



# Bacterial bioremediation of heavy metals in wastewater: A review of processes and applications

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## ABSTRACT

Heavy metals, a treasure of nature, turns to be toxic at high concentrations in water. Among several methods adopted to alleviate heavy metal pollution, bioremediation is considered to be a sustainable, cost-effective technology. Bioremediation largely relies on bacteria, apart from other microbes and plants. The inherent and adaptive mechanisms evolved in bacteria to defend the metal toxicity include bioadsorption/biosorption, bioaccumulation, bioprecipitation and bioleaching. Heavy metal resistant bacterial strains are easy to culture and maintain, and even dead cell biomass display high heavy metal remediation potential in solution. All the heavy metal remediation mechanisms exhibited by bacteria in water is comprehensively reviewed with recent research outputs and in-situ and ex-situ techniques. The cellular mechanisms of heavy metal remediation are discussed, considering efficient bacterial strains, physicochemical parameters, nutrient supplementation and design of novel microbial techniques. Research at omics level would effectuate further manipulation of the cellular process and increase its efficiency. Bacterial heavy metal remediation technique provides double benefit of metal recovery and water purification, along with reuse prospects for both water and metal resources. Technological intervention could meet the challenges of process acceleration, resist biofouling, compete with native wild bacterial species in wastewater, design for commercialization. Industrial translation of the technology is the pivotal avenue to be tackled. Ultimately, understanding of bacterial heavy metal remediation process is essential for the implementation of this promising technology to safeguard the environmental health.

## 1. Introduction

Heavy metals are elements with specific density of more than 5 g cm<sup>-3</sup> [1] and some of which are quintessential at low concentrations for the normal physiological functions of living organisms [2]. Specific amounts of Calcium (Ca), Sodium (Na), Magnesium (Mg), Potassium (K), Copper (Cu), Iron (Fe), Zinc (Zn), and Chromium (Cr) are required for the survival of organisms, while their high concentrations cause toxicity [3]. Several metals like Cadmium (Cd), Mercury (Hg), Nickel (Ni), Arsenic (As) and Lead (Pb) are toxic at any quantity causing deleterious effect on the wellbeing and survival of living organisms [4]. And it causes ecological, nutritional, genetic and evolutionary impacts. Heavy metals reach the environment by natural and anthropogenic activities [5]. As the heavy metals are non-degrading and bio accumulating with well-delineated toxic effects; government agencies, has

imposed rules and legislations to limit the use and discharge of toxic metals to environment. World Health Organization [6] has defined the permissible heavy metal concentration in safe drinking water. Whereas Food and Agriculture Organization has stipulated the maximum permissible limit of toxic heavy metals in irrigation water [7].

To comply with the regulations and standards, many strategies and techniques have been investigated and implemented to alleviate heavy metals from water. The generally employed techniques are coagulation, ion exchange, membrane filtration, chemical precipitation, adsorption, electrochemical treatment, flocculation and bioremediation [8]. Bioremediation is gaining tremendous attention since a few decades due to its less or no requirement of chemicals, cost-effectiveness, absence of solid sludge by-products and eco-friendly operating techniques [9].

Bioremediation can be defined as the process that utilizes and depends on biological mechanisms to transform/degrade/detoxify

*Abbreviations:* ATP, Adenosine triphosphate; ABC, ATP binding cassette transporter; FTIR, Fourier transform infrared spectroscopy; GTP, Guanosine triphosphate; LPS, Lipopolysaccharide; NADH, Nicotinamide adenine dinucleotide hydride.

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pollutants to innocuous state, and ultimately mineralize to carbon dioxide, nitrogen, and water etc., using live or dead biomass [10]. Plants, fungi, bacteria, algae, and cyanobacteria are widely researched and employed for heavy metal bioremediation. Microorganisms are most suitable for the purpose as they are easier to handle, culture and implement. In recent years, microorganisms especially bacteria reckoned recognition with their ability to adsorb, solubilize and precipitate heavy metals by bioadsorption (biosorption), bioaccumulation, bioleaching and bioprecipitation. Omni presence, abundance, diversity, small size and unique capacity to grow and propagate under controlled and uncontrolled conditions with environmental resilience make bacteria the best candidate for bioremediation [11].

Bacterial bioremediation of heavy metals in solution mostly occur by means of adsorption on bacterial cell surface. It is the first line defence of bacteria to resist metal toxicity. Wherein, negatively charged carboxyl, amino, phosphoryl, and sulfo groups on bacterial cell wall acts as potential ion exchange sites and metal sinks. Thus, adsorption occurs on cell wall by redox process, ion-exchange, complexation, electrostatic attraction, and precipitation. Bioadsorption or biosorption is an inactive process, where heavy metals are adsorbed on to the cell surface devoid of energy expenditure (metabolism independent) until equilibrium is achieved. While bioaccumulation is predominantly metabolism dependent or active uptake of heavy metals by living biomass/cells [10]. Bacteria bind heavy metal ions by means of exopolymers/polysaccharides to their cell surface, further circumscribe or internalise these metal species into the cell for various metabolic functions, besides chelate the metals by producing several metabolic ligands [12]. Bioleaching is the bio-recovery of metals by releasing/excreting ligands, like organic acids, cyanide, thiosulphate and phenol derivatives. These ligands interact/react with metals in its vicinity, bind and form mostly soluble complexes directly or indirectly. Bioprecipitation of metals is a metal sequestering process exhibited by metabolically active cells, where metabolites produced react with metals present in water and form metal precipitates. Microorganisms, particularly anaerobic bacteria, convert the metals present or dissolved in aqueous solution into solid precipitates via sulphate, carbonate, phosphate or hydroxide precipitation [13].

### 1.1. Significance of the study

To explore and understand the underlying mechanisms by which bacteria remediate heavy metals is vital for the development of feasible technologies and treatment strategies using bacteria, for heavy metal removal from polluted environments. Better knowledge on cellular process leading to hazardous heavy metal removal is quintessential for

- The selection of appropriate syntrophic bacterial strains for effective elimination and extraction of heavy metals from wastewater.
- Suitably modulate or enhance the remediation process by controlled supplementation of necessary nutrients (nitrogen/carbon sources), or electron acceptors (O<sub>2</sub>, Fe, and S), thereby conditioning the cellular metabolism.
- Adjust physiochemical working parameters like, pH, temperature, media composition, etc., to achieve maximum remediation.
- Choose compatible bacterial species or strains with specific metabolic features, distinct competencies, and synergistic potentialities to design and develop microbial consortia/biofilm to detoxify heavy metals by bioaugmentation.
- Proper and productive construction of microbial fuel cells, which elide heavy metals with greater redox potential than the existing anode and deposit it at cathode chamber, facilitating metal recovery.
- Determine and incorporate the precise bacterial candidates in novel techniques like constructed wetlands [14], particularly floating treatment wetland and combined ecological floating beds. The efficiency of the process is inevitably related to the microbial community attached to the floating mats and rhizomes.

- Expand the knowledge spheres to omics (genomics, transcriptomics, proteomics, and metabolomics) and suitably manipulate the process by gene editing and engineering for enhanced expression of heavy metal transporter proteins, sequestering proteins and enzymes of relevant metabolic pathways [15].
- Capacitate fruitful interventions in microbial nanotechnology, especially bacterial synthesis of metal nanoparticles [16].

Therefore, the present review delineates the remediation mechanisms and its underneath cellular processes exhibited by bacteria, that aid in the removal of heavy metals in aqueous media. Relevant reports of previous researchers are discussed. Additionally, very recent research findings are briefly summarised and tabulated. Diagrammatic representations are provided to support the descriptions.

The keywords used for data retrieval from scientific sources (*Scopus, Web of Science, PubMed*) were - bioremediation of heavy metals using bacteria, biosorption, bioadsorption, bioaccumulation, bioprecipitation and bioleaching. The publications that deal with heavy metal remediation from aqueous medium using bacteria were included for the study. Other microbial (fungi, algae and nanomaterials) remediation procedures were excluded. Even bacterial remediation in solid substrates like soil was also not considered. Publications from the year 2000 to 2022 were scrutinized and the bacterial remediation mechanism elucidated in each research paper were probed and discerned. As most of the literature available were confined to bioadsorption and bioaccumulation, and both seldom differentiated, emphasis was given to discrete and delineate each mechanism precisely. The papers published from 2018 to 2022 on bioremediation of heavy metals from water with specified bacterial strains and mechanism were compiled and tabulated. The practical applications of bacteria mediated heavy metal bioremediation is provided, mentioning the bacterial species involved and its removal efficiency.

## 2. Mechanism of bioremediation

Several bacterial strains have evolved diverse, discrete mechanisms to adapt, interact, acclimate and thrive in environment rich in minerals, especially heavy metals. These include uptake of heavy metals on to the cell surface by biosorption, intracellular sequestration by accumulation, extracellular sequestration as insoluble compounds by precipitation, and production of metabolites that solubilize and chelate metal compounds that leads to leaching. The different mechanisms and its several modes, exhibited by bacteria to remove and annihilate heavy metals is depicted in Fig. 1 and discussed in following section.

### 2.1. Bioadsorption/biosorption

Biosorption/bio adsorption is the non-directed active or passive physiochemical interaction between inorganic and organic metal/minerals with cellular substances [17,18]. The major processes involved in biosorption mechanism are surface adsorption, physisorption, chemisorption, ion exchange, and surface complexation. Surface adsorption involves electrical attraction between negatively charged ligands present on cell wall and the positively charged metal ions in medium and is often an "exchange" reaction [19]. Physical adsorption majorly comprises Van der Waals forces, whereas in "chemical" or "activated" adsorption, attraction occurs between the adsorbent and adsorbate. An assemblage of all these mechanisms, either functioning together or independently, leads to the overall metal adsorption on microbial cell surface [20]. Heavy metal binding, possibly a two-stage task, comprises interaction between reactive groups on bacterial cell surface and the metal ions, followed by deposition of metal [19].

Spectroscopic and chemical modification studies have depicted that, the cellular radicals like-hydroxyl, carboxyl, sulfate, sulfhydryl (thiol), thioether, phosphate, phosphonate, phosphodiester, amino, imine, amide, imidazole, and carbonyl (ketone) possess high metal binding potential [21]. Table 1 elucidates the chemical structure, occurrence

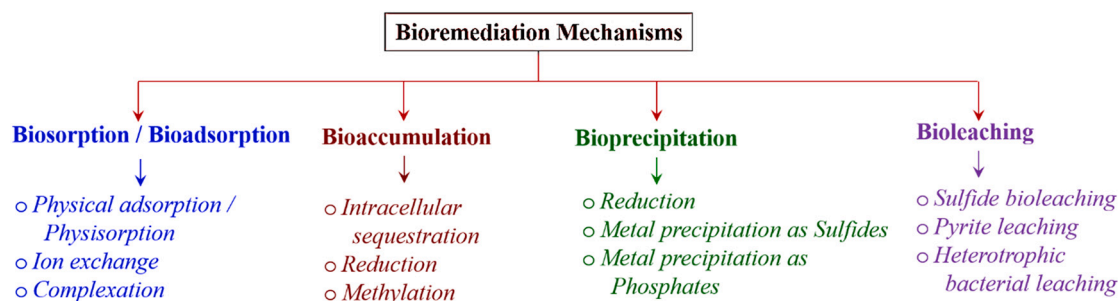


Fig. 1. Different mechanisms involved in heavy metal remediation by bacteria in liquid medium.

and position of these active radicals in bacterial cell structure. Most of these groups are present on bacterial cell wall. Ionization of these functional groups turns the bacterial cell surface negatively charged, viable for cationic metal attachment [20].

Cell walls of bacteria are primarily formed of peptidoglycans, which constitute linear chains of sugar; the disaccharide *N*-acetylglucosamine- $\beta$ -1-4-*N*-acetylmuramic acid interconnected with peptide chains. The peptide chains are tetramers composed of L-alanine, D-glutamic acid, D-amino pimelic acid or L-lysine and another L-alanine. Apart from that, a glycopolymer teichoic acid is embedded in gram positive bacterial cell walls increasing its gross negative charge. Cell walls of gram-negative bacteria are not profusely cross-linked and composed of thinner peptidoglycan layer than gram-positive bacteria. Gram-negative bacteria harness an outer membrane formed of lipopolysaccharides (LPS), lipoproteins and phospholipids. Gram-positive bacterial cell wall contains glycoproteins on its outer side, dispensing more accessible binding sites than LPS and phospholipids [19].

### 2.1.1. Physical adsorption/physorption

It is the phenomenon of transferring ions from one phase to another namely, liquid phase to solid phase, involving: (i) bulk transfer to peripheral layer; (ii) inward diffusion or massive transmission through pores and/or random motion of atoms by solid diffusion; and (iii) adhesion or adsorption to the exterior surface of bacterial cells. Physical adsorption, is due to non-specific, rapid and reversible attraction forces like Van der Waals forces. Or, it occurs by electrostatic adsorption on account of coulombic attractive forces arising between charged solute particles and bacterial cell surface [18]. Ahalya et al. [22] reported that copper biosorption on *Zoogloea ramigera* bacterium occurred through electrostatic interaction. Physicochemical interactions between the cellular radicals on the bacterial cell wall and membrane, and the metal species in solute are the prime factors responsible for rapid and reversible metabolism-independent biosorption in dead cell biomasses. FTIR studies by Hasan et al. [23] elucidated that amine, hydroxyl and carboxyl groups existing on the cell surface (dead dried biomass) of *Aeromonas hydrophila* was the fundamental cause of Pb (II) sorption, besides the passage through pores. Anionic groups,  $-C-O$ ,  $-COO$ ,  $-NH$ ,  $-OH$  and  $-C=O$  present on dead *Streptomyces rimosus* bacterial cell walls exhibited fairly high adsorption towards  $Pb^{2+}$  ions [24].

### 2.1.2. Ion exchange

In ion exchange mechanism, metal cations bind to a vacant site, previously occupied by another cation. Divalent ions of metals are adsorbed by exchange with polysaccharide counter ions present on the cell wall and outer membrane of bacteria [25]. This process relies on several factors like the different kinds and number of sites present on the cell surface and their ionization pattern, which is eventually determined by the pH and pKa value of respective groups. Protonated amine groups are positive in charge; and turns neutral while deprotonated. Addition of protons convert phosphate, carboxyl, and sulfate groups into neutral, and in deprotonated condition turns negatively charged [26]. Nickel biosorption by ion exchange was reported in *Pseudomonas fluorescens*

4F39 [27]. *Pseudomonas pseudoalcaligenes* and *Micrococcus luteus*, adsorbed significant quantities of lead and copper, which further increased with pH from 2 to 6 [28]. Hasan et al. [23] has inferred that Pb (II) ions compete with  $H^+$  at low pH and get adsorbed to *Aeromonas hydrophila* by ion exchange.

### 2.1.3. Complexation

Remediation of metals could also occur by formation of complexes over cell periphery by the interaction of reactive radicals on cell wall and metal ions in solution. Amino, carboxyl, hydroxy, thiol, phosphate, and hydroxyl-carboxyl groups interact in coordination with heavy metal ions [29]. ‘Complex or co-ordination compound’ is a poly-atomic molecule with neutral, or negative or positive charge, and it consists of single or numerous central atoms (generally metal cations) bounded and joined to ligands (other negative or neutrally charged atoms or groups). If a ligand is connected through two or more coordinating atoms to central atom, then the complex is termed as ‘Chelate’ [26]. Magnesium, calcium, cadmium, copper, zinc, and mercury complexation were found to occur in *Pseudomonas syringae*. Complexation is the major biosorption process involved during alkaline pH. Mercury and uranium complexation has been reported at pH above 7 in *Pseudomonas fluorescens* 4F39 by López et al. [28]. Hydroxyl, phosphoryl, carboxyl and amino groups of proteins and polysaccharides on the extracellular matrix of *Shewanella putrefaciens* was found to complex with Cr(VI) [30].

Recent research outcomes on bacterial biosorption of heavy metals are tabulated (Table 2). Environmental factors like temperature, pH and composition of wastewater, concentration and oxidation state of heavy metals, organic and inorganic entities present, colloids and emulsions, may influence biosorption, along with the type of bacterial strain and mechanism of metal removal. Use of dead cells is more propounded, as dead biomass is hardly susceptible to heavy metal toxicity, and nutrients are not required for the growth of bacterial cells [19,31], lowering operational cost. The biomass waste from fermentation industries could be used for the purpose. Metal loading on biomass is very rapid, as the non-living biomass is an efficient ion exchanger. Sterilization is not mandatory, with storage options and pretreatment of bacterial biomass may further improve the biosorptive capacity. Moreover, operating conditions like pH, temperature, time etc. could be controlled with possibility for metal recovery. However, the improvement of biosorption potential by genetic engineering or biological altering of metal valency state is limited in dead biomass [19].

Bioadsorption is a simple and fast process that could utilize alive or dead bacterial cells or exopolymers alone, to retrieve heavy metals from aqueous solution. Though physical and chemical parameters of media and oxidation state of metal ions determine the efficiency of the process, metals are adsorbed within few hours. Rather resorption is easy, and biomass could be reused. This is the most common heavy metal defence mechanism exhibited by mesophilic bacterial strains.

## 2.2. Bioaccumulation

Bioaccumulation is the influx and accretion of metals within

**Table 1**  
Heavy metal binding radicals present in bacterial cell structure.

Metal binding cellular radicals	Chemical structure	Presence in cellular compounds	Position in cell structure
Carboxyl	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C} \\ \backslash \\ \text{OH} \end{array}$	Fatty acids, proteins, organic acids	Cell membrane, cell wall, cytosol
Hydroxyl	$\text{R}-\text{OH}$	Alcohols, carbohydrates	Cell membrane, cell wall, cytosol
Sulfate	$\begin{array}{c} \text{O} \\ \parallel \\ \text{O}-\text{S}-\text{O}^- \\ \parallel \\ \text{O} \end{array}$	Aminoacids-cysteine, methionine	Cell membrane, cytosol
Sulphydryl (Thiol)	$\begin{array}{c} \text{H} \\   \\ \text{R}-\text{C}-\text{SH} \\   \\ \text{H} \end{array}$	Amino acid-cysteine	Cell membrane, cytosol proteins
Phosphate	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{O}-\text{P}-\text{OH} \\   \\ \text{OH} \end{array}$	DNA, RNA, ATP	Cell membrane, cytosol, chromosomes
Amino	$\begin{array}{c} \text{H} \\   \\ \text{R}-\text{C}-\text{NH}_2 \\   \\ \text{H} \end{array}$	Proteins and nucleic acids	Cell membrane, cell wall, cytosol
Amide	$\begin{array}{c} \text{H} \\ \parallel \\ \text{R}-\text{C}-\text{NH}_2 \\   \\ \text{H} \end{array}$	Fattyacid-aminoacid bonds	Cell membrane lipids
Imine	$\begin{array}{c} \text{R} \\ \backslash \\ \text{C}=\text{N}-\text{R} \\ / \\ \text{R} \end{array}$	Deaminated aminoacids	Cell membrane, cell wall, cytosol
Ester	$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{O}-\text{R} \end{array}$	Lipids	Cell membrane, cytosol
Thioether	$\begin{array}{c} \text{R} \\ \backslash \\ \text{S} \\ / \\ \text{R} \end{array}$	Aminoacids	Cell membrane, cell wall, cytosol
Carbonyl (ketone)	Internal $\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{C} \\   \quad   \\ \text{O} \quad \text{O} \end{array}$ Terminal $\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{H} \end{array}$	Polysaccharides, ketones Polysaccharides, aldehydes	Cell membrane, cell wall, cytosol
Imidazole	$\begin{array}{c} \text{H} \\   \\ \text{C}=\text{N} \\ / \quad \backslash \\ \text{C} \quad \text{C} \\ \backslash \quad / \\ \text{N} \\   \\ \text{H} \end{array}$	Aminoacids and nucleic acids	All protein components in cell membrane, cytosol, ribosomes and nucleic acids
Phosphonate	$\begin{array}{c} \text{H} \\   \\ \text{C}-\text{P} \\   \quad \backslash \quad \backslash \\ \text{H} \quad \text{OH} \quad \text{O} \end{array}$	Lipid fractions, phosphonolipids	Exoploysaccharides
Phosphodiester	$\begin{array}{c} \text{H} \\   \\ \text{H}-\text{O} \\   \\ \text{O}=\text{P}-\text{O}-\text{CH}_2 \\   \\ \text{O} \end{array}$	DNA, RNA	DNA, RNA, ribosomes

bacterial cells by the uptake of non-metabolic metals through the same carrier pathway involved for metabolically essential metals. The mechanism constitutes, binding of metal ions to the reactive radicals at the outer surface of bacterial cell wall, likewise and furthermore to its internal region through energy independent mechanism. Subsequently, metals diffuse into the cell cytoplasm, by means of energy dependent/independent process. The pathway for the transport of metabolically pivotal ions like sodium (Na), potassium (K), magnesium, etc. would be

relied for the transport of heavy metals through microbial cell membranes for intracellular accumulation. The cation transport systems will bind with heavy metal ions of identical ionic radius and charge as of essential metal ions [29].

### 2.2.1. Intracellular transport

Heavy metals are imported to the bacterial cell by means of channels, secondary carrier proteins and primary active transporters present on

**Table 2**  
Recent research reports on bacterial adsorption of heavy metals.

Bacterial species	Heavy metals adsorbed	Mode of adsorption	Ref.
<i>Bacillus cereus</i>	Pb	Ion exchange, complexation	[32]
<i>Bacillus cereus</i>	Pb, Ni	Complexation, physisorption	[33]
<i>Oceanobacillus profundus</i>	Pb, Zn	Ion exchange, complexation	[34]
<i>Bacillus thuringiensis</i> , <i>Pseudomonas stutzeri</i> , <i>Micrococcus yunnanensis</i>	As	Ion exchange, physisorption	[35]
<i>Lactobacillus plantarum</i>	Cd, Pb	Complexation, physisorption	[36]
<i>Pseudomonas aeruginosa</i> , <i>Bacillus subtilis</i> , <i>Azotobacter chroococcum</i>	Cu, Ni, Pb, Cd, Cr	Ion exchange, physisorption, complexation	[37]
<i>Agrobacterium tumefaciens</i>	Pb, Cd, Ni	Ion exchange	[38]
<i>Sinorhizobium sp</i>	Cr	Ion exchange, physisorption	[39]
<i>Bacillus xiamenensis</i>	Pb	Physisorption, ion exchange	[40]
<i>Pseudomonas stutzeri</i> , <i>Bacillus subtilis</i>	Cd	Physisorption, complexation, ion exchange	[41]
<i>Pseudomonas koreensis</i> , <i>Patonea sp</i>	Cd, Cr, Pb	Ion exchange, physisorption	[42]
<i>Ochrobactrum sp</i>	Cu	Physisorption, complexation	[43]

bacterial cell membrane. Channels are  $\alpha$ -helical proteins that serve for the passive diffusion of heavy metals across the membrane according to concentration gradient. These channel proteins belong to Major intrinsic proteins super family and are found to transport arsenic and mercury in a series of bacterial species like *E. coli*, *Cornebacterium*, *Streptomyces coelicolor*, *Serratia* and *Pseudomonas* [44].  $\beta$ -barrel proteins and porins present in gram-negative bacteria have also been found to translocate heavy metals. Uniporters, symporters and antiporters are the secondary carrier proteins involved in heavy metal accumulation. Transporter-Opsin-G protein coupled receptor super family and major facilitator super family proteins form the chief carrier proteins behind this function. These proteins rely on proton motive force for metal shifts into the bacterial cell. Likewise, uptake of Ni, Co and As has been reported in *Helicobacter pylori* [45], *Rhodospseudomonas palustris* [46], *Novosphingobium aromaticivorans* and *Staphylococcus aureus* [47]. Primary active transporter proteins are multi domain tertiary proteins embedded in the plasma membrane of bacterial cells, which comprises a transmembrane portion and cytosolic ATPase coupling component. Periplasmic solute binding domain is also sometimes present. P-type ATPase proteins and ABC transporter proteins are the major protein super families that constitute primary active transporters [44]. These transporters transit heavy metals against concentration gradient using energy derived from the hydrolysis of ATP or GTP reserves in cell. *Lactobacillus plantarum* [48], *Thlaspi caerulescens* [49], and *Enterobacter hirae* [50] bioaccumulated Cd into the cell through primary transporters.

Apart from the membrane carrier proteins, interaction of metals with bacterial surface ligands subsequently leads to its slow transport into the cell. Bacteria including cyanobacteria release high-affinity, low-molecular-weight (200–2000 Da), coordination molecules termed as siderophores, that bind iron atoms. The synthesis of siderophores in bacteria is a stimulatory response towards iron scarcity in environment. In addition to iron radicals, siderophores are able to join and bind with other metals also e.g. Thorium (Th), Uranium (U), Ni, Gallium (Ga) and Cu [51]. Bacterial siderophores constitute catecholates (e.g., enterobactin), carboxylates (e.g., rhizobactin), and hydroxamates (e.g., ferrioxamine B) [52]. Bacteria use different siderophore-mediated iron (Fe) transport systems and it varies in gram-positive and negative bacteria. Gram-negative bacteria like *Escherichia strains* possess outer membrane

TonB-dependent receptors which recognize Fe(III)-siderophore complexes accessible at the cell surface and bind them thenceforth. Fe(III)-siderophore bound to the TonB-dependent outer membrane receptors, crosses the outer membrane to periplasmic space via high-affinity periplasmic binding protein, which accompanies the Fe(III)-siderophore complex up to cytoplasmic membrane, and is relieved back to periplasmic space to resume its carrier function. Fe(III)-siderophore complexes are shuttled across the cytoplasmic membrane to cytoplasm by ATP-binding cassette (ABC) transport system. Finally, Fe(III) is dissociated from the siderophore complex via reduction of Fe(III) to Fe(II). Subsequently, siderophores are either degraded or recycled, through a shuttle mechanism using specific efflux pump [53] (Fig. 2). Whereas, in gram-positive bacteria like *Bacillus*, no outer membrane receptors are present due to the lack of outer membrane.

Therefore, the Fe(III)-siderophore complexes directly attach to the periplasmic siderophore binding proteins embedded on cytoplasmic membrane. After which, the Fe(III)-siderophore complexes are transported to the cytoplasm as like in gram-negative bacteria, by the ATP-dependent ABC transport system [54].

### 2.2.2. Intracellular fate of bioaccumulated heavy metals

Growing bacterial cells are capable of eliminating metals perpetually by internal detoxification methods. Biotransformation and reduction of metals using enzymes, methylation, sequestration by metal-organic complexion, and production of metal chelators-metallothioneins are the different mechanisms exhibited by bacteria to defend metal toxicity [55].

**2.2.2.1. Intracellular sequestration.** Intracellular sequestration is the process of formation of composites of metal ions with various cellular cytoplasmic compounds. Upon entry to the cells, heavy metal ions are translocated, attached, or imbedded in cellular organelles, depending on the element concerned and the bacterial strain. Chelation is the process of binding of metals to ligands or compounds. Cytoplasmic metal chelation is mostly aided by metallothioneins-peptides rich in cysteine,  $\gamma$ -glutamylcysteine- a glutathione analogue present in haloarchaea, and by polyphosphate-the chelating molecule produced in most halophilic bacteria and archaea species. Iron-storage protein ferritin, copper and zinc-storing metallothioneins are the common metal sequestering proteins found in bacterial cells [56]. Metal stress induces high expression of metallothionein genes and its overproduction in bacteria, resulting in augmented metal binding and sequestration [57].

This metal accumulation potential of specific bacterial strains has been manipulated mainly for effluent treatment. *Rhizobium leguminosarum* with the aid of glutathione, manifested considerable quantity of intracellular cadmium ion sequestration [58]. Jroundi et al. [59], reported intracellular accumulation of lead in polyphosphate grains within the cells of *Bacillus* species isolated from Mediterranean sea. Cyanobacteria, *Anabaena cylindrica* and *Plectonema boryanum*, accumulated cadmium and aluminium in polyphosphate bodies [19].

**2.2.2.2. Reduction of heavy metal ions.** Bacteria make use of metals and metalloids as electron donors or acceptors for energy generation. Bacterial cells can transform the oxidation state of metal ions, thus reducing its toxicity. Oxidized metals act as terminal electron acceptors in bacteria during anaerobic respiration. Many anaerobic and facultative anaerobic bacterial species use oxidized metallic elements like Fe(III), Mn(IV) or Cr(VI) as terminal electron acceptors [60]. Reduction of selenium and arsenic coupled to organic substrates, lactic acid, acetic acid and aromatics is observed in certain bacterial species. Oxyanions of selenium and arsenic are also used as terminal electron acceptors during anaerobic respiration by bacterial strains, that provide energy for metabolism and growth [61].

Enzymatic reduction results in the generation of less toxic forms of mercury and chromium. Mercury detoxification is accomplished in

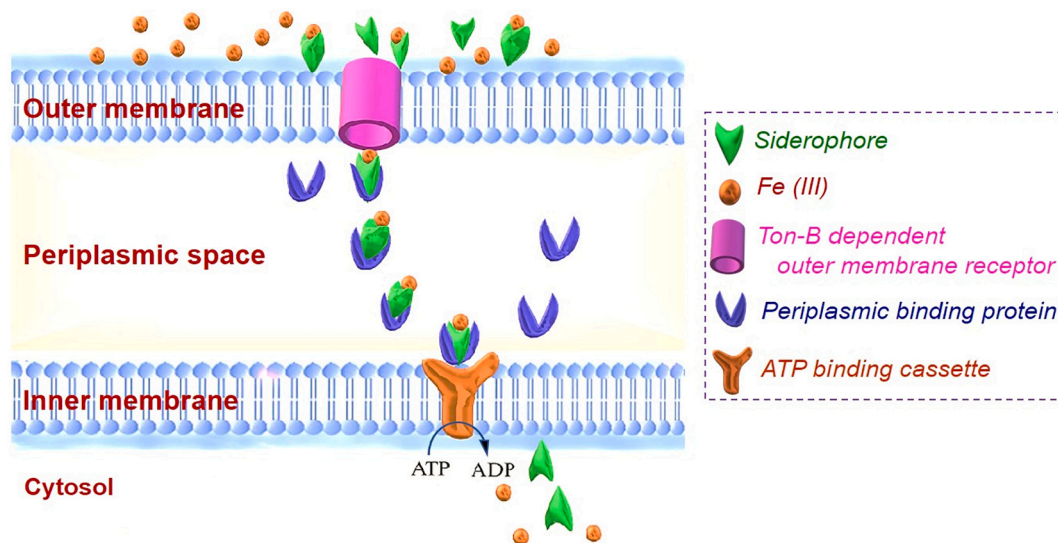


Fig. 2. Intracellular transport of ferric [Fe(III)] ions by siderophores in bacteria.

bacterial strains by organomercurial lyase (MerB) and mercuric reductase (MerA) enzymes. Enzymatic chromate reduction that involves copper-dependent reductase was observed in haloalkaliphilic *Amphibacillus* sp. [62]. Membrane-associated NADH-dependent chromate reductases catalysed chromate reduction is prevalent in halophilic *Halomonas* strains [63]. NADH-dependent nitrate reductase catalyses ionic silver ( $Ag^+$ ) reduction to inactive metallic colloidal silver nanoparticles in *Bacillus licheniformis* [64]. Arsenate reduction in prokaryotes is carried out by soluble arsenate reductases (ArsC) [65]. Several bacterial strains of *Bacillus*, *Pseudomonas putida* and *Pedomicrobium* reduces

manganese- Mn (III and IV) to Mn (II) and further oxidize it to be used as terminal electron acceptor in electron transport chain [66].

2.2.2.3. *Methylation of metals.* Certain bacterial strains are proficient in methylating various metal, metalloid and organometallic compounds to methyl derivatives, which is often volatile and evaporates [19]. Methylation is one of the fundamental biochemical pathways of bacterial cell. Intracellular metal and metalloid methylation occur by any of the three pathways namely involving-S-adenosylmethionine, methylcobalamin and N-methyltetrahydrofolate. Sulphate or iron reducing

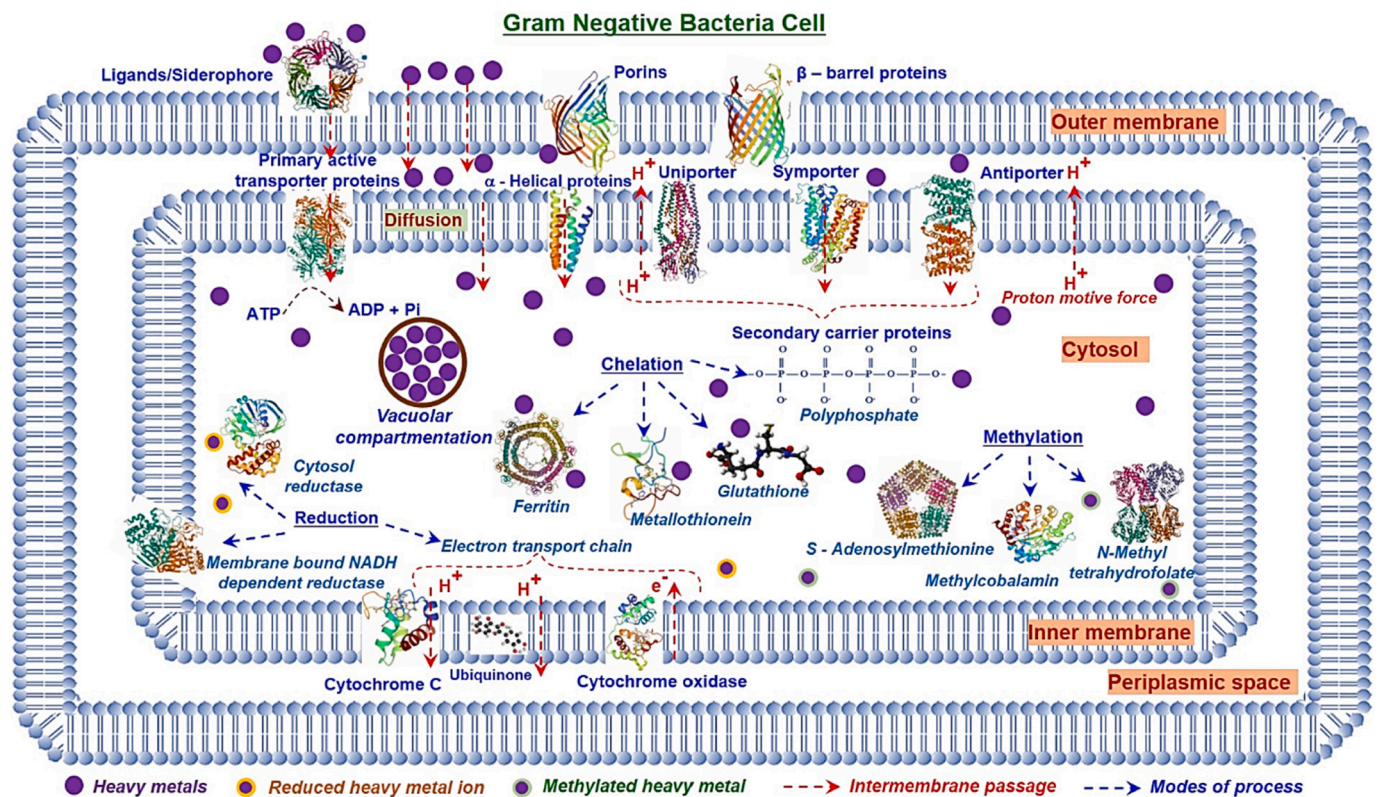


Fig. 3. Illustration of intracellular passage of heavy metals by different channels and its bioaccumulation within bacterial cell through various mechanisms (i) Vacuolar storage, (ii) Sequestration by ferritin, metallothionein, glutathione and polyphosphate molecules, (iii) Reduction by receiving electrons from Electron transport chain or by enzymatic action, and (iv) Methylation. (Protein structures according to PDB format).

bacteria utilize methylcobalamin to methylate mercury (Hg). Whereas, methylation of Arsenic (As) occurs via S-adenosylmethionine [67]. Metal compounds, when methylated turns to be explosive mostly; for instance, *Escherichia sp*, *Bacillus sp*, *Pseudomonas sp*, and *Clostridium sp* biomethylate Hg (II) to gaseous methyl mercury. Bio methylation of As to gaseous arsines; selenium (Se) to volatile dimethyl selenide, and lead (Pb) to decomposable dimethyl lead has been observed in solutions stagnant on polluted top soil containing bacteria [55]. Each methylation pathway is distinct, and the pathways involved vary among bacterial species and oxidation state of metals in aqueous solution [67]. Schematic representation of intracellular transport of heavy metals and its cellular accumulation process is depicted in Fig. 3.

Table 3 outlines the recent research findings on heavy metal accumulation by bacterial species. Bioaccumulation is a toxicokinetic process influenced by the sensitivity of living cells to heavy metals. It depends on the type of bacteria, concentration and metals involved; and could be inhibited by metabolic inhibitors like low temperatures and nutrient deficiency. Moreover, metal ion uptake is dependent on cell age, pH of metal solution and composition of media, initial metal concentration, contact time, and finally the concentration of bacterial cells in aqueous solution. Kinetics of bioaccumulation of metal ions is biphasic with an initial rapid phase rendering 90% of metal uptake, which last for about 10 to 30 min and a gradual second phase of approximately 4 h [68].

Bioaccumulation is a heavy metal bioextraction process with numerous application prospects. The essential metal ions are accumulated rapidly than the non-essential ones, and this process could only be realized with live bacterial cells. Genetic engineering works are more focused in this aspect of bacterial process by redesigning the transporter proteins for enhanced passage of preferred metals. Proteomics and its manipulation of metal sequestering proteins are also underway. Evolving research could brighten up the possibilities of bacterial bioaccumulation in heavy metal extraction and recovery.

### 2.3. Bioprecipitation

Removal of metals from solution by means of precipitation is often coupled with the defence mechanism of bacteria. Cellular metabolism-independent precipitation is due to the chemical interaction of bacterial cell surface and metal ions [22]. Precipitation occurs by reduction, sulfide formation and as phosphates depending on the bacterial species and environment.

#### 2.3.1. Reduction

Reduction process occurs in bacterial species as mentioned earlier. Numerous bacterial strains catalyse the reduction of hazardous selenite [Se(IV)] and selenate [Se(VI)] ionic forms to elemental selenium, which gets deposited as red precipitate over bacterial colonies. Biofilm of *Desulfomicrobium norvegicum*, was shown to precipitate elemental selenium with sulfur [72]. A strain of *Alteromonas (Shewanella) putrefaciens* that normally reduce Mn(IV) and Fe(III), was also found to reduce U(VI),

**Table 3**  
Recent research reports on bacterial bioaccumulation of heavy metals.

Bacterial species	Heavy metals bioaccumulated	Mode of accumulation	Ref.
<i>Lactobacillus plantarum</i>	Ni, Cr	Reduction	[36]
<i>Bacillus megaterium</i>	Pb, Ni, Cd	Reduction	[69]
<i>Bacillus sp</i>	Pb	Polyphosphate chelation	[59]
<i>Pseudomonas stutzeri</i>	Cd	Sequestration, chelation	[41]
<i>Bacillus xiamenensis</i>	Pb	Sequestration, chelation	[40]
<i>Pseudomonas taiwanensis</i>	As, Cd	Sequestration, enzymatic reduction	[70]
<i>Cupriavidus necator</i>	Cu, Zn	Chelation, reduction	[71]

producing black precipitate of U(IV) carbonate [73]. *Shewanella oneidensis* MR-1 and two *Geobacter* species reduced Hg(II) to Hg(0) in the presence of electron acceptors [74]. The bacterial species involved in uranium reduction and the mechanisms involved has been thoroughly reviewed by You et al. [75].

#### 2.3.2. Metal precipitation as sulphides

Sulphate-reducing bacteria are heterotrophic, obligate anaerobes which oxidizes organic compounds or hydrogen for energy metabolism using sulphate as terminal electron acceptor. It reduces sulphate to sulfide which further combines with available metals in the cell and environment leading to the formation of metal sulphides (Fig. 4). Metal sulphides except alkali and alkaline-earth metals are insoluble and the resultant precipitation of sulphides protect sulphate-reducing bacteria from metal toxicity, and metals protect the organisms from sulfide toxicity, vice versa [19]. Sulphate-reducing bacteria also generate an extremely reducing condition which could bio-chemically reduce metals like U(VI) and Cr(VI) [76].

Enzymatic sulfide formation with the aid of thiosulfate reductase has been reported in *Salmonella typhimurium*. When thiosulfate reductase gene (*phsABC*) of *S. typhimurium* cloned and highly expressed in *E. coli*, metal-sulfide precipitation happened due to sulfide production from inorganic thiosulfate compounds [77]. *Klebsiella planticola* Cd-resistant strains cultured in thiosulfate supplemented media precipitated substantial quantities of cadmium sulfide (CdS) [78].

#### 2.3.3. Metal precipitation as phosphates

In this precipitation method, the enzyme phosphatase liberates inorganic phosphate (Pi) from cellular organic phosphate like glycerol-2-phosphate, and Pi in turn precipitates metals/ radionuclides as phosphates on the cell (Fig. 4). Immobilized cells of *Citrobacter sp* precipitated Cu, Cd, Pb, and U from glycerol-2-phosphate enriched solutions. Here, phosphatase catalysed glycerol-2-phosphate cleavage released hydrogen phosphates, which precipitated metals extracellularly as insoluble metal phosphates [17]. A mixture of hydrated zirconia (ZrO<sub>2</sub>) and Zr(HPO<sub>4</sub>)<sub>2</sub>, was obtained upon mineralization of zirconium by *Citrobacter sp*. [19].

Precipitation can be either metabolically dependent or independent. In metabolically active precipitation, reaction between the molecular compound(s) produced by the bacterial defence system and target metal (s) in medium results in crystallisation of metals. On the other hand, metabolism-independent precipitation is due to chemical interaction between the dissolved heavy metal ions and the reactive radicals on bacterial cell surface (Fig. 4). Metal crystallisation occurs in media/wastewater/effluent and on the peripheral region of bacterial cells during bioprecipitation, and is dependent on cell metabolism and metal concentration [22]. Recent reports on bioprecipitation of heavy metals by bacteria is comprehensively presented in Table 4.

Alive and dead bacterial cells do exhibit heavy metal precipitation; but dead biomass is less efficient in the process, as only passive interaction of bacterial surface ions and the metal ions in medium is the sole cause of precipitation in latter. The metal recovery is comparatively easy in case of precipitated metals and is suitable to treat mine effluents and electroplating industry sewage.

### 2.4. Bioleaching

Bioleaching is the solubilization of metals from its natural parent materials such as ore substrates or its crystals suspended or present in contact with the bacterial medium. It occurs directly as a part of bacterial metabolism or indirectly by bacterial metabolic by products. Bioleaching is more often a bio hydrometallurgical method used to recover metals like gold, copper, zinc, arsenic, antimony, cobalt, uranium, bismuth, nickel, molybdenum, lead and vanadium. Mostly, chemolithotrophic, mesophilic bacteria which require metabolic energy sources like ferrous sulphate, pyrite and sulfur are involved in metal

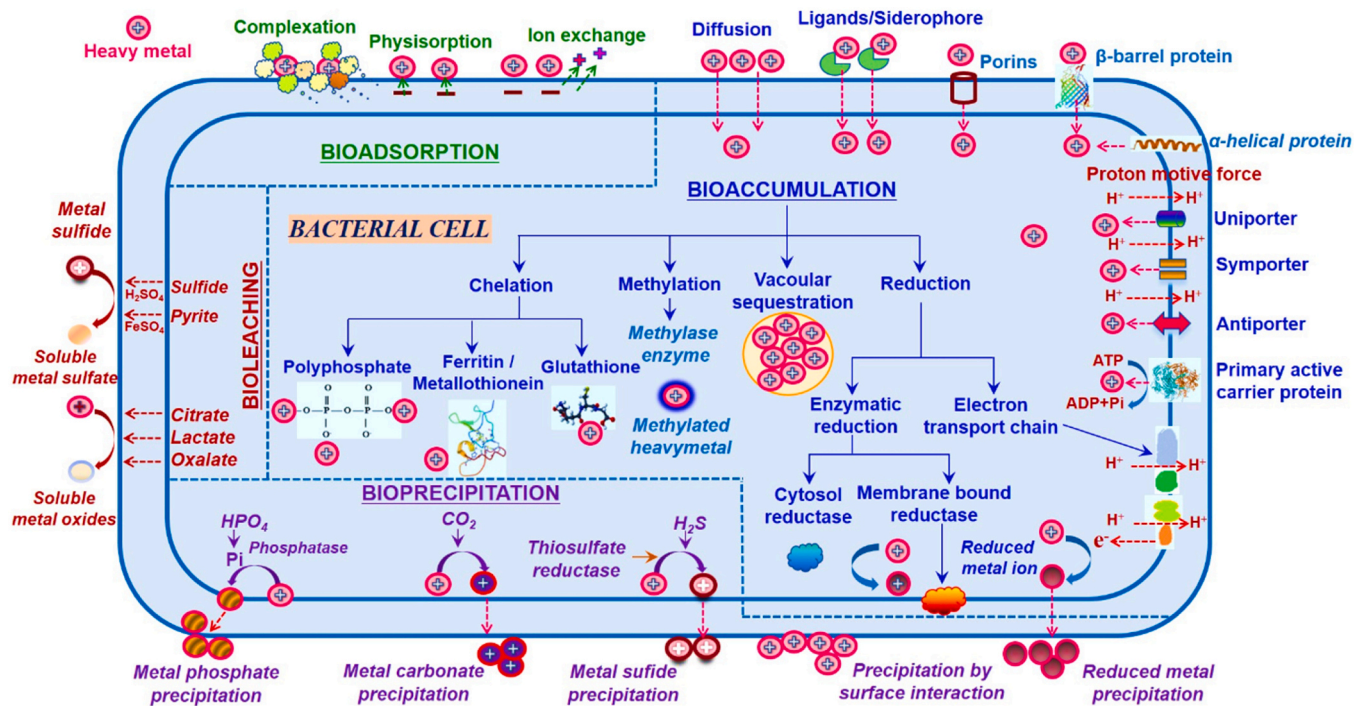


Fig. 4. Schematic representation bioremediation mechanisms exhibited by bacteria. Dotted line compartmentation is provided to differentiate each process separately.

**Table 4**  
Recent research reports on bacterial bioprecipitation of heavy metals.

Bacterial species	Heavy metals bio precipitated	Mode of precipitation	Ref.
<i>Sporosarcina sp</i>	Cu, Zn, Ni, Cd	Enzymatic reduction (Urease)	[79]
<i>Geobacter, Desulfovibrio sp</i>	Fe, Zn, Cu, Mn	Sulphide precipitation, reduction	[80]
<i>Sphingobium sp</i>	Ni	Sulphide precipitation	[81]
<i>Escherichia coli mutant strains</i>	Mn	Passive interaction on cell surface	[82]
<i>Sporosarcina pasteurii, Stenotrophomonas rhizophila, Variovorax boronicumulans, Stenotrophomonas sp</i>	Zn, Pb, Cd	Enzymatic reduction (Urease)	[83]
<i>Bacillus, Micrococcus</i>	Zn, U, Cu, Cr, Fe	Phosphate precipitation passive interaction on cell surface	[59]
<i>Bacillus atropharous, B.subtilis, B.aryabhatai, B. amyloliquefaciens, Proteus mirabilis</i>	Cd, Ni	Enzymatic reduction (Urease)	[85]
<i>Brochothrix thermosphacta, Vibrio alginolyticus, Staphylococcus epidermis</i>	Al	Passive interaction on cell surface	[86]
<i>Staphylococcus epidermis</i>	Cr, Pb	Enzymatic reduction (Urease)	[87]
<i>Desulfovibrio desulfuricans</i>	Zn	Sulphide precipitation	[88]
<i>Achromobacter xylosoxidans</i>	Pb	Phosphate precipitation	[89]
<i>Bacillus cereus</i>	U	Phosphate precipitation	[90]

leaching. Predominantly iron-oxidizing *Acidithiobacillus ferrooxidans* and sulfur-oxidizing bacteria *Acidithiobacillus thiooxidans* are used in bioleaching. Besides, *Acidophilium, Nirospira, Leptospirillum ferrooxidans, Ferroplasma,* sulfur-oxidizing bacteria-*Acidithiobacillus albertis* and

*Acidithiobacillus caldus,* are also used in mine sludge bioleaching. Thermophilic bacteria *Sulfobacillus thermosulfidooxidans* are used for faster bioleaching rate at higher temperatures. Several heterotrophic bacterial species like *Acidophilum, Arthrobacter, Acetobacter, Trichoderma* and *Pseudomonas* are capable of producing organic acids like citric acid, malic acid and oxalic acid. These acid moieties when released to the medium join with metals by supplying both protons and metal complexing anions, thus leading to metal leaching [91].

#### 2.4.1. Sulfide bioleaching

Direct and indirect mechanisms are involved in sulfur based leaching process by bacteria leading to the formation of metal sulfides and its dissolution (Fig. 4). In direct leaching process, bacteria directly contact and react with metal sulfide and oxidize it to soluble metal sulfates like NiS, ZnS, CuS, etc. On the contrary, in indirect bacterial leaching, the sulfur-oxidizing bacteria oxidize reduced sulfur compounds and elemental sulfur to sulfuric acid, thus reducing the pH of the persisting medium consequently augmenting the solubility of metals in solution [91]. Sulfate-reducing bacteria, *Sulfobacillus thermosulfidooxidans, Sulfobolus sp., S. acidophilus, Thiobacillus denitrificans,* and *T. thioparus* were successfully applied in the remediation of mine seepage water [92]. Successful removal of Fe, Cu, Zn, As and Ni sulphates from mine drainage using *Desulfovibrio desulfuricans* and *Desulfomicrobium baculum* strains, has been reported by Sahinkaya et al. [93].

#### 2.4.2. Pyrite leaching

In iron based bioleaching mechanism, reduced sulfur and iron compounds are oxidized through direct or indirect process. Non-iron metal sulphides are oxidized directly by iron-oxidizing bacteria like *Acidithiobacillus ferrooxidans* into soluble metal sulfate (Fig. 4). In direct leaching, bacterial cells adhere selectively to the surface structure of minerals, by chemotactic behavior. On the other hand, in indirect pathway, the bacteria oxidize Fe<sup>2+</sup> to Fe<sup>3+</sup>, and the Fe<sup>3+</sup> in turn react with metals and leaches through chemical reaction. The bacterial cells are not in need of direct contact with minerals during this process. The production of sulfuric acid as a by-product of this mechanism, further



enhances the solubilization process [91]. *Gallionella ferruginea* has been reported to oxidize iron in mine water drainage [94]. Bacteria such as, *Acidithiobacillus thiooxidans*, *At. ferrooxidans*, *At. cryptum*, *Acidiphilium caldus*, *Acidianus brierleyi*, *Ochrobactrum anthropic*, *Citrobacter*, *Cronobacter*, *Clostridium*, *Ferroplasma acidiphilum*, *Ferribacterium limneticum*, *Leptospirillum ferrooxidans*, *L. ferriphilum*, have demonstrated excellent ability in leaching metals from their corresponding ore waste exudates [95]. Latorre et al. [96] has found that *L. ferriphilum* and *At. thiooxidans* leach ores of copper sulfide, and metals like copper and arsenic with better efficiency, by oxidizing iron and thereby reducing inorganic sulfur compounds.

#### 2.4.3. Heterotrophic bacterial leaching

Heterotrophic bacteria, namely *Pseudomonas sp* have the potential to produce acidic metabolites that are capable to extract and solubilize metals from non-sulfidic minerals present in sewage sludge via acidolysis (the formation of organic or inorganic acids), complexation (excretion of complexing agents) and reduction (oxidation and reduction reactions). Currently, heterotrophic bioleaching is mostly applied in metal recovery of gold, silver, titanium, aluminium, nickel, copper, manganese, chromium and uranium from sewage [97]. Heterotrophic bacteria release organic acids, like lactic acid, citric acid, oxalic acid, gluconic acid and phenolic derivatives that have at least two hydrophilic reactive groups capable of dissolving heavy metals by displacing metal ions with hydrogen ions. This process is supplemented by the production of soluble metal chelates and complexes (Fig. 4). Role and interactions of heterotrophic bacterial strains of *Staphylococcus*, *Rhodococcus*, *Pseudomonas aeruginosa* PAO1 and *Cupriavidus metallidurans* with autotrophic bacteria during bioleaching of copper has been delineated by Jeremic et al. [98]. Heterotrophic bacteria, *Bacillus*, NS-1 has been found to increase metal leaching efficiency in electroplating sewage treatment [99].

The solubilised metals could be recovered or removed from solution by using ligands, electro winning, biosorption or solvent extraction. Different factors like temperature, pH, contact time, composition of medium, tolerance towards the metal, biomass concentration, types and quantity of metabolites produced and released by bacterial strains into the medium, affect the bacterial leaching mechanism [100]. Recent research reports on heavy metals leached by bacterial species are tabulated (Table.5).

Bioleaching is a bio-extraction technique to retract metals from its

**Table 5**  
Recent research reports on Bacterial bioleaching of heavy metals.

Bacterial species	Heavy metals bioleached	Mode of leaching	Ref.
<i>Pseudomonas putida</i> , <i>P. fluorescens</i> , <i>P. azotoformans</i>	Zn, Mn, Cu, Al	Heterotrophic leaching	[101]
<i>Leptospirillum sp</i> , <i>Acidithiobacillus sp</i> , <i>Acidithiomicrobium sp</i> , <i>Sulfobacillus sp</i>	Ni, Co, Zn, Cu	Sulphide and pyrite leaching	[102]
<i>Acidithiobacillus ferrooxidans</i>	Cu, Ni	Sulphide and pyrite leaching	[103]
<i>Acidithiobacillus ferrooxidans</i>	Fe, Cu, Pb, Zn	Pyrite and sulphide leaching	[104]
<i>Streptomyces albidoflavus</i>	Al, Cu, Cd, Fe, Ni, Zn, Ag, Pb,	Sulphide leaching	[105]
<i>Acidithiobacillus thiooxidans</i>	Ni, Mo, Al	Sulphide leaching	[106]
<i>Acidithiobacillus thiooxidans</i>	Co, Mn	Sulphide leaching	[107]
<i>Acidithiobacillus ferrooxidans</i>	Cu, Ni, Zn, Pb, Cr, Cd	Sulphide and Pyrite leaching	[108]
<i>Sulfobacillus acidophilus</i> , <i>S. thermosulfidooxidans</i> , <i>Acidithiobacillus caldus</i>	Mn, Zn, Ni, Cu, Cr	Sulphide leaching	[109]
<i>Leptospirillum ferriphilum</i> , <i>Sulfobacillus thermosulfidooxidans</i>	Cu	Sulphide and pyrite leaching	[110]
<i>Bacillus sp</i> , <i>Thiobacillus ferrooxidans</i> , <i>T. telloxidans</i>	Cd, Cu, Pb	Heterotrophic and Sulphide leaching	[99]

ores. The metals could be biologically dissolved and abstracted. Though the process is gradual, it is eco-friendly, sustainable technique with potential to retrieve precious metals.

Bacteria has turned to be a potent bioremediating agent of heavy metal contaminants from effluents. Bacterial biosorption, accumulation, precipitation and leaching of heavy metals, are inexpensive methods to remediate or stabilize heavy metal pollutants from contaminated water. The boon and bane of each mechanism is enlisted in Table 6. The whole process of bioremediation mechanisms is collectively represented in Fig. 4. Appropriate understanding of cellular mechanism is essential for the accurate selection of strains, for urban and industrial wastewater treatment, which is the most challenging task of degradation and retrieval of heavy metals from e-waste processing by-product exudates. Bacterial heavy metal remediation mechanisms are easy to understand when it refers to a single metal through single pathway or process; but it becomes complicated in the presence of multiple ions, generally encountered in effluents. Standardization of physicochemical parameters that imparts maximum remediation is highly essential for better results. Furthermore, molecular level studies are required to discover the exact and apparent cellular mechanisms exhibited by bacteria to eliminate toxic heavy metals at different conditions and environments. Researchers are exploring novel bacterial species with great heavy metal removal potential and subsequent genetic engineering works are also in

**Table 6**  
Advantages and disadvantages of bacterial heavy metal bioremediation mechanisms.

Mechanism	Advantages	Disadvantages
Bioadsorption	<ul style="list-style-type: none"> <li>Both live and dead bacterial biomass perform adsorption</li> <li>Could be carried out exclusively using bacterial exopolymers</li> <li>Comparatively simple and fast process</li> <li>Common heterotrophic and aerobic bacteria could be used for the purpose</li> </ul>	<ul style="list-style-type: none"> <li>More efficient in alkaline pH</li> <li>Emulsion and colloids in waste water interfere adsorption process</li> <li>Oxidation state of metals influence adsorption efficiency</li> </ul>
Bioaccumulation	<ul style="list-style-type: none"> <li>Accumulation accomplished within 4 to 5 h</li> <li>It is a bio extraction process without huge sludge production</li> <li>Genetic engineering could modulate the process and preference of metals to be accumulated</li> </ul>	<ul style="list-style-type: none"> <li>Only possible with alive bacterial cells</li> <li>Dependent on concentration gradient of metals on either side of bacterial cell membrane</li> <li>Optimum physio-chemical parameters and nutrient availability essential for efficient accumulation</li> <li>Essential metal ions are accumulated faster than non-essential metals</li> <li>Cells are disrupted for metal recovery, hence cannot be reused</li> </ul>
Bioprecipitation	<ul style="list-style-type: none"> <li>Both alive and dead cells exhibit precipitation process</li> <li>Genetic manipulation feasible to enhance the process</li> <li>Metals could be crystallized in either water or bacterial cell surface</li> <li>Metals could be easily recovered even by filtration</li> </ul>	<ul style="list-style-type: none"> <li>Precipitates as sulfides, phosphates carbonates or oxides of metals</li> <li>Multimetal contamination hinder the process</li> </ul>
Bioleaching	<ul style="list-style-type: none"> <li>Retrieve metals from its ores</li> <li>Mostly employed for recovery of precious metals (gold, silver titanium, uranium, etc.,) from mine effluent</li> </ul>	<ul style="list-style-type: none"> <li>Iron and sulfur bacteria are proficient and efficient for leaching</li> <li>Gradual process, mostly occurring in low pH</li> </ul>

progress. At the same time various bioremediation strategies and protocols has been designed and devised, relying on the inimitable decontamination prospects of bacteria. These techniques have proved application potential in almost all industrial sectors like tannery, textile, electroplating, printing, and municipal sewage, where wastewater treatment is pivotal.

### 3. Applications of bacterial bioremediation in wastewater treatment

Several bacterial based processes have been developed, implemented and practised for heavy metal remediation in wastewater treatment, which includes both ex-situ and in-situ approaches. The ex-situ techniques crafted and materialized are microbial bioreactors, microbial fuel cell or microbial electrolysis cell and microbial desalination units (Table 7). While, constructed floating wetlands and bioaugmentation are the in-situ bacterial based heavy metal remediation systems (Table 8). Research have evolved from proof-of-concept study, to pilot and full-scale approaches and these remediation protocols and practises have recognized immense application in domestic sewage, landfill leachate, industrial effluent treatment and polluted natural water resource (lakes, ground water, reservoir, etc.) decontamination.

Bioreactors are specially engineered devices that provide optimum conditions to foster the growth and biochemical activity of specific

**Table 7**

Bacterial based ex-situ techniques developed and practiced for heavy metal remediation.

Technology and bacterial species involved	Heavy metals removed with efficiency in percentage (%)	Ref.
Bioreactors		
<i>Desulfovibrio sp</i>	Cu-96.4, Cd-92, Zn-79.8, Fe-71, Pb-61.5, Ni-47.5,	[111]
<i>Desulfovibrio sp</i>	Cu-98.5, Zn-96.3, Fe-95.2, Mn-93.8	[112]
<i>Pseudomonas aeruginosa</i>	Cu-100, Cd-100, Zn-100, Fe-62, Pb-47	[113]
<i>Syntrophobacter, Methanoseta, Geobacter, Anaerolinea, Longilinea</i>	Cu-99.3, Zn-99.4, Fe-99.9	[114]
<i>Acidothiobacillus ferroxidans</i>	Cu-100, Zn-100, Fe-85, Ni-90, As-95, Co-75, Cr-100	[115]
<i>Kosmotogal, Ruminococcus, Clostridium</i>	Ni-99	[116]
<i>Proteobacteria, Cyanobacteria, Bacteroidetes, Firmicutes, Acidobacteria, Chlorobium, Acinobacteria, Spirochaetes Nitrospirae, Armatimonadetes</i>	Cu-99, Cd-99.7	[117]
<i>Desulfovibrio halophilus</i>	Fe-85.3	[118]
<i>Comamonas, Pseudomonas, Desulfomicrobium, Burkholderia, Halomonas</i>	Hg-88.9	[119]
<i>Ferrovum, Delftia, Acinetobacter, Metallibacterium, Acidibacter, Acidiphilium</i>	Fe-93.7	[120]
<i>Pseudogulbenkiania</i>	Zn-83, Fe-50	[121]
<i>Desulfovibrionaceae</i>	Zn-95, Fe-95, Pb-95, Mn-80	[122]
Microbial fuel (electrolysis) cell		
<i>Desulfovibrio</i>	Cu-98	[123]
<i>Pseudomonas, Geobacter</i>	Cu-87.7, Hg-97.3, Ag-98.5	[124]
<i>Pseudomonas</i>	U-90	[125]
<i>Enterococcus avium</i>	Cu-89.2, Fe-77, Cd-57.5, Pb-97.1, Ni-98.1, Cr-12.4, Tl-91	[126]
<i>Bacillus, Klebsiella, Enterobacter</i>	Cd-88, Pb-90.14, Cr-90.34	[127]
<i>Corynebacterium vitaeruminis</i>	Cr-98.63	[128]
<i>Ochrobactrum, Halomonas, Achromobacter</i>	Cd-87, Ni-92	[129]
<i>Serratia marcescens</i>	Cr-100	[130]
<i>Castellaniella</i>	Cu-99.89, Cd-99.91, Cr-99.59	[131]
Microbial desalination cell		
<i>Acaligenes aquatilis</i>	Cu-91.8, Ni-92.2, Mg-68.5	[132]
<i>Desulfomicrobium, Aquamicrobium, Paracoccus, Stappia, Alcaligenes, Rhodobacterales</i>	Zn, Ca, Mg-99.85(Collective removal)	[133]

**Table 8**

Bacterial based in-situ techniques developed and practiced for heavy metal remediation.

Technology and bacterial species involved	Heavy metals removed with efficiency in percentage (%)	Ref.
Constructed (floating) wetlands		
<i>Bacillus cereus, Aeromonas salmonicida, Pseudomonas gessardii</i>	Fe-72.5, Pb-40.9, Ni-70.3, Cr-77.7, Mn-83.5	[134]
<i>Acinetobacter junii, Rhodococcus sp, Pseudomonas indoloxydans</i>	Fe-90, Cd-60, Cr-90, Ni-80	[136]
<i>Serratia, Pseudomonas</i>	Cd-99.6, Zn-94.41	[137]
<i>Bacillus endophyticus, Bacillus pumilus, Microbacterium arborescens, Pantonea sp</i>	Fe-89, Cd-72, Cr-97, Ni-88	[138]
<i>Acinetobacter junii, Rhodococcus sp, Pseudomonas indoloxydans</i>	Cu-77.5, Zn-89.7, Fe-81.0, Pb-73.3, Ni-86.9, Mn-70	[139]
<i>Aeromonas salmonicida, Pseudomonas indoloxydans, Bacillus cereus, Pseudomonas gessardii, Rhodococcus sp</i>	Fe-85.7, Pb-91.6, Cr-98.1, Ni-75.3, Mn-85.3	[140]
<i>Pseudomonas aeruginosa, Onchrobactrum sp, Enterobacter</i>	Cr-88	[141]
<i>Bacillus cereus, Paenibacillus alvei, Aeromonas caviae, Paenibacillus taiwanensis, Achromobacter spanius</i>	Cu-95, Pb-93.4	[142]
<i>Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Nitrospirae, Spirochaetes, Cyanobacteria</i>	Cu-97.6, Zn-80.1, Cd-74, Ni-69.8, Co-67.1	[143]
<i>Acetanaerobium, Exiguobacterium</i>	Cu-99	[144]
Bioaugmentation		
<i>Desulfovibrio desulfuricans</i>	Zn-100	[145]
<i>Bacillus, Enterobacter aerogenes, Bacillus pumilus</i>	Cr-96	[146]
<i>Pseudomonas sagittaria</i>	Mn-95	[147]
<i>Aeromonas hydrophila</i>	Cr-93.71	[148]
<i>Klebsiella pneumoniae, Enterobacter cloacae</i>	Cu-81, Zn-77, Fe-39, Cd-19, Cr-51, Pb-50, Ni-73, Mn-53	[149]

bacterial species for desired remediation purpose. Polluted water samples are fed to the reactor for the removal and retrieval of contaminants. The reactor design and bacterial strain selection depends on the purpose and polluted source, which can be slurry phase, packed bed, fluidised partitioned, suspended carrier, airlift, up-flow anaerobic stage reactor, continuous flow, sequence batch, stirred tank, biofilter based and membrane bioreactors [111–113]. This can be installed at industrial and open leachate sites and its operational procedures and treatment duration varies with the input wastewater parameters and the desired product quality [111].

Microbial fuel cells employ microbes especially bacteria to generate electrons from metabolic reactions. These electrons generated are used for the concomitant reduction/oxidation of heavy metal contaminants, thereby its precipitation at anode chamber; and/or for electron transport to cathode. The electrons reaching cathode combines with the available oxygen; and protons are released and transferred back. A continuous flow of these entities produce energy that could be used for varied purposes [128]. Microbial desalination cell is the integrated process of electro dialysis using microbial fuel cell for the effective treatment of wastewater with simultaneous desalination, electricity generation and metal recovery. Mixed or pure culture of exoelectrogenic bacteria is used for this procedure. *Shewanella putrefaciens, Proteobacteria, Actinobacteria, Pseudomonas, Bacillus subtilis* are the major bacterial strains used in microbial desalination cells [132]. Ship spillage was found to be effectively treated using this technique. The ex-situ bacterial bioremediation techniques and its heavy metal removal efficiency are tabulated in Table 7.

Constructed wetland or floating treatment wetland is a novel phyto-microbial technique for the treatment of water bodies to mitigate pollution. These artificial wetlands consist of floating mat, over which vegetative plants are grown and its roots hang in the water column. Bacterial biofilms are attached to these hanging roots and desired endophytic bacteria are also inoculated to the plants [134,135]. These bacterial colonies aid heavy metal adsorption and water

decontamination. This procedure is implemented in several regions and sites, and fruitful results were achieved. Bioaugmentation in wastewater decontamination is the addition of selective bacteria (single strain or consortia) to the polluted water bodies. This is done according to the prevailing physiochemical conditions of polluted source, and the heavy metal to be removed. The bacterial strains used and the removal efficiency of in-situ remediation methods is compiled in [Table 8](#).

#### 4. Challenges to meet

Selection of most suitable bacterial strain according to its remediation potential, and process relied is instrumental for the design, development and implementation of bioremediation techniques [150,151]. Genetic engineering has enhanced the efficiency of the technology; but, treatment of huge volumes of wastewater is still a challenge. Though genetic engineering efforts have been practiced for over two decades, its industrial translation faces several impediments [152]. The genetic stability of recombinant species is always in speculation, and the incorporation of unnatural amino acids in designed proteins seldom confer unexpected functions, turning the venture risky in distant future [153]. The competency of genetically modified bacteria to thrive in real wastewater effluents and to remediate metals, like that of native wild species is yet to be demonstrated. The capacity of genetically modified species to resist competitive exclusion has to be revealed. Substantial bacterial load of treated water is another challenge to be addressed. Thus, inception to abate the same is a perpetual need to scale up the technology to successfully meet the technoeconomic, environmental risks and assessments. These are the unexplored avenues of research. To advance and promote bacterial based heavy metal remediation techniques, bioprocess level development is recommended. Protooperation of bacteria with algae has demonstrated better heavy metal remediation in waste water [154]; and more reliable research is essential in this sphere. Research could be routed towards metal recovery as major destination with remediation as the direction to attain the same. A strong understanding of the mechanism inevitably aids to modulate the research in right path. Implementing faster operation procedure and metagenomic manipulation of bacterial remediation process would facilitate to achieve more recognition and popularity to these eco-friendly techniques.

#### 5. Future prospects

Despite several proven research works; bacterial heavy metal remediation technique is mostly confined to laboratory. The major challenge and pitfall of this technology is the lack of large-scale production and commercialization. Development of integrated technologies is imperative to scale up the efficiency and meet the sustainable development goals. Progressive and exploratory research in this regard is essential with in-depth investigations on molecular level remediating mechanisms, modelling of site-specific and adaptive bacterial strains with regeneration capacity. More research works are to be directed towards customising bacterial remediation for bulk quantities of water with faster rate of action, by suitably manipulating the inherent capacity of bacterial strains to remediate heavy metals. Economic feasibility of the bacterial based bioremediation techniques has to be addressed in future research. The multiple benefits of bacterial aided heavy metal remediation have to be popularized, at the same time bacterial kits are to be commercialized; as better availability will obviously promote the prevalence of usage.

#### 6. Conclusions

Bacterium is one of the most versatile microbes with immense bioremediation potential that has to be widely explored and implemented. Bacteria rely on any one of the aforementioned mechanisms or a combination of different mechanisms based on the heavy metals

present, prevailing physiochemical factors and the strains involved.

- Bioremediation of heavy metals by bacteria is an eco-friendly, economically feasible and sustainable process and could also act as a treatment process that enhances the efficacy of industrial wastewater treatment.
- Selection and utilization of proper bacterial strains that exhibit tolerance to heavy metals with exceptional remediation properties and adaption skills to the environment are critical for successful bioremediation.
- Research should be further channelled to increase the remediation rate thus to decrease the treatment time concurrently achieving high efficiency. Technical interventions are necessary to elevate the 'bacterial remediation platform' with engineering support. The threats of biofouling and competitive exclusion by the nascent bacteria and microbes in the effluents are to be resolved.
- Procedures to recover metals from bacteria has to be standardised, simplified and effectuated as non-destructive, for the possible reuse of cells.
- Bacterial strains or consortiums proficient to remediate each metal and its different ions are to be well differentiated; at the same time, strains that could act in multi-metal environment have to be recognized and commercialized.

Scientific outputs have to be channelled to industrial sector and have to be publicized, as this technology has proven potential to decontaminate polluted natural water bodies. Expansion and implementation of both ex-situ and in-situ techniques is imperative in different sectors, at the same time, research has to be undertaken to establish its potential in more arenas.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the works reported in this paper.

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