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A Systematic Review on Microbial Bioremediation and Nanobioremdiation of Toxic Heavy Metals Pollution

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Abstract: Heavy metal pollution in soil, water, and the environment poses a huge risk to human health and well-being. Most often, they are non-biodegradable, leading to accumulation and entering the ecosystem and food chain, affecting water, vegetation, and aqueous systems. Conventional physiochemical methods are not environmentally friendly and suffer from several setbacks. Alternatively, bioremediation of toxic heavy metals using natural sources is safer, efficient, rapid, and environmentally friendly. Microbial bioremediation (MBR) mechanisms such as oxidation/ reduction, biosorption, biomineralization, biotransformation, bioaccumulation, bioprecipitation, biocrystallization, bioleaching or biomining, bioaugmentation, biofilm formation, production of biosurfactants and immobilization with nanoparticles have huge potential for effective cleanup of the toxic heavy metal's pollution. This review highlights the recent updates in bioremediation (BR), microbial bionano remediation (MBNR) process of heavy metals through biodegradation methods, and the scope of the metagenomics approach to screening the microbes for novel genes involved in BR and MBNR. The future aspects of BR and MNBR processes for effective degradation, removal, and management of heavy metals pollution in soil and water are also discussed.

Keywords: heavy metals pollution; bioremediation; microbial bionano remediation; biodegradation.

1. Introduction

Heavy metals (HMs) pollution is a worldwide problem due to contamination of the Earth's environment. Anthropogenic activities are responsible for the production of toxic metals worldwide. Currently, co-contamination is also a major problem that poses a great challenge. Hence, less-polluting industrial processes, restrictions on the use of non-degradable compounds, proper disposal of hazardous wastes, and remediation of contaminated sites must be followed to protect our environment. Remediation has the most significant role in environmental restoration as it cleans preexisting contaminated sites. Remediation of the contaminated sites can be achieved by a number of methods. Bioremediation using microorganisms is preferred over other physico-chemical methods as it is cost-effective and eco-friendly [1]. Organisms use organic substances as sources of carbon and energy; they have degradative abilities and co-exist with many organic compounds. The evolution of microbial gene(s) for degradation of many unrelated natural organic compounds is developed due to its growth on different substrates. Composting was the first biological treatment process effectively used for remediating military sites. Microorganisms have also been used in conjunction with physical methods, combined with UV-biological degradation of polynuclear aromatic hydrocarbons (PAHs) with 90% removal. Gene clusters encoding for the biodegradation of several pollutants, simple aromatics, nitroaromatics, chloroaromatic, polycyclic aromatics, biphenyls, polychlorinated biphenyls, components of oil, etc., have been cloned to create genetically engineered microorganisms (GEMs) [2]. The construction of GEMs by reshuffling the gene(s) and promoters has been experimentally proven to be an effective BR agent under laboratory conditions.

Pseudomonas fluorescens is reported to be very efficient in bioremediating As, Cd, Cr, Co, Cu, Hg, Ni, and Pb [3], and algal species *Spirogyra hyalina* bio remediates As, Cd, Cr, Co, Hg, Ni, and Pb [4] and fungal consortium A. *flavus*, *A. gracilis*, *A. penicillioides*, *A. restrictus*, *Sterigmatomyces halophilus* bio remediates Cd, Cu, Fe, Hg, Mn, Ni, Pb and Zn [5]. BR of metals is based on their conversion to a less toxic form and/or immobilization in order to reduce their availability. There are many HMs that not only affect the environment but are also toxic to humans and cause several diseases. Numerous conventional methods have been employed for the sequestration of HMs, which are associated with a high yield of toxic waste, residual effects, and high cost. A combination of microbial BR methods, nanobioremediation methods, and metagenomic methods would certainly increase the effective removal of THMs manifold from soil and water. In this review, the importance of the microbial multifunctional BR process, the mechanism involved, the role of nanotechnology/ nanobiotechnology, and recent methods, including genetic engineering and metagenomic sequencing of potential microbes for the effective control of THMs pollution at contaminated and co-contaminated sites are discussed.

2. Bioremediation (BR)

Many bacterial species are very effective in bioremediating toxic heavy metal pollutants (THM) pollutants (Table 1). Immobilization, bioaccumulation, biosorption, precipitation, reduction, siderophore formation, and enzymatic activity are some common mechanisms microorganisms adopt [6]. Microbes also employ detoxification, degradation, mineralization, and transformation processes to eliminate HMs [7]. Actinobacteria showed great BR potential in pesticides, HMs, and co-polluted soils.

Table 1. Bioremediation of heavy metals by bacterial species reported recently.

Bacterial species	Heavy metals	Mechanism	References
Cupriavidus metallidurans CH34	Heavy metals	Resistance genes	[9]
Klebsiella Enterobacter	Arsenic, cadmium & Lead	Resistance genes	[10]
Bacillus megaterium	Chromium (VI)	Tryptophan residues proteins	[11]
Achromobacter sp. L3	Cadmium	Immobilization	[12]
Desulfobulbaceae, Desulfobacteraceae, Syntrophobacteraceae, Desulfovibrionaceae and *Desulfomicrobiaceae*	Cadmium	Dissimilatory sulfate reduction	[13]
Geobacter sulfurreducens	Chromium (VI)	Bioaccumulation	[14]
Lactobacillus plantarum MF042018	Nickel & Chromium(VI)	Biosorption	[15]
Pseudomonas aeruginosa Azotobacter chroococcum Bacillus subtilis	Lead, Copper, Nickel, Cadmium & Chromium (VI)	Biosorption	[16]
Geobacter sulfurreducens	Chromium	Biosorption	[17]

Fungal species have been used as effective biosorbents for the bioremoval of metals from polluted soil. The contribution of fungal consortium *P. subtephropora*, *D. starbaeckii*, *P. concrescens*, *C. aurantiopora*, *F. equiseti*, *Polyporales sp.*, *A. niger*, *P. lilacinus*, *A. serialis*, *A. fumigatus*, *P. cataractum*, *T. versicolor*, and *F. chlamydosporum* for removal of HMs (As, Mn, Cu, Cr, and Fe) by bioaugmentation process was already reported [8]. Effective removal of Cr (VI) (70%) and Cd (II) (74%) by fugal strains *Aspergillus flavus* and *Aspergillus fumigatus* in a liquid medium was recently reported [18].

Microalgae remove metals by biosorption and bioaccumulation process, and the cell wall polysaccharides (cellulose and alginate), lipids, and proteins containing amino, carboxyl, hydroxyl, imidazole, phosphate, sulfonate, and thiol groups also involved metal binding [19]. Functional groups present in the biomass, such as amide, carbonyl, carboxylic acid, ether, and hydroxyls, also contributed to the biosorption. Surface adsorption of metals on microalgae depends on covalent bond formation between HMs and ionized cell walls. Transport of metals into the cytoplasm, diffusion, and binding with proteins (GSH), peptides (metal transporter), and oxidative stress-reducing agents are the major processes involved [20]. Extracellular adsorption, reduction, volatilization, complex formation, ion exchange, intracellular accumulation, chelation, and bio-methylation are microalgae's effective metal removal methodologies [21].

All types of microorganisms, including bacteria, archaebacteria, protozoa, fungi, algae, and lichens, can be bioremediate HMs [22]. HMs strongly affect the activities and survival of microbes. High concentrations of many HMs harm microbial cells and toxic heavy metals (THMs) are toxic to many microorganisms, even at the lowest concentrations [22]. HMs harm cells by mimicking essential elements and inhibiting biological function, producing reactive oxygen species (ROS), and destabilizing biomolecules like DNA, RNA, and protein [23]. Microorganisms resistant to HMs, with their varying phenotypic expressions, also affect the concentration of HMs in the environment. However, microorganisms affect the concentration of HMs through removal, recovery, precipitation, and detoxification. Biotransformation (oxidation-reduction), biomineralization, bioprecipitation, bioleaching, biosurfactant production, biomethylation, biovolatilization, biosorption, bioaccumulation, and microbe-assisted phytoremediation are BR mechanisms adopted by microbes (Figure 1).

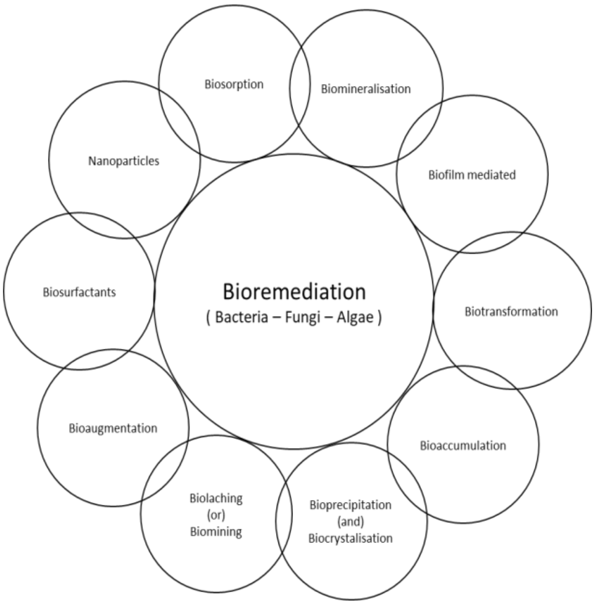

Figure 1. Microbial bioremediation of toxic heavy metals.

In biosorption, functional groups like–SH, –OH, and–COOH on the surface of microbes are involved in the adsorption of metals [24]. By excreting metal-chelating substances or by adopting a particular transport system, microbes develop resistance to HMs and metal accumulation. Alternatively, binding metal ions with intracellular molecules such as metallothionein, vacuole, or mitochondria leads to changes in the distribution of metal ions. Also, metal ions interact with microorganisms through metals associated with the cell wall, resulting in intracellular accumulation, formation of metal siderophores, extracellular polymeric reactions with transformation, extracellular mobilization or immobilization, and volatilization of metals [25]. The environmental conditions required for the removal of HMs by microbes are crucial. In intracellular and extracellular sequestration, metal ions form a complex with various compounds present in the cell cytoplasm. The interaction of metal ions with surface ligands (peptidoglycan, polysaccharide, and lipid) of cell wall containing numerous metal-binding ligands (e.g., -OH, -COOH, $-HPO_4^{2-}$, SO_4^{2-} $-RCOO^-$, $R_2OSO_3^-$, $-NH_2$, and -SH) followed by transport into the microbial cells has been exploited for HMs removal. In extracellular sequestration, metal ions form insoluble complexes with cellular components present in the periplasm [26].

Eukaryotes, bacteria, or archaea form biominerals such as carbonates (aragonite and calcite), silicates (opal), Fe- and/or Mn-oxides. It often leads to the hardening or stiffening of the mineralized materials such as silicates in algae and diatoms, carbonates, and calcium phosphates in vertebrates. In biotransformation, catalytic properties of enzymes and physiology of microbes and several environmental factors like pH, temperature, co-metal ions, and nutrient sources are the main factors that govern the mobility and bioavailability of HMs for microbial transformation [27]. Bioaccumulation deals with the entry of metals into a food chain and accumulation in biological tissues of aquatic organisms acquired from water, food, and suspended sediment particles [28]. During the bioaccumulation process, these concentrations of metals increased continuously in the organism.

Accumulation in living organisms usually occurs if the metals are taken in more concentration and stored as well than metabolized or excreted. Bioaccumulation is a dynamic process that plays a role in protecting humans and other organisms from HMs toxicity. Apart from the bioaccumulation of HMs, the uptake, bioavailability, bioconcentration, and biomagnification are also important. Uptake is the entry of a chemical into an organism. Bioavailability is the intracellular availability of a metal in the organism maintained in a culture medium. Bioconcentration is the concentration of foreign chemicals in the organism (greater than its concentration in the environment). Bioconcentration in fish is the uptake through the gills or through the skin. In biomagnification, the concentration of pollutants increases as they move from one trophic level to the other in the food chain.

In bio-precipitation or biomineralization occurs in organisms. Nucleation of cell surfaces or associated extracellular polymers alters fluids' chemical composition, thereby increasing the supersaturation followed by mineralization. In biocrystallization, crystals are formed in living organisms using organic macromolecules. It may be due to a response to stress or as a part of a metabolic response to the waste disposal or pathological response. Bacteria or archaea are used for bioleaching (or) biomining of valuable metals from low-grade ore and can also be used to clean mining sites. Bioleaching is used to extract gold, silver, uranium, nickel, copper, cobalt, and zinc. Sulfide minerals are extracted using *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* bacteria. In direct bioleaching, microbial enzymes are used to oxidize minerals to separate the metal from the ore. In indirect bioleaching, leaching agents produced by microbes are used for the oxidation of the ore. The emission of toxic sulfur dioxide is avoided by bioleaching sulfate toxins from the mine. Bioleaching is a more preferable and cost-effective method than smelting processes. In bioaugmentation, cultured microorganisms are added to the contaminated source to biodegradate the contaminants. Easily cultivable, fast-growing, and contaminant-resistant microorganisms need to be added to the contaminating source for removal. Bioaugmentation of HMs employing microorganisms to reduce heavy metal contents and transform them into unavailable forms has already been reported. Soil contaminated with Zn was augmented by using bacteria *Bacillus licheniformis*, *Bacillus* sp., *Gordonia amicalis*, and *Leifsonia* sp. and fungi *Penicillium raperi*, *Penicillium janthinellum*, *Penicillium glabrum* and *Trichoderma harzianum* [29].

Biofilm-forming microorganisms are widely used to BR heavy metal environmental pollutants [30]. Exopolysaccharides (EPS) of biofilm are responsible for immobilizing metal ions, and increased EPS concentrations increase heavy metal detoxication. Biofilms can survive in toxic environments due to the presence of certain genes and in-built resistance. The high biomass of bacterial biofilm was helpful for the efficient removal of HMs and dyes through bioaccumulation, biosorption, and enzymatic activities [31]. The binding of EPS and biosurfactants of biofilm can transform the toxic HMs and dyes. The formation of organometallic complexes between polyionic EPS and metal ions was reported. The sequestration of HMs by EPS is facilitated by electrostatic interactions between the negatively charged functional groups of EPS and positively charged metal ions [32]. Different mechanisms, such as biochelation, bioaccumulation, biosorption, bioprecipitation, bioreduction, complexation, and irreversible adsorption, are involved in the biofilm-mediated removal of metals and dyes [33]. Bio-surfactants produced by various microbial cells contain hydrophilic and hydrophobic groups used to remediate metal-contaminated soils. Biosurfactants enhance the desorption of HMs from the soil in two different ways. Metal ions also form 'biosurfactant micelle' with biosurfactants. The tight bonds between the soil and metals are disturbed by biosurfactants, which increases the metal's bioavailability for removal. Biosurfactants have low toxicity, selectivity, and biodegradability over conventional chemical surfactants. Based on the chemical composition, biosurfactants can be classified as glycolipids (trehalolipids, sophorolipids, rhamnolipids), lipoproteins, and lipopeptides (lichenysin, surfactin), fatty acids, phospholipids (corynomycolic acid) and polymeric biosurfactants emulsan). HMs present as surface contaminants on vegetables are effectively removed by treatment with lipopeptide biosurfactant from *Bacillus* sp. (MSI 54) [34].

3. Nanoparticles in Bioremediation

Many microorganisms are used to remediate the HMs contaminants in the presence of nanoparticles (NPs) and nanomaterials (NMs) [35]. Iron oxide nanoparticles (IONPs) loaded with sodium alginate beads are used for the remediation of hexavalent chromium [Cr (VI)]; it reduces 90% of Cr (VI) at pH 2.5. In the microbial bionano remediation process, NPs and microorganisms are used to remove HMs from polluted sites. Various NPs are used to enhance the microbial degradation of HMs. Polyvinylpyrrolidone (PVP)-coated iron oxide NPs were used for the removal of metals, i.e., Cd (II) and Pb (II) by interaction with the Gram-negative bacteria *Halomonas* sp. (combined approach involving bacteria and NPs) [36]. Microbial cells are immobilized in magnetic nanoparticles and are used to remove HMs. Encapsulation, covalent binding/crosslinking, entrapment, and adsorption are some of the immobilization methods. Carbon nanotubes (CNTs) and multi-walled carbon nanotubes (MWCNTs) are also employed to remove HMs. Silica-based nanomaterials or nanocomposites (nano-silica) with modified surface functional groups are nontoxic and found to be more effective for removing HMs [37]. Metal oxide, bimetallic, and graphene oxide nanoparticles are used to remove HMs [38]. Nanomaterials effectively eliminate the HMs due to their increased adsorption capacity metals [39]. Various nanotechnological methods used for the remediation of toxic heavy metals are shown in Figure 2.

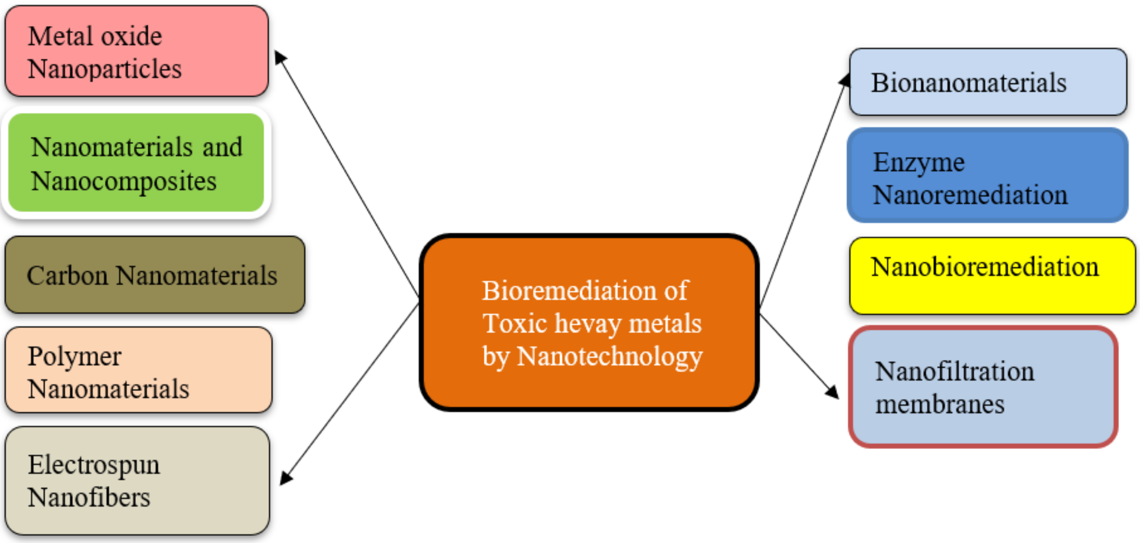

Figure 2. Application of nanotechnology in bioremediation of toxic heavy metals.

4. Bioremediation of Heavy Metals by Microorganisms

4.1. Genetically engineered microorganisms in heavy metal bioremediation.

Several strains were produced by manipulating microorganisms' genetic material (gene exchange) through genetic engineering techniques (recombinant DNA technology). These strains are called genetically modified microorganisms (GMMs) or genetically engineered microorganisms (GEMs). GMM or GEM has been very effective in BR of HMs recently. But some of the problems associated with GEM organisms are the growing strains and microbial species used are not the same to perform a bulk of the BR process, field conditions, hostile field factors, horizontal gene transfer (affect the native microflora) and its survival in harsh environmental conditions [40]. The presence of heavy metals resistance genes alkB, alkB1, alkB2, alkM, xylE, nidA, and ndoB in microbes serves as a marker for biodegradation potential. High-throughput and next-generation sequencing are also helpful in identifying genes involved in the degradation of HMs. Genetic alteration of microbial membrane transporters is also used to remove HMs. CRISPR-Cas gene-editing technology can also be used to modify microbial genes to effectively remediate HMs [41].

5. Metagenomics Approach for Screening of Microbes

Screening of microbes for novel genes involved in BR by conventional methods is a time-consuming and challenging task. Hence, the metagenomics approach and in silico tools are rapid techniques more frequently used. SmashCommunity (Simple Metagenomics Analysis SHell), MG-RAST (Rapid Annotation using Subsystems Technology for Metagenomes), IMG/M (Integrated Microbial Genomes and Metagenomes), RAMMCAP (Rapid analysis of Multiple Metagenomes with Clustering and Annotation Pipeline), MEGAN (Meta Genome

Analyzer), QIIME (Quantitative Insights Into Microbial Ecology), MOTHUR and RemeDB are some of the tools used for metagenomic analysis [42]. The Metagenomics approach is used in analyzing the functional and structural characteristics of microbes involved in BR of toxic heavy metals [43]. Metagenomic sequencing is often used to study the metabolic processes, genome assemblies, and conserved sequences of microbes; pyrosequencing and ligation sequencing are also used to study the potential strains involved in HM remediation. Metagenomic read analysis using a rapid annotation search tool was also used for the functional analysis of metagenomes [44]. The presence of metal resistance genes in microbes is also evaluated by the metagenomic approach [39]. The meta-omics approaches through sequence-based, function-based, and metabolic-based methods are used to identify the target enzyme and strains from environmental microbiota [45]. More genomic research would help better understand the microbial metabolic pathways and mechanisms involved in heavy metal tolerance and detoxification [46].

6. Conclusions and Future Prospects

HMs affect soil and the environment, and there is a need to develop environmentally friendly and cost-effective methods. Many in-situ and ex-situ remediation approaches have been used to manage and remediate HMs accumulation in the environment and the soil. Among these approaches, microbial BR is the most environmentally friendly, efficient, and cost-effective method. Most microbes that resisted HMs are cultured in the laboratory for successful employment in the BR process. Molecular manipulation of heavy metal-resistant strains by genetic engineering techniques such as insertion of metal-resistant genes or operons into bacteria, microbial chromosomal mutation, peptides to the surface proteins, inducing the expression of metal transport systems, etc., are some of the recent strategies to increase the heavy metal resistance and thereby increased BR. Hence, developing hyper-absorbing, hyper-accumulating, or hyper-biosurfactant-producing indigenous strains is the need of the hour for an ecofriendly BR process. The mixed culture microorganisms approach has been reported to be very effective due to their high efficiency, adaptability, and capability of handling multiple contaminations [47].

The recent BR approach is stabilizing heavy metal-resistant bacterial colonies by using carbon microspheres [48], use of clay, chitin, peat, microbial biomass, and agricultural wastes (activated carbon) as bioadsorbents [49], BR by synthetic biology approach uses microbial models such as synthetic microbial community (synthetic genetic circuit), data mining, genome editing, metabolic engineering, synthetic microbial biosensor system are effective in HMs removal [50]. Immobilization of microbial cells in magnetic nanoparticles by encapsulation, covalent binding/crosslinking, entrapment, and adsorption for HM removal has been reported to be a very effective method. Apart from nano bioremediation by NPs, several NMs such as nanocrystals, nanopowders, nanomembranes, nanocomposites, nanotubes, nanosponge, etc., are also used along with living cells (plant/ microbes) for removal of HMs [35]. The selection of NMs, nano iron, and its derivatives, dendrimers, carbon-based NMs, single-enzyme NPs, engineered polymeric, biogenic uraninite, and metals other than iron is based on the pollutant to remediate. Screening microbial metagenomic sequences using bioinformatics tools and RemeDB bioinformatics tool to identify BR enzyme sequences is another approach to selecting suitable organisms for the BR process [43]. Harnessing the BR potential of indigenous microorganisms using the above techniques is greatly useful in saving the environment and soil from heavy metal pollution. Effective remediation of heavy metal contamination by microbial-induced carbonate precipitation (MICP) methods has been reported recently [51]. Microbial bioremediation and nanobioremediation methods for removing toxic heavy metals are shown in Figure 3.

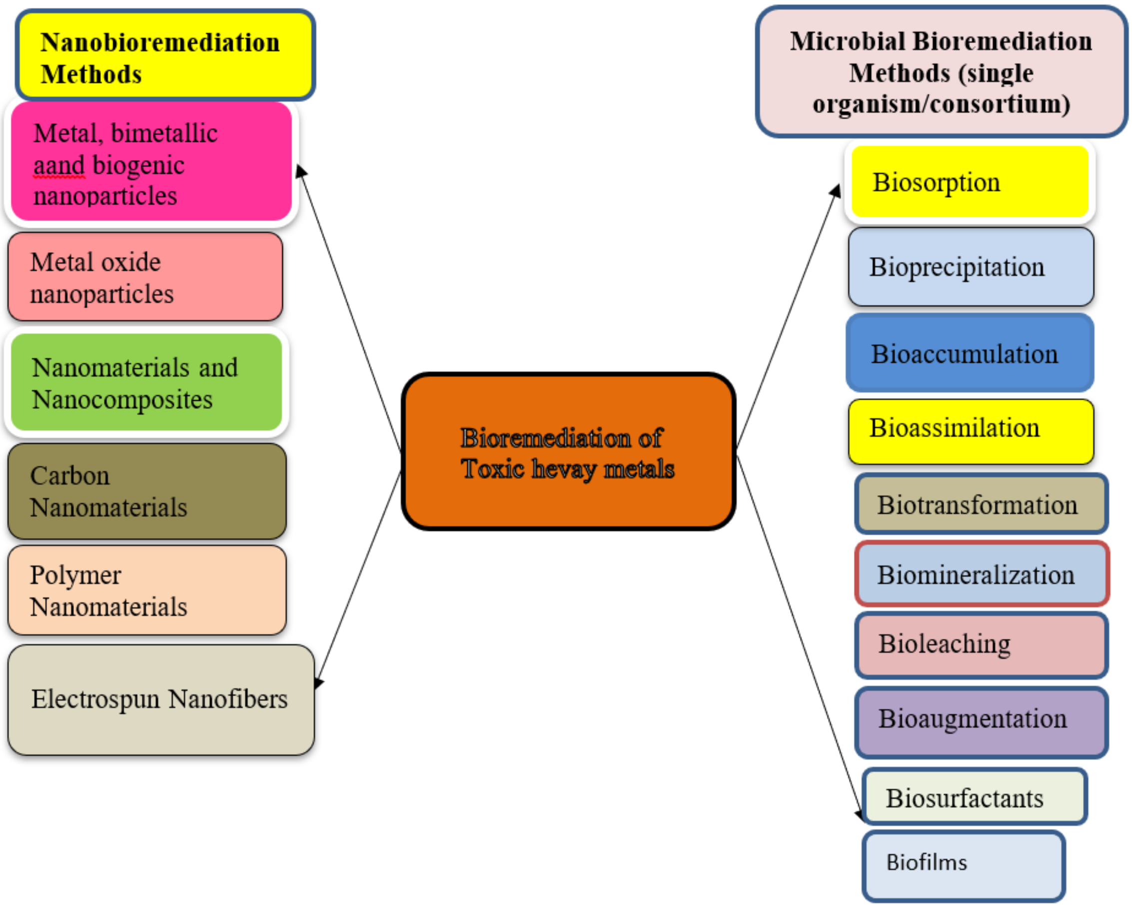

Figure 3. Microbial bioremediation and nano bioremediation methods for removal of toxic heavy metals.

HMs pollution is considered to be a serious threat to human well-being. Accumulation of these toxic HMs in the ecosystem causes life-threatening diseases to all living creatures, including mankind, which need to be addressed on a war footing to save the lives of all living creatures. MBR offers more promising solutions to degrade these toxic HMs by a single organism or a group of organisms. The use of conventional methods to screen the BR potential is time-consuming. Hence, genome sequencing by NGS followed by sequences by in silico bioinformatics tools to identify the novel genes for the production of enzymes, surfactants, exopolysaccharides, etc. This could help us identify potential strains for large-scale production and scale up the bioremediation process to clean up the impact of HMs toxicity on the ecosystem.

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Conflicts of Intrest

The authors have no conflict of interest.

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