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Bioremediation: Genetically Engineered (GE) Organisms for Detoxification of Heavy Metal Contaminated Soils

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Abstract

This review outlines the overview of bioremediation, telling the gravity of present scenario and exploring natural approaches to mitigate the problems. Expounding the mechanisms adapted by naturally occurring organisms to abate the heavy metals from environment. Underlining the advances in biotechnological engineering of plants and microbes to enhance their metal accumulation and tolerance capabilities from contaminated soils addressing potential, limitations and physiological mechanisms. Furthermore, few genes for degradation of pollutants are also mentioned, emphasizing the recent attempts for development of transgenics using these genes, which have resulted in generation of superior genetically modified organisms for detoxification processes.

Keywords- *Bioaugmentation, Biopiling, Bioremediation, Biosparging, Hyperaccumulators, Phytoextraction,Phytostabilization, Phytovolatization, Rhizodegradation*

1. Introduction

Heavy metals or ions are naturally occurring compounds with partially or completely filled d-orbital and having density more than 5 g/cm^3, their concentration in environment is maintained *via* biogeochemical cycles (Gupta *et al.*, 2016). But with the boom in the global economic industrialization, environmental homeostasis has been severely compromised. Anthropogenic activities have disturbed the optimum levels of these metals in the ecosystems that has not only threatened environment but also severely affected human health. Skin exposure, breathing, drinking, or eating are all plausible subjection routes of metal contaminants. Heavy metal toxicity can damage functioning of lungs, kidney, brain, blood composition, and other important organs (Jaishankar *et al.*, 2014). Whereas long-term subjection can lead to degenerative operations that stimulate diseases like muscular dystrophy, Alzheimer's disease, Parkinson disease and multiple sclerosis. Constant long term exposure can even cause Cancer (Jaishankar *et al.*, 2014). The immense issue of environmental pollution and

degrading human health is tremendous now and the present situation screams for critical action to restore the functioning of biogeochemical cycles.

Nature has spellbinding rejuvenating potential and bioremediation is one of the paradigm. The term "bioremediation" is employed in context of utilizing living organisms for the reclamation/cleaning up of a contaminated medium. "Remediate" literally means to repair something and "Bio-remediate" means using biological organisms to rectify the polluted environments like contaminated atmosphere, water bodies and soil. A green ecological technological approach to ameliorate the effects of the accumulated waste products utilize living organisms such as plants (phytoremediation) or microbes which is interweaved under comprehensive term bioremediation.

Bioremediation has been highly valued for environmental cleanup in past few decades, as it not only decontaminates the site, but also reduces the cost of remediation as plants and microbes, the cleanup machinery, utilize solar energy and chemical energy respectively. This saves natural resources and reduces cost expenses. Furthermore, these biological cleaners have capabilities to alter their environments mildly and by developing unique biochemical systems for nutrient acquisition and detoxification, they also control and regulate local geochemical conditions (Sridhar *et al.*, 2002).

2.Mechanisms of Bioremediation

Bioremediation of heavy metals is a technique which enlists natural processes to abolish toxic pollutants from contaminated sites. Its applications are characterized as either *in situ* or *ex situ*. As the name suggests, *in situ* application treat infected soil or water at the same location in which they are found whereas *ex situ* techniques need excavation of infected soil or water before treatment. *In situ* technique is the least expensive one.

2.1. *Bio-remediating Bacteria*

Microorganisms are known to sustain metals since early times.Wide range of transition metals are present at active centres of enzymes. The chemical properties of the metal have been acquired for catalyzing reactions or for sustaining protein structure. These metals are required in minute amounts for normal cell operations and their absorption is subject to intrinsic homeostatic mechanisms (Valls *et al.*, 2002). Thus, from a physiological standpoint, metals fall under three broad categories i.e. a) essential or non-toxic (e.g. Mg or Ca) b) essential but fatal at higher concentrations (e.g. Cu, Fe, Mn, Zn etc.) and c) toxic due to their ability to denature proteins (e.g. Cd or Hg).Different microorganisms display varied responses to toxic ion which is based upon a number of definite resistance mechanisms like impermeability of metals, inactivation of metals, alteration of site of inhibition or bypass means.. These tolerance mechanisms are often plasmid borne, which promote dispersion from one cell to another (Valls *et al.*, 2002).

Taking into consideration bacterial responses to different metals enabled us to utilize this natural potential in bioremediation techniques. In situ bioremediation uses non-engineered naturally occurring microorganisms which can be bio stimulated by addition of nutrients, such as N and P, surfactants or oxygen (Watanabe, 2001). But in such treatments, the nature of microbial ecological niches are unforeseeable. There are different in situ bioremediation practices which are popular these days like (1)*biosparging*, which imply introduction of air under pressure below water table to enhance ground water oxygen concentrations and intensify the rate of biological degradation of contaminants by indigenous bacteria, (2)*bioventing*, it uses low air flow to spare enough oxygen to support microbial activity at the residual contamination sites, (3)*biopiling*, in which burrowed soils are mixed with soil amendments, positioned on treatment area and bioremediated using forced aeration (Das *et al.*, 2014).

Diverse strains of *Pseudomonas putida, Escherichia coli, Ralstonia eutropha, Sphingomonas desiccabilis, Mycobacterium marinum, Bacillus idriensis*, etc., have genes in their genomes, which allow them to selectively bio-remediate toxic metal compounds (Valls et al., 2000; Ackerley et al., 2004; Kube et al., 2005; Parnell et al., 2006; Schue et al., 2009; Liu et al., 2011).

A chief example of heavy metal resistant bacteria is *Cupriavidus metallidurans* CH34 (renamed from *Ralstonia metallidurans* and formerly known as *Alcaligenes eutrophus*) discovered by Frank Reith (1976)is a gram negative, non-spore forming bacteria. It is known for its distinctive property of precipitating metallic gold and forming gold nuggets from gold (II) chloride. It is an aerobic chemolithoautotroph and is of industrial importance used in heavy metal remediation and sensing (Gupta *et al.*, 2016).

Lysinibacillus sphaericus CBAM5 is a gram positive, spore forming bacteria found in soil. It is a heavy metal tolerant stain isolated from Easter Planes of Colombia. *L.sphaericus* biomass has been known to bioremediate metals such as cobalt, copper, chromium and lead (Peña-Montenegro TD *et al.*, 2015).Native Colombian isolates of *L.sphaericus* OT4b.31 and IV (4)10 showed heavy metal biosorption in living and dead biomass, both

expressing the S-layer proteins (Peña-Montenegro TD *et al.*, 2015).

Microbacterium profundi strain Shh49^T is a gram positive, aerobic bacteria isolated from a deep-sea sediment sample collected from the East Pacific polymetallic nodule region (Wu *et al.*, 2015). It is believed that this strain comprises genes associated to oxidation/reduction of metals. This strain have possible potential to oxidize Fe from ferrous to ferric form on the basis of detection of two ferroxidases. Genome of strain Shh49^T was sequencedby using Solexa paired-end sequencing technology by a whole-genome shotgun (WGS) strategy (Robinson *et al.*, 1990).

Geobacter spp, an anaerobic bacteria has been reported for bioremediation of Uranium from groundwater. The apparatus used for degradation is electrically conductive pili build between the bacteria and pollutant material, utilizing it as an electron source. Pili expression increased the value and development of uranium deduction for each cell and culminate the fixation of hexavalent uranium U (VI) to tetravalent uranium U (IV) (Mirlahiji *et al.*, 2014).

2.2. Phytoremediation by Hyperaccumulators

Weyens and co-workers (2009c) defined phytoremediation as: *"An in situ solar powered remediation technology that requires minimal site disturbance and maintenance resulting in low cost and high public acceptance."* Phytoremediation is a process in which plants assimilate contaminants (heavy metals) from the polluted environment into their roots and leaves. The phytoremediation of heavy metal contaminated soil primarily involves their extraction from soil and assimilation into plant organs (mainly in roots and leaves) or their inactivation in the soil (Lombi et al., 2001). Surprisingly, phytoremediation process was registered by man more than 300 years ago, however the scientific intervention for selection and development of potential plants was not initiated until early 1980's (Lasat, 2000).

In the past 2 decades, a number of workers have addressed the role of plants in remediating contaminated soils and ground waters (Paterson *et al.*, 1990; Shimp *et al.*, 1993; Schnoor *et al.*, 1995; Simonich and Hites, 1995; Watanabe, 1997). Using plants to remove toxins from contaminated ecosystems proved to be an environmentally safe and cost-effective technology. This necessitated a deep understanding of mechanism of phytoremediation in order to select highly effective plant species as all plants are not suitable for remediation. Such plant species with high metal accumulation potential are termed as hyperaccumulators. Chang and Corapcioglu (1998) entailed the basic processes for soil remediation, these include: (1) modification of physical and chemical properties of contaminated soils; (2) release of root exudates, that increases organic carbon; (3) improve aeration by releasing oxygen directly to the rhizosphere, as well as increase the porosity of the upper soil layer; (4) inactivation and stabilization of chemicals; (5) alteration of co-metabolic microbial and plant enzymatic transformations of recalcitrant chemicals; and (6) minimize the vertical and lateral penetration of pollutants to groundwater by extracting available water and reversing the hydraulic gradient.

Enumerating the factors influencing toxin accumulation within living plants, include: (1) physical and chemical properties of the chemical (e.g. water solubility, vapor pressure, and molecular weight); (2) environmental conditions (e.g. temperature, pH, organic matter content, and soil moisture content); (3) plant characteristics (e.g. type of root system, and type of enzyme exudates). Some of the mechanisms for phytoremediation of heavy metal contaminated soils include:

2.2.1.Phytoextraction/phytoaccumulation

In phytoextraction the contaminant is not degraded rather it is accumulated in plant tissues. Baker and Brooks (1989) defined heavy metal hyperaccumulation as accumulation of a minimum of 0.1% dry weight in plant tissues for Co, Cu, Cr, Pb and Ni, while for Cd as 0.01% and for common elements like Fe, Zn and Mn as 1% of the element by dry weight in plant tissues. More than 400 hyperaccumulator plants have been registered including members of the Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Fabaceae, Lamiaceae, Poaceae, Euphorbiaceae to name some. Amongst these, several Brassicaceae species are capable to hyperaccumulate more than one metal (Prasad and Freitas, 2003). Metals can be recovered from such plants after harvesting them which can later be used for various industrial purposes.

2.2.2 Phytostabilization

Phytostabilization is a method that can be practiced to minimize migration of contaminants in soils, and implies alteration of soil conditions, such as pH and soil moisture content by plant roots. Many root exudates cause precipitation of metals, thereby reducing bioavailability. This mechanism has an advantage over phytoaccumulation, that the disposal of the metal-laden plant is not needed. By selecting and maintaining a suitable cover of plant species, along with desired soil modifications, it may become feasible to stabilize certain metals in the soil (Cunningham *et al.*, 1995), and thus, averting the negative interaction of these metals with other biota of that niche.

2.2.3. Phytovolatilization

This method involves conversion of a contaminant into a less toxic volatile form, thus removing it from the soil or water (Terry *et al.*, 1995) at an adulterated site. For instance, some plants, in association with microorganisms, can convert selenium to dimethyl selenide, which is a less toxic, volatile form of selenium. This accounts for the usefulness of phytovolatilization as a cheap means of removing selenium from sites dumped with high concentration selenium wastes.

2.2.4. Rhizodegradation

The biological treatment of a toxin by enhanced bacterial and fungal activity in the rhizosphere of certain vascular plants is stated as rhizodegradation (Sridhar *et al.*, 2002). Microorganisms are often symbiotically associated with plant roots in rhizosphere, an area of very active microbial activity (Anderson *et al.*, 1993, 1994; Schwab *et al.*, 1995; Jordahl *et al.*, 1997; Siciliano and Germida, 1998).

Many plant exudates are chemically similar to the contaminants and can be used as co-substrates. To elucidate, phenolic substances secreted by plants have been found to stimulate the growth of PCB (polychlorinated biphenyls) degrading bacteria (Donnelly and Fletcher 1994a; Fletcher and Hegde, 1995; Fletcher et al., 1995). Some studies have described enhanced degradation of pentachlorophenol in the rhizosphere of wheat grass (*Agropyron cristatum*) (Ferro *et al.*, 1994), increased initial mineralization of surfactants in soil-plant cores (Knabel and Vestal, 1992), and enhanced degradation of TCE (trichloroethane) in soils collected from the rhizospheres. Anderson et al., (1993) provides a review of microbial degradation in the rhizosphere.

2.3. Bioremediation via Mutualistic Plant-endophytic Bacteria

The intrinsic decontaminating properties of plants alone are not sufficient for cleaning of heavily polluted sites, in such cases, mutualistic endophytic bacteria play a crucial role in enhancing the phytoremediation prospect of indigenous plants. Endophytic bacteria also protect plants from the toxic effects of pollutants accumulated in them. Arachevaleta *et al.*, (1989) reported that during phytoremediation, endophytic bacteria through its metal resistance and sequestering system lessens the metal toxicity in host plants and escalates metal translocation to aerial parts, hence minimizing the stress in niche. Some examples of endophytic bacteria and their corresponding plants are enlisted in Table 1.

3. Genetically engineered (G.E) vs Natural Bioremediating organisms

Phytoremediation is an inexpensive, green technology, albeit challenging. As stated by Alkorta *et al.*, (2004), phytoremediation does not imply to simply plant some hyperaccumulators in any heavy metal contaminated soil, it is rather a highly specific and exacting scientific application. It requires

profound knowledge and field experience to select apposite species according to the type of metal and degree of contamination, considering the geo-climatic conditions of that area (Alkorta *et al.*, 2004). The ideal species for practical use must certainly have a substantial magnitude of metal uptake and accumulation within a minimum duration (Gratão *et. al.*, 2005).

In nature, copious metal hyperaccumulating plants are found, belonging to a wide range of families of vascular plants (Reeves and Baker, 2000; Prasad and Freitas, 2003). However, most of the known species are metal selective, have slow growth rate, produce smaller amounts of biomass, and can be cultivated in their natural habitats only (Kamnev and van der Lelie, 2000). Additionally, the use of natural hyperaccumulators is further restricted, if little is known about their agronomic characteristics, physiology, breeding potential, and pest management, as they grow often in remote areas and in certain cases, their niche is threatened by mining, industrialization and others activities (Cunningham *et al.*, 1995).

These constraints therefore engender the development of transgenic plants with enhanced metal uptake and accumulation capacities and also increased tolerance of toxicity that can remediate different geological sites in considerable time scale. Such characteristics could be achieved by overexpressing natural or modified genes encoding antioxidant enzymes or those that are involved in the biosynthesis of glutathione and phytochelatins (Gratão *et al.*, 2005). The overexpression of a gene encoding a rate limiting gene product would be expected to lead to a faster overall rate of the pathway and to more efficient phytoremediation (Pilon-Smits and Pilon, 2002). However, it must be considered that metals rarely occur alone in the environment and an adaptive tolerance may be essential for several metals simultaneously (Karenlampi *et al.*, 2000).

Likewise, though naturally occurring microbes have significant potential to eradicate many environmental pollutant without any external

intervention, the arrival of genetic engineering in 1970's enabled the possibility of reasonable designs of bacteria to catabolize specific compounds, which could be released in environment as a potent bioremediation agents.

Table 1.

Plant species	Endophyte	Contaminant	Reference
Brassica juncea	*Rhizobium leguminasarum*	Zn	Adediran et al.(2015)
Sedum alfredii	*Sphingomonas* SaMR12	Zn	Chen et al. (2014)
Salix alba	*Pseudomonas putida* PD1	Cd	Khan et al. (2014)
Salix alba	*Burkholderia sp.* HU001, *Pseudomonas sp.* HU002	Cd	Weyens et al. (2013)
Festuca arundinacea	*Neotyphodium coenophialum*	Cd	Soleimani et al. (2010)
Orychophragmus violaceus	*Flavobacterium sp.*	Zn	He et al. (2010)
Brassica juncea	*Enterobacter aerogenes*	Ni, Cr	Kumar et al. (2009)
Ricinus communis	*Pseudomonas sp.* M6, *Pseudomonas jessenii* M15	Ni, Cu, Zn	Rajkumar et al.(2008)
***Brassica napus, Microbacterium sp.*G16**	*Pseudomonas fluorescens* G10	Pb, Cd	Sheng et al. (2008)
Lycopersicon esculentum	*Methylobacterium oryzae*	Cd	Madhaiyan et al. (2007)

Examples of endophyte-assisted phytoremediation of different heavy metals

4. Genetically engineered (GE) organisms for augmented bioremediation

Microbes contain a plethora of catabolic genes for detoxification of contaminants that can be incorporated into target organism through means of biotechnological applications. (Figure 1.)

4.1.Genetically engineered microbes

The vital entity responsible for large-scale transformation in the environment are microorganisms and their metabolic pathways. Microbes eliminate toxic metals *via* absolute mineralization or co-metabolism in aerobic or anaerobic conditions (Dvořák P et al., 2017). The advancement of recombinant DNA technology accommodated the transformation of bioremediation from suppositious practices to empirical one. The objective of this advancement is to engineer complete microbe, their biodegradation pathways and the involved enzymes for mineralization of pollutant. These constructed *superbugs* are expected to provide an environmental friendly, economical and feasible technologies for heavy metal removal. In prokaryotes, intracellular-to-extracellular transporters encoded by plasmids are responsible for the resistance against metals (Qiu *et al.*, 2014). To illustrate, the ability of *Enterobacter* to grow in the presence of Cd and Zn is speculated to be controlled by a plasmid (Gerhardt *et al.*, 2009).

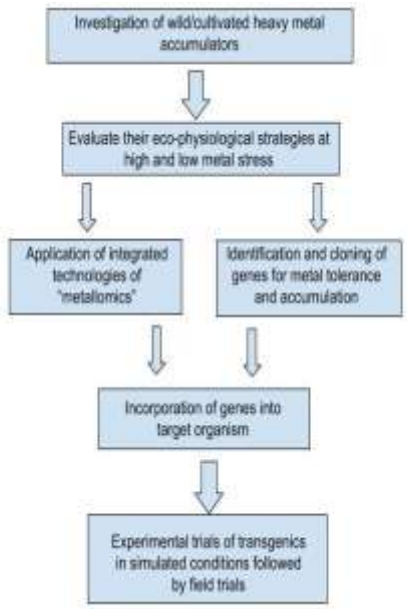

Fig. 1: The use of genetic engineering to breed superior bioremediating organisms with escalated potential (Modified after Karenlampi et al., 2000, Prasad, 2004b).

In late 1980's Chakrabarty and co-workers described the preparation of recombinant *Pseudomonas putida* strains in breaking down crude oil by the *plasmid-assisted molecular breeding*, which involved propagation of groundbreaking catabolic capabilities *via* directed bacterial conjugation and plasmid transfer. It is a Gram-negative, rod-shaped, saprotrophic soil bacteria and sometime called as multi-plasmid hydrocarbon-degrading *Pseudomonas.*

There are many approaches for construction of genetically modified microorganism for bioremediation application. First and foremost approach is – the identification of microbial host suitable for modification. Over the years, many microbial hosts have been scrutinized for application in bioremediation process, but no single, naturally obtained bacterial strain possess all desired characteristics of an optimal degrader. Though soil bacteria could fulfil many of these requirements due to the conditions they face naturally in the niches in which they live, which include exposure to environmental contaminants. These microorganism have versatile metabolic lifestyle that allow them to adapt changes, sometime adverse (temperature, osmotic imbalance, oxidative stress) (Dvořák P et al., 2017).

The second approach is pathway construction, extension and regulation. The tenacity of pollutants is attributed to the absence of complete degradative pathway in a single organism or in wild strain (Kulshrestha S, 2013). Construction of genetically engineered microorganisms have the degradation capabilities of different microbial communities which improve the efficiency and efficacy of any catabolic pathway. Third approach is qualification of enzyme specificity. Any metabolic pathway is majorly mediated by enzymes that is produced by transcription and translation of specific genes. Genetically engineered organisms (GEM's) are developed by hybrid genes clusters which reshape their enzymatic activity and substrate specificity.

With the onset of technology, new approaches will lead us to techniques like *bioagumentation*, in this process bacteria are introduced into the contaminated sites which may embrace genetically engineered (GE) bacterial strains. Genetic engineering may prove to be efficacious tool that assist in optimization of bioremediation. Key genes vital for heavy metal bioremediation can be obtained from nature for raising the engineered bacteria without caring the primal host. The GE bacteria have elevated degradative capacity and have been indicated for degradation of many pollutants under controlled conditions. Genetically engineered bacteria involved in bioremediation are listed in Table 2.

4.2. Genetic engineering of plants

Transgenic plants, engineered with desired detoxifying catabolic genes either from prokaryotic or eukaryotic source, have been proved to have accelerated remediation characteristics for decontamination of heavy metal rich ecosystems. To elucidate, Glutathione synthase (GSH), a substrate for synthesis of phytochelatins, is produced by translation of *gcsgs* gene via ATP-dependent reactions in cytosol or chloroplast in the plants (Válega et al. 2008). Phytochelatins (PCs) are heavy metal binding proteins that are known to equip the organism to take up metals by providing them resistance to a wide range of heavy metals (examples include Cd, Hg, Cu, Ni, Zn, Ag, and Pb) (Bhargava et al. 2012; Yang et al. 2005). The genes conferring metal chelators like phytochelatins, metallothioneins and GSH have been successfully expressed in transgenics and have shown to boost sequestration and compartmentalization of heavy metals in the host's tissues (Roy et al. 2015). An encapsulation of various transgenics with magnified phytoremediation amplitude developed in recent decades is provided in Table 3.

Transgenic grey poplars (*Populus tremula x P. alba*) modified to over express *gshI* gene from *E. coli* are proved to have two to four times increased production of GSH in leaves and thus, have enhanced PC production (Peuke and Rennenberg 2005). Through greenhouse experiments, it was eminent that these transgenic poplars have much higher decontaminating potential when compared to wild types. Another stratagem for the accumulation of mercury is based on cloning and expression of metallothionein gene, *mt*, from mouse (Ruiz *et al.*, 2011) and gene for mercuric ion binding protein, *MerP*, and *ppK* gene (Nagata *et al.*, 2009). Furthermore, alfalfa plants modified to co-express human *GST* and *CYP2E1* genes are found to clean soils with mixed contamination (heavy metals (Cd, Hg)/ organic pollutants) (Zhang and Liu 2011; Zhang *et al.*, 2013). Overexpression of λ-ECS gene from *Saccharomyces cerevisiae* in transgenic *A. thaliana* plants by increasing GSH synthesis escalated remediation of as polluted soils (Guo *et al.*, 2008).

4.3. Genetic engineering of endophytic bacteria

Even though successful manipulation for phytoremediation has been achieved for both plants and bacteria, bioengineering of bacteria is much simpler than that of higher plants (Doty 2008; Weyens et al. 2009c). Thus, it is more feasible to manipulate endophytes with desired detoxifying catabolic genes in order to decimate the toxins which are taken up by the host plants (McCrady et

al. 1987; Trapp et al. 2000). The fundamental employment of transgenic endophytes for enhanced phytoremediation is to supplement the activity of natural hyperaccumulators. In soil, these transgenic endophytes can undergo genetic recombination i.e., can exchange a gene segment and thus naturally transmit these desired catabolic genes to other endogenous soil bacteria (Taghavi *et al.*, 2005).This horizontal gene transfer (HGT) is a natural method of bringing novelty in evolution and adaptation in bacteria (Ochman and Moran 2001). Phytoaccumulation of metals is predicated on similar genetic manipulating techniques in both plants and their endophytes (Doty 2008; Kärenlampi et al. 2000; Kuffner et al. 2008). A recent experiment focused integration of GSH gene, *gcsgs*, in *Enterobacter* sp. CBSB1, an endophyte of *Brassica juncea*, not only increased tolerance to metal but also intensified hosts potential to phytoremediate Pb-Cd contaminated soils via increased secretion of GSH in plant tissues (Qiu et al. 2014).

Table.2 Engineered bacteria involved in remediation of heavy metals.

Bacteria	Gene of interest	Heavy metals	Reference
***Synechococcus* strain PCC 6301**	*smtA* and *smtB*	Zn and Cd	Soleimani *et al.*, (2015), Stetter *et al.*, (1996)
HgR *E. coli*	*merA*	Hg	Gomes *et al.*, (2013)
***Salmonella choleraesuis* strain 4A**	*smtAB*	Pb	Naik et al. in (2012)
***Proteus penneri* strain GM10**	*smtAB*	Pb	Naik et al. in (2012)
Deinococcus radiodurans strains	Hg (II) resistance gene (*merA*)	Hg(Radioactive waste sites from nuclear weapons)	Brim et al. (2000)
***P. putida* strain**	Chromate reductase (ChrR)	Cr(Bacterial cultures, as well as cell suspensions)	Ackerley et al.(2004)
***E .coli* strain**	PCS gene expression (*SpPCS*)	Cd^{2+}(Microbial sorbents for Cd removal)	Kang et al. (2007)
E. coli JM109	Cd transport system and MT(namely M4)	Cd(Removal of Cd from aqueous solution)	Deng et al. (2007)
Mesorhizobium huakuii B3	Phytochelatin synthase (PCS)gene expression	Cd^{2+}(From rice fields)	Sriprang et al.(2003)
Pseudomonas fluorescens OS8; Escherichia coli MC1061; Bacillus subtilis BR151; Staphylococcus aureus RN4220	*MerR/CadC/ZntR/Pmer/PcadA/PzntA* (expression of *luxCDABE* genes)	Cd, Zn, Hg and Pb (water-suspensions and extracts of soils)	Bondarenko et al. (2008)
Achromobacter sp AO22	Hg reductase expressing *mer* gene	Hg (In situ bioremediation of contaminated sites)	Ng et al. (2009)
Sphingomonas desiccabilis and Bacillus Idriensis strains	Overexpression of *arsM* gene	As (Laboratory conditions)	Liu et al. (2011)
Methylococcus capsulatus (Bath)	CrR genes for Cr (VI) reductase activity	Cr (VI) (Cell-associated Cr removal in laboratory conditions)	Hasin et al. (2010)
E. coli strain	Metalloregulatory protein ArsR (overexpressing *ELP153AR*)	As (Contaminated drinking and ground water)	Kostal et al. (2004)
***Enterobacter* sp. CBSB1 (endophyte of *Brassica juncea*)**	*Gcsgs*	Pb, Cd	Qiu et al. (2014)

Table 3. Examples of Genetically Engineered Organisms for enhanced bioremediation

Transgenic Plant/Bacteria	Gene/Protein of Interest	Metal Ameliorated	Reference
Populus deltoides	*merA*	Hg	Che et al. (2003)
A. thaliana	*merA & merB*	Methyl mercury	Bizily et al. (2000)
Cottonwoods	*merA & merB*	Phenylmercuric acetate	Lyyra et al.(2007)
Spartina alterniflora	*merA & merB*	Mercuric chloride	Czako et al. (2006)
A. thaliana	Selenocysteine lyase	Se	Pilon et al. (2003)
Nicotiana glauca	Phytochelatin synthase	Cd,Pb,Cu and B	Martinez et al. (2006)
A. thaliana	*YCF1*	Pb and Cd	Song et al. (2003)
Poplars	*gshI*	heavy metals	Peuke and Rennenberg (2005)
A. thaliana	*AsPCS1,GSH1*	Cd, As	Guo et al.(2008)
Nicotiana tabacum	*mt1*	Hg	Ruiz et al, (2011)
Nicotiana tabacum	*ppK*	Hg	Nagata et al.(2009)
Medicago sativa	*GST* and *CYP2E1*	Cd	Zhang et al.(2011)
Brassica juncea	*STM* gene	Se	LeDuc et al.(2004)
Brassica juncea	*gshI*	Cd	Zhu et al.(1999b)
A. thaliana	*PsMTA*	Cu	Evans et al.(1992)
A. thaliana	*tyMT*	Cu,Cd	Zhang et al.(2004)
Vicia faba	*AtMT2a, AtMT3*	Cd	Lee at al.(2004)
A. thaliana	*ZntA*	Pb, Cd and Zn	Lee et al.(2003a)
Arabidopsis	*ZAT1*	Zn	van der Zaal et al. (1999)
Arabidopsis	*AtNramp1*	Fe	Curie et al. (2000)
N. tabacum	*NtCBP4*	Ni	Arazi et al. (1999)
Arabidopsis thaliana	*ArsC and γ-ECS*	As	Dhankher et al. (2002)
Liriodendron tulipifera	*merA*	Hg	Rugh et al. (1998)
N. tabacum	*CAX4*	Cd, Zn, Mn	Korenkov et al. (2007b)

5. *Conclusion*

The present alarming scenario of high concentration of heavy metals in soil demanded the development of specifically designed GE organisms for bioremediation. A review of pertinent scientific research present us with fortunate experiments of GMO's under controlled laboratory conditions. But it is toilsome to test their potential in natural field conditions, due to the instability of transgenes in plants as they tend to lose introduced gene. Though in microbes, there are lesser chances of losing transgene but they are capricious in nature and cannot be precisely predicted for end results. Furthermore, it will be interesting to see the role of the public biosafety authorities in receiving regulatory acceptance for successful implementation of bioremediatory practices using genetically modified plants and bacteria.

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