



CLIMATE
SURVIVAL
SOLUTIONS

MICROBIAL DEGRADATION OF HEAVY METALS

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Abstract:

Environmental contamination due to anthropogenic activities has led to intrinsic environmental degradation globally. Environmental degradation has substantially deteriorated the quality of natural resources, such as air, water and soil, to an extent that these natural resources could no longer support life. Strikingly, high levels of environmental degradation due to anthropogenic activities have pushed the earth towards the sixth mass extinction. The earth has reached its tipping point with no hope of return. The loss of biodiversity due to overpopulation, habitat destruction and fragmentation, and over-consumption of natural resources remains a global concern. The increasing global food crisis and climate crisis are the by-products of environmental degradation. Owing to the onset of several severe conditions due to environmental contamination, it becomes the need of the hour to detoxify the environment, as even cutting down the use of fossil fuels now, would hardly have any beneficial impact on the environment. The prime pollutant, with extremely high environmental degradation capabilities, occurs to be the “*Heavy metals*”. These metals enter the environment through various sources and remain persistent in the environment with little to no degradability. Several physical and chemical techniques have been explored to eliminate heavy metal contamination from the environment, however, the feasibility of such methods remains quite meagre. This paper discusses the utilization of microbes for degrading the heavy metal contamination from wastewater, soils and biosolids (obtained from the digesters). The microbes occur to utilize several different biochemical pathways to bioremediate the heavy metals from the environment and attain resistance to them.

Keywords: *Bioremediation, Microbial Degradation, Environmental Contamination, Heavy Metals Contamination, Wastewater, Biosolids.*

Introduction

The ever-increasing urbanization and industrialization globally form the major determinants of pollution. Pollution refers to the introduction of a contaminant into nature, which leads not only to adverse alteration of the environment but also its degradation. Various anthropogenic activities conducted for utilizing environmental services selfishly have resulted in the pollution of air, water and soil. Polluted natural resources fail to support life and lose their potential for various purposes. For example, contaminated soil, drops its ability to sustain plantation and drives towards desertification. The contaminants (the pollution-causing agents), readily interact with nature, undergoes physical and chemical alteration and get integrated into the environment. The environment thus serves as an accumulator of waste.

Historically, the waste generated followed an equilibrium with their natural degradation in the environment, resulting in negligible or zero pollution of the environment. However, in the post-industrialization era, several xenobiotics ended up in an environment that was foreign to the natural ecosystem and were subjected to the least degradation.

Environmental pollution has a greater cruciality not only in terms of ethical concerns for future generations but also because environmental degradation poses a serious threat to human wellbeing. It leads to disorderly national economies, triggering political instabilities and fueling violent conflicts by

aggravating inequalities in resource scarcities regions. Most industrial contaminants and human activities press the environment to deal with excessive and ridiculously high levels of contaminants, leading to unanticipated environmental risks and irreversible impacts.

The contaminants don't remain restricted at the site of pollution but could demonstrate their ill impacts, miles away from the polluted sites. This literally means that the most prolific polluters could harm the health of the people not involved in creating much pollution, residing in places far from the pollution origin site. For example, as per research published in one of the elite science journals, the air pollutants such as ozone, particulate matter, nitrogen dioxide and sulfur dioxide arising from China readily make their way to the west coast of the United States. Likewise, owing to westerly winds, many of the air pollutants find their way across the Pacific to the West Coast. The most potent air pollutants – sulfur dioxide and nitrogen oxides could effortlessly spread throughout the globe, (irrespective of their site of origin), rising into the sky and mixing with clouds. These clouds pass across the borders and lead to acid rain.

Additionally, the bioaccumulation of contaminants/toxins in the foods – plants, meat and seafood, and the exportation of these contaminated food items causes health havoc within the importing nations.

As the oceans connect the world, a slight variation (from the normal) in the physiochemical characteristics of the oceanic water could be experienced at greater distances.

Environmental degradation apparently occurs to be the major determinant of a healthy society, both in terms of its functioning and firm framework.

Hence, the control of the release of contaminants in the environment becomes a global cumulative effort, as it is the root cause of several global issues and concerns.

Various conservational techniques (eco-friendly and green approaches) currently have been adopted worldwide, to reduce environmental contamination. However, these approaches and techniques fail to restore the already degraded nature. The degradation of the recalcitrant components in nature becomes crucial to cease further pollution as well as revert nature back to homeostasis. This crucial step of nourishing nature back to the healthy stage remains the monopoly of the microbes – one of the most important entities of nature.

This paper discusses the untiring role of microbes in the degradation of the most persistent contaminants of nature making the process of recycling natural resources more sustainable.

Causes of Environmental Contamination

The environment and living entities on the earth share intrinsic and inseparable links. The environment has a self-repair mechanism to revert to its natural form. However, this repair has its restriction and requires a stipulated time to perform the troubleshooting process. Humans, in the meanwhile, generate millions of tons of contaminants, which overpowers the self-replenishing mechanism of the environment. Failure of these mechanisms leads to the climate crisis, extreme weather events, food and water crisis, health issues, loss of wildlife and crops and economical downfall.

The constant struggle of improving their lifestyle has pushed humans to an extent where they themselves have paved their way to extinction. Humans have ached their destructive and environmentally damaging

activities, touching the global tipping points, with no point of return. The working of the earth stands immensely influenced by human activities. The movement of rocks, soil, and sediments by humans globally, overpowers the cumulative action of all the natural processes.



Figure 1. Major Causes of Environmental Contamination (illustration by author after [1]).

Impacts of Environmental Contamination

The constant struggle of improving their lifestyle has pushed humans to an extent where they themselves have paved their way to extinction. Humans have ached their destructive and environmentally damaging activities, touching the global tipping points, with no point of return. The working of the earth stands immensely influenced by human activities. The movement of rocks, soil and sediments by humans globally, overpowers the cumulative action of all the natural processes.

The industrial revolution of the 19th century led to a whole new consumption pace of fossil fuels in the mechanized production of goods. The excessive and unexercised utilization of fossil fuels constitutes the major factor for environmental contamination. Environmental contaminants include the substances present in the environment above the permissible limits of concentration, and impacts negatively the health of humans, plants and animals [2]. These contaminants could be either in solid, liquid or gaseous form, which are generated by human activities, for short-term benefits, having the potentiality of creating long-term nuisance to ecology resulting in environmental degradation. Such extensive environmental degradation due to contamination, not just impacts the abiotic factors of the ecosystem but has a more commanding influence on the biotic factors of the ecosystem. Figure 2. illustrates in detail the impacts of environmental degradation in varied sectors.

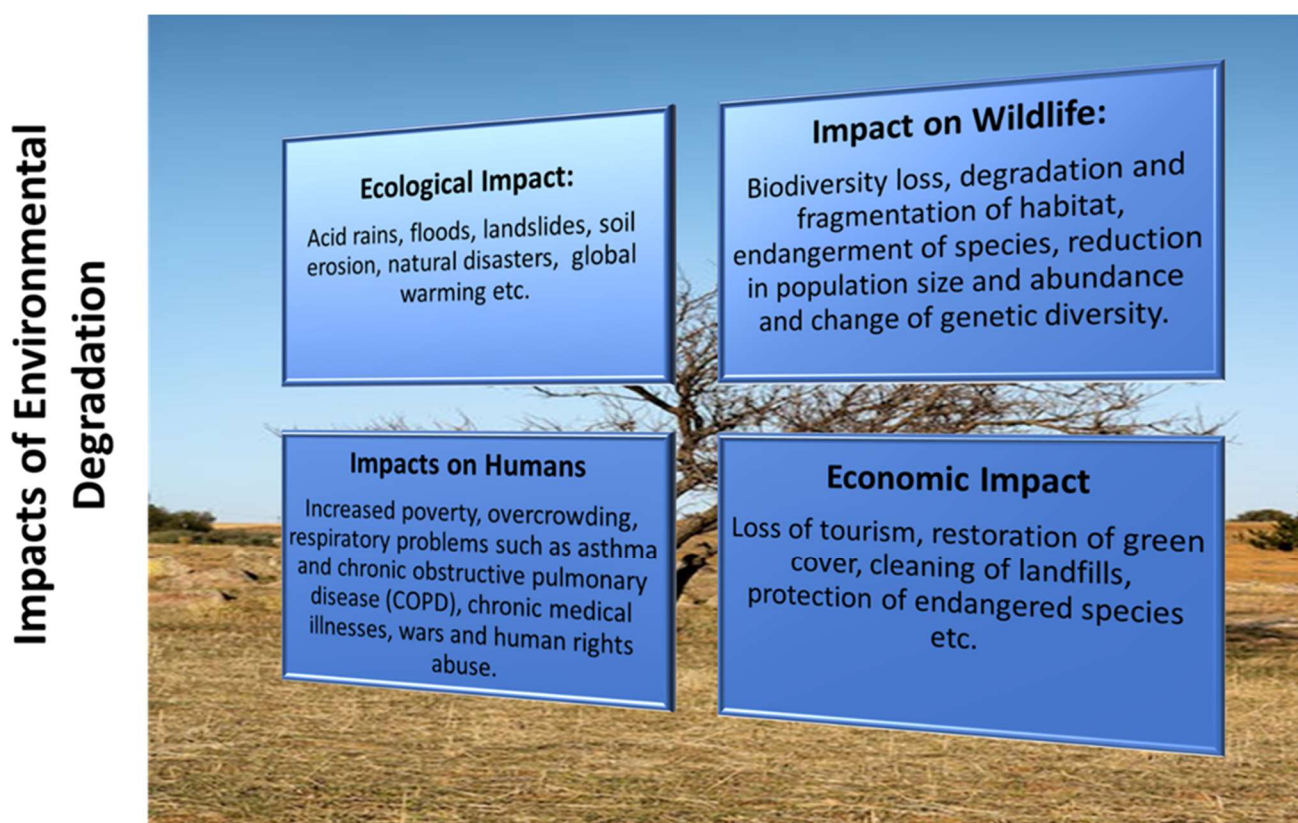


Figure 1 Impacts of Environmental Degradation in varied sectors (Image designed by author after [3], [4])

Heavy Metal Contamination

Out of the several environmental contaminants, heavy metals (type of contaminants) released in the environment forms the major global issue. Heavy metals comprise higher atomic numbers with a density at least 5 times higher than that of water [5]. Heavy metals remain persistently in the environment and undergo bio-accumulation within the food chains, ultimately possessing a risk to human health and the ecosystem [6]. Heavy metal causes several chronic disorders owing to their high concentration in the environment due to extensive industrialization and anthropogenic acts. Due to the higher toxicity of

arsenic, cadmium, chromium, lead, and mercury, they hold a prime spot among metals that causes severe public health consequence [5].

The heavy metals have profusely contaminated the air, water and soil. Several sources of heavy metal contamination include mining, industries (chemical industries, refineries, petrochemical plants), untreated sewage sludge, metal piping, and combustion of by-products from the coal-burning power station.

Human exposure to heavy metals leads to several disorders including kidney dysfunction, nervous system disorder, skin lesions, vascular damage, immune system dysfunction, birth defects and cancers [7]. Heavy metals frequently interact with biological systems by losing either electron(s) or forming metal cations having affinity to the nucleophilic sites of crucial macromolecules [7]. Several acute and chronic toxic impacts of heavy metals affect different organs of the body. Exposure to a higher dose of heavy metals, predominantly mercury and lead, may trigger various health issues such as abdominal colic pain, bloody diarrhoea and kidney failure [8], [9]. On the contrary exposure to lower doses of heavy metal may prove to be a hidden threat and elusive, unless repeated regularly, which could be diagnosed by the manifestation of fatigue, anxiety, and reduction in IQ and cognitive development in children [10].

These heavy metals have emerged as potent carcinogens, with the most probable mechanism of altering the genome and gene expression. The metals occur to disrupt DNA synthesis and its repair. Likewise, these metallic elements adversely impact cellular components and organelles such as cell membrane, mitochondria, lysosomes, endoplasmic reticulum, nuclei and several enzymes playing a noteworthy role in metabolism, detoxification and damage repair [5]. The toxicity of these heavy metals usually occurs due to the reactive oxygen species (ROS) generation, enzyme inactivation, and suppression of the antioxidant defence.

Table 1, below, depicts the sources of exposure of five most commonly found heavy metals in the environment and their mechanism of toxicity.

Sr. No.	Type of heavy metal	Sources	Mechanisms of toxicity
1.	Arsenic (As)	<ul style="list-style-type: none">• Industries.• Drinking water gets contaminated with As through pesticides and unexercised disposal of arsenical chemicals in the water.	<ul style="list-style-type: none">• Affects the sulphhydryl group of cells resulting in malfunctioning of cell respiration, cell enzymes and mitosis [11].• As inhibits the pyruvate dehydrogenase, blocking the Krebs cycle and restricting oxidative phosphorylation. This results into decrease ATP production and causes cell damage [12].• As damages, the capillary endothelium by increasing

			vascular permeability, resulting in vasodilation and circulatory breakdown [13].
2.	Lead (Pb)	<ul style="list-style-type: none"> • Industrial processes. • Food and smoking. • Contaminated drinking water and domestic sources. • Storage batteries. • Plumbing pipes, faucets and fixtures. • Exhaust from automobiles. 	<ul style="list-style-type: none"> • Pb leads to oxidative stress in a living cell by disturbing the balance between the generation of free radicals and antioxidants (components responsible for cellular repair). Pb increases the levels of free radicals while decreasing the levels of antioxidants in the cell. • Higher levels of free radicals account for damage of cells, proteins, nucleic acids, lipids and membrane [14].
3.	Mercury (Hg)	<ul style="list-style-type: none"> • Agriculture, municipal wastewater discharges, mining, incineration, and discharges of industrial wastewater [15]. 	<ul style="list-style-type: none"> • Hg damages the tertiary and quaternary protein structure. • Hg alters cellular functioning by attaching to selenohydryl and sulphhydryl groups which reacts with methyl mercury and disrupts the cellular framework [16]. • Hg hinders the translation and transcription process, adversely impacting the number of ribosomes, endoplasmic reticulum and activity of natural killer cells [16].
4.	Cadmium (Cd)	<ul style="list-style-type: none"> • Incineration of municipal waste, usage of phosphate fertilizers, sewage sludge and certain industries such as plastic industries, NiCd batteries 	<ul style="list-style-type: none"> • Cd possesses the capability of binding with cysteine, glutamate, histidine and aspartate ligands leading to iron deficiency [16]. • Cd effectively replaces zinc present in metallothionein,

		manufacturing industries, plating industries etc.	thereby, inhibiting it from serving as a free radical scavenger within the cell.
5.	Chromium (Cr)	<ul style="list-style-type: none"> Burning of oil and coal, excessive usage of fertilizers, oil well drilling, sewage, paper and pulp industries and metal plating tanneries. 	<ul style="list-style-type: none"> The reaction between hexavalent Cr and the biological reductants such as thiols and ascorbate results in the generation of reactive oxygen species such as superoxide ion, hydrogen peroxide, and hydroxyl radical causing oxidative stress in the cell with DNA and protein damage [17].

Table 1. *Sources of five most found heavy metals in the environment and their mechanism of toxicity.*

The list mentioned in the table 1. is indicative of the types of heavy metals found in the environment due to contamination. Other heavy metals which make their way into the environment include antimony, arsenic, bismuth, cerium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium, and zinc [18].

Impact of Heavy Metals on Soil and Plants

Heavy metal contamination of soil generally occurs by several metals, particularly Cu, Ni, Cd, Zn, Cr, and Pb [19]. Heavy metal contamination drastically alters the soil's properties in terms of organic matter, clay content, porosity, water-holding capacity, mineral composition and pH, which largely affects the soil's physical and chemical properties [20]. Likewise, heavy metal adversely impacts the enzymatic activities of the soil by shifting the microbial communities responsible for the production of the required enzymes [21]. The contaminated soil has the potential of reducing the biomass and diversity of normal microbial flora of the soil subsequently impacting the soil respiration rate and nutrient cycling.

Different metals elicit varied effects on the enzymes. For example, Cd is more toxic to enzymes than Pb owing to its greater mobility and lower affinity for soil colloids [22]. Likewise, Pb significantly decreases the activities of urease, catalase, invertase and acid phosphatase. Cd illustrates a negative effect on the activities of urease, protease, alkaline phosphatase and arylsulfatase [22].

The heavy metal contamination in the soil directly impacts the plants' growth. Some of these impacts include a decrease in the seed germination and lipid content, inhibition of photosynthesis, reduction in the seed germination, diminution of chlorophyll production and plants' growth [23]. Similarly, high levels of toxic heavy metals in the soil lead to the prevention of nutrient uptake and physiological & metabolic processes, resulting in chlorosis, damaging the root tips, minimizing the uptake of water and impairing several enzymes. Higher accumulation of heavy metals in the plants leads to disorganization of the grana structure and reduces the root and shoot growth [24]. Under heavy metal stress conditions, several plants

are reported to undergo necrosis and programmed cell death [24]. Generation ROS remains the chief culprit of toxicity caused to plants by heavy metals.

The heavy metal accumulation in soil not only causes functional disorders in soil but even harms human health through contamination of the food chain. The heavy metals could readily get bioaccumulated in the living systems, wherein, their concentration steeply increases as they pass from one organism to another, i.e., from lower trophic levels to higher.

Certain heavy metals such as zinc (Zn), nickel (Ni), manganese (Mn) and iron (Fe) are classified as essential heavy metals and are required by plants for several physiological and biochemical processes, while heavy metals such as lead (Pb), arsenic (As), cadmium (Cd), chromium (Cr) and mercury (Hg), possesses no reported physiological role [25]. However, the presence of all the mentioned heavy metals beyond the critical limits proves hazardous to plants and human health.

Impact of Heavy Metals on Aquatic Life

Untreated or insufficiently treated wastewater when released into the environment causes ultimate environmental degradation. The wastewater forms the major source of heavy metal contamination. The heavy metals readily reach the aquatic organisms and thereby, adversely impact them. The fish absorbs the heavy metal into the body tissues which gets transferred to the humans upon consumption of the affected fish. Some of the reports suggest high mortality of juvenile fish and a decrease in the breeding potential of adult fish due to long-term exposure to heavy metals [26]. A study conducted to understand the impact of heavy metals on *Labeo rohita* indicated several physiological alterations in the fish including: wrinkle cell membrane/ total damage of cell membrane, damage of nucleus, shift in nucleus position, reduced or enlarged size of the nucleus and complete destruction of the nucleus [27]. The exposed aquatic animals likewise, illustrated low levels of protein content. Similarly, anaemic conditions in the exposed aquatic animals were reported by several researchers.

Humans consuming the affected fish suffer damage to liver, kidney, heart and joints. The liver and kidneys get stimulated to produce metallothioneins leading to toxicity, proving fatal for humans [27].

Thus, the impacts of heavy metals in the environment, disrupt the ecological balance, ultimately compromising life on the earth. Due to the several ill impacts of heavy metals (as discussed above), it becomes a priority to eliminate the heavy metals from the environment in a sustainable manner, without further degrading nature.

Ways to Eliminate Heavy Metals from the Environment

Several methods have been utilized for eliminating/ recovering heavy metals from polluted environments. Some of the well-recognized conventional procedure for heavy metal removal/recovery from solution includes adsorption processes, chemical oxidation or reduction reactions, chemical precipitation, electrochemical techniques, evaporative recovery, ion exchange, reverse osmosis, and sludge filtration [28]. However, these techniques are quite expensive, often impractical, unsustainable and not environment friendly. Furthermore, the production of toxic waste, high reagent obligations and the uncertain nature of metal ion removal, involves some of the disadvantages of the conventional techniques [29]. Thus, there occurs an immediate requirement for the exploration and implementation of sustainable

alternatives, which should be suitable appropriate, and applicable to local conditions, to eliminate the heavy metals from the environment to meet the established permissible limits.

Bioremediation is the most emerging technique to manage the removal of heavy metal toxins from the environment by utilizing biological systems such as bacteria, algae, fungi or plants. This paper largely focussed on the bioremediation my microbes. The technique has proved to be quite effective for the removal of heavy metal contamination from soil and wastewater. The method proves to outweigh the disadvantages of the chemical and physical techniques of heavy metal removal. The utilization of microbes for the restoration of contaminated sites creates a sustainable solution with negligible waste production and occurs to be a cost-effective approach.

Microbial Degradation of Heavy Metals from the Environment

Microbes ubiquitously occur in the environment. The microbial diversity in the soil defines the character of the soil and dictates the plants' growth. Similarly, the microbes present in the wastewater form an effective network clearing up the pollutants and treating the water. Several normal microbial flora of soil and water gets disrupted by the presence of heavy metals. For the microbial degradation of heavy metals, certain specific groups of microbes play a noteworthy role. These microbes have either evolved or genetically modified to tolerate the high levels of heavy metals and degrade them. The choice of microorganism may be the native organism of the polluted environment or isolated from the polluted site and utilized at the remediation site [30]. The microbes accumulating the heavy metals should display tolerance against one or two heavy metals at higher concentrations, must possess improved transformational abilities – transforming the highly toxic component into a harmless form, and contain the heavy metal [31].

Microorganisms employ the techniques of biotransformation, biosorption, bioaccumulation, and biomineralization for potentially lowering the concentration of heavy metals in the environment. Several factors affect the microbial remediation of metals, which includes: the bioavailability of the metal to the microorganisms, the concentration of heavy metals, electron acceptors, moisture content, nutrients, osmotic pressure, oxygen, pH, redox potential, soil structure, temperature, and water activity [32].

The microbial cell has the potential of trapping and accumulate heavy metal ions and subsequently absorb them through cell wall, due to the presence of functional groups such as -SH, -OH, and -COOH, which aid the absorption of metals [33]. This technique, commonly referred to biosorption, remains independent of the metabolic cycle. Several mechanisms involved in biosorption include electrostatic interactions, ion exchange, precipitation and surface complexation[34]. The fragments of cells and tissues, as well as dead biomass of living systems, could undergo biosorption via surface complexation onto the cell wall and other outer layers.

Another method, referred to as bioaccumulation, involves the passage of heavy metal ions across the cell membrane and into the cytoplasm through the cell metabolic cycle.

Similarly, microbes may trigger the biotransformation mechanism by which highly toxic heavy metals are converted into non-toxic forms. For example, in mercury-resistant bacteria, organomercurial lyase converts the methyl mercury to Hg(II), which is 100 times less toxic than methyl mercury [35]. Mercury-resistant bacteria utilize MerA enzyme to reduce Hg(II) to the volatile form of Hg(0). Likewise, Cr(VI) could

be reduced to Cr(III), which has less mobility and toxicity. Even the reduction of Se(V) to elemental Se(0) has been proven to be quite significant during the remediation of contaminated water and soils.

The process of biomineralization involves the microbial formation of minerals, which results in the removal of heavy metals from the contaminated sites. The major biominerals precipitated by microbes include oxides, phosphates, sulphides and oxalates, all of which have certain desirable traits such as high metal sorption capacity and redox catalysis [36].

Several microbes could possibly utilize the above-mentioned mechanism to remove/recover/ transform the heavy metals from contaminated soil and wastewater.

Microbial Degradation of Mercury (Hg)

Mercury is quite resistant metal largely available in nature owing to industrial pollution and the irrational release of wastewater into the environment. It becomes pretty vital to remove the high levels of Hg from the soils and aquatic bodies, as it extensively disrupts the ecological balance and the biotic factors of the environment. The biomagnification of Hg has been largely observed in nature, where fish, occur to be the primary step of mercury accumulation, and consequently pass to humans. Several chemical and physical treatments attempted to remove the Hg from the soil and water, only leads to more pollution and forms unsustainable approaches. In order, to develop and implement an appropriate and sustainable approach to bioremediate the Hg from the contaminated soil, wastewater as well as bio-solids (obtained from the anaerobic digesters treating the wastewater), microbes play a noteworthy role.

A study by (B. Pushkar, et.al, 2018), illustrates the isolation of mercury-resistant bacteria (MRB) from the river sample, which was able to eliminate around 90% of mercury in 48 hours at 50 ppm of mercury. The homology analysis of the 16S rDNA gene identified the bacterium as *Bacillus thuringiensis* strain RGN1.2 [37].

The mercury-resistant bacteria harbor the *mer operon* within their genome [38]. This operon includes several functional genes such as *merA* and *merB*, coding for mercuric ion reductase and organomercurial lyase, respectively. The lyase controls the reduction of highly toxic organomercurial compounds such as methylmercury and phenyl mercuric acetate into non-toxic elemental mercury with the help of the enzyme reductase [38]. While acting together, *merA* and *merB* in bacteria result in broad-spectrum mercury resistance. However, *merA* alone establishes only a narrow spectrum of resistance against mercury. Other genes encoding organomercury resistance have been designated as *merG* and *merE*, located between *merA* and *merB* in *mer* operon of broad spectrum mercury-resistant bacteria.

The process begins with the transport of Hg^{+2} into the cell by the protein-dependent transport system, through the series of transporter proteins. Initially, Hg^{+2} binds with a pair of cysteine residues on the MerP protein (in periplasm), then gets transferred to cysteine residue on MerT (in the cytoplasmic membrane) and finally to a cysteine pair at the site of MerA. The gene *merA* encodes the enzyme mercuric ion reductase (a type of flavoprotein) that catalyses the NADPH-dependent reduction of Hg^{+2} to Hg^0 [39]. Elemental Hg gets volatilized readily due to high vapor pressure and the environment tends to be free from mercury contamination. However, the biochemical pathway of reduction of organomercurial compounds follows a slightly varying path. These compounds are selectively transported across the membrane by MerE and MerG in gram-negative bacteria [40] [41]. Once the organomercurial compounds

reach the cytoplasm the C–Hg bond gets cleaved by the organomercurial lyase (coded by *merB*) to release Hg^{+2} , which then gets reduced to Hg^0 by mercuric ion reductase (coded by *merA*) by the same NADPH-dependent mechanisms (as mentioned above) to eliminate mercury from the contaminated sites [38].

Similar studies by (A. Essa, et.al, 2002), indicated a few more pathways for the removal of mercury from the wastewater by utilization of *Klebsiella pneumoniae* M426, such as aerobic precipitation of ionic Hg as insoluble HgS , leading to H_2S and production and biomineralization of Hg as insoluble mercury-sulphur complex other than HgS . The resultant compounds were believed to be formed due to the aerobic production of volatile thiol compounds. Likewise, the studies illustrate a high level of Hg removal at varying pH and salinity levels [42].

Research conducted by (K. Saranya et al., 2017), illustrated the high bioremediation capabilities of mercury chloride by *Vibrio fluvialis*. The highest mercury-remediation rate of 60% was observed at a mercury concentration of 100 $\mu\text{g/ml}$ after 42 hrs of incubation [43]. Few other species reported effective against mercury removal include *Bacillus* sp., *Bacillus thuringiensis*, *Pseudomonas aeruginosa*, *Brevibacterium casei*, *Tetrahymena rostrate*, *Pseudomonas* sp., *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Eubacterium casei*, *Tetrahymena rostrate*, *Pseudomonas* sp., *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* [43] and *Alphaproteobacteria Xanthobacter autotrophicus* Py2 [44]. Likewise, fungi *A. flavus* strain KRP1 was reported to be a potential strain for bioremediation of Hg(II) from the aqueous substrate by biosorption mechanism [45].

Microbial Degradation of Arsenic (As)

Arsenic occurs to be one of the most toxic heavy metals occurring in the environment while inorganic arsenic has been confirmed as a carcinogen. Long-term exposure to arsenic has been associated with cardiovascular disease and diabetes. In utero and early age exposure to arsenic has been likewise linked to adverse impacts on cognitive development and a higher number of deaths within the young adult population. High-level arsenic contamination of groundwater has been found in more than 105 countries of the world [46]. Therefore, it becomes quite crucial for the removal of arsenic from contaminated sites.

Bacteria have devised several mechanisms to degrade this toxic metal and reduce its toxic effects. These mechanisms include arsenite oxidation, arsenate reduction and methylation [47]. The bacteria utilize the arsenic for their own growth through energy-generating processes such as arsenite serving as an electron donor while arsenate acts as an electron acceptor [48]. Research conducted (by F. Wolfe-Simon et al., 2011) indicated that the bacterial strain GFAJ-1 (a strain of the rod-shaped bacteria belonging to the family of Halomonadaceae), during the process of arsenic degradation could effectively replace the phosphate with arsenate in its DNA to support its growth[49].

Utilization of arsenite oxidation mechanism to get rid of excess arsenic occurs in both heterotrophic and chemoautotrophic bacteria [47]. During the heterotrophic bacterial oxidation, the bacterial species oxidises the toxic As(III) to harmless As(V) prior to its release out of the cell. On the other hand, the chemoautotrophic bacteria utilize As(III) as an electron donor while undergoing aerobic oxidation, thereby, converting As(III) to As(V) , and obtaining energy. The oxidation of the arsenite occurs within the bacterial periplasm. During the oxidation process, a sensor kinase – *aoxS*, identifies arsenite and rapidly stimulates a regulatory protein – *aoxR* which aids the transcription of arsenite oxidase responsible for detoxifying the arsenic present in the bacterial surrounding [50]. A purple sulphur bacterium -

Ectothiorhodospira sp. strain PHS-1 and MLHE-1, harbours a novel arsenite oxidase – ArxAB, which utilizes As(III) as an electron donor in anoxic conditions [51].

Arsenate-reducing bacteria utilize varied reduction systems to eliminate the arsenate from their environment. They basically convert the arsenate to arsenite prior to its release from the cell. One of the reduction strategies involves the cytoplasmic reduction system (ArsC) [52]. Once As(V) enters the bacterial cell through the phosphate transporters, an enzyme – arsenate reductase (ArsC) catalyses the reduction of As(V) to As(III), utilizing ferredoxin or glutathione as an electron donor, thereby, forcing As(III) out of the cell through ArsB efflux pump [53]. Certain bacteria possess an ATPase called ArsA attached to ArsB for the release of arsenite [54]. Yet, another mechanism by which arsenate gets reduced involves certain groups of bacteria which utilize As(V) during anaerobic respiration as a terminal electron acceptor obtaining energy. These bacteria are referred to as dissimilatory arsenate respiring prokaryotes (DARPS) [55], for example, *Chrysiogenes arsenatis*, Firmicutes γ -, δ -, and ϵ Proteobacteria [50].

Certain bacteria likewise, utilize the arsenic methylation mechanism to detoxify their surroundings. During this process of detoxification, arsenite gets methylated to methylarsonic acid, which further undergoes a series of reduction and methylation reaction steps resulting in dimethylarsinic acid, dimethyl arsine and ultimately being extruded as trimethylarsine oxide [47]. Trimethylarsine oxide gets volatilized, facilitating the removal of arsenic by bacteria. S-adenosylmethionine methyltransferases occur to be the major enzyme responsible for the methylation of arsenic. Bacteria such as *Methanobacterium formicium*, *Desulfovibrio gigas*, *Clostridium collagenovorans* and several other aerobic and anaerobic bacteria have been observed to be involved in arsenic methylation [56] [57].

A few examples of reported arsenic-degrading bacteria include *Herminiimonas arsenicoxydans* [58], *Bacillus*, *Delftia*, *Pseudomonas*, *Agrobacterium*, *Firmicutes*, *Acinetobacter*, *Crenarchaea*, *Stenotrophomonas*, *Achromobacter*, *Citrobacter*, *Rhodobium*, *Clostridium*, *Methanobacterium* and *Cyanobacteria* [47]. Likewise, a few specific fungal strains of *Aspergillus oryzae*, *Aspergillus nidulans*, *Rhizomucor variabilis*, *Emericella* sp possess the high potential of As bioremediation through bioaccumulation [59].

Microbial Degradation of Lead (Pb)

Lead occurs to be highly toxic heavy metal occurring in the environment, owing to several sources (as mentioned in table 1). It occurs as a cumulative toxicant, specifically dangerous to young children and pregnant women. Inside the body, Pb has the potential of causing permanent damage to the neurological and cardiovascular systems. *The World Health Organization's 2021 update of the public health impact of chemicals: knowns and unknowns*, indicates that half of 2 million lives lost to known chemical exposure in 2019, were due to lead exposure. Worldwide figures estimates around 30% of idiopathic intellectual disability, 4.6% of cardiovascular diseases and 3% of chronic kidney disease are due to lead exposure [60].

The commonly utilized physical and chemical techniques to remove lead from the environment includes an electrochemical deposition system, ion exchange, flotation, filtration, precipitation, sedimentation and granulated activated carbon [61]. However, like any other chemical and physical techniques, these techniques possess sustainability, economic and large-scale practicality issues.

Bacterial remediation of lead forms a feasible approach for eliminating lead from soil, wastewater and biosolids. Biosorption of lead by bacteria forms a highly effective method for lead removal. Both live and dead bacteria could act as a good absorbent. The bacterial cell surface comprises several anionic ligands such as carboxyl, amine, hydroxyl, phosphate and sulfhydryl groups which could strongly bind to lead [62]. Bacteria could be readily immobilized on solid material for the biosorption procedure which involves recycling the bio-absorbed lead for safe disposal or further utilization [63]. The biosorption treatment could be carried on out in a continuous fashion in a bioreactor on site. The immobilized cells utilized for the biosorption could be repetitively utilized as the cells don't get washed out through the process. [64]. Reports suggest that *Bacillus megaterium* illustrates high biosorption characteristics under alkaline contaminated environment [65].

Biomineralization occurs to be yet another mechanism conducted by bacteria for the removal of Pb from the contaminated sites. Biomineralization causes the chemical alteration of heavy metals into solid-state minerals biologically. Research (by Zhang et al., 2019) concluded the biomineralization of Pb from the contaminated soil by *Bacillus* sp. with high efficiency. The results likewise, indicated that during fermentation, the bacteria could readily decompose the phosphate-containing organic compounds present in the culture medium to produce a large amount of phosphate groups on the surface of the bacterial cells. Pb^{+2} , was observed to be precipitated in form of $Pb_3(PO_4)_2$ stable minerals [66].

Lately, microbial-induced calcium carbonate precipitation (MICP) based on biomineralization has been a widely utilized technique especially, for soil remediation. This technique utilizes the capability of ureolytic bacteria to completely remove the lead from their surroundings, by their ability to effectively hydrolyse urea to produce carbonate ions and increase the pH to around 8.0-9.1. The alkaline pH enhances the precipitation of lead and calcium carbonate. For example, *Sporosarcina pasteurii* removes lead from the environment, by induced mineralization, depending upon the surrounding environmental conditions [67]. Out of several pathways utilized by the MICP technique, the use of urea as the substrate forms the most common pathway. Several extracellular polymeric substances produced during bacterial growth have negatively charged functional groups for example, ($-COOH$, $-OH$, and $C=O$). These functional groups excellently absorb the heavy metals from the site/solution. Ca^{+2} (from the source) and CO_3^{-2} combine to form $CaCO_3$ precipitating under alkaline conditions with co-precipitation of Pb ions. Further, Pb ions take up the position of Ca^{+2} , in $CaCO_3$ lattice, resulting in the formation of calcium salt-lead composite precipitation [67]. Some of the bacteria utilized in the MICP technique for bioremediation of Pb includes *Kocuria flava*, *Sporosarcina koreensis* UR47, *Pseudomonas stutzeri*, *Stenotrophomonas rhizophila* A323, *Variovorax boronicumulans* C113, *Enterobacter cloacae* KJ-46 [67].

One of the studies (by Y. Jing et al., 2021), illustrates the use of edible fungal residue (EFR) (which occurs as a type of base waste from the cultivation of edible fungus and its safe disposal still remains a concern), for adsorption of Pb(II) ions from wastewater to drastically reduce the environmental pollution and Pb toxicity [68].

Microbial Degradation of Cadmium (Cd)

Cadmium, a non-essential metal, causes large-scale environmental degradation adversely impacting life. Cd largely impacts the kidneys and human skeleton. Cd chiefly enters the environment from the unexercised use of phosphorous fertilizers. It readily enters the food chain through the contaminated

crops causing chronic renal, pulmonary, and cardiovascular disorders. Exposure of 30-50 µg/day to adults has been reported to link with an increased risk of bone fracture, cancer, kidney dysfunction and hypertension [69]. Likewise, cadmium leads to oxidative stress, epigenetic changes in DNA expression, inhibition of transport pathways, impairment of mitochondrial function by inducing apoptosis etc [70].

The microbial remediation of Cd in the environment majorly depends on the bioprecipitation and Cd(II) binding technique. Certain microbes generate carbonate, H₂S, phosphate and oxalate which react with Cd(II) to form insoluble cadmium salts. Similarly, the urease-producing bacteria, possess ureases which decompose urea into carbonate to precipitate Cd(II) [71]. Certain sulphate-reducing bacteria produce H₂S to generate intracellular/extracellular CdS precipitates [72]. Furthermore, some bacteria elevate the levels of soluble phosphate and phosphoric acid in the soil, which reacts with Cd(II) to form insoluble cadmium phosphate [73]. The precipitated salts could be removed from the site through membrane separation. Additionally, some bacteria produce a metal-binding protein (such as bacterial metallothionein – BmtA) to reduce the damage of Cd(II) [74]. Bacterial exopolysaccharides (EPSs) illustrate excellent Cd(II) binding ability either through negative charge adsorption or by forming a complex with functional groups such as amine and hydroxyl of EPSs [75].

Pseudomonas aeruginosa remains one of the most versatile microbes with a high tolerance of cadmium, and its effective utilization as biosorbent, forms a most accepted approach for the removal of cadmium from the soil, wastewater and biosolids. *P. aeruginosa* PU21 biomass occurs as the most effective biosorbent for the removal and recovery of Cd from polluted water [76]. Likewise, even the dead cell biomass of *P. aeruginosa* possesses a higher ability to adsorb Cd from the aqueous solution [77].

A study indicated a high Cd removal property of *Bacillus safensis* (JX126862) from heavy metal-contaminated mangroves sediments [78]. A few more bacteria such as *Proteus vulgaris* and *Acinetobacter* have been reported for bioremediation of cadmium [79]. Likewise, the fungi *Trametes versicolor* plays a significant role in reducing Cd from contaminated environmental sites [80].

Microbial Degradation of Chromium (Cr)

Chromium occurs as yet another heavy metal responsible for contaminating the environment. Hexavalent chromium occurs to be the most dangerous form of chromium leading to several health concerns such as allergic reactions, skin rashes, DNA damage, kidney and liver disorders and could even lead to death. The health damage from chromium largely varies and depends upon the dose, exposure levels and duration [81].

The conventional means for removal of Cr(VI) from wastewater involves several physicochemical techniques such as chemical reduction, adsorption on porous surfaces along with sites for ion exchange and electrocoagulation [81], however, these methods have limitations owing to sludge production, a large amount of chemical requirements and risk of secondary pollution [82]. Therefore, like other heavy metals mentioned in the paper, bioremediation of cadmium forms a sustainable approach for the removal of Cd from contaminated sites.

Cr(VI) generally enters the bacterial cell through the active sulphate transporters. Once inside the cell, Cr(VI) undergoes reduction processes. Bacteria capable of reducing Cr(VI) largely belong to nitrate-reducing, Fe(III) reducing and sulphate-reducing bacteria. Several gram-positive bacteria such as

Bacillus, *Deinococcus*, and *Arthrobacter* have been reported to possess Cr(VI) reduction capability [83] [84] [85]. Similarly, certain yeasts, fungi and algae could effectively reduce Cr(VI) to a less toxic form. Cr(VI) reduction occurs both enzymatically as well as chemically through reducing agents such as Fe(II) and H₂S generated by bacteria [86]. Cytochrome c (a heme protein) present on the inner and outer membranes, causes a direct reduction of Cr(VI) to less toxic Cr(III) through electron transfer across the respiratory chain [81]. NADH (a coenzyme) mediates the activity of chromate reductase to detoxify Cr(VI).

The enzymatic reduction mechanism appears to be different under aerobic and anaerobic conditions. The major enzyme involved in the aerobic reduction of Cr(VI) occurs to be Cr(VI) reductase, classified into ChrR, OYE, Nema, YieF, NfoR and NfsA families [87]. For example, ChrR utilizes a two-step electron transfer pathway, where one electron gets transferred to Cr(VI) generating Cr(V). This Cr(V) then accepts another two electrons to form Cr(III) along with forming reactive oxygen species which could lead to DNA damage [87]. However, this damage could be repaired by the utilization of SOS repair systems, RuvRCAB and other repair proteins.

Additionally, several other membrane enzymes have been found to be linked with the reduction of Cr(VI) in aerobic conditions. For example, In *Bacillus* sp. MSM1, *Serratia proteamaculans* CRB1 and *Alishewanella* sp. WH16-1, some membrane proteins could effectively reduce Cr(VI) [88] [89]. One of the reports likewise, indicates the presence of extracellular enzymes involved in Cr(VI) reduction in *Candida utilis* M20 [90].

The anaerobic reduction of Cr(VI) have been linked to extracellular electron transport (EET). Cr(VI) reducing bacteria undergoes one of the three pathways (as indicated below) where Cr(VI) acts as the terminal electron acceptor [87]

- 1) *Shewanella oneidensis* MR-1 typically utilizes cytochrome *c* – the electron transfer chain for anaerobic Cr(VI) reduction. The electrons get transferred from NADH to ubiquinone via dehydrogenase, and subsequently flow to CymA, MtrA and MtrB. Next, MtrB and get transfers to MtrC and OmcA. Cr(VI) accepts the electrons from MtrC and OmcA [91].
- 2) Electron shuttles (ESs) (which could reversibly oxidised – reduced and thus transferring electron from microbes to Cr(VI). The bacterial *c* – types cytochromes such as MtrC, OmcA, OmcB and OmcS effectively reduces ESs which are then reduced by Cr(VI) [92].
- 3) Microbial nanowire, consisting of electrically conductive proteins which transfer electrons produced by bacteria to electron acceptors, plays a critical role in the reduction of Cr(VI) [93].

Several microbes reported from the degradation of chromium involves *Bacillus cereus* [94], *Acinetobacter haemolyticus*, *Pannonibacter phragmitetus* LSSE-09, *Burkholderia cepacia* MCMB-821, *Bacillus sphaericus*, *Bacillus thuringiensis* [95]. *Aspergillus* sp. have been reported to have high degradability of Cadmium [96].

Conclusion

The environmental contamination leading to its degradation due to heavy metals must be ceased to conserve the environment. However, the degradation already done could be effectively restored by the utilization of non-conventional methods, i.e., the biological methods to detoxify nature. Several different

microbial candidates form a large network of saviours of the planets. The bioremediation potential of different microbial must be employed rationally to eliminate the toxins from the environment. However, the genetic engineering of several potential bioremediating microbes needs to be conducted to expose and utilize their underrated capabilities of biodegradation. The biodegrading capabilities and the biochemical pathways of several crucial microbes remain to be explored further.

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