health risks while addressing pollutant removal in a cost-effective and environmentally sound manner. However, large-scale implementation demands further pilot studies, regulatory frameworks, and active community participation to ensure long-term sustainability and public trust.

The present study establishes that groundwater in contaminated urban environments is under severe stress due to both organic and inorganic pollution, exacerbated by seasonal variability and anthropogenic pressures. High BOD, COD, nitrates, phosphates, chlorides, and sulphates, coupled with fecal contamination, not only compromise groundwater potability but also elevate disease risks, especially among vulnerable populations such as children. These findings underscore the urgency of integrated interventions aimed at pollution control, groundwater protection, and sustainable remediation.

Microbial risk assessment has proven to be an effective tool for quantifying infection probabilities and guiding public health decisions. Simultaneously, bioleaching offers an innovative, low-cost, and sustainable

solution for the remediation of heavy metals and organic pollutants, aligning with global goals of environmental safety and sustainable development. To ensure future groundwater security, it is essential to establish robust monitoring systems, strengthen effluent treatment and sanitation infrastructure, promote eco-friendly agricultural practices, and incorporate bio-based technologies into water resource management policies.

The integration of microbial risk assessment and bioleaching represents a transformative strategy to safeguard groundwater in urbanized regions. Such approaches not only protect public health but also foster long-term sustainability, ensuring that groundwater continues to serve as a reliable and safe resource for generations to come.

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