



Review on Microbial Mechanism of Heavy Metal Bioremediation: A Global Concern

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ABSTRACT

We all contribute to environmental degradation every day, whether intentionally or not. The pharmaceutical and industrial sectors create a wide variety of medications and other materials, but they may also release toxic heavy metals into the environment. Heavy metals from many sources can contaminate agricultural soil, harming our crops and even altering the metabolic processes of animals and plants. It has been my understanding from my research that heavy metal contamination can be eradicated by an ion exchange or electrochemical process. As a result, a biological approach may prove to be an efficient substitute for standard methods of treating heavy metals pollution. It has been discovered that many different types of bacteria, fungus, and algae, from a wide range of taxonomic families, all contribute to the detoxification of these insoluble substances through their enzymes. Although the heavy metal-tolerant strains discussed in the current study may survive and even thrive in the presence of these chemicals, it has been shown that their interactions with them may lead to physiological or metabolic changes in the bacteria.

Keywords: Environment, Heavy metal toxicity, Biofilter, Bio absorption, Industry effluents.

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INTRODUCTION

That the environment, public health, and pollution are all interconnected is a view that we share wholeheartedly¹. Effluents from factories are often untreated and dumped into the environment, where they can pollute the land and the water. Most heavy metals are toxic and carcinogenic^{2,3} thus they constitute a serious risk to aquatic life and the human population in the watersheds they enter. Cadmium (Cd), mercury (Hg), zinc (Zn), and chromium are all examples of extremely poisonous heavy metals (Cr). Similarly, even at low concentrations, lead (Pb), iron (Fe), arsenic (As), and nickel (Ni) can be harmful. Since many factories lack adequate wastewater treatment systems, their waste water simply flows into nearby bodies of water. Toxic metals can penetrate bacterium cells and bind to their outer membranes. Heavy metals may undergo chemical changes within the bacteria when the microbe uses chemical processes to breakdown food². Effluents containing heavy metals pose a threat to the metabolisms of the millions of individuals who drink them, increasing their risk of diseases including hypertension, skin cancer, stroke, chronic renal disease, and anemia⁴.

Physicochemical and biological treatments are both effective in reducing the harmful effects of industrial

waste, including the presence of heavy metals. The efficacy of modern biofiltration methods, such as genetic engineering, in the removal of toxic metals². Heavy metal involving microorganisms can be bioremediated by techniques such as bio-accumulation, biosorption, chemical extraction, polymer micro-encapsulation, leaching, hydrolysis, bioprecipitation, and absorption by isolated biopolymers from microbial cells^{2,1}. For lead and cadmium analysis, atomic absorption spectrometry has been used⁵. Arsenic polluted water⁶ can be treated by any of the methods indicated in Table 1 for getting rid of heavy metals, including coagulation-precipitation, adsorption, ion exchange, and membrane filtering.

Table 1: Heavy metals and their removal techniques followed

Heavy metals	Techniques of Removal	Reference
1. Cadmium (Cd)	Atomic absorption spectrometry	6
2. Mercury (Hg)	Reverse osmosis, ion exchange	7
3. Lead(Pb)	Atomic absorption spectrometry	6
4. chromium (Cr)	Biosorption	8
5. Arsenic (As)	Conventional purification method	9
6. Zinc (Zn)	Biosorption	10
7. Nickel (Ni)	Adsorption process	11

Bioremediation and biofiltration, on the other hand, have emerged as effective replacement methods for cleaning up polluted water and soil¹. Several microorganisms can form



a thin coating on the surface of filter media, which is referred to as biofilm. Biomass in a biofilter is maintained by microbial activity, which includes algae, fungus, aerobic bacteria, anaerobic bacteria, and facultative bacteria. Degradation of Cd, Hg, Pb, and Ni is aided by *Rhodospirillum* species of bacteria². Brown algae are necessary for Cr breakdown², while *Desulfovibrio* species (bacteria) eliminate Zn toxicity. Acid-producing lactobacilli (LAB) are responsible for detoxifying As from toxic byproducts of industry⁹.

Bioremediation of heavy metals :

Of course, human-made factories aren't the only places where heavy metals may be found; the lithosphere and other terrestrial ecosystems can be as rich in the stuff. Volcanoes are a significant contributor to environmental heavy metal levels, and xenobiotic chemicals have been found in abundance.

Heavy metals and their short breakdown mechanisms during bioremediation are listed in Table 2.

Cleansing polluted areas with microorganisms including bacteria, yeast, and fungus is called bioremediation. Promoting the growth of certain microflora or microbial consortiums that are adapted to contaminated environments and capable of performing necessary tasks is central to this strategy.¹²

- This technique makes use of active microorganisms.
- Reduce the toxicity of environmental pollutants by degrading them.
- The microbes might have originated at the polluted site or been brought in from elsewhere.
- Depending on the circumstances, it may be in- or out-of-place.

Heavy metal	Contaminates	Degrading microorganism	Aerobic	Anaerobic	Mechanism	Organization	Guidelines	Effect	Reference
Cadmium (Cd)	1.Drinking water	<i>Pseudomonas aeruginosa</i>	-	+	Producing new proteins and RNA/DNA from scratch. Chemical mutagenesis	1.EPA	There are no long-term health risks associated with this level of exposure in drinking water. Problems with bottled water	Loss of nerve development, decreased IQ, impairment of renal function, and respiratory issues are all part of the cardiovascular and pulmonary difficulties that youngsters face.	13,14,15,16,17,18,19
	2.Bottled water	<i>Pseudomonas fluorescens</i>	+	+		2.FDA			
		<i>Enterobacter agglomerans</i>	+	-					
Mercury (Hg)	1.Drinking water	<i>Pseudomonas aeruginosa</i>	-	+	proteins covalent bond formation as well as antioxidant depletion.	1.WHO	Recommendations for the purity of drinking water in the presence of all organic mercury forms. Legal maximum levels of methylmercury	Toxins that transcend the blood-brain barrier and have an effect on the digestive system and the kidneys can cause damage, but they are not, by themselves, carcinogenic.	13,20,21,14,22,19
	2.Seafood	<i>B. Revundimonas sp</i>	+	-		2.FDA			
Lead (Pb)	1.Drinking water	<i>B. lactis</i>	-	+	Interrupts DNA synthesis and repair, calcium metabolism, and tumour suppressor protein synthesis and cycling by mimicking calcium.	1.EPA	hydration action level. Limitations	Effect on reproductive system, CNS, Leads to anemia, probably carcinogenic.	13,23,24,25,26,27,28,19
	2.Packaging water	<i>Pseudomonas marginalis</i>	+	-		2.FDA			
		<i>T. longibrachiatum</i>	+	+					

Chromium (Cr)	1. Drinking water 2. packaging water	<i>Acinetobacter haemolyticus</i> <i>Staphylococcus simulans</i>	+ -	- +	Chromosomal abnormalities and DNA strand breakage are brought on by Cr(VI).	1. WHO 2. FDA	Drinking-water quality standards for total chromium are 0.05 mg/L. Not to rise above the maximum amount of chromium	Most toxic from of Cr (vi) its affect on respiratory tract, cardiovascular , renal, may causes asthma. allergies, carcinogenic.	[13,25,29,30,31,19]
Arsenic(As)	1. WORKPLACE WATER 2. DRINKING WATER	<i>Pseudomonas putida</i> <i>Desulfo microbium spp</i> <i>Shewanella spp</i>	+ + -	- + +	Enzymatic biomethylation of inorganic compounds may be carcinogenic; As(III) may render about 200 enzymes inactive; As(V) may replace phosphate; hinder the DNA repair process; stop cellular respiration	1. OSHA 2. WHO/ EPA	In locations that use inorganic arsenic, the airborne arsenic limit is eight hours. Drinking water limitation.	Increases level of diabetes, neuro behavioral disorders .	[13,32,33,34,35,36,37,19]
Zinc (Zn)	Drinking water	<i>Morganella morganii</i> <i>Stenotrophomonas maltophilia</i>	+ +	- -	Exogenous zinc is typically not taken up by cells due to cellular regulatory mechanisms, whereas endogenous zinc is important in cytotoxic events in individual cells. Here, zinc affects apoptosis by interfering with a number of the caspases and proteins from the Bcl and Bax families that regulate programmed cell death.	1. WHO	Drinking water limitation	Zinc is usually harmless. Toxic consequences only occur with large amounts of exposure. Dermal or skin problems, neuron disorders, not carcinogenic	[13,3810,39,40,19,41,42]
Nickel (Ni)	Drinking water	<i>Azotobacter vinelandii</i> <i>Aspergillus flavus</i>	+ +	- -	The kidneys and lungs are the major organs that are targeted. outcomes in humans from inhalation or absorption through the digestive system. Nickel's toxicity is primarily mediated through the reduction of glutathione levels and interactions with protein sulfhydryl groups.	1. WHO	Present in 0.02 mg/L obey quality guild line	lung damage that is permanent, aberrant pulmonary functions, renal tubular necrosis, anemia, eosinophilia, and ulceration of the nasal septum. Additionally, skin absorption or ingestion of nickel are both possible.	[13,43,44,39,42,45 ,19]

Cadmium (Cd):

Cadmium may be found in the II-B group of the periodic table ¹. The molecular weight of silver is 112.41, and its atomic number is 48 ⁴⁶. Bone loss, kidney failure, and cancer are among side consequences of their toxic nature ⁶. Cadmium may be removed from pharmaceutical waste water by certain types of bacteria, fungus, and algae. The results showed that when Cd concentrations rose from 20 to 60 ppm, different types of fungus began to absorb it. Fungi such as *A. awamori*, *P. chrysosporium*, *A. niger*, and *mucor rouxi*³ and bacteria may break down cadmium (*Rhodospirillum* species³, *Lactobacillus rhamnosus*, *Lactobacillus casei*). Uptake increases to 40 ppm in *B. cereus*. When it comes to Cd absorption from liquid media, *T. fasciculatum* (18.59 mg/g) is superior than *A. terreus* (5.41 mg/g) ³. Fungi belonging to the genus Deuteromycetes, such as *T. viride*, have chitin and glucan polymers encoded in their cell walls, which aid in Cd binding ³. *Pseudomonas aeruginosa* is widely distributed in polluted environments, including Cd- and Hg-contaminated sediments and water. Studies have identified *P. aeruginosa* as cadmium-resistant bacterium due to its ability to withstand the buildup of the hazardous metal, despite having its cell wall plasma membrane complex altered and its DNA methylated. Cd can enter bacterial cells via Mn²⁺ gene amplification, active Cd efflux, and increased transcription of metallothionein genes, all processes involved in the absorption of divalent cations. Among Gram-negative bacteria, Cd resistance is best understood in the context of detoxification systems that are RND-driven, such as Czc, which primarily exports

zinc. Cd resistance was shown in the gram-negative bacteria that carried the plasmid containing the *czc* gene cluster. The *czr* homologous gene cluster (*czc*) was found on the chromosome of *P. aeruginosa* strain CMG103. The chromosomal resistance marker *czr* is present in *P. aeruginosa* strain CMG103. Sequencing the amplified fragment confirmed the presence of the cadmium-resistant gene on the chromosome, proving that the gene was really present on the chromosome by amplification using chromosomal DNA (*czc*). The cadmium-resistant gene in EP-Cd1 is called efflux pump. *P. aeruginosa* was shown to be highly successful in removing 94.7 percent of pollutants when isolated from active sludge, as reported in the author's study¹⁵. According to a study, *P. agglomerans* JCM1 and *Enterobacter aburaage* JCM 6051 may be able to remove heavy metals from polluted effluents before they are released into a river. Five stages make up the bacterial resistance mechanism to heavy metals: (1) the production of compounds that can bind and detoxify metals within the cell; (2) the excretion of metals via secretion transport systems; (3) the absorption of metal by exploiting the structure of the cell envelope; (4) the release of cadmium chelators into the extracellular; and (5) the successful completion of these processes via intra- and extracellular pathways ¹⁶. The sample was spun at 6000 rpm for 10 minutes, and then the heavy metal concentration was analyzed using atomic absorption spectroscopy. The efficiency of the elimination process. Between all, a maximum removal rate of 50 ppm (or 90%) was found to be feasible ⁴⁷.

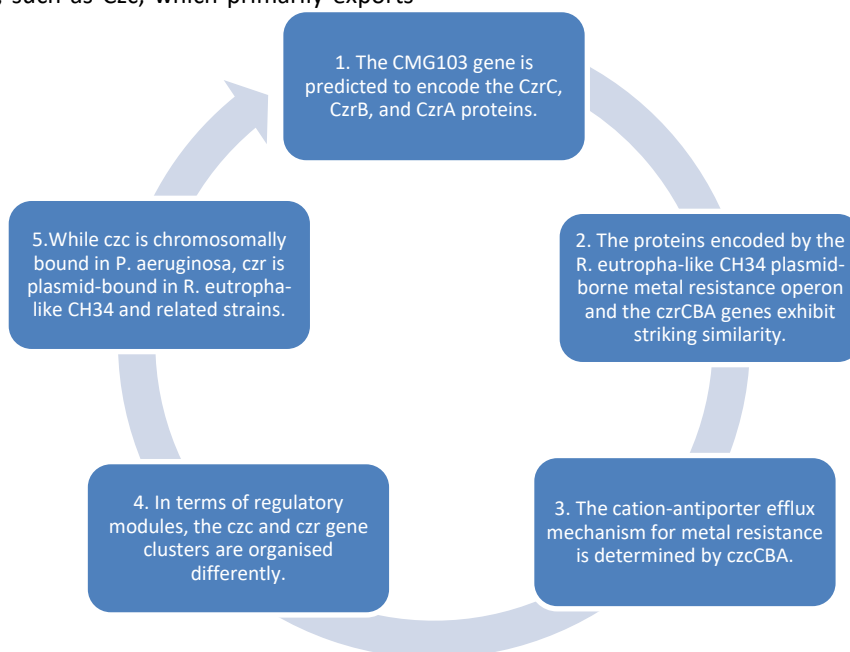


Figure 1: Mechanisms of cadmium bioremediation by microorganisms.

Mercury (Hg):

Mercury exists in biological and inorganic forms in the hydrosphere, biosphere, and lithosphere. Organomercury is formed when Hg binds to the methyl group. Microorganisms are responsible for the transformation of

organomercury into inorganic Hg ⁴⁸. *Pseudomonas aeruginosa*, a Gram-negative bacterium, has a slimy polysaccharide layer composed of glucose, glucuronic acid, and acetyl groups. The purpose of this research is to evaluate the efficacy of Gram-positive bacteria that produce capsules and slime layers in the process of

removing mercury from contaminated liquids and to characterise some physicochemical aspects of mercury biosorption by local isolates of *K. pneumoniae ssp. pneumoniae* and *P. aeruginosa*²⁰. Seven mercury-resistant *Pseudomonas* bacteria were immobilised from pure cultures in the confines of a bioreactor bed. Seven mercury-resistant *Pseudomonas* strains were grown in pure culture on carrier material in a 700 L packed bed bioreactor. Neutralized chloralkaline electrolysis wastewater (0.7 m³/h-1.2 m³/h) containing 3-10 mg/L of mercury was continuously fed into the bioreactor. There was a 97% efficiency in mercury retention within 10 hours of inoculating the bioreactor. At maximum capacity, the mercury content in the bioreactor's outflow was below 50 g/L, meeting the limit for mercury release from industrial wastewater⁴⁹. It has been reported that *B. revundimonas* sp flourishes in HgCl₂, making it a useful tool for purifying water. Heavy metals can't break down the defences that these microorganisms have developed. These processes have the potential to be utilised in the elimination of environmental contamination and the removal of heavy metals. Cells from this bacterium, which produces metallothionein and polyphosphate kinase, aggregated, precipitated, and darkened in colour when cultivated in high mercury concentrations. Metallothionein suggested that transgenic bacteria's high mercury tolerance and accumulation might be responsible for these effects. Bacteria that are resistant to mercury are essential for the detoxification process, which involves two sequentially functioning enzymes, notably organomercurial lyase, which cleaves the carbon-mercury bonds of certain organomercurials, and mercuric reductase, which reduces to the volatile mercury²¹.

From what I've seen in my prior research, the mercury-reducing microbial biofilm in the technical scale bioreactor was subjected to a number of stressful conditions, including high mercury and salt concentrations, high flow rates, and an average temperature of 42 °C. At 40 °C, much below *Pseudomonas*' ideal growth temperature of 30 °C, only a few of our inoculant strains grew poorly (data not shown). Given that the whole mercury retention efficacy was obtained on the day of inoculation, we may conclude that the microbial community there was so active that almost full mercury detoxification was performed even in the less-than-ideal circumstances witnessed in the pilot plant⁵⁰. Shake-flask cultures were analysed to see how a cloned strain of *E. coli* K-12 with a high tolerance to Hg²⁺ grew and eliminated the heavy metal. In these analyses, increasing the Hg²⁺ concentration appears to extend the lag phase without significantly affecting the growth rate. Later studies employing batch cultures revealed that relatively high concentrations of Hg²⁺ would not kill the resistant bacteria, but showed that a significant reduction in the Hg²⁺ content in the medium is necessary before growth is observable. Due to the wide range of Hg²⁺ concentrations seen in industrial waste water, this is a very significant finding⁵¹.

Lead (Pb):

Battery acid contains lead (Pb), a metal often used in the production of automobiles.

Lead has a Boiling point of 174°C/318°F and a melting point of 327°C/621°F (48). The toxicity of pb can be neutralised by a variety of microorganisms, including the bacterium *Thauera selenatis*, the fungus *Aspergillus Niger*, the brown algae and the green algae⁽²⁾. Lead was removed at a lower rate by *Rhamnosus* GG and *L. fermentum* ME3 strains (up to 97 percent). Research done in the past has shown that lead's specific binding increases with rising initial metal concentration. The maximum binding value of lead was measured to be between 32.3 and 175.7 mg/g. At low metal concentrations, binding efficiency by boiling *B.Lactis* Bb12 decreased, while at higher concentrations, it rose erratically⁶. Even low levels of lead were enough to disrupt the enzyme activities of bacteria (23). Lead also damaged protein and nucleic. Lead enters cells via Fe²⁺ and Ca²⁺ transporters, where it binds to metalloproteins and is then excreted as a poison. *Bacillus megaterium*, a Gram+ve bacterium, causes a build-up of internal cytoplasm, while *Pseudomonas marginalis* seems to live outside the cell. The second component of the operon, designated PbrB, was found to be a phosphatase that enhanced lead resistance. In *Cupriavidus metallidurans* CH34, a P-type ATPase is responsible for the cytoplasmic removal of Pb²⁺ ions, while a phosphatase creates inorganic phosphate for lead sequestration in the periplasm. Gene clusters including neighbouring P-type ATPase and phosphatase genes were revealed after searching databases and a wide variety of different bacterial species, which indicates that Pb²⁺ detoxification via active efflux and sequestration may be a common resistance mechanism²⁴. Observing the concentration rise from 20 ppm to 60 ppm as a result of bacterial activity, we find that *T.Longibrachiatum* (with the greatest concentration)>*P.Chrysosporim*>*A.Terreus* absorb Pb. Previous studies found that the bacterium *B. cereus* had the largest absorption of Pb. This occurred because of the bacterium's poor affinity for metal ions, which is caused by electrostatic contact. Based on XRD analysis performed after Pb²⁺ removal, we know that the bacterial suspension converts Pb²⁺ to Pb₅(PO₄)₃OH; in the case of intact cells, Pb²⁺ is changed into Pb(Mgw)S₃O₃, and the metabolites convert to Pb₉(PO₆). Based on the results of the XRD examination, it was determined that the bacteria had broken down phosphorus into phosphate as part of their metabolic process, discharging the resulting phosphate into the fluid ²⁵.

Chromium (Cr):

Bioconcentrated plant and aquatic animal species rely on chromium (Cr) for survival. The element can be eliminated from the food chain at low concentrations, but not at greater ones. These bacteria are able to withstand chromium exposure: *Bacillus* sp ⁵², *Agrobacterium species*⁵³, *Staphylococcus simulans* ²⁹, and *Lysinibacillus macroides*⁵⁴. Intercellular bioaccumulation, periplasmic biosorption, and direct and indirect enzymatic metabolism have all been



linked to Cr (vi) reduction⁵² *Acinetobacter haemolyticus* has been employed on an industrial scale for analytical tolerance and reduction capacity, according to a recent study from World Microbial⁵² We focus on the process of altering genetic material and changing the metabolic response of bacteria as the primary means by which toxicity can be mitigated. Due to bacterial chromium (vi) tolerance reduction due to oxygen diffusion in the replica plate approach. WM's in-depth research has revealed that the surface activity of metal binding involves amino, hydroxyl group, and carboxyl group these functional groups. The typical peaks of COOH-groups were found to be alternating in chromium-treated cyanobacteria⁵². Microorganisms may tolerate harsh environmental conditions because they remove oxyanions both directly and indirectly. Adsorbing microorganisms used for DNA methylation and updating convert chromium (iv) to chromium (iii) enzymatically. Chromate-tolerant bacteria catalyse electron transfer from NAD(p)H electron donors to chromate (cr) (vi) and (iii) reductases (chr R, Nema, nfs A, and yieF)⁵³. Analysis of the 16S rRNA sequence from blast was performed using the parameters primer 518 F (CCAGCAGCCGCGTAATACG) and 800F (TACCQGGGTATCTAATCC) to zero in on the taxonomic identification of Archie and eubacteria, as shown in research⁵³. Bacterial genomic DNA is isolated by PCR in the phenol-chloroform-ethanol-isoamyl procedure. The author decided to amplify his or her fragments by length, therefore they employed the universal primer 27F (5'AGAGTTTGATCMTGGCTCAG -3')⁵⁴. All of these sequences are produced in a Malaysian lab and subsequently uploaded to the NCBI⁵⁵.

Arsenic (As):

Arsenite (As5+) and arsenate are the hazardous oxidation forms of arsenic that damage the environment. Arsenate is more toxic than arsenite; it interferes with cellular phosphorylation and might have long-lasting effects on RNA and DNA synthesis⁴⁸. Several studies show that *Acidithiobacillus ferrooxidans*,⁵⁵ *Alcaligenes faecalis* (Bacterial strain)², *Bacillus cereus*⁵⁶, and *Pseudomonas* species⁵⁷ may remove arsenic from water. In order to purify water from arsenic, bio methylation and biosorption are two common methods. Recently, the chromosome of *Pseudomonas putida* KT244'0S was changed to allow for the arsenite As (iii) s- adenosylmethionine methyltransferase (ArsM) gene, which might be used for bioremediation of environmental arsenic. The structures of the As (iii) S-adenosylmethionine methyltransferases were determined by X-ray crystallography. The basic framework was outlined. at the 72nd, 174th, and 224nd places of the centimetre Crystal structure of the *cyanidioschyrion* sp. ArsM ortholog, resolving the connection between the arsenic and s adenosylmethionine binding sites to an eventual resolution of 1.6 Å. Binding of AS (iii) has a little effect on the conformation. Helix 4 and a loop (residues 49-80) are involved in SAM binding, and they both point in the direction of the As(iii) binding domain, where the methyl group can be transferred³² Thanks to the ars c gene Plasmid DNA in bacteria is responsible for the reduction of aspartate from

aspartate+5⁵⁸. Examining the Sher 2020 In As 6, the first bacterial strain to be submitted for cultivation by the Bank of Pakistan. Shifts in peaks in the FTIR spectrum of bacteria treated with 15 mM arsenite. In order to absorb As3+, bacteria use a wide variety of functional groups, such as hydroxy groups, peptide and protein carboxylic acids, and others. As seen in the results, 93% of the metal contamination was eliminated⁵⁸. One of these organisms, *Desulfomicrobium* Ben - RB, acted as an As(v) terminal electron acceptor. The second SRB, *Desulfovibrio* Ben-RA, used an as resistance technique to reduce AS(v). Though it lacked the ability to carry out dissimilatory As(v) reduction, *Desulfovibrio* Ben - RA was still able to promote As 2S3 precipitation. All cultures of *Desulfomicrobium* um Ben-RB and *D. auric* pigment were grown in conditions with a pH near to neutral³³ *Haemophilus*, *Micrococcus*, and *Bacillus* were all proposed as possible sources for the bacteria that caused AOB-1, AOB-5, and AOB-6, respectively. Researchers believe these bacteria types are mostly responsible for oxidising As (iii). *Brevudimonas diminuta*⁵⁹ produces siderophore and is involved in IAA and ACC deaminase. action, and phosphate solubilization, too. when grown in As-containing soil (v). Bacterial strains introduced by rhizoneculation decreased As(v) accumulation in aerial proteins, particularly those found in edible parts of the plant, and promoted increased plant development⁶⁰. This work built a microbial-mediated reactive transport model to investigate the microbial reduction of As(V) by *Shewanella* sp. and their subsequent precipitation without accounting for the oxidation of Fe(II) or As (III). described the use of reactive transport modelling to predict the outcome of an abiotic Fe(II) oxidation and subsurface As removal system. To the best of our knowledge, no previous studies have taken into consideration the synchronised effects of Fe(II) and As(III) oxidation on microbial response kinetics. Since the chemical/biological oxidation of Fe(II) significantly affects the fate and transport of As, comprehensive reactive transport modelling can properly predict the biochemical reaction to concurrent Fe(II) and As(III) oxidation by IOB⁴⁴

Zinc(Zn):

Zinc ranks as the twenty-third most common element in the planet's crust(38) Zinc has an atomic mass of 65,3 and an atomic number of 30. (Element 11-B) a metal with a bluish white colour⁴⁸. The bacterium *Morganella morganii* has been identified as the source of zinc tolerance, as reported on pages 869-876 of the aforementioned pdf. The 16S rRNA sequence for the *Morganella morganii* ACZ05 strain may be found in the GenBank under the accession number NJ8 307541, as determined by phylogenetic analysis. To "emphasise the phylogenetic connections," alignments of *M. morganii* Azo5 16s RNA were employed. The bootstrap confidence interval had 1,000 replicates. Growth suppression at high concentrations of heavy metals is confirmed by a strain sensitive to heavy metals, whereas strains resistant to heavy metals exhibit no such inhibition. Based on this speculation, *M. morganii* ACZ05 was shown to be a competent isolate that demonstrates zinc resistance



³⁸. *Serratia* sp., a kind of zinc-resistant bacterium (ZRB). The ZTB strain generates gluconic acid, which is used as a chelating agent for metals. The ZTB was genetically profiled using amplification and sequencing of 16s r DNA. With the help of the universal primers 27F (5'AGAGTTTGATCMTGGCTCAG-3') and 1492R, (3'TACGGYTACCTGTACGACTT '5), the 16s r DNA was successfully amplified. Gel extraction kit from sigma was used to further purify the PCR product, and then an automated DNA sequencer was used to sequence the product right away (genetic Analyzer, Applied Biosystems)⁶¹. The 16S rRNA gene amplification product was isolated and digested using Alu I and Rsa restriction enzymes. Since the ARDRA method was employed to find a pattern of limitations, but none of the candidates demonstrated 100 percent vigilance, sequencing of the 16rRNA gene is required for definitively identifying the bacterial strain ⁴¹. Microbial fuel cells (MFCs) have been the subject of recent studies because of their potential to be utilised for the recycling or purification of heavy metals found in soil and wastewater ⁵⁸. *Stenotrophomonas maltophilia* is a bacterium that lives in the feed unit cell, and its strain xzNA has Zn tolerance. We anticipate increased use of microorganisms, leading to a concomitant decrease in microbial biomass. the amount of Zn²⁺ taken by bacteria decreases with increasing usage times, indicating a positive link between the two variables. With each subsequent usage, the bacteria's ability to eradicate decreased, and the microbial population decreased, indicating that three replications was the limit. By the fifth round of repetition, however, the bacteria have become resistant to the removal process and the microbial amount is almost zero¹⁰. To purge the *Botrytis cinerea* (*B. cinerea*) biomass of Zn²⁺ ions, a dilute HCl solution was applied over time. Recent research into desorption and reusability has revealed that the bio sorbent may be recycled five times through biosorption-desorption cycles without losing any of its absorbing capacity^{62,63}

Nickel: Nickel, antimony, and arsenic are the white metals. Nickel often takes on the +2(Ni²⁺) oxidation state⁶⁴. Nickel-resistant bacteria include *Escherichia coli*, *P. chrysogenum*^{3,4}, and *Scenedesmus* genus algae^{3,4}. According to study 46, researchers evaluated the effects of different carbon sources on the biological elimination of Ni (ii) using a number of different bacterial strains. If the phosphate-biodegrading *Azotobacter vinelandii* isolate is given glucose as a carbon source, it grows rapidly and effectively. Using glucose as a carbon source favoured bacterial growth for enzyme and organic acid production. Significant differences in bacterial growth were observed across different carbon sources⁴³. An academic research compared the efficiency of two bacterial strains, *S.pasteurii* 586s and *B.megaterium* 1295s, in bio-removal of Ni²⁺ ions from sewage sludge. The results showed that the former was less effective than the latter. *S. pasteurii* 586S shows no significant changes between live and dead cells, which may be related to characteristics of the bacterial cell wall⁶⁵, but *B. megaterium* 1295S shows a large variation in removal

efficiency between living and dead cells. DNA sequencing has shown 45,16s RNA in nickel-isolated strains (SNI-1), (SNI-2), (SNI-3), and (CNNI-1). In a bidirectional DNA sequencing process, the 8F and 1492R primers were used to sequence PCR amplicons with the BDT.⁶⁶ The majority of fungal species demonstrate reduced removal and absorption of the nitrate -nitrogen source because *Neurospora crassa* and *Aspergillus flavus* are unable to utilise the restricted nitrogen supply while harbouring the nitrate reductase enzyme. The chemicals produced by different fungal strains facilitate the accumulation of metals outside of cells, or the accumulation might occur after a chemical reaction between the metal and the cell surface and be physiologically unrelated. It is possible to think of the exceptional activities that take place on the surface of cell walls and load the metal ions onto the fungal strains as a result of cell surface sorption⁶⁷. Arupite deposition on pre-existing precipitation foci may be facilitated by the metal resistance mechanism of *R. metallidurans* CH34, which involves a chemiosmotic efflux system of metal ions and leads to high exocellular pH ⁴⁴.

DISCUSSION

Based on what has been said above, it is clear that further research is required to improve the ability of microorganisms to remove heavy metal ions from wastewater. Bioremediation strategies utilize microorganisms to decrease, remove, contain, or modify toxins in soil, sediments, water, and air³. The ability of living cells to bioaccumulate is affected by factors such as their age, physiological condition, and development stage²¹. Heavy metal bioremediation makes use of several microorganisms. Strains of *Rhodospirillum species* and *Mucor rouxi* are able to reduce cadmium's toxicity. *Pseudomonas sp.*, *Aspergillus sp.*, *Staphylococcus sp.*, *Bacillus sp.*, *Serratia sp.*, and *Azotobacter sp.* have all been shown to reduce the toxicity of mercury (Hg), lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), and nickel (Ni) in laboratory tests. Based on a comparison of the sorption uptake represented by the *k_e* parameter from the Freundlich model, the following rank was established: Pb(ii)>Cu(ii)>Zn(ii)>Ni(ii)>Cd(ii)⁶⁸. This process's primary objective is to develop a system that integrates biodegradation and nanotechnology to achieve the aforementioned goals of enhanced biocatalyst activity and stability, as well as the much-desired recycling feature, as reported for wastewater bioremediation by photosynthetic bacteria⁶⁹. The crystallinity of both new and previously used nanoparticles was assessed through XRD analysis⁷⁰. Increased metal absorption is achieved thanks to the peptidoglycan and polysaccharide component of the bio sorbents' cell wall. The advantages of this technology include low cost and little impact on the environment, as well as rapid kinetics, high metal binding throughout a broad pH and temperature range, and so on²³. According to reports, at a concentration of 60 ppm, *B. cereus* was able to remove the most Pb (16.66 mg/g), Cd (49.95 mg/g), and Ni (2.51 mg/g) from the water than any other bacterium. The problem is that *P. aeruginosa* is one of the ideal bacteria for



cadmium bioremediation, yet its use and study constitute a classic case of dual debt in a scientific context. It's the leading cause of nosocomial infections and a major opportunistic pathogen⁴⁵. Since mercury and its derivatives have such a strong affinity for the thiol groups in proteins, they pose a serious threat to living organisms. Toxic HgCl₂ stops bacterial growth and can kill them if it gets into their system. If *Brevundimonas sp.* HgP1 and HgP2 could thrive in the presence of extremely concentrated HgCl₂²¹, it might be useful in the waste water treatment process. During the metabolic process, phosphate was biomineralized from organic phosphorus by bacteria. More study is needed to comprehend the mechanisms behind bio-mineralization and the roles played by microorganisms in the mineralization process²⁵. Cr-resistance and Cr-reduction ability in *A. haemolyticus* (VI), Cr(VI) reductase activity was shown to be associated with the soluble cell fraction found within the cell itself⁵². *Desulfomicrobium's* use of

autotrophic metabolism successfully reduces metal contamination in As(III) oxidizers may be preferable since they do not necessitate the use of organic carbon sources. The only microorganisms shown to exhibit this metabolism to date are those isolated from extreme environments³³. Several growth-promoting characteristics were also present in these *Serratia sp.* strains. In addition, ZTB strains generate gluconic acid, which is used as a natural chelating agent for heavy metals and contributes to the formation of metal complexes, as shown by experimental evidence. Findings suggest that ZTB can be used as microbial inoculants to bioremediate heavy metals at damaged industrial sites and to boost agricultural output in Zn-contaminated soil⁶¹. How beneficial a bacteria's biomass is for biotechnology depends on its bio-removal capability. Since *B. megaterium* 1295S kills cells more efficiently than *S. pasteurii* 586S, it is the superior strain⁶⁵.

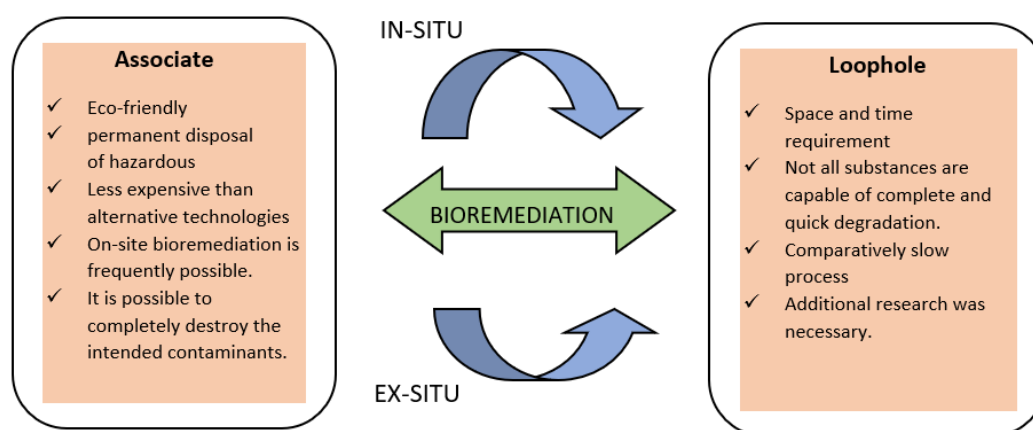


Figure 2: Microbial Bioremediation has 2 major process for the removal of heavy metal contaminants. Figure shows various associates and some loopholes with this process and other techniques.^{52,71}

FUTURE ASPECTS

In light of the biotechnological potential of microbes in metal removal and/or recovery, this research found that heavy metal absorption by microorganisms processes have seen a dramatic increase in attention in recent years. Researchers have identified a number of challenges that prevent the broad use of this technique, including the lack of an easily accessible and inexpensive biomass and the negative effects of coexisting metal ions on absorptive capacity.

- Potentially game-changing novel antibiotic-resistant organisms have also been discovered.
- Microbial fuel cells (MFC) are employed in the bioremediation of heavy metals in the environment.
- Bioremediation mediated by biofilms is an area that needs more research.

CONCLUSION

Heavy metals are harmful to human health because they disrupt the function of essential biological components. Chromium (Cr), lead (Pb), cadmium (Cd), mercury (Hg), zinc (Zn), and nickel (Ni), among others, are all metals and metalloids that can be found in the environment. Using a microbial mix for heavy metal removal may prove to be the best solution after optimization procedures are stabilized. This technique, which can effectively remove heavy metals to ppb levels and is less costly, is recommended for use in the treatment of wastewater from companies such as those producing chemicals and textiles, pulp and paper, dyes and pigments, medicines, fertilizer, etc. This research suggests that aerobic bacteria are the most effective in breaking down heavy metals. Biological approaches can be used to safely and sustainably manage heavy metals by capitalizing on the resilience of isolated microorganisms from polluted environments.

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