METAGENOMIC APPROACHES TO MONITORING AND MANAGEMENT OF POTENTIAL TOXIC ELEMENT POLLUTION

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PREFACE

Recently, rapid advances in high-throughput sequencing technologies and computational tools have led to a significant increase in environmental and biological data. This growth has made metagenomics a vital field for understanding microbial communities and their responses to potentially toxic elements (PTEs). Data structures in environmental studies are becoming increasingly complex due to large-scale sequencing outputs, improved bioinformatics platforms, and the expanding use of artificial intelligence and machine learning techniques.

These developments have generated growing interest in reliable and efficient approaches for monitoring, interpreting, and managing environmental contamination. This book presents a set of interrelated chapters that examine the sources and pathways of PTEs, the biological mechanisms of microbial response and resistance, and the application of metagenomic and complementary omics approaches in environmental assessment and bioremediation.

The early chapters focus on the fundamentals of PTE pollution and biological monitoring strategies, while subsequent chapters explore metagenomic methodologies, sequencing technologies, and data analysis frameworks, highlighting their practical applications in sustainable environmental management.

16/12/2025

Prof. Dr. İbrahim ÖZKOÇ

EDITOR

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METAGENOMIC APPROACHES TO MONITORING AND MANAGEMENT OF POTENTIAL TOXIC ELEMENT POLLUTION

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INTRODUCTION

In recent years, the issue of pollution by potential toxic elements (PTEs) has gained significant attention due to its adverse effects on ecosystems and human health. These elements include Arsenic (As), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Lead (Pb), Iron (Fe), Manganese (Mn), Mercury (Hg), Molybdenum (Mo), Nickel (Ni), Selenium (Se) and Zinc (Zn). PTE's are known to disperse through ecosystems and accumulate in sediments and soils, leading to pollution in aquatic environments (Bakan & Özkoç, 2007; Tokatlı et al., 2021; Özkoç & Arıman, 2023; Pant et al., 2023; Kandel et al., 2024; Tırınk et al., 2025; Arıman et al., 2026).

Traditional monitoring methods for PTEs have often proven costly, time-consuming, and limited in scope. Researchers have developed a groundbreaking method to assess the presence of PTEs in the environment by studying microorganisms. The potential of biological technologies as promising alternatives for monitoring and managing contaminated natural sources, particularly in the context of PTEs, is very promising. Microorganisms respond to PTE exposure through distinct physiological and metabolic alterations, making them effective bioindicators for environmental pollution. The new approach focuses on the interactions between microorganisms and PTEs, offering a more efficient understanding of contamination. Different taxa exhibit varied tolerance and detoxification mechanisms, which enable the assessment of contamination levels based on species-specific responses. Studying multiple microbial species simultaneously offers a holistic understanding of pollutant dynamics. Numerous studies have emphasized the utility of microbial communities as reliable indicators of PTE pollution (Devarapalli & Kumavath, 2015; Nazir, 2016; Pan et al., 2018; Tırınk & Özkoç, 2021; Aparicio et al., 2022; Zhang, 2022).

Moreover, metagenomic approaches offer significant promise for environmental monitoring, biomarker discovery, and the development of targeted bioremediation strategies (Pant et al., 2025).

Applications of these technologies extend beyond pollution control, impacting agriculture, industrial fermentation, and the discovery of bioactive compounds and enzymes with pharmaceutical potential.

Next-generation sequencing (NGS) platforms have further enhanced the resolution and accuracy of metagenomic analyses, enabling the identification of keystone microbial taxa and functional genes involved in pollutant degradation (Mishra & Bharagava, 2016; Techtmann & Hazen, 2016; Al-Bayati et al. 2024). As microbial community dynamics directly influence the fate and transformation of environmental pollutants, understanding these interactions is essential for effective remediation efforts.

Metagenomics allows for a comprehensive analysis of the collective genetic material present in environmental samples, providing insights into the metabolic potential and functional diversity of microbial communities involved in bioremediation processes. Moreover, microorganisms are reliable indicators of PTE pollution, guiding efforts to understand concentration and toxicity. This knowledge leads to healthier and cleaner environments (Devarapalli & Kumavath, 2015; Nazir, 2016; Pan et al., 2018; Aparicio et al., 2022; Zhang, 2022; Özkoç et al., 2023).

Metagenomic methods usually analyse the structure and functions of microbial communities more thoroughly during bioremediation in contaminated environments. (Al-Bayatı et al., 2024; Chettri et al., 2024). Metagenomic strategies, leveraging next generation sequencing (NGS) technologies, offer accurate and valuable insights into key enzymes and genes that play a role in the breakdown and detoxification of environmental pollutants (Mishra and Bharagava, 2016). Metagenomics offers novel insights into the functional potential of microbial communities and provides reference genes and genomes for metatranscriptomics (Shi et al., 2011; Carvalhais, 2012). In this context, metatranscriptomics has emerged as a powerful tool for investigating the functional responses of microbial communities to environmental stressors, including pollution by toxic elements. By sequencing and analysing the total RNA transcripts present in a microbial sample, metatranscriptomics reveal which genes are actively expressed under specific environmental conditions. This allows researchers to identify stress responses, metabolic pathway shifts, and potential resistance or detoxification mechanisms employed by microbial taxa. Additionally, metatranscriptomics identifies which genes are actively expressed in a given environment by sequencing and analysing the total RNA transcripts present in a microbiological sample. This enables scientists to detect metabolic pathway changes, stress reactions, and potential resistance or detoxification strategies employed by microbial species (Nebauer, 2024).

This study aims to explore the impact of PTEs on microbial diversity. It further highlights the role of metagenomic and metatranscriptomic technologies in assessing, monitoring, and managing environmental contamination. Emphasis is placed on their advantages, limitations, and future potential in bioremediation process and microbial ecology.

1. POTENTIAL TOXIC ELEMENTS

Potentially toxic elements (PTEs) occur naturally and are widely distributed throughout the human environment, primarily due to the weathering of geological materials containing heavy metals (Pan et al., 2018). These elements are typically characterised by a density greater than 5 g/cm^3 and may include both metals and certain non-metals. While some PTEs are essential micronutrients at trace levels, their persistence and bioavailability in elevated concentrations can have severe toxic effects on plants, animals, and humans.

Non-metal elements, such as As and Se, are also categorised as PTEs. Like other essential micronutrients, Se plays vital roles in both plant and human systems at low concentrations. However, due to their widespread occurrence in both natural and anthropogenically impacted environments, PTEs tend to accumulate in the biosphere, resulting in long-term ecological and health concerns (Ozkoc & Arıman, 2023).

A wide range of human activities significantly contribute to the mobilisation and accumulation of PTEs. These include industrial emissions, improper disposal of hazardous waste from urban and mining operations, the use of lead-based paints and fuels, the application of pesticides and fertilisers (e.g., compost, sewage sludge, and animal manure), irrigation with contaminated wastewater, petrochemical spills, and atmospheric deposition from coal combustion residues (Nieder & Benbi, 2024; Tırınk et al., 2025).

1.1 Sources of Potentially Toxic Elements

The occurrence and accumulation of PTEs in the environment stem from a combination of natural and anthropogenic sources. Understanding the origin of PTEs is crucial for assessing their ecological impact and informing effective mitigation strategies (Wuana & Okieimen, 2011; Nieder & Benbi 2024; Hossain et al., 2024).

1.1.1 Geogenic (Natural) Sources

The presence of PTEs in soils is strongly influenced by the geochemical characteristics of the main rock materials. The mineral composition of bedrock directly affects the elemental profile of the resulting soils. Additionally, pedogenetic processes such as weathering, leaching, and organic matter accumulation further shape the spatial distribution and concentration of PTEs (Nieder et al., 2014; Nieder & Benbi, 2024).

1.1.2. Atmospheric Deposition from Anthropogenic Emissions

Airborne PTEs, primarily derived from industrial emissions, fossil fuel combustion, and vehicular exhaust, contribute significantly to environmental contamination (Rawat et al., 2025). These elements are released as particulate matter through stack and fugitive emissions.

Urban fires, metal smelting operations, and the combustion of petroleum-based products are particularly critical in areas near industrial zones and transportation corridors (Fergusson, 1990; Smith et al., 1995; Zhang et al., 2002; Wuana & Okieimen, 2011).

1.1.3. Mining and Metallurgical Activities

Mining operations represent one of the most significant anthropogenic sources of PTEs. The extraction and processing of metal ores generate large quantities of waste materials, such as tailings and slag, which are often rich in toxic metals. These residues can disperse through wind and water, contaminating surrounding soils and water bodies. Smelter emissions further exacerbate localised pollution (Henriques & Fernandez, 1991; Dudka & Adriano, 1997; Obrador et al., 2007; Nieder et al., 2014).

1.1.4. Agricultural Inputs: Fertilizers and Manure

The prolonged use of both inorganic and organic fertilizers has been shown to increase PTE levels in agricultural soils (Nieder et al., 2018). Phosphate fertilizers, in particular, may contain trace amounts of cadmium and lead. Additionally, manure and other organic inputs can contribute to the accumulation of PTEs, especially in regions with intensive livestock production and inadequate waste management (Verkleji, 1993; Sumner, 2000; Zhang et al., 2005; Ostermann et al., 2014).

1.1.5. Wastewater and Sewage Sludge

Wastewater originating from domestic, industrial, and commercial activities often contains a mixture of metals and metalloids. The application of sewage sludge as a soil amendment is a common practice in many countries, yet it poses a risk of heavy metal accumulation, especially in long-term use scenarios (Angino et al., 1970; Bjuhr, 2007; Nagajyoti et al., 2010; Shankar et al., 2014)

1.1.6. Agrochemicals and Irrigation Practices

Pesticides, particularly those containing copper and arsenic, are major contributors to soil PTE contamination. These agrochemicals are frequently used in orchards and vineyards. Moreover, irrigation with contaminated surface or groundwater introduces additional PTE loads into agricultural ecosystems (Ross, 1994; Kurt & Özkoç, 2004; Nagajyoti et al., 2010; Wuana & Okieimen, 2011).

1.1.7. Arsenic Contamination in Groundwater

Arsenic contamination arises from both natural geological sources and human activities (Ozkoc & Arıman, 2023). Naturally occurring arsenic-bearing minerals in alluvial sediments can result in elevated concentrations of arsenic in groundwater. Meanwhile, anthropogenic inputs such as mining operations, arsenical pesticides, wood preservatives, and fossil fuel combustion significantly intensify arsenic pollution, particularly in vulnerable aquifers (Bissen & Frimmel, 2003; Polizzotto et al., 2006; Shankar et al., 2014).

2. PATHWAYS OF PTE’S IN THE ENVIRONMENT

Potentially toxic elements infiltrate various environmental compartments, including soil, vegetation, atmospheric systems, precipitation, and both surface and subsurface freshwater bodies such as rivers, lakes, streams, and groundwater reserves. Once introduced into these ecosystems, PTEs may persist and propagate through multiple biogeochemical pathways, often resulting in long-term ecological and health implications.

As illustrated in Figure 1, PTEs can enter freshwater systems through both point and non-point sources, resulting in the contamination of aquatic habitats. Within these systems, bioaccumulation of PTEs occurs in aquatic organisms, particularly fish, posing significant risks to higher trophic levels, including humans (Tırınk & Özkoç, 2021). Similarly, in terrestrial ecosystems, PTEs accumulate in soil, where they may be taken up by plants or ingested by soil-dwelling invertebrates and vertebrates. These contaminants can then biomagnify through the food chain, ultimately affecting human health through the consumption of contaminated crops and animal products.

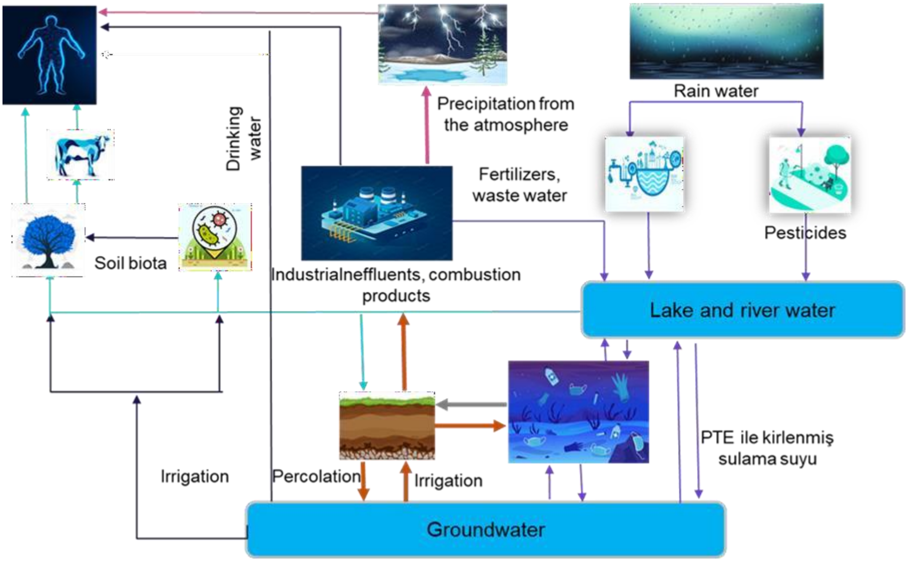

Figure 1. Major PTE's pathways in the environment

Additionally, the discharge of untreated or poorly treated industrial effluents constitutes a major source of PTE pollution in both surface water and groundwater. In aquatic systems, PTEs exist in various physicochemical forms, including dissolved ions, particulate-bound elements, and biologically assimilated phases each displaying different levels of mobility, stability, and bioavailability (Nieder & Benbi, 2024).

To reduce the ecological and human health risks associated with PTE contamination, the use of alternative clean water sources is recommended. These alternatives include properly treated surface water, harvested rainwater, and protected groundwater. Implementation of stringent water quality management practices can significantly reduce exposure to PTEs and prevent their accumulation within ecosystems.

Monitoring PTE's poses a significant risk to both aquatic ecosystems and human health due to their non-biodegradable nature and toxic properties. Elements such as lead, cadmium, chromium, copper, nickel, and zinc pose a significant health risk that can disrupt the balance of the biosphere (Tırınk & Özkoç, 2021). PTEs are introduced into the environment through various sources, including geological weathering, volcanic eruptions, erosion, vehicle emissions, mining operations, metal-based fertilisers, pesticides, runoff, and urban or industrial activities. The continuous accumulation of these contaminants in the environment leads to a decline in environmental quality and disrupts the normal biogeochemical cycle (Bakan & Ozkoc, 2007). In aquatic systems, sediment plays a crucial role in the deposition of PTEs, affecting water quality and serving as a significant food source for microorganisms and larger organisms. Consequently, the presence of PTEs in sediments can lead to their accumulation and magnification in the tissues of aquatic organisms, resulting in various health issues, including life-threatening cancers (Kurt & Ozkoc 2004; Pan et al., 2018; Ozkoc & Arıman, 2023). Therefore, assessing the quantity of PTEs in sediment is essential to understand their ecological risks and determine their sources for effective environmental risk management Various methods, such as contamination factors, enrichment factors, pollution load index, geo- accumulation index, and potential ecological risk index, are used to evaluate the risks of toxic elements and assess sediment quality (Ozkoc & Arıman, 2023). Additionally, statistical analyses such as principal component analysis/factor analysis, hierarchical clustering analysis, and Pearson correlation index are employed to analyse the data, identify contamination sources, and classify sediments based on similar characteristics (Ustaoğlu & Islam, 2020; Tırınk et al., 2025).

3. BIOLOGICAL CONTROL OF PTEs

Advanced biological technologies have emerged as effective, eco-friendly solutions for controlling PTE contamination. These methods leverage natural biological processes to remove, immobilise, or detoxify heavy metals from soil, water, and sediments. Here are some examples of advanced biological technologies used for PTE control (Smith et al., 1995; Ye et al., 2017; Wang et al., 2021; Kumar et al., 2021):

3.1 Phytoremediation

This technique utilises plants to absorb, stabilise, or transform PTEs. Certain hyperaccumulator species, such as sunflowers (*Helianthus annuus*), are known for their ability to uptake lead and cadmium from contaminated soils (Ye et al., 2017).

3.2 Bioremediation

Bioremediation employs microorganisms (bacteria and fungi) to transform or degrade toxic elements into less harmful forms. For instance, specific bacteria can convert mercury compounds into elemental mercury, reducing toxicity (Gadd & Pan, 2016; Yin et al., 2019; Kumar et. al., 2023).

3.3 Mycoremediation

This approach uses fungi to absorb or sequester PTEs. Fungal mycelium has a high metal-binding capacity. Oyster mushrooms have been successfully used to remediate arsenic-contaminated sites (Yin et al., 2019).

3.4 Bioleaching

This technique utilises microbes to extract metals from ores or waste while immobilising or detoxifying PTEs. Sulfur-oxidising bacteria, for instance, can release metals while stabilising arsenic. (Wang et al., 2021).

3.5 Bio-mineralization

Microorganisms facilitate the precipitation of PTEs as insoluble minerals, reducing their mobility and bioavailability. For example, sulfate-reducing bacteria promotes the formation of metal sulfides, sequestering copper and zinc (Gadd & Pan, 2016)

3.6 Genetic Engineering & Metagenomics

Modern genetic tools are used to enhance the metal-accumulating or detoxifying capacity of plants and microbes. Genetically modified organisms (GMOs) and metagenomic approaches are paving the way for more efficient PTE remediation strategies (Ye et al., 2017).

These advanced biological technologies offer sustainable and eco-friendly approaches to PTE control. They are increasingly applied in various environmental remediation projects, providing practical solutions for reducing PTE contamination and mitigating associated risks.

However, the key to efficient bioremediation lies in finding new metabolic pathways and enzymes and tuning them to degrade various pollutants with high proficiency. The metagenomic approach has also been applied to bioremediation, where novel systems for the microbial remediation of contaminants have been discovered (Yadav & Dharne 2024).

It also helps in tracing the pollutant source to understand the nature of the pollutant and develop a treatment approach as well as to check the pollutant generation at the source itself.

4. METAGENOMICS FOR SUSTAINABLE PTE CONTROL

Metagenomics is an advanced molecular technique that involves direct extraction and analysis of DNA from environmental samples. Unlike traditional microbial culturing methods, metagenomics does not require the isolation and cultivation of individual microorganisms, allowing for comprehensive genomic analysis of entire microbial communities — including those that are uncultivable under standard laboratory conditions. This technology enables the investigation of microbial diversity, abundance, functional roles, interactions, and adaptive mechanisms across a wide range of ecosystems (Kumar & Chandra, 2020; Kumar et al., 2022; Sokal et al., 2022).

Metagenomic approaches can be broadly categorised into sequence- based and function-based analyses:

- The sequence-based approach focuses on decoding nucleotide sequences from whole genomes or specific genetic markers, such as the 16S rRNA gene for bacteria or internal transcribed spacer (ITS) regions for fungi. This allows for accurate profiling of microbial diversity and relative abundance in environmental samples.
- The function-based approach, on the other hand, emphasises the identification of microbial genes associated with key biological functions, such as metal reduction, degradation of pollutants, or nutrient cycling. This approach provides insights into the metabolic potential and ecological roles of microbial communities involved in PTE remediation.

By enabling the detection of microbes that thrive in extreme or contaminated environments, metagenomics has proven instrumental in uncovering novel microbial species and functional genes that play crucial roles in biogeochemical processes. These include nutrient cycling, organic matter decomposition, suppression of plant pathogens, and detoxification of pollutants — all vital for maintaining ecological balance and supporting bioremediation efforts.

Metagenomic studies of pristine ecosystems also offer valuable baselines for understanding the structure and functionality of healthy microbiomes. However, it is important to recognise that natural microbial communities are highly dynamic and influenced by both climatic and anthropogenic stressors. Environmental variables, including temperature, salinity, oxygen levels, pH, nutrient availability, biotic interactions, and geographical location, significantly influence microbial diversity and community structure during sampling. Advancements in next-generation sequencing (NGS) technologies have made metagenomics a cost-effective and efficient tool for environmental monitoring and remediation. These techniques facilitate the direct characterisation of complex microbiomes, including those found in wastewater and contaminated soils, enabling researchers to assess microbial ecology, community dynamics, and their interactions with plants, animals, and the surrounding environment (Mishra & Bharagava, 2016).

Overall, metagenomics presents a powerful, eco-friendly strategy for PTE control and environmental restoration, offering practical, data- driven solutions for mitigating contamination and promoting ecosystem resilience.

4.1. Metagenomic Approaches

Metagenomics has revolutionised our understanding of microbial communities by enabling the direct analysis of genetic material from environmental samples, without the need for cultivation. In addition to traditional DNA-based metagenomic analysis, several complementary 'omics' approaches have emerged, offering deeper insights into microbial functions, activity, and interactions within ecosystems (Yadav & Dharne 2024). These include metatranscriptomics, metaproteomics, metabolomics, fluxomics, and advanced sequencing technologies.

4.1.1 Metatranscriptomics

Metatranscriptomics involves the extraction and sequencing of messenger RNA (mRNA) from complex microbial communities in environmental samples. This approach provides crucial information on gene expression patterns, helping to reveal how microbial communities respond to environmental stimuli at a functional level.

Despite its potential, in situ metatranscriptomic studies are technically challenging due to the instability of mRNA, its short half-life, and the difficulty of isolating high-quality mRNA from mixed RNA populations. To overcome these limitations, direct complementary DNA (cDNA) sequencing via next-generation sequencing (NGS) technologies is employed, enabling whole-genome expression profiling and transcript quantification.

Studies using pyrosequencing and metatranscriptomics have identified actively expressed genes in soil microbiomes and uncovered the role of small RNAs in environmental functions (Siman & Daniel, 2011; Aguiar-Pulido et al., 2016; Bharagava et al., 2019). This approach provides valuable insights into microbial activity and its contributions to key biogeochemical processes.

4.1.2 Metaproteomics

Metaproteomics involves the large-scale analysis of the complete protein content (proteome) extracted from environmental microbial communities. It offers a functional snapshot of microbial ecosystems by identifying expressed proteins and their roles in metabolism and environmental interactions.

A notable metaproteomic study by Ram et al. (2005) investigated microbial biofilms in acid mine drainage using mass spectrometry, identifying over 2,000 proteins from the five most abundant microbial species. However, metaproteomics faces challenges due to uneven species abundance, variation in protein expression levels, and genetic diversity within microbial populations.

Despite these complexities, metaproteomics provides a direct link between gene expression, microbial activity, and ecosystem function (Siman & Daniel, 2011; Bharagava et al., 2019).

4.1.3 Metabolomics

Metabolomics focuses on the identification and quantification of small-molecule metabolites produced or consumed by organisms in an ecosystem. The metabolome serves as an indicator of microbial activity and environmental health, with applications in nutrient cycling, pollutant transformation, and microbial communication.

Unlike metagenomics or transcriptomics, metabolomics relies on analytical techniques such as liquid chromatography (LC), gas chromatography (GC), mass spectrometry (MS), and nuclear magnetic resonance (NMR). These methods detect and profile metabolites based on spectral patterns and peak intensities (Aguiar-Pulido et al., 2016; Bharagava et al., 2019).

This approach also sheds light on microbial processes such as quorum sensing—cell-to-cell communication based on population density— which regulates collective gene expression and environmental responses.

4.1.4 Fluxomics

Fluxomics examines the rates of metabolic reactions (metabolic fluxes) within living systems. It provides a dynamic and quantitative understanding of how metabolites flow through metabolic pathways, linking genomic and environmental data to actual phenotypic functions.

Unlike genomics or proteomics, fluxomics benefits from analysing a smaller, more manageable number of metabolites, making it a powerful tool for understanding microbial function under varying environmental conditions (Aguiar-Pulido et al., 2016; Bharagava et al., 2019).

5. METAGENOMICS AND ENZYMES

Metagenomics has emerged as a transformative tool for discovering novel enzymes and biocatalysts from uncultured microorganisms in diverse environmental samples. By bypassing traditional cultivation methods, metagenomic approaches allow direct access to the genetic diversity of microbial communities, leading to the identification of previously unknown enzymes with significant industrial and environmental applications (Lorenz et al., 2002; Nazir, 2016). Numerous enzymes, such as cellulases, lipases, xylanases, amylases, and proteases, have been discovered and characterised through metagenomic screening of environmental DNA libraries. These enzymes play critical roles in biotechnological processes ranging from biofuel production to food processing and waste management.

5.1 Cellulases

Cellulases are a class of glycosyl hydrolases (GHs) that include three main enzymatic activities:

- **Endoglucanases** – which randomly cleave internal β-1,4-glycosidic bonds,
- **Exoglucanases** – which remove cellobiose units from the ends of cellulose chains, and
- **β-glucosidases** – which hydrolyze cellobiose into glucose.

These enzymes are widely used in animal feed enhancement, fruit juice clarification, baking, and paper de-inking. Metagenomics has enabled the isolation of novel cellulases from environments including compost soil, rumen, forest soil, elephant dung, cow rumen, and decaying wood.

Researchers have successfully constructed metagenomic libraries to screen for biologically active clones expressing cellulase activity. For instance, novel genes such as β-glucosidase unglu135B12 and CelEx- BR12, which encode multifunctional enzymes, have been identified and characterized. Many of these enzymes exhibit unique features, such as halotolerance and thermal stability, making them suitable for industrial applications under extreme conditions (Nazir, 2016).

5.2 Xylanases

Xylanases, which degrade the hemicellulose component xylan, have also been identified through metagenomic studies. These enzymes are of particular interest due to their utility in bio-pulping, animal feed, textile, and food industries.

Metagenomic libraries have uncovered xylanase genes from insect guts, thermophilic anaerobic digesters, and dairy farm waste lagoons. Some of these enzymes show exceptional properties, including:

- High catalytic activity,
- Broad pH stability and
- Substrate specificity.

A notable example is a GH11 xylanase gene exhibiting superior enzymatic performance, while a GH10 xylanase from cow rumen showed promising industrial potential due to its robust biochemical profile (Nazir, 2016).

5.3 Next-generation Sequencing (NGS) Technologies

NGS technologies underpin most metagenomic and meta-omics approaches, enabling high-throughput sequencing of microbial DNA and RNA. These platforms provide insights into microbial diversity, gene expression, pollutant degradation, detoxification pathways, and pathogen transmission in environmental settings.

Popular NGS platforms include:

- **Illumina (Solexa)** – high accuracy and read depth for genome-wide studies.
- **Roche 454** – suitable for longer reads and amplicon sequencing (now largely phased out).

- **Ion Torrent (Proton/PGM)** – offers speed and cost-effectiveness for smaller genomes.
- **SOLiD sequencing** – known for high-throughput applications.

NGS technologies are widely applied in environmental microbiology, wastewater treatment studies, and bioremediation research (Kim et al., 2013; Bharagava et al., 2019).

Together, these metagenomic approaches offer a multi-dimensional view of microbial ecosystems — from genetic potential (metagenomics) to functional activity (metatranscriptomics, metaproteomics, metabolomics, and fluxomics). By integrating these tools, researchers can gain a deeper understanding of microbial dynamics, enabling the development of targeted strategies for sustainable environmental management and PTE remediation.

6. METAGENOMICS PROCESS

The metagenomics workflow comprises several essential steps that enable researchers to investigate the genetic content of entire microbial communities directly from environmental samples. The general process includes the following stages (Chen et al., 2022):

- Sample Collection

Environmental samples, such as soil, water, sediment, or faecal matter, are collected based on research objectives and the target microbial community. The sampling strategy influences the downstream quality of genetic material and the representativeness of microbial diversity.

- DNA Extraction

This step involves breaking microbial cell walls to release genomic DNA. Both direct and indirect extraction methods can be used, depending on the sample type and desired yield and purity.

- DNA Fragmentation

Extracted DNA is fragmented into smaller pieces to facilitate sequencing. Fragmentation can be achieved through:

- Physical methods (e.g., sonication),
- Enzymatic digestion (e.g., restriction enzymes).

- Library Preparation

Adapters are ligated to fragmented DNA to create sequencing libraries. This step also involves PCR amplification to enrich DNA fragments for sequencing.

- Sequencing

High-throughput sequencing technologies (e.g., Illumina, Ion Torrent, PacBio, or Oxford Nanopore) are used to generate large volumes of sequence data from the prepared DNA library.

- Data Analysis

Sequencing data undergoes quality control, trimming, and error correction. Reads are assembled into contigs or aligned to reference databases for: Taxonomic profiling, and Gene prediction.

- Functional Annotation

Using bioinformatics tools, DNA sequences are compared with functional gene databases (e.g., KEGG, COG, or Pfam) to infer metabolic pathways, gene functions, and potential biotechnological applications.

- Data Interpretation and Visualization

The final step involves integrating taxonomic and functional information. Visualisation tools such as:

- Heatmaps,
- Bar charts,
- Network graphs, or
- Taxonomic trees are employed to present results in an interpretable and meaningful manner.

Metagenomics continues to expand our understanding of microbial ecosystems while serving as a crucial platform for enzyme discovery, bioprospecting, and environmental biotechnology. Its ability to unlock the genetic potential of uncultured microbes holds promise for novel industrial and environmental solutions.

It is important to note that the specific details of each step may vary depending on the study design, available resources, and the sequencing technologies employed. However, these general steps provide a framework for conducting Metagenomic analysis to explore and understand the genetic content of microbial communities in environmental samples (Figure 2 explain the steps of Metagenomics processes) (Chen et al., 2022).

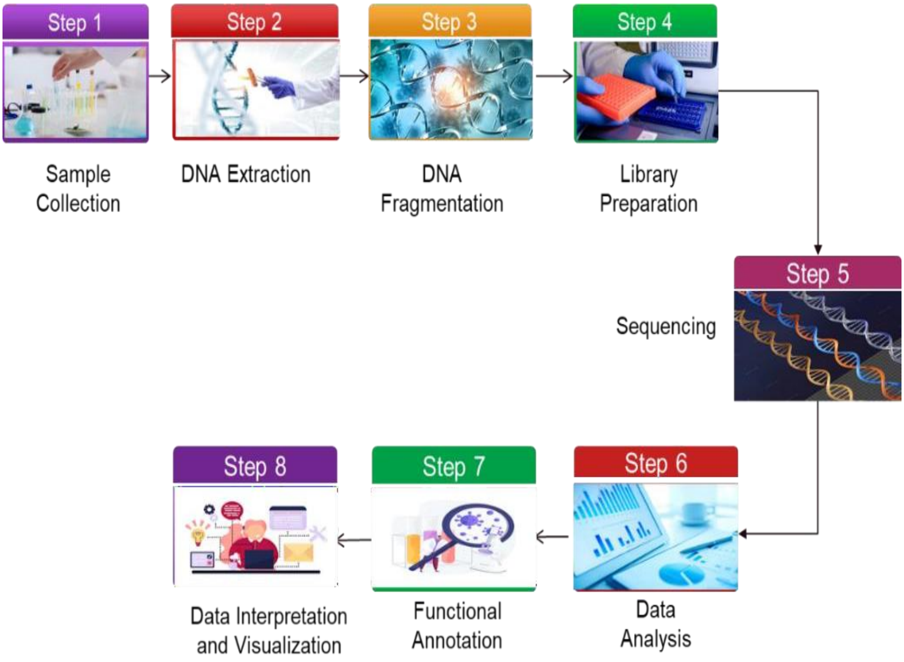

Figure 2. Explain the Steps of Metagenomics Processes

7. ADVANTAGES AND DISADVANTAGES OF METAGENOMIC APPROACHES IN STUDYING PTES

Metagenomic approaches have emerged as powerful tools for exploring microbial ecology and biotechnological potential in environments contaminated with potentially toxic elements (PTEs). Below is a critical evaluation of their advantages and disadvantages (Haiser & Turnbaugh, 2013; Kumar et al., 2022; Lema et al., 2023)

- **7.1 Advantages**

1. **Comprehensive Analysis**

   Metagenomics enables the examination of entire microbial communities, offering a holistic view of their genetic potential and functional roles in PTE transformation, detoxification, and resistance. This includes the identification of novel genes, enzymes, and pathways involved in bioremediation.

2. **High-throughput Screening**

   The approach supports the simultaneous analysis of multiple environmental samples, allowing researchers to efficiently process large datasets across various ecosystems. It helps identify key microorganisms and gene clusters relevant to PTE resistance or remediation.

3. **Culture-Independent Analysis**

Unlike traditional microbiology techniques, metagenomics bypasses the need for culturing, making it suitable for analysing unculturable or rare microbial species. This avoids cultivation bias and expands our understanding of microbial diversity.

4. **Long-term Data Utility**

Metagenomic datasets can be archived and revisited for future research questions. This reusability supports comparative studies, trend analysis, and integrative research across disciplines.

- **7.2 Disadvantages**

1. **Data Complexity and Computational Demands**

Metagenomic datasets are typically large and complex, necessitating the use of advanced bioinformatics pipelines and substantial computational resources. Accurate analysis of PTE-related data requires careful selection and optimisation of analytical tools.

2. **Limited Taxonomic and Functional Resolution**

Metagenomics may not reliably assign functions or resistance traits to specific taxa, especially in environments with high microbial diversity. This can hinder the identification of key microbial species involved in PTE cycling.

3. **Detection Sensitivity**

   The approach may struggle to detect genes or microbes associated with trace amounts of PTEs, particularly in complex environmental matrices, limiting its effectiveness in low-contamination scenarios.

4. **Requirement for Experimental Validation**

   Insights derived from metagenomics often require confirmation through complementary methods, such as chemical analyses or ecotoxicological assays. Without validation, the practical application of findings remains uncertain.

5. **Ethical and Legal Concerns**

   The generation and sharing of genetic data raise important ethical considerations regarding data ownership, privacy, and benefit-sharing, especially when samples originate from sensitive ecosystems or indigenous lands.

Understanding these strengths and limitations allows for the more effective application of metagenomics in environmental microbiology and the development of sustainable PTE management strategies.

8. HOW METAGENOMICS HAS TRANSFORMED THE RESEARCH STRATEGY OF MICROBIAL TREATMENT OF HEAVY METALS

Heavy metal pollution, a byproduct of rapid industrialization, poses significant ecological and health risks due to the toxicity and bioaccumulative nature of metals in wastewater. Traditional physicochemical methods for metal removal are often cost-prohibitive and environmentally taxing. As a result, microbial remediation has emerged as a cost-effective and sustainable alternative. However, understanding the microbial mechanisms underlying heavy metal resistance and reduction has historically been limited.

8.1 The Role of Metagenomics

Metagenomics has revolutionised the field by enabling researchers to explore the genetic and functional composition of microbial communities under heavy metal stress, without the need for cultivation. Key contributions include:

- **Community-Level Insights**:

  Metagenomic studies have revealed significant structural, functional, and metabolic shifts in microbial communities exposed to heavy metals. These adaptations include the enrichment of metal-resistant species and the activation of specific resistance genes.

- **Gene and Pathway Identification**:

  Metagenomics has uncovered genes encoding proteins involved in redox reactions, efflux pumps, metal-binding proteins, and enzymes responsible for transforming metals into less toxic forms. These findings form the basis for developing microbial consortia or engineering strains for bioremediation.

- **Application in Wastewater Treatment**:

  In real-world wastewater systems, where multiple heavy metals coexist, metagenomics has enabled the mapping of complex microbial responses and the identification of functional genes critical to detoxification processes.

- **Guiding Future Research**:

  Although much has been learned, challenges remain in deciphering the collective microbial response in environments contaminated with diverse mixtures of metals. Metagenomics provides the foundation for in-depth investigations using integrative approaches, such as metatranscriptomics and metaproteomics.

8.2 Role of Metagenomics in Understanding Climate-Driven Microbial Dynamics

Metagenomics provides powerful tools for decoding microbial responses to climate stressors at both community and functional levels. Key applications include:

1. Exploring Microbial Diversity

- Reveals previously unknown or unculturable microbial species that are functionally active in subsurface environments.
- Enhances our understanding of biodiversity hotspots and microbial reservoirs that may shift under changing climatic conditions.

2. Identifying Functional Capabilities

- Genome-level analysis connects specific microbes to processes like:
    - Methane production or oxidation
    - Denitrification and nitrification
    - Sulfate reduction
- Allows researchers to track how functional gene abundance changes with environmental stressors.

3. Enhancing Predictive Models

- Incorporating metagenomic data into biogeochemical and reactive transport models improves forecasting of:
    - Greenhouse gas fluxes
    - Nutrient transformations
- Helps anticipate how microbial processes might accelerate or buffer climate change impacts.

4. Supporting Environmental Solutions

- Metagenomic insights guide:
    - Greenhouse gas mitigation strategies
    - Subsurface water quality management
    - Pollution control and nutrient balancing
- Enables evidence-based environmental policy and engineering design (Hossain et al, 2024).

8.3 Metagenomics Applications in Bioremediation

Metagenomic approaches are rapidly transforming bioremediation practices, offering:

- Higher contaminant degradation efficiency
- Improved understanding of microbial communities responsible for detoxification.

Key Benefits of Metagenomic Bioremediation (Kumar, 2022; Chettri et al., 2024);

- Enables identification of microbial consortia that naturally degrade pollutants like heavy metals, hydrocarbons, and pesticides.
- Reveals functional genes responsible for detoxification, resistance, and transformation processes.
- Facilitates the design of targeted microbial treatments for specific environmental contaminants.

8.3.1 Recent Case Studies on Metagenomics Applications in Environmental Monitoring and Bioremediation

Metagenomics has significantly advanced the field of environmental microbiology by enabling the comprehensive study of microbial communities, especially in contaminated ecosystems. Metagenomic- assisted bioremediation yields higher degradation rates compared to conventional methods.

Unlike traditional cultivation methods, which can access less than 1% of environmental microbes, metagenomics analyses the collective genetic material from microbial communities, providing unprecedented insight into microbial diversity, function, and ecology. Integration of metagenomic tools leads to more sustainable and adaptable remediation strategies across diverse environmental settings (Devarapalli & Kumavath, 2015; Nazir, 2016; Al-Bayati et al., 2024).

Recent studies have applied metagenomics to track and remediate pollutants in:

- Marine ecosystems
- Groundwater systems
- Drinking water supplies

These environments often harbour uncultured or rare microorganisms with potential for bioremediation. Metagenomic data reveal novel genes and pathways involved in contaminant breakdown and nutrient cycling, laying the groundwork for water quality management and pollution control (Al-Bayati et al., 2024).

- **Bioremediation of Hydrocarbons and Petroleum Products**

A study by Yergeau et al. (2012) focused on petroleum reservoirs and demonstrated that metagenomic clones could degrade up to 94% of phenanthrene and methylphenanthrene within 21 days. These findings highlight the petroleum-degrading potential of uncultured bacteria, enabling the design of more effective oil spill remediation strategies.

- **Wastewater Treatment: Nutrient Removal and ARGs**

Metagenomics has revolutionised our understanding of biological phosphorus and nitrogen removal in wastewater treatment:

- **Phosphorus Removal**:

Metagenomic binning has expanded knowledge of phosphate- accumulating organisms (PAOs). It identified *Candidatus accumulibacter* as a dominant PAO and revealed the coexistence of *Ca. propionivibrio*, challenging prior assumptions.

- **Nitrogen Cycling**:

The discovery of *Nitrospira* spp. capable of complete nitrification showcases the functional versatility of microbial communities involved in nitrogen transformations.

- **Antibiotic Resistance Genes (ARGs)**:

Metagenomic analysis helps track the distribution and horizontal transfer of ARGs in wastewater systems, identifying how ecological factors influence ARG composition and evaluating removal strategies (e.g., UV disinfection) (Chen et al., 2022).

8.4. Land Use and PTE-Resistant Microorganisms

Saran et al. (2018) investigated how land use practices influence PTE accumulation and the occurrence of resistant microorganisms in watersheds. Findings show that:

- Agricultural and industrial areas have higher PTE levels.
- There's a strong correlation between PTE exposure and microbial resistance, indicating the need for integrated land use and water quality management to reduce contamination and resistance proliferation.

8.5. Heavy Metal Detoxification and Soil Microbial Communities

Al-Bayati (2024) conducted a case study in Iraq, assessing the impact of heavy metals (Cd, Pb, Cr, Ni) on soil microbial ecosystems using pollution indices such as EF, CF, and Igeo. Key findings include:

- Identification of metal-resistant bacteria such as *Pseudomonas* and *Flavobacterium* via 16S rRNA sequencing.
- Use of PICRUSt functional profiling to predict metabolic pathways disrupted by metal stress.
- Insights into soil health and bioremediation potential through integration of microbial ecology and environmental toxicology.

8.6. Advancing Environmental Monitoring Through eDNA and Bioinformatics

Garrido-Cardenas et al. (2017) emphasised the limitations of traditional wastewater monitoring methods and highlighted how metagenomics—including eDNA analysis and next-generation sequencing (NGS)—transforms microbial community assessments. Applications include:

- Detection of pathogens and pollution indicators
- Optimising treatment processes
- Enhancing public health protection

Garlapati et al. (2019) further explored how eDNA metagenomics can support conservation and environmental management, noting its superiority in monitoring microbial dynamics across diverse habitats.

9. ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING IN METAGENOMICS

The convergence of metagenomics and artificial intelligence (AI) marks a transformative era in microbial ecology, environmental monitoring, biomedicine, and biotechnology. As metagenomics enables the direct analysis of genetic material from complex microbial communities—many of which are uncultivable—AI and machine learning (ML) offer the computational power and precision needed to interpret vast, high-dimensional datasets efficiently (Mahajna, 2022; Gupta, 2024).

Key Applications of AI in Metagenomics

1. Taxonomic Classification

AI models, particularly ML classifiers, enhance the accuracy and speed of:

- Operational Taxonomic Unit (OTU) identification
- Species-level classification
- Phylogenetic clustering

Unlike traditional sequence alignment methods, AI can detect subtle genomic patterns and classify sequences even in the presence of noise or incomplete data.

2. Functional Annotation

Metagenomes contain genes with unknown or hypothetical functions. AI models:

- Predict gene functions based on sequence similarity, protein domains, and motif analysis
- Reconstruct metabolic pathways and biochemical networks
- Identify enzymes, antibiotic resistance genes, and secondary metabolite biosynthesis clusters

This facilitates a deeper understanding of microbial roles in biogeochemical cycles, health, and disease.

3. Microbial Community Analysis

Using tools like PCA, clustering algorithms, and deep learning, AI helps:

- Reveal trends in microbial diversity
- Analyses community structure across spatial and temporal gradients
- Track microbial shifts in response to variables like temperature, pH, or nutrient levels

These insights are critical for ecosystem monitoring and predicting microbial ecosystem responses to environmental change.

4. Bioremediation and Environmental Applications

AI models trained on metagenomic data from contaminated environments (e.g., heavy metals, hydrocarbons) can:

- Suggest optimal microbial consortia for bioremediation
- Predict enzymatic pathways for pollutant degradation
- Aid in designing bioaugmentation strategies for polluted soils, water, and sediments

5. Human Health and Personalised Medicine

AI-driven metagenomics enables:

- Microbiome-based disease diagnostics
- Nutritional profiling and intervention
- Personalised probiotic treatments
- Exploration of host–microbiome interactions and identification of biomarkers for disease susceptibility

6. Bioprospecting and Industrial Applications

AI aids in mining metagenomic databases for:

- Novel bioactive molecules, antibiotics, and enzymes

- Metabolic capabilities useful in renewable energy, agriculture, and pharmaceuticals

This AI-guided bioprospecting is faster, more targeted, and capable of identifying low-abundance genetic assets.

7. Integration of Multi-Omics

Modern AI systems integrate multi-omics (e.g., metatranscriptomics, metaproteomics, metabolomics) to provide:

- Systems-level insights into microbial physiology
- Temporal dynamics of microbial communities
- Enhanced predictions of ecosystem functioning under stress conditions

8. Emerging Technologies

- **Federated Learning**: Allows decentralized AI training without exposing raw data, addressing data privacy concerns in human microbiome research.
- **Real-Time AI Analysis**: On-the-fly AI interpretation of metagenomic sequences for rapid clinical diagnostics or environmental monitoring.
- **Interpretable AI**: Emphasis on transparent, explainable models that improve trust and utility in clinical and biological research.

9. Ethical and Computational Considerations

While the integration of AI in metagenomics offers transformative potential, challenges remain:

- Data privacy, especially for human-associated metagenomic data
- Ethical use of predictive modeling
- Need for computational infrastructure, such as GPUs and cloud platforms
- Interpretability and reproducibility of AI-generated outputs

Proper governance and adherence to bioethical standards and data protection laws are essential as AI expands in biological domains (Gupta, 2024).

CONCLUSION

This study underscores the critical importance of understanding PTEs and their impact on ecosystems and human health. Metagenomics has emerged as a powerful platform for exploring microbial community composition and functionality, particularly in polluted or stressed environments.

Metagenomics plays a dual role in addressing global environmental challenges:

1. **Climate Change Research**:

   By decoding microbial responses to changing subsurface conditions, metagenomics helps predict greenhouse gas emissions and informs ecosystem models.

2. **Bioremediation Enhancement**:

   By identifying functional genes and microbial players, it supports the development of precise and effective environmental clean-up strategies.

Together, these applications position metagenomics as a cornerstone of modern environmental microbiology and climate science.

The integration of AI and machine learning significantly enhances the analytical capacity of metagenomics, enabling:

- Deeper insights into microbial ecology
- More precise environmental diagnostics
- Innovations in biotechnology, medicine, and bioremediation

However, the complexity of metagenomic datasets calls for ongoing development of efficient, interpretable, and ethically sound AI tools. Future research should focus on:

- Elucidating microbial stress responses to PTEs
- Discovering novel enzymes and biocatalysts

- Enhancing real-time applications and multi-omics integration

Ultimately, harnessing AI and metagenomics together offers a pathway toward sustainable and precise solutions for environmental monitoring, public health, and industrial innovation.

Metagenomics plays a dual role in addressing global environmental challenges:

3. **Climate Change Research**:

   By decoding microbial responses to changing subsurface conditions, metagenomics helps predict greenhouse gas emissions and informs ecosystem models.

4. **Bioremediation Enhancement**:

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Together, these applications position metagenomics as a cornerstone of modern environmental microbiology and climate science.

GLOSSARY OF TERMS AND KEY CONCEPTS

Term	Definition
16S rRNA gene	A highly conserved gene used in prokaryotic phylogenetic analysis and taxonomic classification in microbial studies.
Actinobacteria	A phylum of Gram-positive bacteria commonly found in soil and known for their ability to degrade complex organic compounds and resist heavy metals.
Bioaccumulation	The process by which organisms absorb and retain toxic substances from their environment over time, often through food intake or direct contact.
Biomagnification	The increase in concentration of toxic substances, including PTEs, as they move up the food chain.
Bioremediation	The use of living organisms, particularly microbes, to remove or neutralise pollutants from contaminated environments.
Contamination Factor (CF)	A quantitative index used to assess the level of pollution by comparing the concentration of an element in sediment with a reference baseline.

Term	Definition
czcABC operon	A set of genes involved in cadmium, zinc, and cobalt resistance in bacteria, often studied in metagenomic assessments.
Efflux pump	Protein complexes that expel toxic substances, including heavy metals, from microbial cells as a defense mechanism.
Enrichment Factor (EF)	A measure of the degree of anthropogenic contamination of an environment by comparing metal concentrations to a natural background level.
Geo-accumulation Index (Igeo)	A metric to determine the extent of metal pollution in sediments relative to geochemical background levels.
Horizontal Gene Transfer (HGT)	The movement of genetic material between organisms other than by vertical inheritance, often contributing to the spread of metal resistance genes.
Metagenomics	The direct genetic analysis of genomes contained within an environmental sample, without the need for culturing.
Metatranscriptomics	The study of the complete set of RNA transcripts in a community, providing insights into microbial activity under

Term	Definition
	specific environmental conditions.
Microbiome	The entire community of microorganisms (bacteria, archaea, fungi, viruses) living in a defined environment.
Mobile Genetic Elements (MGEs)	DNA elements such as plasmids, transposons, or integrons that can move between genomes and carry resistance genes.
Operational Taxonomic Units (OTUs)	Groupings used in microbial ecology to classify organisms based on DNA sequence similarity, often a proxy for species.
Pearson Correlation Index	A statistical measure of the linear relationship between two variables, commonly used to analyse co-contamination trends.
Pollution Load Index (PLI)	An integrated measure of the overall level of metal pollution in a given site or sediment.
Potential Ecological Risk Index (PERI)	A method to assess the ecological risk of heavy metals, accounting for toxicity, concentration, and response factor.
Proteobacteria	A major bacterial phylum containing many metal-resistant genera such as

Term	**Definition**
	Pseudomonas and *Acinetobacter*.
Psychrophiles	Microorganisms that thrive in cold environments, often studied in polar and permafrost-contaminated regions.
Shotgun Sequencing	A metagenomic approach where all DNA in an environmental sample is randomly sequenced to reconstruct microbial community composition and function.
Taxonomic Profiling	The classification of organisms in a sample based on sequence data, typically using databases like SILVA or Greengenes.
Volatilization	The conversion of heavy metals or metalloids into gaseous forms by microbial processes, such as mercury methylation or arsenic volatilization.

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