

Review

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Review

# Bioremediation of Lead in the Water by the Microbiome

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**ABSTRACT:** Anthropogenic activity like Industrialization has unleashed vast quantities of organic pollutants, including heavy metals. The hazardous heavy metals can cause cancer, neurocognitive defects and wide-ranging health problems. The pollutants resist environmental degradation and can often accumulate in the environment. Microbial degradation of pollutants is a potentially efficient solution for pollution remediation. Amongst the heavy metals, lead contamination resulting from anthropogenic activity have resulted in tragic consequences since the advent of human civilization, even in 21<sup>st</sup> century as demonstrated by the Flint water crisis in the US. Bioremediation of lead from environment could be attempted by various strategies, including mitigation of human activity that causes it in the first place. Microbiome, specifically bacteria, mediated bioremediation can serve as a potent platform for remediating environmental pollution with heavy metals such as lead. Herein we will review the mechanisms and methods of microbial bioremediation of environmental heavy metals with a focus on lead and highlight the challenges and opportunities.

**Keywords:** bioremediation; microbiome; water; lead; metal toxicity; environment

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## Overview of Bioremediation and Lead Poisoning

Since the advent of human civilization, anthropogenic activities have altered the environment, often negatively impacting human and environmental health (1). This issue has escalated with industrialization, driving the use of chemicals, pesticides, and heavy metals, causing widespread pollution of soil, land, and water (1). The rise in pollution, coupled with a growing population and varying global standards, has intensified environmental concerns (2). Traditional remediation methods, which involve transporting contaminated soil to landfills, merely shift the problem without solving it (3,4).

Heavy metals can be removed through physical remediation, chemical remediation, or bioremediation (4, 5). Bioremediation is an innovative technology that employs biological agents such as bacteria, fungi, algae, yeasts, molds, and plants to eliminate, detoxify, transform, or neutralize heavy metals (6). Unlike several physicochemical techniques, bioremediation is cost-effective and efficient. Microorganisms play a significant role in bioremediation, proficiently dissolving and participating in the oxidation and reduction of heavy metals (6, 7). Utilizing microorganisms' metabolic abilities for heavy metal pollution eradication is a form of green technology.

Bioremediation uses naturally occurring or deliberately introduced microorganisms, plants, or other life forms to break down environmental pollutants, presenting a promising solution due to its economic advantage, enhanced efficiency, and environmental friendliness (6, 7). This technology can be categorized into in situ and ex situ methods, employing aerobic, anaerobic, or both types of microorganisms. Techniques like rhizoremediation, phytoremediation, and vermicomposting further augment bioremediation. A significant advantage of bioremediation is its ability to operate on-site, reducing transportation costs and detoxifying pollutants into carbon dioxide, water, and biomass, offering a sustainable alternative to expensive cleanup machinery (8).

## Lead Poisoning: a historical perspective

Humankind has utilized lead for nearly 6,000 years, with documented poisoning cases spanning at least 2,500 years (9). The Romans identified the link between lead exposure and toxicity in the 1st century. They used lead pots to preserve and sweeten wine, leading to widespread lead poisoning in ancient Rome. Some suggest that this widespread poisoning contributed to the decadence and eventual fall of the Roman Empire. In the 19th century, lead poisoning reemerged as a significant issue during industrialization, prompting comprehensive clinical studies and initial preventive efforts (9,10). However, understanding of lead poisoning remained clinical until the late 20th century when subclinical and early forms of lead toxicity were recognized, leading to stricter hygiene standards.

Pediatric lead poisoning became a severe concern with the introduction of tetraethyl lead in gasoline in the 1920s (10, 11). Restrictions on lead use in the 1980s significantly reduced blood lead levels (12). However, lead still poses a health hazard in the 21st century, as tragically underscored by the Flint water crisis in the United States (12, 13). Lead levels in the environment have increased more than a thousand times since the 18th century, primarily due to fossil fuel combustion and factory operations (14). The United States is the second-largest producer of refined lead, contributing less than 10%, while China accounts for over 40% of the world's total refined lead production (13, 14). Lead is used in various industries, including the production of lead-acid batteries, ammunition, metal products, and X-ray shielding devices. However, recent events like the Flint water crisis have led to a decrease in global refined lead production (14, 15).

## Sources of Lead in Water

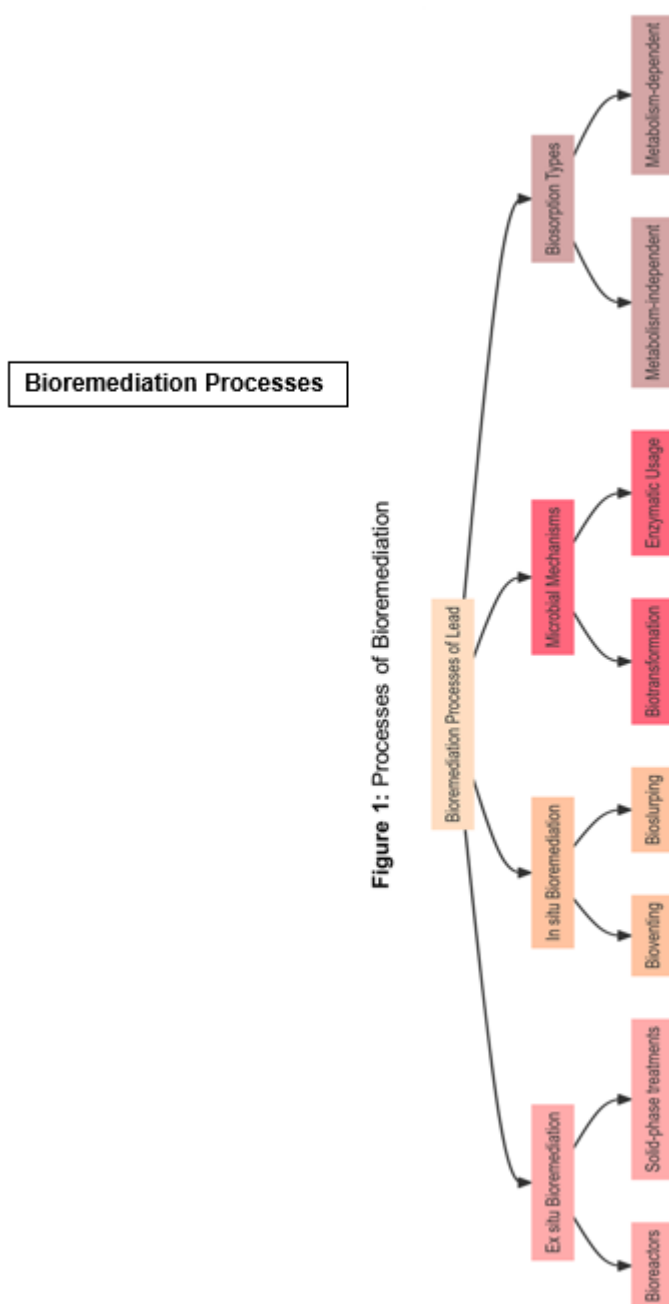
Lead, even at low concentrations, can be highly persistent and toxic, causing serious health hazard and environmental stress (16). It alters the ecological balance. Heavy metals, including lead can occur in freshwater environments in dissolved, colloidal, and particulate forms, differing in bioavailability and toxicity (17, 18). Once present in the aquatic environment, either natural or from human made (lead pipes as in Flint), lead can enter the food web and move up the food chain, thus becoming serious human health hazard (19). Human industrialization has directly linked heavy metal contamination of freshwater systems (**Table 1**). For example, in the Laurentian Great Lakes, heavy metals like copper, iron, lead, and mercury are linked to the area's development (17, 18). Lead contamination in the Great Lakes is primarily due to the burning of tetraethyl-lead gasoline introduced in 1923 (20). Despite discontinuing leaded gasoline in vehicles after the 1970s, alkyl leads are still used in aviation gasoline. Legacy sources of lead in Lake Michigan include mining, smelting of lead and zinc ores, and atmospheric transport (20). Conventional remediation methods are not environmentally or economically sustainable, making bioremediation a more viable solution.

**Table 1: Sources of Lead**

Source of Lead in Water	Description
<b>Natural Sources</b>	Lead occurs naturally in the environment, potentially entering freshwater systems.
<b>Human-made Sources</b>	Lead pipes, industrial activities, and urban development contribute to lead contamination.
<b>Industrialization</b>	Heavy metal contamination in freshwater systems is directly linked to human industrialization.
<b>Burning of Leaded Gasoline</b>	Tetraethyl-leaded gasoline introduced in 1923 and used until the 1970s, particularly in the Laurentian Great Lakes.
<b>Aviation Gasoline</b>	Despite the discontinuation in vehicles, alkyl leads are still used in aviation gasoline.
<b>Legacy Sources</b>	Past activities such as mining, smelting of lead and zinc ores, and atmospheric transport contribute to contamination.
<b>Ecological Impact</b>	Lead can alter ecological balance, enter the food web, and move up the food chain, posing serious health hazards.
<b>Great Lakes Contamination</b>	Specific sources in the Great Lakes include burning of leaded gasoline, mining, smelting, and atmospheric transport.

### Mechanism/Pathway of Bioremediation Processes of Lead

Microbial bioremediation techniques involve identifying novel microbes at contamination sites with significant potential to reduce pollutants (21, 22). Microbial consortia include genetically modified strains to enhance bioremediation by promoting bioaccumulation and biodegradation mechanisms (**summarized in Figure 1**). Bioremediation can be either ex situ or in situ (21, 22, 23). Ex situ involves physically removing contaminated waste to a different location using bioreactors, solid-phase treatments, biopiles, or windrow techniques. In situ bioremediation treats pollutants at their origin with minimal soil disturbance, employing techniques like bioventing and bioslurping to enhance microbial activity and pollutant degradation.



The microbial species have evolved to utilize distinct mechanisms for survival or detoxification and/or developing resistance to heavy metals in the environment. They include, but are not limited to, mechanisms such as gene regulation, chemical transformations such as chelation through the production of specific compounds like metallothionein, altering enzymatic pathways, generating exopolysaccharides and biosurfactants, or undergoing biotransformation (23-29). For instance, bacteria titrate, i.e. either upregulate or downregulate the expression of genes that are responsible for heavy metal efflux or sequestration in direct response to the amounts of heavy metal in their environment (30, 31). They can also regulate chemical interactions that involve ion exchange, redox or electrostatic properties to regulate the uptake or efflux of heavy metals (32, 33). Metal efflux or metal-ligand degradation mechanisms or generation of anionic structures on the cell wall that include NH<sub>2</sub>, COO<sup>-</sup>, thiol, or sulfonyl groups that bind to adsorb the heavy metal cations through biosorption are amongst the processes utilized by bacteria (34, 35). Thus, bacteria utilize a variety of mechanisms to deal with heavy metal toxins, and the preferred mechanism is a function of species and the amount and type of heavy metal in the environment.

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Biosorption of heavy metal bacteria is either dependent or independent of metabolism (36-39). The metabolism-independent biosorption occurs at the exterior of the cell while dependent biosorption is intracellular in nature that include redox reactions, species transformations, and sequestration methods (38, 39). The extracellular sequestration of metallic ions involves components of the cell in the periplasm or the gathering of metallic ions as insoluble compounds through production of genes that promote metal resistance that are often induced by the specific heavy metal. The intracellular sequestration of metals can be exploited for the treatments of effluents (40). Microbes can interchange metal ions from one oxidation state to another, resulting in the reduction of harmfulness (41, 42). Metals and metalloids are used by bacteria as electron donors or acceptors throughout the energy-generation process (43, 44).

**Mechanisms of lead bioremediation by bacteria:** Bacteria are ubiquitous in the natural environment and some can thrive in an environment polluted by heavy metals, including lead (45). The toxic heavy metals are converted by these bacteria into non-toxic forms. Bacteria maintain a defense mechanism in two ways (i) for targeted pollutants develop degrading enzymes (ii) resisting related heavy metals (46). Bacteria utilize several strategies: adhesion, immobilization, oxidation, processing, and volatilization of heavy metals. By understanding the mechanisms that regulates the strategies will enable development of bioremediation processes that can be more effective (47). Microbe-metal mechanisms of interaction allow for various processes such as biotransformation, biosorption, biomineralization, bioleaching and bioaccumulation as modes of bioremediation (**Figure 2**).

Biosorption is a biological physicochemical process that is employed for the removal of recalcitrant compounds, including metal ions (48). The analysis of the supernatant derived from suspension cultures of bacteria revealed that the primary factor responsible for metal sequestration is the soluble exopolysaccharides that facilitate biosorption heavy metals, including lead (Pb) (49). The secretion of exopolysaccharides has been observed in various bacterial strains (discussed below in more detail). They include, but not limited to *Bacillus*, *Paenibacillus* etc (50, 51). The biosorption of the exopolysaccharide molecule depends on the type of the heavy metal. For instance, the capacity to eliminate Pb is distinct for different distinct exopolysaccharide molecules. It is regulated by the electrostatic interactions that occur between the negatively charged functional groups of exopolysaccharide and the positively charged Pb ion (52). It is also influenced by the bacterial immobilization processes, including the attachment and encapsulation methods, are utilized by the bacteria (53).

Bioleaching mechanisms is another process that is employed by the microbes for removal of lead (54). This process is highly dependent on the pH. For example reduction in pH and increase in oxidation-reduction potential creates an optimal environment for the removal of lead. The process of bioleaching can occur either through the direct metabolic activity of leaching bacteria or indirectly through the by-products of bacterial metabolism (55).

Bio-mineralization refers to the process by which various solid minerals, such as carbonates, phosphates, silicates, and sulphates are formed from lead (heavy metal) ions that are subsequently precipitated by microorganisms (56). Bio-mineralization is contingent upon urea hydrolysis, pH, and temperature and utilized not only by bacteria but also by other microorganisms, including photosynthetic microorganisms and other microbes utilizing autotrophic and heterotrophic pathways (57). Genetic engineering of bacteria to affect processes like oxidation-reduction and other desirable traits to enhance biosorption, bioaccumulation, bioleaching etc could be utilized (58). Both intracellular and extracellular mechanisms, in which passive absorption is limited, determine the bioaccumulation process. Metagenomics and metatranscriptomics analysis highlighting the function of major genes and pathways provide for potential targets for genetic engineering to enhance bioremediation by the bacteria. involved in bioaccumulation (59, 60).

### **Bacterial species for bioremediation of lead**

Bacteria are specifically suited for remediation of metals from environment. Several features and mechanisms of bacteria, either individually or collectively, that can be exploited for bioremediation of heavy metals will be reviewed briefly below and summarized in **Table 2**. The nature of the bacterial cell-wall can be exploited for biosorption of heavy metals (61-63). Bacteria, that survive in metal-stressed environments evolved multiple cell-wall related mechanisms to tolerate metal ion uptake. Biosorption to cell walls and entrapment in the extracellular capsule, pre-precipitation the efflux of metal ions outside the cell, encasing them in extracellular capsules, precipitation, complexation, in addition to enzymatic oxidation-reduction reactions, buildup of metal ions in a less poisonous state, and chemisorption of metal ions are some of the mechanisms (64). Because of the presence of novel catabolic enzymes, bacterial strains can survive in a variety of ecological niches (65).

**Table 2: Bacterial Species for Bioremediation of Lead**

Bacterial Species	Optimal Conditions
<i>Bacillus subtilis</i>	3 mg/L Pb within five days
<i>Bacillus megaterium</i>	3 mg/L Pb within five days
<i>Penicillium sp.</i>	3 mg/L Pb within five days
<i>Lactobacillus brevis</i>	Bacterial concentration of 3 g/L and a contact period of 12 h
<i>Bacillus sps</i>	Increased biosorption capacity with temperature; optimal pH 6
<i>Lactobacillus species</i>	Maximal adsorption capacity at 60.6 mg Pb/g after 30 min of incubation
<i>Bifidobacterium species</i>	Initial metal concentration of 1000 µg/L, pH 6, temperature 37°C
<i>Micrococcus sp.</i>	Distinct adsorption rate and concentration dependence
<i>Alcaligenes sp. BApb.1</i>	Distinct adsorption rate and concentration dependence
<i>Rhodobacter sphaeroides</i>	Highly effective at high Pb concentrations
<i>Pseudomonas</i>	Optimal contact time of at least 10 min for 80% removal of Pb at pH 6.0
<i>Stenotrophomonas</i>	Variable
<i>Serratia</i>	Variable
<i>Staphylococcus</i>	Optimal pH 4.5
<i>Oceanobacillus</i>	Growing interest in extreme conditions
<i>Spirogyra</i>	Growing interest in extreme conditions
<i>Anabaena</i>	Growing interest in extreme conditions
<i>Spirulina</i>	Growing interest in extreme conditions

Numerous bacterial strains have been isolated and characterized for their ability to reduce lead in liquids and in soil. Bioaccumulation is the most efficient method of metal removal from aqueous medium by bacterial strains, and it is predicated on the bacterial cell's metabolic activity (58). Specifically, for instance, *Bacillus subtilis*, *Bacillus megaterium*, and *Penicillium sp.* reduced 3 mg/L Pb within five days (66). By contrast the biosorption of Pb by *Lactobacillus brevis* is best at bacterial concentration of 3 g/L and a contact period of 12 h (67). The biosorption of Pb ions by *Bacillus sps* is greatly impacted by temperature and when temperature was increased, the biosorption capacity also increased (67). Similarly pH also had significant effect on biosorption capacity and when pH was adjusted 3, the biosorption is poor, and it increased as pH climbed and reach its greatest binding of Pb was optimal at pH 6 and hereafter it decreased when pH increased over 6 (56, 58, 68).

Different bacterial species show variation based on not only on pH but also the underlying concentration of lead and the time period of incubation in the laboratory and mechanisms employed by the bacterial species may be distinct (69). For example, some *Lactobacillus* species that had maximal adsorption capacity at 60.6 mg Pb/g after 30 min of incubation (70). Mechanistic studies demonstrated morphology of the strain changed before and after Pb biosorption. The results revealed that Pb was primarily connected with bacterial cell surfaces, and that changes in surface morphology were caused by exopolysaccharide secretion (70). Different *Lactobacillus* species demonstrated that increasing pH and bacterial concentrations have major impact on lead removal capacity (69, 71, 72). Experimental data on lead removal by *Bifidobacterium* species was optimal at initial metal concentration was 1000 µg/L, pH 6 and temperature 37°C (72, 73). By contrast, *Micrococcus sp.* isolated from effluent sample of electroplating industry in India showed ability to produce amylase,

and bioremediate Pb at an adsorption rate and concentration dependence that was distinct from the *Alcaligenes* sp. BApb.1 species isolated from China (74, 75). Another Pb-resistant strain *Rhodobacter sphaeroides* is also highly effective at Pb removal at a high concentrations of Pb (76). Besides *Lactobacillus*, other Firmicutes also possess variable potential to eliminate Pb (77).

It is important to note that certain of these features for lead biosorption are also exhibited by bacteria that are known human pathogens. Bacteria that cause significant human morbidity and mortality such as *Pseudomonas*, *Stenotrophomonas*, *Serratia* and *Staphylococcus* also have capacity for lead biosorption (78-82). Once again, the pH and the concentration of the lead, the contact time remain critical features. For example, biosorption potential of a species of *Pseudomonas* showed an optimal contact time requirement of at least 10 min for 80% removal of Pb at an optimal pH of 6.0 (83). The biosorption potential of *Staphylococcus* for Pb is more acidic (pH 4.5) (84). Thus amongst the bacteria that regulate lead, the temperature, pH, biomass and Pb concentration are critical factors that affect the biosorption process. While several pathogenic bacteria are discussed, other microbial organisms that are pathogenic, especially in immune-compromised individuals, such as fungi, have been described to possess substantial lead bioremediation capabilities, but are not discussed here in order to keep the focus of the review on bacteria.

There is also growing interest and some evidence that other anaerobic microbes and extremophiles, that grow at diverse pH, temperature and other harsh conditions could be leveraged for lead bioremediation (85). Microbes such as *Oceanobacillus*, *Spirogyra*, *Anabaena*, *Spirulina*, and others (85). Furthermore, most studies have been done to explore the characteristics and optimal features in bioremediation capabilities of bacteria in isolation. The role and functions, in the context of whole microbiome- the ecological niches, and the synergies and antagonistic interactions that can impact lead bioremediation will need to be analyzed before the strategies can be reliably applied in diverse lead laden water contamination. Utilization of emerging nanotechnologies with carefully designed artificial intelligence modelling along with genetic engineering may usher in new era of leveraging microbial bioremediation strategies against lead contamination of water to make it a more efficient, broad, cost-effective technique when compared to other physical and chemical methods.

### **Advances in Bacterial Technologies for Lead Bioremediation**

Extensive endeavors have been undertaken over a prolonged period to tackle the persistence of lead contamination in the environment. Notwithstanding changes in industries that used lead, the issue persists as demonstrated by the Flint water crisis even in resource rich country like the United States (13, 14). To mitigate the potential ecological ramifications of lead pollution, it is imperative to develop innovative and robust ecological technologies that can effectively utilized to reclaim lead from contaminated environments, including water. As noted above, the utilization of microbial bioremediation could be a cost-effective approach to address this issue. Several novel technologies might hold promise for bioremediation of lead from water and environment. They include microbial fuel cells, biofilm, nanotechnology, and as mentioned earlier, genetic engineering.

*Microbial Fuel Cells:* Microbial fuel cells (MFCs) have been identified as a feasible approach to mitigate environmental contamination (86). MFCs convert chemical energy into electrical energy via oxidation mechanisms facilitated by microorganisms or enzymatic catalysis (87). These are thus bio-electrochemical devices that facilitate the decomposition of organic waste into smaller molecules, liberating protons and electrons, which production of energy. Briefly, microorganisms, such as bacteria, generate electrons and protons at the anode through the process of oxidizing organic materials and assimilate metal ions into their biomass (88). The transportation of electrons occurs through an external circuit, while the diffusion of protons toward the cathode takes place through the solution while a chemical with a high redox potential such as heme molecules function as the electron acceptor (89). MFCs offer certain advantages over conventional fuel cells because the production of fuel is achieved through the utilization of diverse organic or inorganic materials, including but not limited to soil sediments and organic water waste (86, 87). However, currently the

technology as yet generates suboptimal electrical generation. More research is needed to enhance the approach as a long-term viable strategy across resource rich and poor regions.

*Nano-technology:* The employment of nanotechnology offers considerable opportunities for environmental bioremediation of lead (90). The principal modality by which nanomaterials eliminate heavy metals, including lead, is through their elevated adsorption capacity for them. Carbon nanotubes have demonstrated exceptional adsorption capabilities towards Pb (and other heavy metals) (90, 91). However, its utilization for the purpose of removing lead is likely cost-prohibitive (91, 92). The utilization of silica-based nanomaterials is also a possibility to extract lead (93). Lead biosorption can be achieved through the utilization of nano-silica as the foundation for nanocomposites and the silica-based nanomaterials are non-toxic (90, 93). The use of microorganisms and bio-fabrication of nanoparticles offers a potentially even more viable and environmentally safer bioremediation approach for removal of lead from water. Chemically generated nanomaterials may have are limited when used in aqueous solutions because of potential for self-agglomeration. By contrast, nanomaterials synthesized with natural sources like bacterial, fungal enzymes and plant extracts that may be a viable solution. In aqueous conditions, such nanoparticles achieve greater firmness due to co-precipitation or by putting bioactive compounds and protein to the external face of nanoparticles (90, 93, 94). Thus, in addition to natural bioremediation capability of the many bacteria, they also contribute to enhancing nanotechnology in many ways (94).

*Biofilm and Genetic Engineering:* In the context of environmental duress in the water, such as nutritional deprivation, pH, or temperature alterations, bacteria respond by synthesizing biomolecules called exopolysaccharides (95). These molecules can enhance the capacity of the bacteria to sequester or release heavy metals, which can be exploited for the bioremediation of lead. For instance, the generation of exopolysaccharides from a collection of bacteria from the same or different species, which is influenced by environmental stressors, can form biofilms that are anionic in nature (95, 96, 97). While the formation of biofilms protects bacteria from challenging environments, they also serve to sequester cationic heavy metals (95, 97). The generation of biofilms by bacteria can, therefore, serve as a potential mechanism that can be exploited for the removal of lead from water.

Rapidly evolving genome engineering techniques such as genetic engineering, metagenomics, meta-transcriptomics, CRISPR gene editing and recombinant DNA technology offer tremendous potential for bioremediation with bacteria (97-100). Metagenomics and metabolic investigations offer insights into microbial diversity, population, and functional composition with respect to metal resistance genes (98, 99). These findings can be leveraged to improve the efficacy of microbial strains in the removal or degradation of heavy metals such as lead. The field of genetic engineering will allow for large scale and highly efficient transfer of advantageous traits from one species to another, resulting in the development of specific strains for the purpose of bioremediating polluted water.

### **Challenges of bacterial bioremediation**

Microbe-based remediation is a viable technique with good performance, low maintenance costs, excellent selectivity, and reduced generation of polluted water. Given the multiple advantages provided by microbe biosorption, the possibility for massive-scale application should have been exploited. However much remains unknown. The screening and selection of appropriate microorganism strains are critical in bioremediation have been extensively studied, but in isolation, in limited microenvironments and rarely in the full context of the microbial ecology and also the presence or absence of other organism (algae, plants, fungi etc). As such it is virtually difficult to develop reliable and durable bacterial bioremediations strategies that can be applied effectively to all places. It is frequently noticed that despite the presence of microorganisms in a water stream, their

action is ineffective; this lack of action may be attributed to a shortage of nutrients such as phosphorus or nitrogen. Another drawback of bioremediation as a technology is that it is frequently confined to biodegradable contaminants and is a highly selective process, making it difficult to apply to the treatment of a wide range of pollutants besides selective metals such as lead. The scalability if any specific bacterial bioremediation approach is fraught to extrapolate bench or pilot-scale operations and research to large-scale site operations. Furthermore, little has been explored or studied over longer time scale in the generation of plausible toxic by-products or the end products in the process of bioremediation when compared to the untreated parent compound. These challenges can now be potentially effectively tackled with evolving technologies and deeper understanding.

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