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Current Eco-friendly and Sustainable Methods for Heavy Metals Remediation of Contaminated Soil and Water: Special Emphasis on Use of Genetic Engineering and Nanotechnology

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ABSTRACT

Anthropogenic activities have polluted soil and aquatic ecosystems by introducing harmful heavy metals (HMs) such as cadmium, copper, mercury, lead, manganese, nickel, zinc, and others. These HMs lead to serious health conditions in humans like cancer, skin lesions, birth defects, liver and kidney damage, and mental retardation leading to other disabilities. Conventional methods of HM remediation of contaminated soil and water include physical, chemical, biological, and integrated methods. The use of physical and chemical methods, in isolation, has been reduced in practice, owing to their negative impacts, however, work on suitable integrated approaches, and the use of organisms for HM remediation has been in steady progress since past few decades. These approaches have proved to be eco-friendly, cost-effective, and show reduced negative impacts on the environment and biota. However, there is consistent increase in anthropogenic contribution to this problem, so, to keep pace with it, more recently work is in advancement on exploiting the biological system to increase the efficiency of bioremediation, using the latest technologies such as genetic engineering and nanotechnology. This paper provides an overview of the current methods deployed to address this problem; developments made in this field in past few decades, and evokes a research thrust that might lead to novel remediation approaches in the future.

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INTRODUCTION

HM contamination of soil, water, and air has become a threatening problem for human health and the environment. A variety of anthropogenic activities like mining, smelting, manufacturing, excessive use of fertilizers and pesticides, municipal waste, traffic emissions, and industrial effluents have led to widespread contamination of soil and water bodies (Masindi and Muedi, 2018). According to the United States Environmental Protection Agency (USEPA), there are eight HMs, namely lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg) and nickel (Ni), that are commonly found in the environment (Selvi et al., 2019) and as per the Comprehensive Environmental Response Compensation and Liability Act, USA, the maximum permissible levels of HMs in water and other aqueous media are: Pb (0.015 mg/L), Cd (0.05 mg/L), Cr (0.01 mg/L), argon (Ar) (0.01 mg/L), Hg (0.002 mg/L), and silver (Ag) (0.05 mg/L) (Ojuederie and Babalola, 2017). In several countries like India, China, Italy,

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Germany, Korea, Turkey, Hong Kong, Bangladesh, Iran, etc., significant levels of toxic HMs have been reported in soil, surface water, as well as ground-water (Kaonga et al., 2017).

HMs are non-biodegradable, persistent, hazardous, and adversely affect the quality of soil, and the health of plants. They have the potential to enter the food chain through crop plants, and eventually accumulate in the human body due to biomagnification (Emurotu and Onianwa, 2017; Li et al., 2021; Ali et al., 2021). Cobalt (Co), Cu, Cd, Ni, Pb, Zn, manganese (Mn), and iron (Fe) are found in fertilizers and pesticides, which pollute soil, atmosphere, and water, and affect the health of humans and animals (Rai et al., 2019; Briffa et al., 2020). Humans are exposed to 35 metals, either through the environment or through their diet, and 23 of these are HMs. These HMs are antimony (Sb), arsenic (As), bismuth (Bi), cadmium (Cd), cerium (Ce), chromium (Cr), cobalt (Co), copper (Cu), gallium (Ga), gold (Au), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), tellurium (Te), thallium (Tl), tin (Sn), uranium (U), vanadium (V), and zinc (Zn) (Jaishankar et al., 2014). HM accumulation adversely impacts plants, animals, and humans (Table 1). For example, the accumulation of Pb can damage the nervous, endocrine, skeletal, enzymatic, circulatory, and immune systems, while an excess Cd causes lung cancer, pulmonary adenocarcinomas, prostatic proliferative lesions, kidney dysfunction, bone fractures, and hypertension in humans (Engwa et al., 2019). HMs in general can cause life-threatening conditions like cancer, atherosclerosis, Alzheimer's

Table 1. Some adverse effects of heavy metals

Heavy Metals	Harmful effects of heavy metals on plants and humans	References
Arsenic	Protoplasmic poison; It affects the sulphydryl group of cells and impairs the cell respiration, mitosis and cellular enzymes	Gordon and Quastel, 1948
	Causes many disorders such as cardiovascular, dermal, pulmonary, neurological, renal, and metabolic	Muzaffar et al., 2022
Lead	It disturbs various physiological processes I plants; It causes production of reactive oxygen species (ROSs) and very high concentrations ROS can cause structural damage to cells, proteins, nucleic acids, membranes and lipids.	Balali-Mood et al., 2021; Jaishankar et al., 2014
Mercury	Very toxic and exceedingly bioaccumulative; methylmercury is a neurotoxic compound which causes microtubule destruction, mitochondrial damage, lipid peroxidation and accumulation of neurotoxic molecules.	Balali-Mood et al., 2021
Cadmium	Predominantly found in fruits and vegetables; impairs the enzymatic systems of cells, oxidative stress and induces nutritional deficiency in plants. Long-term exposure can lead to cancer and toxicity in skeletal, urinary, reproductive, cardiovascular, central and peripheral neurological, and respiratory organ systems in animals.	Hassan et al., 2020; Rahimzadeh et al., 2017
Chromium	It causes various types of toxicity in plants and causes chlorosis and necrosis; Cr (VI) is mutagenic and is categorized as group I human carcinogen by International Agency for Research on Cancer	Srivastava et al., 2021; Balali-Mood et al., 2021
Aluminum	It has no biological role but is toxic nonessential metal to microorganisms; it is very harmful to nervous, osseous and hemopoietic cells	Nam et al., 2016; Olaniran et al., 2013
Iron	Free iron leads to lipid peroxidation which damages mitochondria, microsomes and other cellular organelles; conversion of ferrous ion to ferric ions releases hydrogen free radicals which attack DNA and cause cellular damage, mutation, and malignant transformations which can cause several diseases.	Cheng et al., 2021; Engwa et al., 2019

disease, Parkinson's disease, etc. (Bakulski et al., 2020). They interact with other pollutants in the environment and make the situation more complicated.

Given the harmful effects of HMs on humans, plants, animals, and other life forms, it becomes apparent that we reduce their availability in the environment. This is necessary because once, HMs are taken up by organisms, they exert their influence through varied mechanisms which have been mostly understood through laboratory studies, and are outside the scope of this review. HMs, being elemental by nature, can't be chemically degraded. The only way to detoxify them in the environment is to either stabilize them *in situ* or remove them from the matrix like soil or water, which has traditionally been done by conventional methods such as physical, and chemical methods (Figure 1). Physical and chemical methods have their own limitations and drawbacks, show varied side effects on the biota and the environment, and thus, are not sustainable. Hence, biological systems are used, either individually or as part of integrated approaches, for more effective, eco-friendly, sustainable, and economical HM remediation in contaminated soil and water. To increase the efficiency of biological remediation or bioremediation, more advanced techniques such as genetic engineering and nanotechnology have also been explored. In this review, we have focused on the bioremediation, and the use of latest technologies to exploit biological systems for more efficient, eco-friendly, and sustainable methods of HM remediation.

MATERIAL AND METHODS

The articles were searched via platforms such as Google Scholar, ScienceDirect, and Google. The search was conducted using key words such as 'heavy metal remediation', 'phytoremediation', 'microbial remediation', 'genetic engineering and heavy metal remediation', 'heavy metal remediation + contaminated soil', 'heavy metal remediation + contaminated water', 'nanobioremediation' etc. Articles that discussed the bioremediation and associated integrated approaches of HM remediation in contaminated soil and water, and the use of genetic engineering and nanotechnology for bioremediation were considered for this review.

BIOREMEDIATION OF HMs

Bioremediation refers to the use of organisms such as plants, bacteria, fungi, etc. to reduce the levels of HMs in contaminated sites. Remediation of HMs using plants is known as 'phytoremediation', while remediation using microbes is referred to as 'microbial remediation'. Numerous studies have been performed on phytoremediation, as plants are better equipped for HM remediation than microbes. Phytoremediation studies have been performed extensively in countries such as China, India, Italy, Spain, Pakistan, USA, Poland, Iran, Egypt, and France (Yang et al., 2022).

Phytoremediation is an ecofriendly and cost-effective method that uses plants to remove, transfer, stabilize, and destroy HMs from contaminated soil and groundwater. Not all plants can serve as good candidates for phytoremediation, so, there have been several studies that have identified plants with good remediation potential. These plants fix or adsorb contaminants, thus cleaning them so that either they disappear from the site or their effects are reduced. Phytoremediation is a complex process and can be accomplished by many mechanisms, such as phytoextraction (plants extract and remove HMs from soil in harvestable parts), phytostabilization (plants reduce HM bioavailability in soil), and phytovolatilization (plants absorb HMs from soil and release them in the atmosphere as volatile compounds) (Yan et al., 2020). Yan et al. (2020) have given a list of plants, such as *Pteris* sp., *Alyssum* sp., *Brassica* sp., *Helianthus* sp., *Thlaspi* sp., *Deschampsia* sp., *Eleocharis* sp., etc., that can be used for remediation of HMs such as As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, and Zn in contaminated samples. Another method is phytofiltration, which can be done

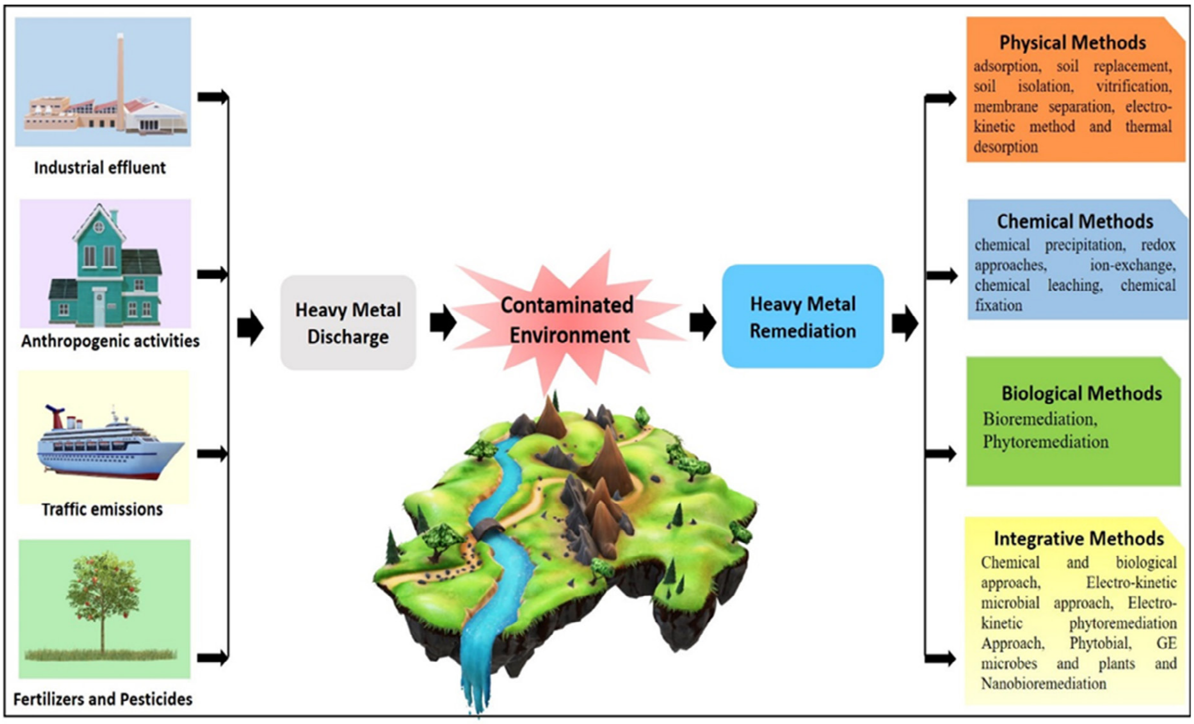

Fig. 1. Major sources of HMs and methods of heavy metal remediation in contaminated soil and water

using roots (rhizofiltration), shoots (caulofiltration) or seedlings (blastofiltration). In this method, the plant parts accumulate HMs from the contaminated groundwater or aqueous waste. Once they become saturated, they are cut off and disposed (Wuana and Okieimen, 2011).

Plants that take up substantial amounts of HMs from the environment are known as 'hyperaccumulators' as they become rich in HMs. Such plants are often annual or semiannual, and after harvesting, they are left in the field for composting, which is the cause of environmental pollution again (Yang et al., 2022). So, efforts should be made such that HMs do not *re-enter* the environment. Hence, new methods have been developed to extract HMs from hyperaccumulators. For example, Zn and Cd can be extracted from the biomass of the plant *Noccacea caerulescens* by hydrometallurgical processes (Hazotte et al., 2017).

A plethora of studies have been performed focusing on the phytoremediation of HMs from contaminated soil and water **(**Ruis and Daniell, 2009; Van Aken et al., 2011; Kubiak et al., 2012; Shim et al., 2013; Das et al., 2016; Mao et al., 2016; Yan et al., 2020) and many reviews have been published in this field (Cherian and Oliveira, 2005; Mahar et al., 2016; Koźmińska et al., 2018; Srivastava et al., 2021; Shen et al., 2022; Venegas-Rioseco et al., 2022; Yang et al., 2022). Some major plants and the HMs they remediate have been included as Annexure 1.

Microbial remediation, using bacteria, fungi, and algae, is another area that has given hope for an environment-friendly method of HM remediation from contaminated soil and water. Bacteria such as *Pseudomonas putida*, *Bacillus subtilis*, and *Enterobacter cloacae* reduce Cr (VI) to Cr (III), and thus reduce its toxicity. *B. subtilis* reduces selenite to less toxic Se, while *Bacillus thuringiensis* and *Bacillus cereus* increase the extraction of Cd from contaminated soil (Chibuike and Obiora, 2014). The formation of siderophore *i.e.,* Fe complexed molecules, is another way by which bacteria facilitate HM extraction. For example, *Azotobacter candidate*sincreases production of siderophore in the presence of Zn (II) (Huyer and Page, 1988). Indirect bioremediation can occur via bioprecipitation using sulphate-reducing bacteria such as *Desulfovibrio desulfuricans* which converts sulphate to hydrogen sulphate which in

Table 2. Consortia for heavy metal remediation of contaminated soil and water

Consortia	Heavy metals	Reference
Pseudomonas pyogenes, Serratia marcescens; Erwnia amylovora; Enterobacter cloacae	Cd, Pb, As, Zn, Cr, Ni, Cu	Nwaehiri et al., 2020
Staphylococcus aureus; Bacillus subtilis	Cd, Pb, As, Zn, Cr, Ni, Cu	Kurniawan et al., 2022
Brochothrix thermosphacta; Vibrio alginolyticus	Al	Purwanti et al., 2019
Bacillus sp.; Rhodococcus sp.; Lysinibacillus sp.	Pb, Cu, Zn	Emenike et al., 2016
Fungi: *Penicillium cataractum; Paecilomyces lilacinus; Antrodia serialis*	Cr, Cu, As, Fe, Mn	Kurniawan et al., 2022

turn reacts with Cd and Zn, and forms insoluble metal sulphides (White et al., 1998). Tarekegn et al. (2020) have summarized list of microbes, which include bacteria, fungi (*Aspergillus* sp., *Candidapara psilosis*, *Saccharomyces cerevisiae*) and algae (*Spirogyra* sp., *Spirulina* sp., *Nostoc* sp.), that can be used for bioremediation of HMs such as Cr, Pb, Cu, Ni, Co, Hg, Zn, Cr, and Cd. Extracellular polysaccharides or exopolysaccharides (EPS), produced by bacteria and fungi can adsorb HMs from contaminated soil and water (Raj K et al., 2018).

Another strategy for HM remediation is to make the soil favorable for bacteria, the process is known as biostimulation, and involves adding nutrients in the form of manure such that bacteria thrive well in the soil and their efficiency for bioremediation increases. The addition of manure decreases the soil pH, which increases the solubility and bioavailability of HMs that can be easily extracted from soil (Karaca, 2004). As against the use of single microbial species, it is better to use microbial consortia, for example algae-bacteria consortia, for HM remediation of contaminated soil and water as they can better tolerate environmental fluctuations and stress due to contaminants (Nguyen et al., 2021) or consortia of more than one genera of bacteria, or bacteria-fungi consortia etc. (Table 2). Bioaugmentation by adding cultured microbes to an existing microbial community is another way to enhance bioremediation potential. Further, bioaugmentation-assisted-phytoremediation, an integrated approach, is another proposed method that has shown better results (Kurniawan et al, 2022).

Microbial bioremediation occurs through several mechanisms. In biosorption, HMs are adsorbed on the cell surface and linked with extracellular polymers, or HMs may infiltrate into the cells. At pH 5-7, several metals such as Cr^{3+}, Cu^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, and Cd^{2+} can be strongly adsorbed on the cell surface, while at pH 2, these metals may be liberated from the microbial biomass (Kisielowska et al., 2010). In bioaccumulation, the rate of absorption of HMs is higher than the rate of losing them. Microbes can tolerate HMs up to a certain concentration, after which they may become toxic to microbes. However, some microbes can tolerate higher concentrations than others and may even biotransform them into less toxic forms (Tarekegn et al., 2020). Thus, such microbes can accumulate large amounts of HMs and assist in bioremediation. Biotranformations using chemical reactions such as oxidation-reduction and methylation-demethylation, bioprecipitation, biocrystallization, and bioleaching of HMs are other mechanisms for microbial bioremediation (Ayangbenro and Babalola, 2017; Tarekegn et al., 2020). Microbial bioremediation is a natural, and cost-effective method. Several studies have been performed in this area and the outcome of many of these studies has been analyzed in many review articles (Dangi et al., 2018; Henao and Ghneim-Herrera, 2021).

In addition to microbes, earthworms can also be used for bioremediation, as they can enhance copper accumulation in plants after earthworm-straw mulching (Hullot et al., 2021) and also act as bioindicators for Pb and Cd in the soil (Hullot et al., 2021; Elyamine et al., 2018).

INTEGRATED METHODS

The limitations of the physical, chemical, and biological methods when adopted individually, encouraged the idea of integrated methods wherein different methods could be brought together to achieve better results with less negative impact on the environment and organisms, including humans.

(i) Chemical and Biological Approach

In this approach, first there is biological treatment of the contaminated site, and then chemical treatment or vice versa can also be adopted. This method is very effective and economical. For example, in one study by Ahmed et al., (2016), for the removal of Cr (VI) from tannery effluent, they adopted chemical precipitation and biological treatment and were successful in recovering 99.3% and 98.4% of total Cr and Cr (VI). Although such studies have been done in research laboratories, their application in the field is yet to be seen.

(ii) *Electro-kinetic and Microbial Approach*

In the electro-kinetic (EK) method, direct current is used to remove fine and low permeability HM particles from the soil without disturbing the composition of the soil. This method is simple to operate, cost-effective, and causes no pollution. But the limitations of this method are the low bioavailability of HMs and the low mass transfer of pollutants from soil to the electrodes. To overcome these limitations, the EK method is integrated with the biological method (Selvi et al., 2019), where both acidophilic and alkalophilic microorganisms are used. The acidophilic bacteria help in the EK process, while the alkalophilic bacteria help in the precipitation of metals. Removal of mercury from the contaminated soil by EK-biological method has been successfully done by using *Lysinibacillus fusiformis* (Azhar et al., 2016) and of Au, Co, and Fe by using gamma Proteobacteria, *Shewanella putrefaciens* CN32 (Varia et al., 2013). For the remediation of As, Cu and Pb, an integrated approach of bioelectrokinetics (bioleaching and electrokinetic method) can be adopted by using *Acidithiobacillus ferrooxidans* (Kim et al., 2012). Bioleaching converts metals to a soluble form, which is favorable for a faster and higher rate of remediation in the EK method.

(iii) *Electro-kinetic and Phytoremediation Approach*

This approach is more recent and more economical than other integrated methods for the remediation of HMs from soil. The laboratory studies have given successful results for EK-phytoremediation of HMs like Pb, Zn, As, Cd, and Cu. In this method, an electric current is passed between electrodes, placed vertically in soil, which separates organic and inorganic molecules. These molecules can then be taken up by plants based on their efficiency, and mechanisms such as phytoextraction, phytoevaporation, rhizodegradation, phytostabilization, or rhizofiltration are involved (Selvi et al., 2019). The plants that have been successfully used in this approach are *Brassica Juncea*- for Cd and Cr (Bhargavi and Sudha, 2015) and *Lemna minor* for As (Kubiak et al., 2012).

(iv) *Phytobial Approach*

This approach is efficient and eco-friendly and utilizes plants as well as microbes. The concept behind this approach is that plants will take up HMs from soil and water, while microbes will degrade the metallic substances. This method is also the cleanest and most cost-effective approach. It can be easily applied to vast areas of contaminated land and groundwater. However, there are certain limitations of this method, like it is restricted to shallow aquifer and soil due to limited length of the roots of plants, fear of transfer of HMs to food chain, need of regular monitoring, requirement of several seasons for remediation to happen, lack of safe disposal practices, difficult metal recovery processes and high cost of recycling. Some of these limitations can be overcome by using plants with deeper roots, transgenic plants that distract herbivores, and suitable evaluation methods. This approach can be integrated with other approaches like EK, bioaugmentation, etc. (Selvi et al., 2019).

The phytobial approach uses free microorganisms to remove HMs. For example, *Sulfurospirillum barnesii, Geobacter*, & *Bacillus selenatarsenatis* (for As removal), *Sporosarcina ginsengisoli, Candida glabrata, Bacillus cereus*, & *Aspergillus niger* have been used under this approach (Littera et al., 2011; Giri et al., 2012). Certain fungi, such as *Glomus mosseae, Glomus geosporum*, & *Glomus etunicatum*, present on *Plantago lanceolata* L., increase the uptake of As by plants (Wu et al., 2009; Orlowska et al., 2012). Algae such as *Dunaliella salina, Ulva* sp., *Enteromorpha* sp., *Cladophora* sp., *Chaetomorpha* sp., *Enteromorpha, Cladophora* a*nd Fucus serratus* can also be used for the remediation of HMs (Gosavi et al., 2004; Al-Homaidan et al., 2011). Aquatic plants such as *Eichhornia crassipes, Pistia stratiotes, Colocasia esculenta, Spirodela polyrhiza,* a*nd Lemna minor* are being used for HM remediation as well (Selvi et al., 2019).

The above discussed integrated approaches hold bright future for HM remediation of contaminated soil and water. However, the HM pollution due to anthropogenic activities will exponentially increase in future, and to cater to this bigger problem, just practicing these approaches will not be sufficient. Thus, the way ahead would be to identify newer plants and microbes with HM remediation potential, and to increase the efficiency of the known plants and microbes. The efficiency of plants and microbes for HM remediation can be enhanced by manipulating their genomes using genetic engineering or using nanotechnology, which are the emerging fields in life sciences, and may well be utilized to address the serious issue of HM pollution in soil and water.

USE OF GENETIC ENGINEERING FOR HEAVY METAL REMEDIATION

Genetic engineering can be used for changing the chemical components of the cell surface and increase the selectivity and adsorption capabilities of plant cells and microbes for HM remediation. Desirable traits such as the ability to endure metal stress, overexpression of metal-chelating proteins and peptides, and enhanced metallic bioaccumulation can be introduced into microbes and plants for HM detoxification and bioremediation. The knowledge from emerging fields such as genomics, proteomics, transcriptomics, synthetic biology, and signaling systems has been used to create genetically engineered microbes (GEMs) and genetically engineered plants (GEPs) (Verma et al., 2021).

Genetically Engineered Microbes (GEMs)

Microbes that can be used for HM remediation may range from bacteria to protozoa, either working individually or in consortium. The members of genera such as *Bacillus*, *Penicillium, Pseudomonas, Flavobacterium, Chlorella, Enterobacter, Micrococcus* and *Aspergillus* are generally involved in bioremediation processes (Diep et al., 2018; Singh et al., 2021). Genetic engineering enhances the bioremediation potential of GEMs, and is carried out by inserting metal-binding proteins and peptides in their extracellular space, screening the microbes with good adsorption properties, & chemically modifying the electrophilic groups on their outer surface (Ueda, 2016; Rangabhashiyam et al., 2014; Ayangbenro and Babalola, 2017). GEMs have been designed to absorb HMs by employing channels, secondary carriers, and primary active transporters. Metallothioneins, phytochelatins, and polyphosphates (polyPs) have been used to sequester HM in these engineered microbes. For example: *Ralstonia eutropha* has been genetically engineered to express mouse metallothionein, a family of proteins that contain cysteine, that enables them to readily bind to and sequester metal ions, on its cell surface, and reduces Cd (II) effects at the contaminated sites (Valls et al., 2000). Metallothionein from the freshwater crab *Sinopotamon henanense* can be genetically modified and expressed in *Escherichia coli* (*E. coli*), resulting in enhanced tolerance and bioaccumulation of HMs like Cu, Cd, and Zn (Li et al., 2021). Similarly, enzymatically synthesized peptides, such as phytochelatins (PCs), from the alga *Chlamydomonas acidophila* can also be genetically modified and expressed in *E. coli* for increased Cd tolerance (Kang et al., 2007).

Further, the *nixA* gene which codes for the nickel transport system in *Helicobactor pylori* was introduced in *E. coli* JM109, which expressed a glutathione-S-transferase pea metallothioneine fusion protein. The resulting transgenic JM109 was able to accumulate four times more Ni (II) than the wild type, without requiring any energy source (Krishnasamy and Wilson, 2000). Similarly, Bang et al., (2000) produced recombinant *E. coli* by introducing the thiosulfate reductase gene (*phsABC*) from *Salmonella enterica* serovar *Typhimurium* which overproduces hydrogen sulfide from inorganic thiosulphate. The activity of thiosulphate reductase enhanced protein synthesis which resulted in the precipitation of cadmium sulphide in the contaminated samples. *Staphylococcus xylosus* and *S. carnosus* have been genetically modified to be used effectively for bioremediation of Ni^{2+} and Cd^{2+} (Samuelson et al., 2000). Endophytic and rhizospheric bacteria in plants at the HM contaminated sites may also be genetically manipulated for remediation (Divya and Kumar, 2011).

Currently, the genomes of many microbes have been genetically manipulated, and as a result, they have become more efficient in removal of HMs from contaminated soil and water samples. However, there are certain concerns about GEMs. The first concern is that, it is challenging to maintain a population of GEMs in the field due to the changing environmental conditions and competition from the native microorganisms (Wu et al., 2006). Secondly, there is danger of GEMs competing with native species when released into contaminated sites, and affecting the biodiversity. There is also the possibility of horizontal gene transfers between them. Further, GEMs might adversely influence the ecological structure of the soil or water ecosystem or get influenced by it themselves. Thus, it is important to understand how GEMs interact with environmental conditions, native microbes, and other biota. We can't rule out the possibility of GEMs becoming invasive and competing with native species, thereby compromising the existence of the latter, and the sustainability of the ecosystem. Moreover, most of the evidences for HM remediation by GEMs have come from laboratory studies, and evidences from field trials are lacking. So, it is uncertain to understand the behavior of GEMs in naturally contaminated sites and their efficacy in HM remediation.

Dixit et al. (2015) have given a list of some genetically modified (GM) bacteria for the remediation of HMs in contaminated sites. However, many other GEMs have also been created (Table 3).

Genetically Engineered (GE) Plants (GEPs)

Plants generally have systems for metal uptake and transport as they supply essential micronutrients such as Zn^{2+}, Fe^{2+}, Cu^{2+}, and Co^{2+}, to their cells. The genes for HM transporters are potential candidates that can be manipulated to increase the efficiency of uptake and accumulation of HMs by various processes such as phytoextraction, phytostabilization, phytovolatization or phytofiltration. Genetic manipulations aim at increasing not only the expression of metal transporters, but also of metal chelators, metallothioneins, and phytochelatins (Ibañez et al., 2016).

In GEPs, also known as genetically modified (GM) plants**,** the desired gene from organisms, such as plants, bacteria, or animals, is inserted into the genome of the target plant by DNA recombination (Van Aken et al., 2011). Apart from introducing new genes, the existing genes can also be modified to increase uptake of HMs, or certain genes may also be silenced to achieve the goal. Genetic engineering allows genomic manipulation of plants with desirable features for

Table 3. Genetically engineered microbes for HM remediation

GEMs	Gene inserted/modified	HM remediation	Reference
Escherichia coli	SpPCS; metalloregulatory protein ArsR; organomercurial lyase; mercury transporter;	Cd^{2+}; As; Hg; Hg	Reviewed by Dixit et al., 2015
Escherichia coli	Mice metallothionein (*pMT-Thio* gene)	Pb^{2+}, Cd^{2+}	Almaguer-Cantú et al., 2011
Corynebacterium glutamicum	ars operons overexpression	As	Mateos et al., 2017
Methylococcus capsulatus	CrR	Cr^{6+}	Hasin et al., 2010
Pseudomonas putida strain	Chromate reductase (ChrR)	Cr	Ackerley et al., 2004
Ralstonia eutropha CH34; *Deinococcus radiodurans*	*mera* gene	Cd^{2+}; Hg	Reviewed by Dixit et al., 2015
Pseudomonas K-62' *Achromobacter* sp AO22	organomercurial lyase gene; mer gene	Hg; Hg	Reviewed by Dixit et al., 2015
Pseudomonas fluorescens 4F39	Phytochelatin synthase (PCS) gene	Ni	Reviewed by Dixit et al., 2015
Deinococcus	Expression of metallothionenin in cell surface proteins	Cd	Misra et al., 2021
Deinococcus radiodurans	Ni/Co Transporter genes (NiCoT): *nxiA* from *Rhodopseudomonas palustris* CGA009 (RP) and *nvoA* from *Novosphingobium aromaticivorans* F-199 (NA)	Bioaccumulation of Co from waste water	Gogada et al., 2015
Deinococcus radiodurans	Expression of *phoK* gene, encoding novel alkaline phosphatase enzyme) from *Sphingomonas* sp.	Uranium (U) from alkaline waste solutions	Kulkarni et al., 2013
Rhodopseudomonas palustris	MerT/P channel MT from *P. sativum*	Hg bioaccumulation	Deng and Jia, 2011
Mesorhizobium huakui subsp. Rengei B3	Human MTL4 gene was fused with nifH and nolB promotors	Increased accumulation of Cd^{2+}	Sriprang et al., 2002

phytoremediation in a much shorter period as opposed to conventional breeding. As a result, fast-growing, high-biomass plants may be developed to improve tolerance against HMs or boost their HM accumulation capabilities, both of which are critical traits of hyperaccumulators. So, understanding the mechanisms of tolerance and accumulation of HMs in plants are imperative to select desirable genes for genetic engineering.

Since HMs induce an excess production of reactive oxygen species (ROS) and induce oxidative stress, their tolerance is generally measured by the strength of the oxidative stress defense system. As a result, increasing the antioxidant activity of plants is the most popular technique for increasing HM tolerance (Kozminska et al., 2018). Metal chelators act as metal-binding ligands that improve HM bioavailability, promote their uptake and root-to-shoot translocation, and mediate their cytosolic sequestration in organelles. Promoting metal chelator production via genetic engineering is another promising approach that may enhance HM accumulation in plants. Further, HM absorption and translocation can be improved by overexpression of genes producing natural chelators (Wu et al., 2010). Metal-detoxifying chelators, such as metallothioneins and phytochelatins, can be used to generate GEPs that are resistant to HMs and have higher efficiency for HM absorption, transport, and accumulation (Ruis and Daniell, 2009).

Table 4. Some examples of genetic manipulation of plants for remediation of HMs

GEPs/Yeast	Gene/s expressed	Remediation of HMs	Reference
Saccharomyces cerevisiae strains (Yeast)	-	Bioremediation and biosorption of Cd^{2+} (pH 3-7) in contaminated water samples	Wei et al., 2016
Beta vulgaris L. (sugar beet)	Glutamylcysteine synthetase-glutathione synthetase (StGCS-GS) gene from *Streptococcus thermophilus* for overproduction of glutathione	Cd, Zn, Cu	Liu et al., 2015
Brassica Juncea	ATP Binding Cassette (ABC) from *Arabidopsis thaliana*	Shoots: Increases accumulation of Cd and Pb	Bhuiyan et al., 2011
Populus alba x. P. Tremula var. *glandulosa*	ABC	Shoots: Increases accumulation of Cd and Zn	Shim et al., 2013
Populus alba x. P. Tremula var. *glandulosa*	ABC from *S. cerevisiae*	Roots: Increases accumulation of Pb	Shim et al., 2013
Arabidopsis thaliana	Iron regulated (IREG)/Ferroportin (FNP) from Psychotria gabriellae	Shoots: Increases accumulation of Ni	Merlot et al., 2014
Arabidopsis thaliana	Copper resistant protein (CoP) from *Pseudomonas* sp.	Shoots: Increases hyperaccumulation of Cu	Rodríguez-Llorente et al., 2012
Nicotiana tabacum	Metal tolerance protein (MTP) from *O. sativa*	Shoots: Increases accumulation of Cd	Das et al., 2016
Nicotiana tabacum	P-1b-ATPase, cation diffusion facilitator (CDF) from *A. thaliana*	Cd transport restricted from roots to shoots	Siemianowski et al., 2014
Manihot esculenta	ZRT/IRT-related proteins (ZIP) from *A. thaliana*	Increases accumulation of Zn in roots; restricted transport to shoots	Gaitan-Solis et al., 2015

There are many hybrid varieties of plants that have been genetically modified to include catabolic genes or specific transporters for more efficient HM remediation (Gullner et al., 2001; Doty et al., 2007). For example, genes for mercuric reductase and γ-glutamylsysteine synthetase may be introduced in plants which increase their resistance to Hg and Cd, and Cu respectively, by accumulating more of them (Gullner et al., 2001; Bittsanszkya et al., 2005). A list of genes that have been manipulated in GEPs to increase efficiency of HM remediation is summarized in Table 4.

Despite the fact that genetic engineering has shown promise in terms of enhancing plant performance in HM remediation, there are still certain concerns and drawbacks. The most important challenge is to design a GE plant as per the ecological conditions of the area (Saravanan et al., 2022) so that it can grow and survive with minimal support. In the long run, the GEPs may become storehouses of HMs, and there could be problems with the disposal of such plants. For example, they can be left in the field to compost or burnt (incineration) (Yang et al., 2022). In both these cases, they will release HM in the soil and air respectively, thus making the problem still bigger. Since, inadequate disposal methods may increase the risk of re-entry of HM in the environment aggravating HM pollution, methods need to be developed that can effectively extract or permanently prevent HM from re-entering the soil or water.

Another concern is that the introduction of GEPs at the contaminated site may threaten the existing biodiversity of the area, by forming superweeds, becoming invasive and outcompeting native species, and cross-pollinating with other plants. They may also change the environment and sustainability of the area (Pasricha et al., 2021).

Because the mechanisms of HM detoxification and accumulation are extremely complex and involve many different genes, genetic modification of several genes to enhance desirable features is time-intensive and ineffective. Another concern is that field testing of GEPs is challenging to carry out in various parts of the world owing to the risk associated with food and environmental safety as there is risk of HMs entering the food chain and leading to biomagnification. This may amplify the toxic impacts of HMs on organisms, including humans.

Moreover, public acceptance of GEMs or GEPs is a major issue that must be addressed before the ‘laboratory technology’ can be brought to the fields.

NANOBIOREMEDIATION

Another alternative for HM remediation that is currently being investigated is nanoremediation. Nanoremediation is a promising technique for removing HMs from a variety of media, including water, soil, and air, and involves the use of engineered nanomaterials (ENMs)/nanoparticles (NPs) to clean up the environment. Nanoremediation has a wide range of potential benefits, including cost effectiveness, environmental friendliness, time saving, increased range of contaminants removal, increased rate of contaminant degradation, a wide range of remedial parameters, short term remediation competence, and synergistic effects when used in conjunction with other methods. Owing to their large surface area and high affinity to different chemical groups, metallic nanoparticles (Fe_2O_3/Fe_3O_4, ZnO, TiO_2 etc.), carbon nanotubes and nanocomposites are most commonly used nanomaterials in HMs remediation (Aragaw et al., 2021) (Table 5). Adsorption, heterogeneous catalysis, employment of electrical fields (electronanoremediation), photodegradation, and the participation of microorganisms (nanobioremediation) are some of

Table 5. Different types of nanomaterials for heavy metal remediation

Nanomaterial Type	Mechanism of remediation	Media	Advantages	Drawbacks
Metal-based	Photocatalysis, adsorption, oxidation, reduction, photodegradation	Soil, water	• Large surface area • Remediation of wide range of HMs and other pollutants • Synergy with other treatments is an important factor to consider	Risk evaluation of nanoparticles for humans and environment is still lacking.
Carbon-based Nanomaterial	Adsorption	Air, soil, water	• Large surface area • Nanocomposites • Environmentally friendly • Compliance with other treatment	• Expensive cost • High saturation • Cytotoxic
Polymer-based Nanomaterial	Filtration	Water	• Use of waste-derived polymer • Synergy with other treatments	• Sensitive to high temperatures • pH-dependent action
Silica-based catalysts	Adsorption	Air, water	• Surface modification versatility • Pore size adaptability • Compliance with other remedial methods	• Dispersed size distribution

the technological procedures used in nanoremediation to remove HMs from polluted soils. For example, FeONPs and CuNPs have been found to adsorb and accumulate Cd from the soil due to their reactivity, electrostatic attraction, and huge surface area (Noman et al., 2020; Manzoor et al., 2021). Similarly, Mateos et al., (2017) reported the impact of carbonaceous nanomaterials like carbon nanotubes to significantly increase immobilization of HM ions like Ni^{2+}, Pd^{2+}, Zn^{2+} and Cu^{2+} in the contaminated soils by improving the adsorption capacity. Other carbonaceous nanomaterials, such as carbon graphene oxide, carry oxygen bonding groups (-COOH and -OH) that improve the electrostatic interaction with Cr (VI) in polluted mediums (Raidongia et al., 2014). Graphene oxide sheets may also conjugate with Cr (VI) due to the presence of a functional group, which leads to an increased rate of HM ion adsorption (Wang D et al., 2017). Recently, nanohybrids have been reported to improve the HM adsorption and removal capacity, for example, decorated zinc oxide (ZnO) nanoparticles (NPs) on graphene oxide nanoparticles can improve Cr (VI) adsorption and removal capacity (Singh et al., 2022).

Because of their simplicity of production, biocompatibility, and redox characteristics, different polypyrrole based absorbants like PPy-polyaniline nanofibers, PPygraphene nanocomposites, PPy-nanoclusters, and PPy-graphene nanocomposites are also employed as an alternative to adsorption for the removal of HMs like Cd (VI) and Pd (II) from contaminated water (Mahmud et al., 2016).

Chemically generated NPs may have drawbacks in terms of chemical usage & self-agglomeration properties. Hence, the usage of nanotechnology is made more sustainable and environmentally safe. Plants, bacteria, yeast, and fungi are emerging as nanofactories with potential use in environmental remediation (Kapoor et al., 2021). The fundamental idea behind nanobioremediation is the breakdown of contaminants by employing nanoparticles that act as catalysts. The incredibly small size of the nanoparticles enables deeper interactions, and since they have a huge surface area per unit mass, more nanoparticles can interact with the environment resulting in the effective bioremediation (Singh and Saxena, 2022). The majority of biogenic nanoparticles that have been examined have been shown to be highly efficient in the remediation of different types of contaminants. The biosynthetic path to nanoparticle synthesis may prove to be a more effective and safer alternative to traditional methods. Hence, the usage of nanotechnology is made more sustainable and environmentally safe through the biofabrication of nanomaterials and the concomitant use of microbes.

The biological synthesis and biomolecule-mediated fabrication of NPs have received a lot of interest in recent years. Biosynthesized silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) from *Nannochloropsis* sp. and *Chlorella vulgaris* have been shown to remove 70% Zn and 60% Pd from wastewater (Adenigba et al., 2020). Further, using AgNPs with the bacterium *Chromobacterium violaceum*, it is possible to recover Ag leached into effluents (Duran et al., 2010). *Noaea mucronata* belonging to subfamily Chenopodiaceae can accumulate Pd, Zn, Cu, and Ni, but if the nanoparticles prepared from *N. mucronata* are used, they can accumulate many folds of HMs (for example, 98% Pd) as tested in water containers by Mohsenzadeh and Chehregani (2012). Biogenic non-toxic CuONPs derived from mint leaves and orange peel extracts have been shown to effectively remove 84% Pb (II), 52% Ni (II), and 18% Cd (II), respectively (Mahmoud et al., 2021). Similarly, nanoparticles derived from *Euphorbia macroclada* have been shown to reduce 92% Pb, 76% Zn, 69% Cd, 75% Zn, and 31% Ni (Mohsenzadeh and Rad, 2011). Iron oxide (FeO) NPs biofabricated from *Aspergillus tubingensis* have been reported to remove over 90% of HMs [Pb (II), Ni (II), Cu (II), and Zn (II)] from effluents with up to five regeneration cycles (Mahanty et al., 2020). In comparison to chemically reduced PdNPs, biosynthesized PdNPs have been reported to exhibit a smaller size and a higher surface-to-volume ratio, resulting in greater catalytic activity for the elimination of Cr (VI) contaminated water (Ha et al., 2016). *Citrobacter freundii* biosynthesized nano-SeNPs were shown to reduce 57% of elemental Hg in polluted soil to insoluble and non-reactive mercuric selenide (HgSe) (Wang X et al., 2017). As a result, these biogenic nanoparticles have the potential to be a versatile and cost-effective remediation strategy for Hg-contaminated soil.

Another study reported, successful nanobioremediation of pharmaceutical effluents containing HMs, primarily chromium (Cr) and lead (Pb), by employing silver (Ag) NPs produced through a more environmentally friendly method assisted by *Bacillus cereus* and supported by alumina. This nano-adsorbent method mediated by microbioal cells has been demonstrated to remove roughly 98.13% (Cr) and 98.76% (Pb) of waste effluents generated from pharmaceutical industries (Kumari and Tripathi, 2020). Nanoremediation may be combined with other methods, such as physical or biological, for a more effective HM extraction. For example, in a study by Akhtar et al., (2020), they tried to remove Cr and Zn from tannery effluents. They adopted several integrated approaches using physical, chemical, biological, and CuINPs and found that the most effective and cost-friendly approach was to use a biologically prepared nanocomposite of CuI.

Thus, nanobioremediation is an emerging technology that can be used for HMs. Future studies must focus on elucidating the mechanisms involved in the synthesis of nanoparticles from biogenic sources, as well as controlling the shape, and size distribution for more efficient nanobioremediation.

CHALLENGES AHEAD

The poor bioavailability of HMs in the environment and the lengthy period (several years) of their existence, make their environmental remediation difficult. Bioremediation is mostly dependent on climate and meteorological conditions, and there is a risk of bioaccumulated HMs entering the food chain if biomass is mishandled. The conventional methods have proved inefficient in present scenario due to the ever increasing usage of HMs. The biological and integrated methods provide some hope, but more advanced remediation methods, *i.e.*, genetic engineering and nanoremediation, are still in their infancy. Techniques such as CRISPR-Cas systems, TALENs, and ZFNs have great potential to tailor unique GEMs and GEPs, having desired characteristics that can effectively carry out HM remediation at contaminated sites, but use of genetic engineering to create transgenic microbes and plants is a costly affair. It has also raised some other serious environmental concerns. Further, with nanoremediation, the extensive usage of NPs has prompted possible concerns about their undesirable impacts on ecosystems and humans. NPs have gained a lot of attention due to their wide range of applications, including nanoremediation. However, their fate & toxicity in the environment have not been sufficiently addressed in the literature. NPs may accumulate in living systems like plants and microbes, and enter the food chain, posing a risk to human health. Nanobioremediation can undoubtedly be a viable method for achieving environmental sustainability once the research gaps regarding their environmental concerns are identified. A more serious challenge is the existence of multiple HMs in one ecosystem, which may require different plants (existing or GM plants) or microbes (existing or GEMs) for remediation. In such circumstances, interaction between various living forms and abiotic components or between living organisms themselves together with abiotic factors is complex, and a subject of future research.

CONCLUSION AND FUTURE PROSPECTS

Biological methods and their integration with other methods offer a more environment-friendly, economical and sustainable approach for HM remediation. Different combinations of consortiums and newer area-specific plants may be explored to exp& the reach of bioremediation, and make the process more economical and sustainable. None of the methods that do not include biological systems may remain sustainable in the long run, hence, the bioremediation potential of organisms can be enhanced by newer techniques such as genetic engineering and nanotechnology. However, these technologies face ethical, social, and practical issues. Basic constraints and knowledge gaps must be addressed in order to ensure public acceptance and safe usage of GEMs and NPs for HM clean-up. As a result, further field studies are required to assess the safety, validity, efficiency, repeatability, fate, intrinsic toxicity, and long-term impacts of NPs, GEMs and GEPs, on HM absorption and bioavailability in polluted soils and water. To reach its promised implications in the environmental remediation, future studies should focus on dosage optimization and effective targeted application of NPs. However, the major challenge for any hypothesis or laboratory study is to validate it through field trials. Without field trials and an actual understanding of the shortcomings and limitations of the method, it is not advisable to accept such methods for their practical applications.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication &/ or falsification, double publication &/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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