

A review of microbial bioremediation with genomic interaction and heavy metal toxicity on human health

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Abstract

On this earth due to rapid industrialization for achieving highest growth rate Man is continuously over exploiting the natural resources without taking care of the future generations. Industries does not treat their effluents before discharging it in the rivers or underground which contaminates the water at below. Contaminants like hexavalent chromium which are highly cancerous and damages various vital organs gets assimilated into the soil and water table thereby creating number of diseases which are beyond treatment too, thus people lose their lives. Hence analysis of Underground Water to test its fitness for human consumption by performing all the standard physio-chemical tests on various drinking water parameters and to test the presence and removal of chromium and other toxic metals in it by using biosorption properties of various agents gained attention. Till now Studies conducted includes the effect of contact time, temperature, solution pH, elution studies, adsorbent dosage and effect of metal concentration by using isotherms along-with Field Emission Scanning Electron Microscope, Atomic Absorption Spectroscopy and Fourier Transform Infrared Spectroscopy on water sample of selected location with Recombinant DNA Technology and various dimensions of Biotechnology. . However More methods and techniques can be conducted to test the toxic level of chromium in environment using various bioremediation agents for its removal. Many researches are going on to provide natural remediation methods which are useful for humans for easy removal of contaminants from our Environment. So it becomes necessary to analyze sources of heavy metals, development of new techniques, status across India along river Ganga, health problems due to heavy metal toxicities, drugs required to treat heavy metal poisoning, biological agents as cheap source of bioremediation when compared to conventional remediation techniques thereby stepping ahead in the field of bioremediation to remove contaminants from environment.

Keywords: Bioremediation; Hexavalent chromium; Ganga river toxicity; Heavy metal drugs; Genetic interactions

1. Introduction

Treatment of water for removal of heavy metal is necessary to control its deleterious effect. Out of them chromium has received great attention due to its toxic effect on living body. Frensius et al. , 1988[1] observed that various sources of chromium are industrial water waste discharged untreated. Earlier ion exchange method was mostly used for removal of toxic heavy metals but it is highly costly, therefore researchers concluded the use of natural adsorbent as they show property of biosorption on their surface due to their surface Affinity and Bioaccumulation to remove these toxic metals. Various research work was conducted and it was found that microbes in modified conditions adsorb Zn Cd and other impurities like nitrates and sulphates etc. from waste water and render water free from impurities[2]. Hence Above technology of Biosorption using Enzymes and surface interactions is seeking attention for biosorption of Chromium From Ground water Bodies using various dimensions of Biotechnology with identification isolation and purification of microbial enzymes for use which will benefit human civilizations. Previously Many bacterial strains, fungal strains have been deployed for removal of heavy metals from Industrial effluents [3].

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Studies conducted on the Heavy metals found in Drinking water according to Titration and Inductive Coupled Plasma Microscopy Method on the Ground Water Samples of Western parts of Uttar Pradesh Region In India toxic metals were far above standard BIS level for Manganese Aluminium Barium Cadmium Chromium Cobalt Copper and lead In Bulandshahar Meerut Muzzafarnagar Saharanpur Aligarh causing Different behavioural physiological and cognitive changes interferes endocrine system and metabolism of the body, impairs natural healing, disrupts coronary arteries Adrenal Glands that reduces production of hormones. Mostly Chromium metal are leaked into the environment when used as alloys, cement, paints, paper and rubber industry. In terms of quantity Low-level exposure of chromium irritates the protective coating of our body that is skin and causes ulceration. [4] It is known carcinogenic metal and leads to problems of respiratory system. Long-term exposure to chromium can lead to liver and kidney damage, and damage of nervous and circulatory system. Researchers have found that chromium gets accumulated in aquatic plants and animals that increases the danger for their life along with of humans who consumes them and hence they get exposed to toxic hexavalent chromium metal [5, 6].

Bioremediation is an innovative environment friendly and latest technology being adopted by developed economies to remove toxic chemicals from our environment however developing economies are still using costly system to clean up the environment.

Microorganisms uptake heavy metals actively. The microbial cell walls contains polysaccharides lipids and proteins along-with active chemical groups such as carboxylate, hydroxyl and amino ions [7, 8].

2. Various sources of toxic heavy metals

On reading various sources of heavy metals it shows that heavy metals are found naturally on crust of the earth. Earth is formed of three layers crust mantle and core. All these layers contains metals as Silicon Aluminium in Crust, silicon Magnesium in Mantle and Nickel Iron in Core. But all these metals were in balanced proportion since the formation of the earth but Due to huge rise in the use of heavy metals, it has caused heavy increase in the quantity of metallic substances in both aquatic and terrestrial lives affecting floras and faunas. Heavy metal pollution has risen mainly due to activity caused by humans which is the major reason of pollution, mainly of mining of the metal, metal casting factories, smelting that is extraction of metals from their ores by applying heat and chemical treatments. Activities like dumping of wastes, land fillings, excretion, faulty livestock managements and chicken manure, roadworks and automobiles also causes addition to heavy metal rise. Along with it another cause of heavy metal contamination in agriculture has been the use of fertilizers, pesticides, and other agricultural product which contains heavy metals in binding forms and get released when it comes in contact with air soil and water. Various nature assisted corrosion and evaporation of metal from water and soil, suspension of sediments, erosion of soil, geological weathering, volcanic eruptions are another major causes of heavy metal contamination [9]. Going through various journals and news articles it is also found that leather and tanning industries excrete large amount of heavy metals like chromium into main rivers thereby contaminating the water table available for human consumption.

Arsenic (As)	• Pesticides, fungicides, metal smelters
Cadmium (Cd)	• Welding, electroplating, pesticides, fertilizer, batteries, nuclear fission plant
Chromium (Cr)	• Mining, electroplating, textile, tannery industries
Copper (Cu)	• Electroplating, pesticides, mining
Lead (Pb)	• Paint, pesticides, batteries, automobile emission, mining, burning of coal
Manganese (Mn)	• Welding, fuel addition, ferromanganese production
Mercury (Hg)	• Pesticides, batteries, paper industries
Nickel (Ni)	• Electroplating, zinc base casting, battery industries
Zinc (Zn)	• Refineries, brass manufacture, metal plating, immersion of painted idols

Figure 1 Sources Of Heavy Metals From Various Industries

3. Status of heavy metal toxicity across India

Present review article also analyses the status of of heavy metal pollution found in different cities of Ganga across India. Study Areas Included Allahabad, Bhagalpur, Diamond Harbour, Haridwar, Ganga Sagar Kanpur, Mirzapur, Varanasi Rivers like Kali, Ramganga, Yamuna, Gomti Ghagara, Son, Damodar etc. According to Reja et al. , 2009 [10] It is found that the level of various heavy metal are far above the acceptable limit due to which through the food chain from aquatic life to Human bioaccumulation and biomagnification occurs posing serious threat on the life of human civilization. So it is utmost necessary for the Industries to minimize their metallurgical load before discharging into River.

Table 1 Concentration of heavy metals (micrograms per Litre) across the cities of the Ganga at different Sites

	As Cd	Cr	Cu	Co	Fe	Hg	Mn	Ni	Pb	Zn
Allahabad	- ND-10 -	ND-18	ND-30	--	--	--	--	--	18-86	26-122
	- -- -	5-68	8-46	--	6300 -11, 900	--	18-94	--	9-181	4-79
	- 20-330 -	3-290	--	--	536-1939	--	--	60-345	166-284	10-55
Berhampore	- 1-2 -	10-18	3-7	--	365-1744	--	181-712	41-84	8-21	65-95
Bhagalpur	- ND -	BDL-1090	ND-120	--	--	-	-	BDL-120	-	BDL-870
Dakshineswar	- ND-3 -	16-22	4-8	--	792-1413	--	85-436	35-44	5-97	42-83
Diamond Harbour	- -- -	--	5-90	--	30-560	150	90-350	-	12-62	150-710
						-620				
Gangasagar	- -- -	--	2-90	--	40-320	100	60-290	--	11-38	30-520
						e490				
Haridwar	- -- -	43-196	101-178	--	--	--	28. 7e16	--	108-690	113-219
Kanpur	- -- -	ND-390. 8	0. 6-52. 1	--	59. 3-27, 956	--	17. 7-272. 6	ND-63. 7	4. 3-57. 5	0. 1-49. 49
	- 75 -	20	20	--	--	--	--	700	10	150
Kaushambi	- -- -	--	ND-1000	--	ND-600	--	--	--	ND-9	ND-980
Kolkata	- -- -	--	1-49	-	90-420	160	90-490	-	17-76	20-280
						-950				
	- 5-6 -	ND	3-33	--	13-5490	-	22-1780	45-240	50-530	5-293
Mirzapur	- 13. 37 -	--	38. 0	10. 50	19. 7-72. 77	--	34. 25	67. 25	34. 25	94. 25
	e32. 73		-157. 80	-26. 77			-105. 55	-176. 13	-185. 75	-423. 75

Palta	-	ND-3	13-21	4-7	--	884-2345	--	123-417	35-53	5-15	68-111	
Rishikesh	-	--	--	32. 1-58.1	--	--	--	--	BDL-36.7	BDL	BDL-1349.7	
Rishikesh-	-	600-13100	--	ND-36000	--	--	--	--	--	2400-26900	ND-106300	
Allahabad												
Rishra-Konnagar	-	0. 043	0. 281	0. 155	--	--	--	--	--	0. 041-0. 058	0. 545-0. 691	
	-	-0. 088	-0. 391	-0. 322								
Uluberia	-	ND-3	13--24	3-6	--	353-1584	--	139-172	34-83	3-52	58-84	
Varanasi	-	100-160	160-1090	1700-2000	--	120-150	--	--	100-900	--	500-600	
	-	--	-	-	--	--	0. 23	--	--	--	--	
	-	ND - 8. 4	1. 2-29. 6	2. 4-18. 1	--	--	--	--	1. 6-60. 8	--	31. 2-185. 2	
	-	ND-51	17-72	47-168	--	342-1981	--	23-67	-	36-86	15. 8-217	
	-	--	100-480	273-305	--	2950-6870	-	1000-2800	643-1120	20-240	9440-15320	
	-	43	20	20	--	--	--	--	550	10	200	
	-	11. 41-39. 24	41. 8-70. 16	19. 42-43. 72	--	83. 17-117. 7	--	40. 62-68. 83	31. 28-61. 11	80. 55-134. 8	31. 73-71. 37	
	-	20-150	30-90	20-80	--	590-680	--	--	220-305	60-190	200-270	

3.1. Ranges of various parameters of Water Quality

In India and across world various Environment Protection Agencies are responsible for enforcement of environmental laws. National Green Tribunal formed in 2010 is presided over by Environmental Specialists who regulates air water and soil protection laws and hence conserves rights of Life available to citizens. Many Regulating agencies are responsible for proper Monitoring of Different parameters to check water quality Index as per standard Protocols.

These are calculated as permissible ranges to analyze water quality across the globe. These parameters include Physical and chemical parameters for drinking water purposes. Agencies Like WHO EPA EU etc. provides standards for testing and estimation of water quality. These parameters are directly or indirectly relates to the health of humans and other living beings .

Table 2 Permissible limits of Different Heavy Metal Toxicity in Water

Parameters	WHO	EPA	EU	Egyptian
PH	6.5-8.5	6.5-8.5	6.5-8.5	6-9
Cond., $\mu\text{S}/\text{cm}$	-	-	400 GV*	-
Colour	-	15 units	-	-
TDS, mg/L	1000	500	500	1500
PO_4^{3-} , mg/L	0.3	-	-	0.3
SO_4^{2-} , mg/L	400	500	250	400
Cl^- , mg/L	250	250	250	600
NO_3^- , mg/L	45	45	-	45
Ca, mg/L	200	-	100 GV	200
Mg, mg/L	150	-	50	150
Na, mg/L	200	-	150	-
K, mg/L	-	-	12	-
Al, mg/L	-	0.05-0.2	0.2	-
B, mg/L	0.3	-	1 GV	-
Fe, mg/L	0.3	0.3	0.2	1
Mn, mg/L	0.05	0.05	0.05	0.5
Cu, mg/L	1	1	0.1	1
Zn, mg/L	5	5	0.1 GV	5
As, mg/L	0.01	0.01	0.05	0.05
Cd, mg/L	0.005	0.01	0.005	0.01
$\text{Cr}_{(\text{total})}$, mg/L	0.05	-	0.05	0.05
Hg, mg/L	0.001	-	0.001	0.001
Ni, mg/L	-	0.1	0.05	0.1
Pb, mg/L	0.05	0.005	0.05	0.05
Se, mg/L	0.02	0.05	0.01	0.01
<i>WHO 1993; US.EPA 2001; EU 1997; ECS, 1994; GV*: Guide value.</i>				

4. Molecular mechanism of bioremediation

According to research work conducted by Dixit et al. , 2015[11] Microbes have evolved an elaborate mechanism to tolerate heavy metal poisoning in their genome. Bacteria which are mercury resistant harbours the mer operon in its genome. It contains some functional genes along-with other genes as regulator, promoters and operators. Out of them most common are mer A and mer B which is responsible for coding of Mercuric ion reductase and organo-mercurial lyase compounds. Lyase enzymes are mainly responsible for removal of toxic organo-mercurial compounds into elemental mercury which is in almost non toxic states with the help of reductase enzyme. Similarly Chromate Reductase enzyme is found in chromium Resistant bacteria that catalyses the enzymes are ChrR YieF Nema which are all located in the cytoplasm. Enzymatic Reduction of CrVI to CrIII involves transfer of electrons from electron donors.

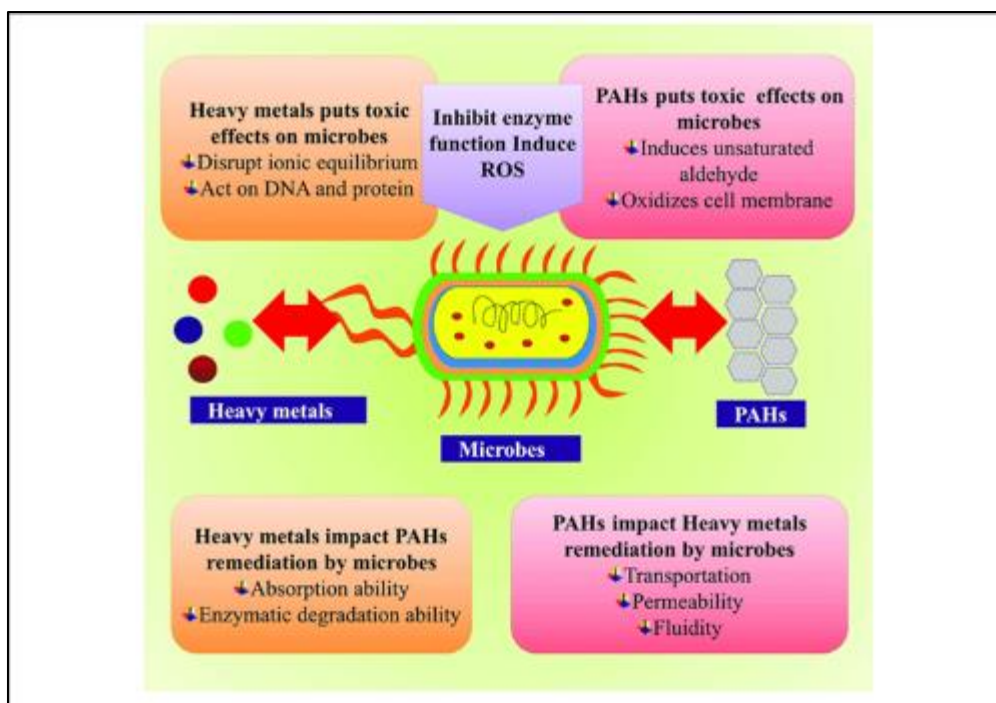


Figure 2 Interaction of Heavy metals with Microbes

4.1. Mechanism Associated with Bioremediation By Microorganism

Microbes are unicellular organisms with a very simple life cycle. They breathe and carry out various metabolic processes through common body surfaces. Microscopic and detailed analysis of the cell wall of the body shows that there are several functional groups on the surface of the bacterial cell wall, such as carboxylimidazole sulfhydrylphenol and others, through which bacteria interact physicochemically and metal ions are absorbed. These metal ions join the functional groups and after the interactions are formed. inactive which will eventually be deleted.

4.2. Bioremediation by Adsorption

Research shows that microbes can absorb heavy metals from binding sites in the cell structure without energy. Among the various reactive compounds associated with bacterial cell walls, extracellular polymeric substances are particularly important and can have significant effects on acid-base properties and metal adsorption. Studies on the metal binding behavior of extracellular polymeric substances (EPS) have revealed a high capacity to complex heavy metals through various mechanisms, including proton exchange and metal microprecipitation. Recent studies have characterized and quantified protons and adsorbed metals in bacterial cells and EPS-free cells to determine the relative importance of EPS molecules in metal removal. In the current scenario, bioremediation research and practice is still hampered by the lack of understanding of the genetics and genome-level characteristics, metabolic pathway and kinetics of the organisms involved in metal adsorption. This leads to the inability to model and predict process behavior and develop a natural bioremediation process.

4.3. Bioremediation by Physio-Bio-Chemical Mechanism

Based on the research done, biosorption is a process in which the biosorbent has a higher affinity for sorbate (metal ions) and continues until a balance is reached between the two components. *Saccharomyces cerevisiae* acts as a biosorbent for the removal of Zn(II) and Cd(II) through an ion exchange mechanism. *Cunninghamella elegans* became a promising sorbent against heavy metals released from textile wastewater. The breakdown of heavy metals involves energy in cellular metabolism. The combined active and passive way of bioremediation of toxic metals can be called bioaccumulation. Fungi have emerged as potential biocatalysts to access heavy metals and convert them into less toxic compounds. Some fungi such as *Klebsiella oxytoca*, *Allescheriella* sp., *Stachybotrys* sp., *Phlebia* sp., *Pleurotus pulmonarius*, *Botryosphaeria rhodina* have metal binding potential. Fungal species such as *Aspergillus parasitica* and *Cephalosporium aphidicola* can degrade Pb(II)-contaminated soils through the biosorption process. Hg-resistant fungi (*Hymenoscyphus ericae*, *Neocosmospora vasinfecta* and *Verticillium terrestre*) were able to biotransform the Hg(II) state to a non-toxic state. Many pollutants are hydrophobic, and microbes appear to take up these substances through biosurfactants and direct cell-pollutant interactions. Biosurfactants form stronger ionic

bonds and complexes with metals before desorption from the soil to the water phase due to the low surface tension[12].

Bioremediation can also involve aerobic or anaerobic microbial activity. Aerobic degradation often involves the addition of oxygen atoms to reactions mediated by monooxygenases, dioxygenases, hydroxylases, oxidative dehalogenases, or chemically reactive oxygen atoms generated by enzymes such as ligninases or peroxidases. Anaerobic degradation of pollutants involves initial activation reactions followed by oxidative catabolism mediated by oxygen-free electron acceptors. Techniques that reduce the mobilization of heavy metals from polluted places by changing the physical or chemical state of the toxic metals are called immobilization. Solidification treatment involves mixing chemical agents in contaminated areas or precipitation of hydroxides. Microbes mobilize heavy metals from contaminated sites through dissolution, chelation, methylation and redox transformation of toxic metals[13]. Heavy metals can never be completely destroyed, but the process changes their oxidation state or organic complex, making them more soluble in water, less toxic and precipitable []. Microorganisms use heavy metals and trace elements as final electron acceptors or reduce them through a detoxification mechanism used to remove metals from a polluted environment. Microorganisms remove heavy metals through mechanisms they use to obtain energy from metal redox reactions, process toxic metals through enzymatic and non-enzymatic processes.

Looking at different studies, it was concluded that the two main mechanisms of bacterial resistance are detoxification (changing the state of the toxic metal and making it inoperable) and active outflow of the toxic metal from the cells. In the soil there is a fundamental reaction (oxidation and reduction) between toxic metals and microorganisms; Microorganisms act as oxidizers of heavy metals and cause them to lose electrons, which are accepted by alternative electron acceptors (nitrates, sulfates, and iron oxides). Under aerobic conditions, oxygen acts as an electron acceptor, while under anaerobic conditions, microbes oxidize organic pollutants by reducing electron acceptors. The microorganism takes energy for growth by oxidizing the organic compound Fe (III) or Mn (IV) as an electron acceptor. Anaerobic degradation of organic pollution is stimulated by the increased availability of Fe(III) for microbial reduction. Metals used as terminal electron acceptors are called dissimilar metal reduction. Biodegradation of chlorine from pollutants occurs through reductive dechlorination, where pollutants such as chlorinated solvents act as electron acceptors during respiration. Microorganisms reduce the state of metals and change their solubility, such as *Geobacter* species, and transform uranium from a soluble state (U6+) to an insoluble state (U4+). Various defense systems (exclusion, partition, complexation and synthesis of binding proteins and peptides) reduce stress caused by toxic metals. Accumulation of heavy metals by microorganisms can be studied by expression of metal-binding protein and peptides (phytochelatins and metallothionein). These metal binding protein transcription factors can mediate the hormonal and redox signaling process in the context of toxic metals (Cd, Zn, Hg, Cu, Au, Ag, Co, Ni and Bi) []. *Synechococcus* sp. (cyanobacterial strains) have been reported in relation to *smtA* gene expression and metal-binding protein production [81]. *Ralstonia eutropha* was genetically modified to express murine metallothionein on the cell surface and reduce the toxic effects of Cd(II) at contaminated sites. Expression of different proteins and peptides in *Escherichia coli* regulates cadmium accumulation. Coexpression of the precursor glutathione (GSH) and phytochelatins (PC) resulted in a 10-fold increase in PC, ultimately increasing cadmium accumulation twofold. The natural resistance pathways of heavy metals (Hg and Ar) in microorganisms are regulated by the metal regulatory protein[13].

5. Health problems associated with heavy metal poisoning

On examining the ground data it is inferred that due to industrial activities human civilisation have been exposed to large amount of toxic metals leading to human poisonings. Out of them Lead Arsenic And Chromium Leads the race. The sources of toxicity are water food and air through which we come in contact with these metals. Biological process like bioaccumulation increases the quantity in human body leading to interference in the growth proliferation damage repairing process etc in the body. These metals generate reactive oxygen species. It weakens the antioxidant defence mechanism of the body, inactivates enzymes and causes oxidative stress. These metals causes genetic instability gets binded to specific macro and micro molecules. DNA repairing gets impaired as these toxic metals damages the DNA structure. They bind with the hydrogen bonds of the DNA and causes unwinding which is popularly known as Carcinogenecity. Presently we have sufficient information of metal carcinogenicity which requires immediate preventive and effective treatment. This is the Chelation mechanism of microbes through which heavy metals get inhibited by them. [14]

In the below given diagram mechanism of DNA toxification due to heavy metal is discussed.

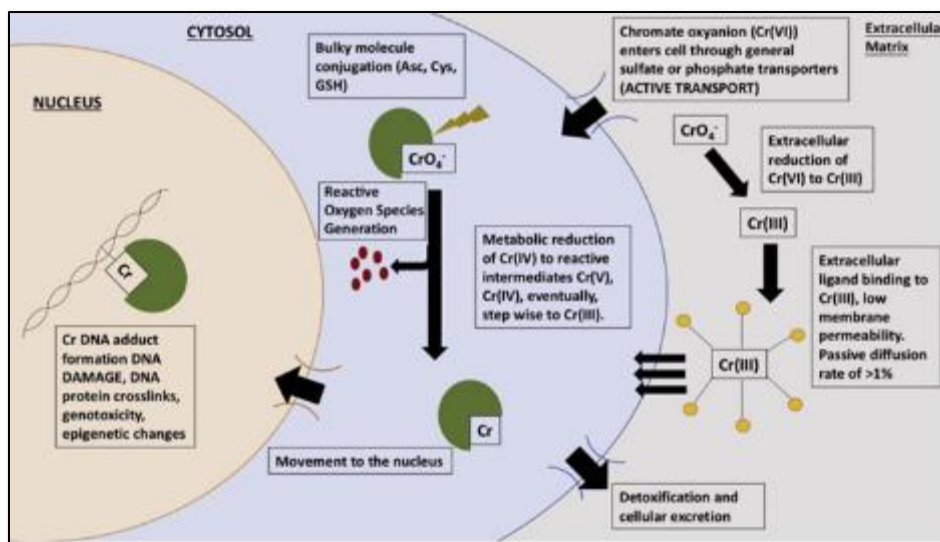


Figure 3 Chromium- DNA interaction

5.1. Drugs to treat heavy metal poisoning and its limitations

On contrary heavy metals which are important for different biochemical process, but they become toxic when found in above concentrations. To be specific they create oxidative stress in the molecular structure of the body by reducing the antioxidant levels. They also alter the general conformation of DNA and protein thereby inhibiting their functioning. Mainly Chelation therapy is involved in the removal treatment of heavy metals toxicity. It is a chemical process that takes place when ligand and ions of the metal interacts which leads to creation of a complex structure which is Ring-like. The ligand molecule has an ion or molecule which behaves as a donor, containing electrons in lone pairs and occurs as monodentate to polydentate. Every metal has a different chemical reaction with a ligand, so a specific chelating agent is required for every metal. Chelating agent with an antioxidant led to improved results. Mode of action of drugs primarily involved in the removal of metal poisoning are Dimercaprol Calcium Disodium Versenate Penicillamine Succimer. Dimercaprol is used in the treatment of lead Arsenic and Mercury toxicity which is administered via deep Intra Muscular injections only mixed in peanut oil base. It is excreted out through urine and bile. Very effective in treatment of renal failure due to metal Toxicity. Similarly Calcium Disodium Versenate And Penicillamine are effective for Pb and As Poisoning[15].

6. Role of biotechnology in heavy metal removal

Biotechnology plays a vital role in the field of bioremediation. Many bacteria, fungi etc are responsible for environmental restoration. Science associated with such studies is known as Environmental Biotechnology. Many biophysical techniques generally used in biotechnology like northern and southern blotting helps in recognition of DNA and RNA structures. Microbial activities are involved in the removal immobilisation and detoxification of heavy metals found in the environment as a part of Microbial Remediation[16]. These activities can be utilised to clean toxic metal wastes before they get entered into the wider natural environment. Advanced process used in Biotechnology are based on Biosorption and Bioprecipitation, however other process that uses binding at specific sites of specified macromolecules have future potential too. Technologies using these phenomena are currently used to control pollution from diverse sources, like mine workings and smelters[15]. With the recent advances in Genetic Engineering it is now Relatively easy to construct (GEM) through Reshuffling of genes promoters etc. To bioremediate Heavy metals Recombinant DNA technology, PCR, Antisense RNA technique and Site directed Mutagenesis are used like *Pseudomonas aeruginosa* is used for remediation of naphthalene compound[16, 17]. Biotechnology provides us various techniques of Protein and DNA sequencing which are used for remediating the metals from contaminated zones. Similarly gene knock-out process is very useful in creating microbes for creation of microbes which can reduce contaminations. In the field of water treatment trickling filters are used that contains biofilms where microorganisms grows and removes heavy metals. Apart from above mentioned Field Emission Scanning Electron Microscope, Atomic Absorption Spectroscopy and Fourier Transform Infrared Spectroscopy are used to minutely study the interactions of microbial structure with heavy metals.

7. Isolation and identification of bacteria

One of the main procedure to perform any bioremediation process is to first select suitable microbes for remediation. Many chemicals are available in laboratories but selection of specific medium is very necessary. Under standard lab Manuals and Regulations various nutrient medium are used. The nutrient medium provides all the necessary ions for the growth and development of microbes. The nutrient medium are used under well sterilised conditions in calculated amount for proper estimation. For Isolation and identification Nutrient agar medium, McKonkey Agar, Wilson Blair Soyabean casein Digest Agar Medium, blood agar etc are used for Biocharacterisation and Isolation. All these nutrient growth medium are specific for particular microorganism and hence supports growth[18 19].

8. Bacteria identified for bioremediation

Review conducted shows list of many microbes involved in metal ion removal. *Bacillus licheniformis*, *Pseudomonas fluorescence*, *Escherichia Coli*, *Arthrobacter* spp, *Pseudomonas veronii*, *Burkholderia* spp, *Kocuria flava*, *Bacillus cereus*, *Sporosarcina ginsengisoli* *Bacillus subtilis*, *Pseudomonas*, *Aeromonas flavobacteria*, *Chrobacteria* *Nocardia* *Corynebacteria*. These are the various bacteria which are capable of breaking down metallic ions through interaction of their membrane and hence neutralises them. They are the chelating agents which ultimately decreases the quantity of toxic ions and they can be easily identified by specific stains by measuring the dried cells and minimum inhibition coefficient. Presently research work is widely in progress to understand the genome of microbes through protein and DNA sequencing methods which provides the essential data on the structure and protein forming capabilities of microbes while dealing with metals in environment. Analysis of genes of microbes before and after interaction with metallic ions gives us opportunity to easily recognise the proteins and hence enzymes which can be used in ready made form to reduce metal contamination. If humans are aware of genes responsible for chelating the metals those enzymes can be easily immobilised and extracted out which would help in direct application over the contaminated zones. Likewise many microbes are now available in lyophilised state which when sprinkled over contamination areas readily absorbs the metals of interest rendering those areas contamination free. In order to study the interactions of bacterial culture with heavy metals various parameters like inoculations, serial dilutions, Ph of the solution, contact time, temperature of the reacting mixture, adsorbent dosage isothermal studies are minutely experimented to derive a specific conclusion[20].

Alongwith this their is a list of Genetically Engineered Microbes for Remediation:-

Table 3 Genetically engineered bacteria for remediation of heavy metals

Heavy Metal	Initial Conc. (ppm)	Removal Efficiency (%)	Genetically Engineered Bacteria	Expressed Gene
As	0.05	100	<i>E. coli</i> strain	Metalloregulatory protein <u>ArsR</u>
Cd ²⁺	-	-	<i>E. coli</i> strain	<u>SpPCS</u>
Cr ⁶⁺	1.4–1000	100	<i>Methylococcus capsulatus</i>	<u>CrR</u>
Cr	-	-	<i>P. putida</i> strain	Chromate reductase (<u>ChrR</u>)
Cd ²⁺ , Hg	-	-	<i>Ralstonia eutropha</i> CH34, <i>Deinococcus radiodurans</i>	<u>merA</u>
Hg	-	-	<i>E. coli</i> strain	<u>Organomurcurial lyase</u>
Hg	7.4	96	<i>E. coli</i> JM109	Hg ²⁺ transporter
Hg	-	-	<i>Pseudomonas</i> K-62	<u>Organomercurial lyase</u>
Hg	-	-	<i>Achromobacter</i> sp AO22	<u>mer</u>
Ni	145	80	<i>P. fluorescens</i> 4F39	Phytochelatin synthase (<u>PCS</u>)

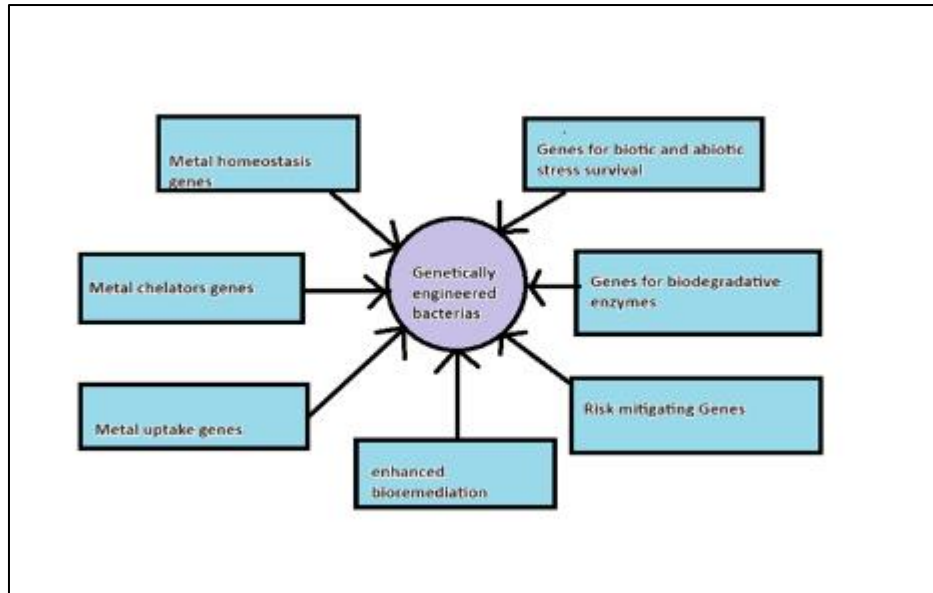


Figure 4 Diagrammatic Representation of Process of Bioremediation of Genetically engineered Bacteria and Role of its various genes

9. Conclusion

Massive industrialization have adverse effects like water, air, food and soil contamination leading to degradation of soil health. All of the above components are important as biotic and abiotic parts of ecosystem. As so much complexity is involved in the usual methods deployed for bioremediation, the use of microbial cells has arisen and given most suited alternative as a time-saver for bioremediation[21]. However, it is also admitted that Bioremediation Technology has limitations too; many microorganisms are unable to break toxic heavy metals into harmless metabolic products, and these causes inhibition of microbial activity itself. Hence Modification techniques should be developed so that the external membrane proteins of the microbes should be capable enough to bio-absorb the heavy metals in their cell. For future purpose, Researchers should focus on all those parameters which affects the improvisation of in-situ techniques which involves the use of genetically engineered microorganisms their evolution, applicability and adaptability to counter adverse conditions while dealing with heavy metal polluted regions. [22]. Lab techniques should be evolved in such a way that it could recognise easily the amino acids or peptide bonds which are directly involved in remediating the heavy metals The public should also accept bioremediation technology as best alternative for removing heavy metals as they are non toxic and can easily remove heavy metals from the environment restoring its natural composition leading to sustainable development agenda of Global world[23].

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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