

Review Article

## Enhancing efficacy of microbial bioremediation by intervention of nanotechnology and metabolic engineering: A review

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### Abstract

Ever since the start of the Industrial Revolution, environmental pollution has significantly increased. The prominent cause of most diseases in humans, animals, and plants is the presence of toxic materials, pollutants, contaminants, and hazardous compounds released by industries. One of the major factors is the presence of heavy metals in the air, water bodies and soil. Heavy metals have biomagnification and bioaccumulation characteristics, making them hazardous for flora and fauna on a large scale. Recently, biological sources such as bacteria, fungi, algae, etc., have been used to bioabsorb these heavy metals. The microbial properties of these cell walls are utilized for effective and low-cost absorption of metals. Bioaugmentation, biosorption and biostimulation are effective strategies for reducing the toxicity of hazardous contaminants in the soil and facilitating bioremediation. The mechanism of biosorption is mainly based on ions and functional groups present in the microbes. Fungal species are advantageous over bacteria as they are easier to handle, cost-effective and, most importantly, non-pathogenic, making them ideal candidates for biosorption. This review provides a comprehensive overview of various microbial strains utilized in bioremediation. Further, the review highlights the application of nanotechnology and metabolic engineering approaches to improve the efficacy of Biosorption, Biostimulation and Bioaugmentation. It provides insights on the role of microbial nanoparticles in bioremediation and prospects in the forte of microbe-assisted bioremediation.

**Keywords:** Bioaugmentation, Bioremediation, Biostimulation, Metabolic Engineering, Nanotechnology

### INTRODUCTION

The rapid industrial revolution, burgeoning population, climate change, and urbanization have collectively intensified pollution levels by releasing various hazardous elements, including organic compounds, inorganic substances, petroleum derivatives, and heavy metals (Alahmadi, 2022). Among these pollutants, heavy metals such as arsenic, lead, mercury, and cadmium stand

out due to their toxicity and carcinogenicity, posing significant risks to human health and ecosystem integrity (Shahjahan *et al.*, 2022; Rahman *et al.*, 2020; Mitra *et al.*, 2022). Heavy metals exhibit non-biodegradable properties, exacerbating their persistence in the environment and causing detrimental effects on aquatic life. Notably, heavy metals possess the ability to bioaccumulate and biomagnify in aquatic organisms, amplifying their toxicity as they ascend the food chain (Shahjahan

*et al.*, 2022; Rahman *et al.*, 2020; Mitra *et al.*, 2022). Bioremediation has emerged as a promising approach to address heavy metal contamination in wastewater, employing various processes such as biosorption, bioaugmentation, biostimulation, bioprecipitation, and bioleaching to remove metals via absorption, precipitation, or solubilization (Atuchin *et al.*, 2023).

Microbial bioaugmentation presents viable strategies for heavy metal remediation, with bacteria such as *Pigmentiphaga* spp. and *Paenanthrobacter* spp., alongside the fungus *Trametes versicolor*, showing promise in this regard (Lopes *et al.*, 2022). Noteworthy microbial examples include *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus* for mercury remediation, *Micrococcus luteus* for lead and copper, *Bacillus* sp. MNU16 and *Aspergillus niger* for chromium, and *Rhizopus stolonifera* and *Bacillus megaterium* for cadmium and nickel (Xu *et al.*, 2021; Imron *et al.*, 2019; Njoku *et al.*, 2020).

In recent years, nanotechnology has introduced innovative wastewater treatment techniques, while metabolic engineering has provided novel technologies to enhance treatment efficiency (Mohapatra *et al.*, 2022). Nanotechnology has shown potential in improving microbial bioremediation efficacy by using nanomaterials like nanoparticles and nanocomposites to enhance pollutant uptake, transport, and degradation. Iron, titanium, and zero-valent metal nanoparticles have been used to enhance heavy metal remediation efficiency by facilitating microbial processes as they provide a larger surface area for microbial attachment and can act as electron shuttles to facilitate redox reactions (Guerra *et al.*, 2018; El-Kalliny *et al.*, 2023). Metabolic engineering techniques have also revolutionized microbial bioremediation strategies by genetically modifying microorganisms to optimize pollutant degradation (Sharma *et al.*, 2023). Engineered bacteria with enhanced metal ion uptake mechanisms or increased metal-chelating agents have shown improved heavy metal remediation capabilities (Mahdizade Ari *et al.*, 2024). Recently, novel microbial strains with strong bioremediation potential have been reported (Mahdizade Ari *et al.*, 2024). These include bacterial species like *Sphingomonas* spp. and fungal strains like *Aspergillus fumigatus*, which are highly efficient in degrading various pollutants, including heavy metals (Mahdizade Ari *et al.*, 2024; Amobonye *et al.*, 2023). Furthermore, using consortia or mixed cultures of microorganisms has shown synergistic effects, increasing pollution removal efficiency compared to individual strains (Amobonye *et al.*, 2023). By integrating nanotechnology and metabolic engineering approaches, microbial bioremediation can be further optimized, leading to more efficient and sustainable treatment of polluted environments.

## MATERIALS AND METHODS

### Different heavy metals and their effects on environmental toxicity

Arsenic is found as As (0), As(III) and As(V) and arsenic gas states. It reaches the environment through natural phenomena such as volcanic eruptions and weathering and anthropogenic activities like mining and smelting of metals (Muzaffar *et al.*, 2023).

As(V) can replace phosphate in several metabolic pathways, leading to ATP depletion. Arsenite, As (III), reacts with sulphhydryl and thiol groups, disrupting proteins structure and regulation of proteins and enzymes, such as pyruvate dehydrogenase (PDH), which, when altered, affects ATP formation cellular respiration, causes dilation of capillaries and thus increased permeability. The most toxic form is arsine gas. Its inhalation at 10 ppm is lethal; at 25 ppm, it is lethal in less than an hour; and at 250 ppm, it is lethal at instance (Kuivenhoven and Mason, 2023). Arsenic exposure leads to various cancers such as skin, bladder, lung cancer etc (Goswami *et al.*, 2022a). Coal mining is the major source of entry of mercury in the environment. Its majorly binds with components containing sulphur and, through plants and food reaches higher trophic levels due to its bioaccumulation property (Raj and Maiti, *et al.*, 2019). In anaerobic and aquatic conditions, it converts into an organic form, its most toxic form, possessing carcinogenic and genotoxic characteristics (Goswami *et al.*, 2024). Mercury toxicity can cause epigenetic alterations and various types of heart disease in humans (Khan *et al.*, 2019) neurotoxic and reproductive adverse effects in marine fishes. In plants, studies have shown to affect the height of rice plants, metabolic activities, induce closure of stomata and induce oxidative stress (Zheng *et al.*, 2019).

Lead (Pb) is used in industries such as mining, agrochemicals, paint, etc. and it enters the environment through natural phenomena such as volcanic eruptions, weathering of rocks etc. It affects growth, hearing capacity, cognitive behavior, neurological and cardiovascular diseases, kidney disfunction, and human reproductive health. Lead in plants is shown to affect morphology and growth. It also affects seed germination, obstructs photosynthetic pathways, and causes plant oxidative stress (Kumar *et al.*, 2020). Lead toxicity in broilers causes weight loss, anorexia, and wing drop and affects organs such as liver and kidney.

Cadmium (Cd) has mainly entered our environment through agricultural means; other sources are mining, combustion, sewage, traffic, contaminated food, water, cigarette smoking etc. It affects the bone and liver in humans. The presence of cadmium has the potential of causing cancers such as breast cancer, kidney cancer, prostate cancer, and lung cancer. In plants, Cd is

known to affect root and shoot growth, metabolic activities, alteration in several elements and water uptake, disturbance in pigment metabolism and plasma membrane activity. Cadmium also has epigenetic effects (Haider *et al.*, 2021; Genchi *et al.*, 2020).

Chromium (Cr), Cr (IV) is a toxic form. Chromium enters the environment through various activities such as chrome plating, mining and industries such as dye and leather, and naturally from soil and rocks. It affects plants by degrading photosynthetic pigments, reducing seed germination, and reducing the growth of shoots, and is also known to affect the nucleic acids through DNA disruption and epigenetic changes (Coetzee *et al.*, 2020; DesMarais and Costa, 2019). Table 1 summarizes different heavy metals and their hazardous effects on the environment.

**Mechanism of bioremediation by microbial species**

Bacterial biomass is employed in the biosorption of metals from wastewater, while dead biomass is used as adsorbent. Charges play a significant role in bioremediation as the negatively charged microbes readily bind with the metals in a cationic state.

For a mixture of several metals received from industrial waste, multimetal biosorption is applied, which is affected by factors such as the number of metals, order in which the metal is added, concentration of the metals etc. The mechanism of biosorption is majorly based on equilibrium isotherms and adsorption kinetics (Priyadarshane and Das, 2020).

The relationship between the concentrations of metal ions adsorbed on the surface of the biomass and the isotherm represents the concentrations of the solution's

**Table 1.** Common heavy metals and their hazardous effects on the environment

| Name of heavy metal | Effects on the environment  | References  |
|---------------------|---|---|
| Arsenic (As)        | ATP depletion<br>Disrupt protein structure and regulation of proteins and enzymes.<br>Alters PDH, pyruvate dehydrogenase affecting ATP formation, cellular respiration, cause dilation of capillaries and thus increased permeability.<br>Inhalation at 10 ppm is lethal.<br>Hemolysis when absorbed by lungs.<br>Cancers such as skin, bladder, lung cancer<br>Hypertension, diabetes, neurodegeneration | Kaur, S <i>et al.</i> , 2011; Pakulska D <i>et al.</i> , 2006 |
| Mercury (Hg)        | Epigenetic effects<br>Heart diseases<br>Neurotoxic and reproductive effects in marine fishes<br>Height of rice plants<br>In plants affects metabolic activity, induces stomatal closure and oxidative stress.   | Khan <i>et al.</i> , 2019; Zheng <i>et al.</i> , 2019         |
| Lead (Pb)           | In humans:<br>Growth hearing capacity cognitive behavior neurological and cardiovascular diseases kidney disfunction reproductive health<br>In plants:<br>morphology and growth seed germination obstructs photosynthetic pathways. causes oxidative stress in plants<br>In broilers:<br>weight loss anorexia wing drop affects organs such as liver and kidney.  | Kumar <i>et al.</i> , 2020                                    |
| Cadmium (Cd)        | In humans:<br>affects the bone and liver several kidney related disorders potential of causing cancers such as breast cancer, kidney cancer, prostate cancer and lung cancer<br>In plants:<br>affect root and shoot growth metabolic activities alteration in uptake of several elements and water disturbance in pigment metabolism and plasma membrane activity<br>Epigenetic effects                   | Haider <i>et al.</i> , 2021; Genchi <i>et al.</i> , 2020      |
| Chromium (Cr)       | Carcinogenic and mutagenic properties<br>In plants:<br>Degradation of photosynthetic pigments<br>reduction in seed germination<br>reduction in growth of shoot<br>DNA damage and epigenetic effects.  | Coetzee <i>et al.</i> , 2020; DesMarais and Costa, 2019       |

metal ions at equilibration. Kinetic analyses to identify the mass transfer and chemical reactions that control the rate of biosorption. Models of pseudo-first and pseudo-second order are used to analyse the kinetics of biosorption. Humic compounds serve as plant signal molecules and are also included in biostimulant products. The mechanism involves isolating the bacteria, conducting phylogenetic analysis, analysing the tolerance and inhibitory properties, and eventually selecting the appropriate strategy (Ijoma *et al.*, 2019). Different mechanisms of Bioremediation utilized by microbial species are summarized in Fig. 1.

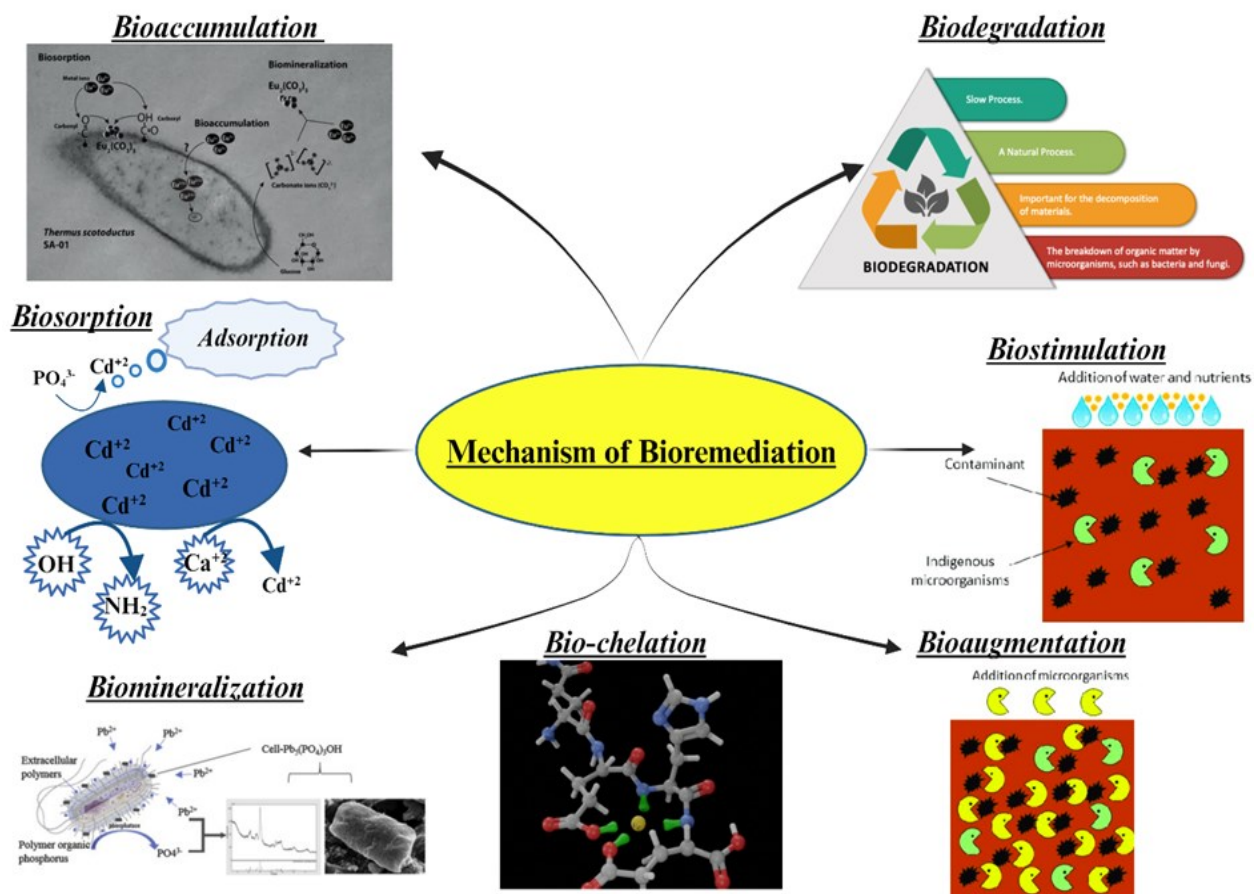
*Pseudomonas aeruginosa* is an example of a microorganism that is frequently employed in biochelation. This bacterium produces siderophores and can chelate or sequester heavy metals like lead, cadmium, and mercury. The bacteria may draw the metal from the environment and concentrate it inside their cells once it has been attached to the siderophore. Biochelators are another potential option for enhancing phytoremediation effectiveness. Moreover, due to its single carboxyl group, the biosurfactant rhamnolipid (RLs) made by *Pseudomonas* bacteria demonstrates excellent selectivity, biodegradability, and biocompatibility as well as a

strong affinity to metals, including Cd (Wang *et al.*, 2021). Resistance bacteria can endure exposure to harmful heavy metals and eliminate them biologically through evolving mechanisms, including biotransformation, bioreduction, bio-oxidation, biosorption, and bioaccumulation.

**Role of bacteria and fungus in bioremediation**

Bacterial activity against the pollutants also depends on many physiochemical properties like temperature, pH, moisture, oxygen concentration, nutrient availability, and type of pollutant or xenobiotic compound to be degraded. It is observed that the bacteria are comparatively less effective than the fungus in acidic pH. Many bacterial enzymes also play an important role in enhancing the remediation process. For example, when used in industrial effluent remediation, a ligninolytic bacterial enzyme laccase becomes more efficient (Panwar *et al.*, 2023). Table 2 summarizes the role of a few of the bacterial species in bioremediation.

Fungi also play a vital role in bioremediation, as mycoremediation. White rot fungi can degrade lignin and various other pesticides like DDT, lindane, polychlorinated biphenyls, etc. (Prajapati *et al.*, 2022). Marine fungi have a huge potential for producing enzymes and



**Fig. 1.** Different mechanisms of bioremediation utilized by microbial species (Image sources: <https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2019.00081/full>; <https://www.mdpi.com/1996-1073/13/18/4664>; <https://www.hello-nature.com/us/key/>)

**Table 2.** Role of the bacterial species in bioremediation

| Name of bacteria  | Role in bioremediation  | Reference                        |
|---|---|----------------------------------|
| <i>Pseudomonas aeruginosa</i>   | Detoxify Cd <sup>2+</sup> through production of intracellular cadmium-binding proteins            | Mei <i>et al.</i> , 2024         |
| <i>Rhodococcus chlorophenolicus</i> , Flavobacterium sp. and Arthrobacter sp.   | Degrade pentachlorophenol in soil   | Khalil and Omara, 2023           |
| <i>Bacillus stearothermophilus</i>  | Use the hydrocarbons of crude oil as their source of nutrition                                    | Novik <i>et al.</i> , 2019       |
| <i>Pseudomonas putida</i>   | Removes heavy metals like 100% of Ti, 96% of Pb, 83% of V, 57% of Ni, 71% of Co,                  | Ali <i>et al.</i> , 2023         |
| <i>Bacillus licheniformis</i>   | Absorbs Zn (53%), Cd (39% and Al (23%)  | Ali <i>et al.</i> , 2023         |
| <i>S. paucimobilis</i>  | Removes Cu, Fe, Pb, Cd and Cr from industrial wastewater by 60, 63, 54, 57 and 53% respectively   | Ali <i>et al.</i> , 2023         |
| <i>Bacillus subtilis</i>  | Removes Cu, Fe, Pb, Cd, and Cr from industrial wastewater by 51, 36, 41, 34 and 37%, respectively | Wrobel <i>et al.</i> , 2023      |
| <i>Rhizobium radiobacter</i>  | Removal was 49, 51, 45, 40 and 50%, respectively  | Wrobel <i>et al.</i> , 2023      |
| <i>Pseudomonas reidholzensis</i>  | Useful in the degradation of diesel   | Vidal-Verdu <i>et al.</i> , 2022 |
| <i>Raoultella ornithinolytica</i> , <i>Serratia marcescens</i> , <i>Bacillus megaterium</i> , <i>Aeromonas hydrophila</i> | Efficiently degrades acenaphthene and fluorene  | Mekontchou <i>et al.</i> , 2024  |
| <i>Brevibacterium frigoritolerans</i> , <i>Bacillus aerophilus</i> , <i>Pseudomonas fulva</i>                             | Bioremediation of organophosphorus pesticide phorate in soil                                      | Kilonzi and Otieno, 2024         |
| <i>Candida viswanathii</i>  | Helpful in degrading Phenanthrene and benzopyrene   | Tao <i>et al.</i> , 2024         |
| <i>Coprinellus radians</i>  | PAHs, methylnaphthalenes, and dibenzofurans   | Ren <i>et al.</i> , 2023         |
| <i>Bacillus cereus</i>  | Diesel oil  | Elumalai <i>et al.</i> , 2024    |

secondary metabolites that can be further used in the degradation of recalcitrants. They also can produce nanoparticles, which have a huge application in various industries. Extremophilic fungi play a pivotal role in the remediation of industrial effluent due to their tolerance to the harsh environment (Singh *et al.*, 2021).

### Metabolic engineering approaches to improve bioremediation

There are several approaches through metabolic engineering, including heterologous expression of entire gene clusters, engineering regulatory networks, gene insertions and deletions, redirecting metabolic pathways, stimulating by precursors, and genetic knocking out of loci.

In *S. rimosus* NRRL 3016, heterologous expression of entire gene clusters was successful in manufacturing secondary metabolites such as tetrangulol and tetrangomycin (Zheng *et al.*, 2021). In another case, the genes responsible for *Ralstonia eutropha*'s polyhydroxy butyrate (PHB) biosynthesis pathway were transferred to *Saccharomyces cerevisiae*, a more practical organ-

ism for industrial application (Thu *et al.*, 2023). The resulting recombinant yeast could produce PHA and PHB, which can be used to make biodegradable bioplastics (Deng *et al.*, 2024).

*Streptomyces coelicolor*, has been displayed to fabricate secondary metabolites (actinorhodin) more productively by constant expression of SARP (Antimicrobial Administrative Protein) positive controllers. In *S. griseus*, for example, inactivation of pathway-specific repressors increased chromomycin production (Zhang *et al.*, 2023). It is critical in metabolic engineering to guarantee that the expression of non-native pathways does not result in a metabolic imbalance. 2-keto acids, for example, are used as intermediates in the amino-acids synthesis in the bacteria *Escherichia coli* (*E. coli*). Keto acids can be transformed into higher alcohols that can be utilised as fuel by adding genes from the yeast *S. cerevisiae* that encode 2-keto acid decarboxylase and alcohol dehydrogenase (Kumar *et al.*, 2023).

Rerouting the metabolic pathway requires multiple changes to a pathway to produce a specific product,

such as succinic acid, which *E. coli* produces as a minor fermentation product (Thakker *et al.*, 2011). However, during anaerobic fermentation, *E. coli* preferentially forms acetic acid, formic acid, lactic acid, and ethanol instead of succinic acid. To increase succinic acid production and reduce the formation of other metabolites, metabolic fluxes need to be redirected. (Zhang *et al.*, 2009; Liu Xiutao *et al.*, 2022)

To promote new metabolic pathways and characterise new mutants to synthesise desirable fuel-grade products, systems biology methods such as transcriptomics, proteomics, metabolomics, and fluxomics are combined (Corrales *et al.*, 2024). Computational analysis and different models are applied during fermentation to improve strain optimization. Making culture media that can improve *P. pastoris* performance uses genome-scale metabolic models and analysis. A functional genomics approach is also a crucial tool for the overproduction of folate, where genes are expressed in *Lactobacillus plantarum* WCFS1 (Russo, 2023).

Some amino acids can serve as both stimulatory precursors and inducers, such as tryptophan for dimethylallyl-tryptophan synthetase in the production of ergot alkaloids (Borkar, 2023) and leucine for bacitracin synthetase (Seyfi *et al.*, 2020). Genetic knockout of loci can coition cells and alter their intracellular architecture to respond to environmental cues and improve product stability (Jo *et al.*, 2023). Antisense technology can also be used to temporarily reduce a gene's activity and increase the activity of a recombinant enzyme.

### **Impact of bioremediation by strategies of metabolic engineering**

Metabolic engineering has significantly improved bioremediation by impacting several key strategies, including bioaugmentation, biosimulation, and biosorption. Bacteria have been genetically altered to express enzymes that can break down various organic pollutants, such as pesticides, polychlorinated biphenyls (PCBs), and hydrocarbons (Bala S. *et al.*, 2022) Biosimulation models can be used to predict the performance of different remediation strategies and optimize the conditions for biodegradation. Metabolic engineering has enabled the development of more accurate models by providing detailed information on the metabolic pathways of microorganisms and the enzymes involved in biodegradation. Genetic engineering has been used to modify microorganisms to express high levels of binding proteins or enzymes that can capture and degrade specific contaminants (Goswami and Gupta, 2020). It is effective in the elimination of heavy metals, dyes, and other toxic pollutants.

### **Role of nanotechnology to improve bioremediation by microbes**

Incorporating nanomaterials such as nanoparticles,

nanotubes, and nanofibers can enhance the bioremediation process's physiological, chemical, and biological characteristics, increasing efficiency and effectiveness. Research has shown that the use of nanomaterials in bioremediation can have significant positive effects on the elimination of various contaminants such as heavy metals, organic pollutants, and even radioactive elements. For example, a study demonstrated that using iron oxide nanoparticles in conjunction with microbes effectively removes hexavalent chromium from contaminated water (Vázquez-Núñez *et al.*, 2020).

The nanomaterials bind to the contaminants, making them more accessible to the microorganisms responsible for their degradation. The nanomaterials' increased surface area and reactivity allow for faster and more efficient degradation of the contaminants. The nanomaterials can also stimulate the growth and activity of the microorganisms, further enhancing the bioremediation process. Nano bioremediation offers several advantages over conventional remediation technologies, including reduced toxicity, increased effectiveness, and cost-effectiveness. However, there are also potential hazards related to nanomaterials, including their potential toxicity and environmental impact, which must be carefully considered and addressed (El-Kalliny *et al.*, 2023).

The elimination of heavy metals present in wastewater using chitosan nanoparticles is one example of how nanomaterials are used in biosorption. Heavy metals, including copper, lead, and cadmium, have been found to have a strong affinity for the biopolymer chitosan, which is derived from chitin. When chitosan is converted into nanoparticles, its surface area is increased, leading to improved adsorption efficiency. In a study, chitosan nanoparticles were found to be effective in removing lead from aqueous solutions, with an adsorption capacity of 141.84 mg/g (Zhang *et al.*, 2023). In biostimulation, iron nanoparticles have been shown to stimulate microbial growth and activity, leading to increased degradation of polycyclic aromatic hydrocarbons (PAHs) in contaminated soils. In a study, iron nanoparticles were used to treat PAH-contaminated soil, resulting in a significant increase in microbial activity and PAH degradation compared to the control group (Hao *et al.*, 2020).

One example of using nanomaterials to remove heavy metals is titanium dioxide (TiO<sub>2</sub>) nanoparticles. The study found that the removal efficiency increased with increasing TiO<sub>2</sub> concentration, and the elimination efficiency of 99% was achieved at a concentration of 0.5 g/L (Modwi *et al.*, 2023). Another illustration is the elimination of mercury using carbon nanotubes (CNTs). Mercury was removed from tainted water using CNTs. With a maximum adsorption capacity of 186.2 mg/g (Fayazi *et al.*, 2020), the study discovered that the ad-

sorption capacity of CNTs for mercury was much higher than that of activated carbon. AgNPs, or silver nanoparticles, have also been investigated for removing heavy metals, including lead and cadmium. Lead and cadmium were taken out of contaminated water using AgNPs. The study found that the removal efficiency of AgNPs for cadmium and lead was 95% and 90%, respectively, at a concentration of 0.2 g/L (Negi *et al.*, 2021).

### Challenges in bioremediation

Because some substances are not biodegradable, the applicability of bioremediation for removing pollutants present in polluted locations is constrained. Even if a substance is biodegradable, its subsequent processing and decomposition may produce harmful metabolites. Furthermore, deploying the same bacterial strain to several sites is difficult since its effectiveness at a particular site is determined by a quantity of site-specific characteristics. The properties of the contaminants, the type of biological processes involved in bioremediation, and the availability of suitable nutrient levels are all considered to involve the biological processes' complexity. Bioremediation is time-consuming and labor-intensive because it calls for soil excavation, unique site layouts, and customization. The usage of large equipment like pumps and machines can produce noise and other disruptions that might affect nearby communities. In addition, ethical issues regarding the use of particular bacterial strains in bioremediation raise doubts about the impact they have on regional microflora (Vishwakarma *et al.*, 2020).

Activity of groundwater bioremediation is extremely determined by geochemistry, geology, hydrology, and pollutant concentration. Low baseline pH and temperature may impact biological treatments, whereas bedrock strength and soil porosity may affect plume movement. Furthermore, precisely anticipating the direction and velocity of groundwater flow is critical for managing pollutant transfer. Pollutant bioavailability, pollutant stability reversal, microbial adaptation, metabolic routes, enzymatic investigations, pollutant interactions, and end-product quality are some of these difficulties. A pesticide or polycyclic aromatic hydrocarbon (PAH) may be less bioavailable for microbial degradation since they are embedded in the soil. Exogenous microorganisms' poor compliance with polluted soils might impair their capacity to adapt to a particular site and degrade contaminants, necessitating proper techniques to solve this issue (Narayanan *et al.*, 2023). Ageing of pollutants also contributes to reduced bioavailability over time, creating a need for solutions that maximize pollutant accessibility.

The metabolic pathways in the bioremediation of organic pollutants and heavy metals remain incompletely understood, making it necessary to explore and under-

stand microbial communities and metabolic dynamics. Molecular biology techniques such as sequencing and synthetic biology approaches and technology can provide valuable tools for understanding the genomic organization of indigenous microbes and addressing limitations to pollutant removal (Goswami and Sharma, 2022b). Enzymatic studies are required to comprehend the catalytic action of several enzymes involved in pollution breakdown. More research is needed to investigate kinetics, molecular structure, activity, and inhibitory processes.

The interactions of contaminants from similar soil or compost composition on their degradation are poorly known, and additional research is required to overcome this obstacle. Finally, it is critical to guarantee that bioremediation results are devoid of harmful organic compounds and metals beyond a certain level.

### Future prospects in bioremediation

Bioremediation is an increasingly popular approach to cleaning up contaminated soil and groundwater. However, there are several challenges in translating laboratory-based results to the field. For bioremediation techniques to be widely adopted, three major constraints are impeding their spread: a lack of comprehensive understanding of how microbes react in the field, difficulty stimulating microbes, and difficulty ensuring proper contact with contaminants (Mondal *et al.*, 2023). Despite these obstacles, scientists are working on novel engineering ways to excite bacteria, such as gas sparking, which has increased the aerobic breakdown of petroleum compounds. Cell genetic manipulation is expected to improve through the development of bioaugmentation technologies which remove microbiological constraints. With a complete knowledge of biotransformation at the environmental and chromosomal levels, advanced bioremediation strategies will be developed. These technologies can also be used to develop methods for treating contaminants like polychlorinated biphenyls and chlorinated solvents, which were previously thought to be impossible to degrade or difficult to degrade (Mondal *et al.*, 2023). To increase microbial bioavailability, methods such as garbage solubilization by heat injection using hot air, steam, or flushing hot water, intense-pressure subsurface matrix fracturing, and the use of surfactants are being studied. Developing efficient and feasible tactics and assessing existing approaches' functionality and usefulness is critical. Many protocols have been developed to evaluate bioremediation technologies and ensure that cleaning objectives are accomplished. Characterization of physiochemical parameters in situ also appears promising, potentially revolutionising field assessment examinations. Despite the promise of bioremediation, it is necessary to handle the microbes with caution and continuously monitor their activity in the subsurface. The de-

velopment of quick technologies that overcome current obstacles and help the world move towards a cleaner, greener environment will determine the future of bioremediation (Khan, 2024). Future prospects entail advanced engineering approaches, improved microbial stimulation methods, and thorough field assessments to realize the full potential of bioremediation in creating a cleaner, greener environment.

## Conclusion

The heavy metals like arsenic, lead, mercury, and cadmium are significant pollutants due to their toxicity and carcinogenicity, posing substantial risks to human health and ecosystems. They persist in the environment, accumulating and magnifying in aquatic organisms, exacerbating their harmful effects up the food chain. Bioremediation emerges as a promising solution, employing various microbial processes such as biosorption, bioaugmentation, biostimulation, bioprecipitation, and bioleaching to remove metals from wastewater. Microbial bioaugmentation showcases viable strategies, utilizing bacteria and fungi with promising heavy metal remediation capabilities. Nanotechnology enhances bioremediation efficacy by employing nanomaterials like nanoparticles and nanocomposites to facilitate pollutant degradation and microbial activity. Metabolic engineering further revolutionizes microbial bioremediation, genetically modifying microorganisms to optimize pollutant degradation. The review highlights novel microbial strains and consortia with strong bioremediation potential, paving the way for more efficient pollution removal. Despite the progress, challenges such as non-biodegradable pollutants, microbial adaptability, and site-specific complexities persist.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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