

Microbes as Potential Tool for Remediation of Heavy Metals: A Review

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Abstract

Environmental pollution caused by heavy metals has received increasing attention worldwide. The recalcitrant and tenacious nature of heavy metals leads to severe threat to environment superiority and life of both plants and animals, counting serious diseases in humans. There exists a wider scope with stress on cost-effectiveness, suitability and sustainability of the techniques to mitigate the influence of environmental change, contamination of food products and biological systems, influence of anthropogenic activities on the environment, and exploration of the aforesaid prospects along with new ingenuities for the restoration of environment. Bioremediation is measured as one of the safer, cleaner, cost operative and environmental friendly technology for decontaminating sites which are contaminated with extensive range of pollutants which is due to the unawareness concerning production, use and disposal of hazardous materials. Bioremediation uses numerous agents such as bacteria, yeast, fungi, algae and higher plants as main tools in treating oil spills and heavy metals existing in the environment. An unceasing search for the new biological forms is essential to regulate increasing pollution and environmental problems faced by man residing in an area. As microorganism demonstrations wide range of mechanisms, there are still few mechanisms which are not known, consequently bioremediation is still measured as a developing technology. Thus, there is a vital need for us to review and amend the available options for environmental clean-up. The objective of this paper is to conduct a comprehensive review on various sources of bioremediation agents and their limitations in treating pollutants present in the environment.

Keywords: Bioremediation; Phytoremediation; Anthropogenic activities; Biosorption

Introduction

Environmental microbiology is an area of growing interest both to microbiologists and to the general public. Currently, more and more people ponder that the scale of pollution problem in our soils and water calls for instantaneous action. Among toxic substances attainment hazardous levels are heavy metals, including mercury (Hg), lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), uranium (U), selenium (Se), silver (Ag), gold (Au) and nickel (Ni). The danger of heavy metals is intensified by their almost indefinite persistence in the environment due to their absolute nature.

The related anthropogenic activities lead to substantial release of toxic metals into the environment purposely. Heavy metal pollution is generating hype in recent years. With the rapid expansion of many industries (mining, surface finishing, energy and fuel producing, fertilizer, pesticide, metallurgy, iron and steel, electroplating, electrolysis, etc.), wastes comprising heavy metals are unswervingly discharged into the environment causing severe environmental pollution and threatening human life. Heavy metals such as As, Cr, Pb, Hg, Cd and U, etc. are persistent components of industrial effluents. Even the aquatic ecosystem is triggered by the heavy metals pollution from industrial and domestic foundations, due to which there has been an increased bioaccumulation and exaggeration of toxicants in the food chain (Table 1). The occurrence of these heavy metals in the environment has been a topic of great worry due to their toxicity, non-biodegradable nature and the long biological half-lives for their removal from biological tissues [1].

Heavy metals are distinct as the ions with partially or completely filled d-orbital. They are elements having atomic weights between 63.5 and 200.6 and a precise gravity greater than 5. Living organisms necessitate trace amounts of some heavy metals including cobalt, copper, iron, manganese, molybdenum and vanadium. Extreme levels of essential metals though can be harmful to the living organism.

Additional heavy metals of specific concern to surface water systems are cadmium, chromium, mercury, lead, arsenic and antimony. These heavy metals are mostly transported by runoff water and contaminate water sources downstream from the industrial site. Heavy metals can bind to the surface of microorganisms and may even breach inside the cells. Inside the microorganism, the heavy metals are chemically transformed as the microorganism uses chemical reaction to digest food (Figure 1).

These metal pollutants pose opposing health effects to those who live near these polluted sites. Metal waste is frequently found in soil, sediments and water. Breathing, eating, drinking, and skin interaction are all likely exposure routes for metal contaminants. Metals such as mercury, lead, and arsenic, can be toxic to the kidneys, diminution mental capabilities and cause weakness, headaches, abdominal cramps, diarrhoea and anaemia [2]. Chronic contact to these pollutants can reason permanent kidney and brain damage [2]. On the other side a metal such as cadmium is tremendously toxic and was shown to induce the DNA breakage. Industrial sewages contaminated with metals liquidated into sewage treatment plants might show the way to high metal concentrations in the activated sludge. Particular of these metals are valuable to the body in low concentration like arsenic, copper, iron, nickel, etc. but are toxic at high concentration [3]. Heavy metals contamination execute various health problems like headache, irritability, abdominal pain and numerous symptoms associated to

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Metal	Industry
Chromium (Cr)	Mining, industrial coolants, chromium salts manufacturing, leather tanning
Lead (Pb)	lead acid batteries, paints, E-waste, Smelting operations, coal-based thermal power plants, ceramics, bangle industry
Mercury (Hg)	Chlor-alkali plants, thermal power plants, fluorescent lamps, hospital waste (damaged thermometers, barometers, sphygmomanometers), electrical appliances, etc.
Arsenic (As)	Geogenic/natural processes, smelting operations, thermal power plants, fuel burning
Copper (Cu)	Mining, electroplating, smelting operations
Cadmium (Cd)	Zinc smelting, waste batteries, e-waste, paint sludge, incinerations and fuel combustion
Molybdenum (Mo)	Spent catalyst
Zinc (Zn)	Smelting, electroplating

Table 1: Sources of heavy metals resulting from anthropogenic activities.

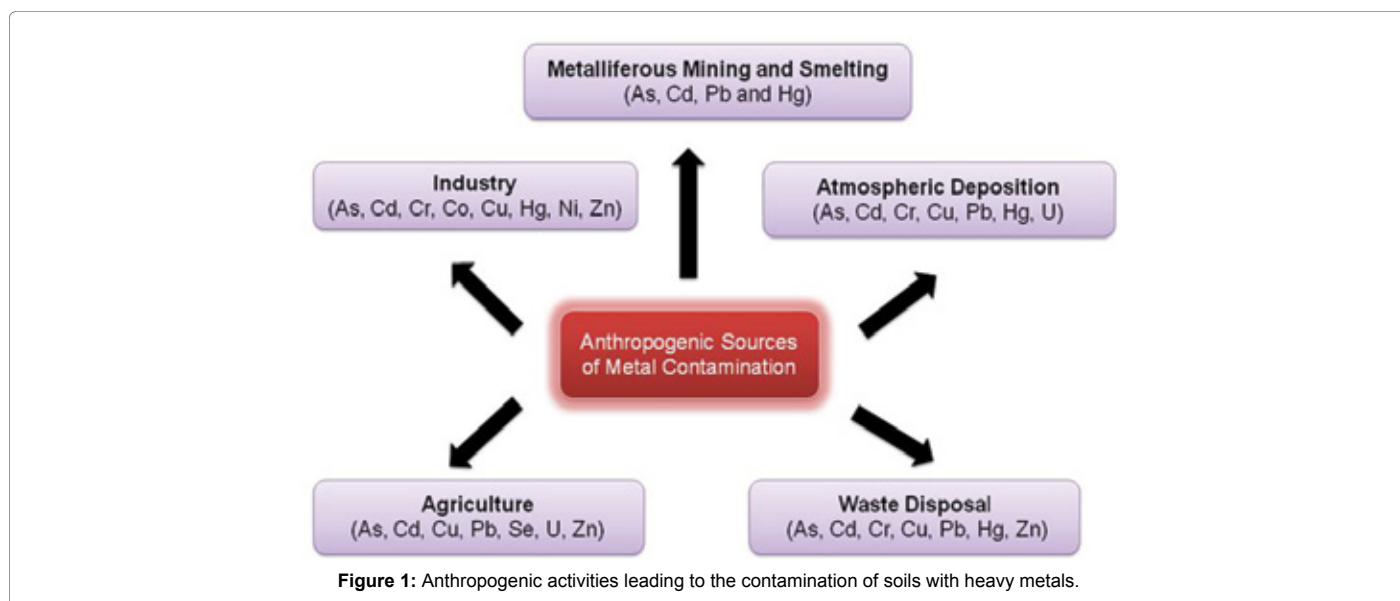


Figure 1: Anthropogenic activities leading to the contamination of soils with heavy metals.

the nervous system, anxiety, bladder and kidney cancer [4] either by transferring the vital nutritional minerals from their original place, thus, hindering their biological function or accumulating, so interrupt function in vital organs and glands such as the heart, brain, kidneys, bone, liver, etc. [5]. Microbial world is varied and due to this microorganisms have altered to the noxious concentrations of heavy metals and become “metal resistant” [6].

Current scenario is this; metal-polluted industrial wastes are regularly treated by conventional methods, such as chemical precipitation, electrochemical treatment, and ion exchange. These methods supply only to some magnitude active treatment and are expensive to put into operation and use, particularly when the metal concentration is low. The substitute use of microbe-based biosorbents for the elimination and recovery of toxic metals from industrial wastes can be an economical and active method for metal elimination. More complex procedures of this type include single or multiple steps: 1) precipitation with hydroxides, carbonates or sulfides; 2) oxidation/reduction reactions; 3) sorption (adsorption with activated carbon/ion exchange); 4) use of membranes (ultrafiltration, electrodialysis and reverse osmosis-RO); 5) electrolytic recovery; 6) evaporation; 7) liquid-liquid extraction; 8) electrodeposition. On the other hand, bioremediation is progressively gaining prominence as an alternative technology, due the benefits it offers: simplicity, efficiency and low cost [7-11].

Bioremediation comprises the use of plants or microorganisms, non-viable or viable, natural or genetically engineered to treat environments polluted with organic molecules that are problematic

to break down (xenobiotics) and to mitigate toxic heavy metals, by altering them into elements with little or no toxicity, henceforth forming innocuous products [12,13]. With the objective of improving the process of bioremediation, diverse approaches can be employed, dependent of the type of the contaminated environment. One of these approaches, bio-stimulation, involves encouraging the growth of indigenous microorganisms by addition of nutrients at the polluted site, in order. As a consequence, the rate of biodegradation/bioremediation can be amplified. Another approach, bio augmentation or bio addition, is the accumulation of microbial populations to indigenous, alien or genetically modified organisms (GMO), in places where there is an inadequacy of indigenous microorganisms or they fail to compete.

Microbes have progressed diverse approaches to overcome the toxic effects of metals and metalloids, utilizing accumulation, resistance or, more interestingly, by reducing their bio-availability or toxicity through biomethylation and transformation. The higher concentrations of waterborne heavy metals have been predictable as an environmental problem in aquatic ecosystems all over the world. Several of these heavy metals reach ground water and others accumulate in seafood or in plants and represent a major toxic source for humans. The rhizosphere is a site of amplified microbial activity that may enhance accumulation, transformation, degradation, and biomethylation of Se and other trace elements. Microbes in the rhizosphere are known to simplify the removal of toxic heavy metals or metalloids originated from wastewaters over and done with biosorption, sulfide-precipitation, and biotransformation (reduction, volatilization).

Presently, there is extensive variability of microorganisms (bacteria, fungi, yeasts and algae) that are actually studied for use in bioremediation processes, and some of these have already been employed as biosorbents of heavy metals [14-16]. The chief benefits of biosorption over conventional treatment methods comprise: low cost; high efficiency; minimization of chemical and biological sludge; selectivity to specific metals; no additional nutrient requirement; regeneration of the biosorbent; and the likelihood of metal recovery [17,18]. Numerous studies have shown that several organisms, prokaryotes and eukaryotes, have diverse natural capacities to biosorb toxic heavy metal ions, giving them altered degrees of intrinsic resistance, mainly in diluted solutions (between 10 to 20 mg/L), due to their mobility, as well as the solubility and bioavailability capacities of these metal ions [19-22]. The search for new technologies for the elimination of toxic metals from contaminated sites has dedicated on bio sorption, which is founded on the metal binding capacities of numerous biological materials.

It is so necessary to separate bacterial strains with novel metabolic abilities and to start degradation pathways both biochemically and genetically. Powerful metal biosorbents in the bacteria class comprise the genus *Bacillus* [23,24], *Pseudomonas* [25,26] and *Streptomyces* [27,28]. Biosorption is created on passive (metabolism-independent) or active (metabolism-dependent) accretion processes [29], in mixtures that fluctuate qualitatively and quantitatively, depending on the type of biomass, its origin, feasibility, and type of processing. Besides biosorption, there are some other beneficial methods for example ion exchange, complexation, precipitation, adsorption, siderophores, biosurfactants, oxidation-reduction (redox), biomethylation, metal-binding cysteine-rich peptides, metallothioneins (MTs), glutathione (GSH), natural phytochelatin (PCs) and synthetic phytochelatin (EC20) and the "cell-surface display" system. We truly need to refine these techniques for the remediation of heavy metals for the welfare of manhood (Figure 2).

Bioremediation a Promising Tool

In the past few years, anthropogenic activities have triggered a severe pollution problem due to the uncontrolled or deliberate discharge of sewage and industrial effluents to water bodies. Not like many other pollutants, heavy metals are hard to remove from the environment [30]. Heavy metals are the potent inhibitors of biodegradation events [31]. These metals cannot be degraded, and are eventually persists in the environment. The toxic properties of heavy metals result mainly from the interaction of metals with proteins (enzymes) and inhibition of metabolic procedures. Heavy metals such as copper, cadmium, lead, zinc, nickel, mercury and chromium when collected in soils, water bodies above their threshold value becomes toxic to plants, animals, humans and aquatic life [32]. Each heavy metal has exclusive bio functions or bio toxicities. Heavy metals gain entry into waste water from domestic, and industrial sources, such as electroplating, chemical works, textile wet dispensation tanneries, photographic industries and mining content, from metal piping (lead and copper), galvanic corrosion (Zn), cosmetics and house-hold cleansing agents [33,34].

Amongst heavy metals Pb, Cd and Hg are deliberated possibly important environmental pollutants due to their trends to gather on vital organs of humans and animals. The most common metals found at polluted sites are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu) and mercury (Hg) (U.S. EPA, 1996b)

Bioaccumulation is a lively process in which the removal of metal needs the metabolic energy of living organisms. It includes the transport

of metal across the cell membrane and its following transformation [35,36]. Microbes have high surface area to volume ratio that offers a large contact area which assistances in the interaction with metals in the surrounding environment. It has established much consideration in the last few years due to the possible use of microorganisms for cleaning polluted water. Metal uptake by microorganisms is a complex procedure that depends on the chemistry of metal ions, the precise surface properties of the organisms, cell physiology and physio-chemical parameters (such as pH, temperature and metal concentration). The same metal ions seem to be sequestered through numerous mechanisms by a diversity of microorganisms [37-39].

Dissimilar studies based on combined sewer sediments have publicised that heavy metals with diverse speciation are usually related with sewer water [40,41]. More specifically, Zn was normally detected in natural water and sediments [42]. Normally, with pH declining in sediment, the antagonism between H⁺ and the dissolved metals for ligands (e.g. OH⁻, CO₃²⁻, SO₄²⁻, Cl⁻, S²⁻, and phosphates) becomes more and more important. The adsorption capabilities and bioavailabilities of the metals consequently decrease and then upsurge the mobility of heavy metal [43]. Moreover, H⁺ (or H₃O⁺) occupies more adsorption sites at lower pH values [44], which results in soluble and carbonate-bound heavy metals precipitated more easily than at higher pH values. Both of these processes result in faster heavy metal release rate with lower pH.

Microorganisms use numerous means to control intracellular metal levels; it includes various influx and efflux mechanisms and metal complexation by cellular components [45,46]. These comprise heterotrophic aerobes and anaerobes from domain bacteria and heterotrophic sulphur reducers from domain Archaea [47]. Adsorption of metals by cell wall components is one of the more significant interaction mechanisms and therefore numerous surface complexation models have used to define the degree of metal adsorption by bacteria [48]. The curiosity procedure frequently comprises adsorption of metal ions at the cell wall or cell membrane via interaction with numerous functional groups and transport into the cell wall with subsequent transformation. Shumate and Stanberg in 1978 have reported the important heavy metal uptakes by pure cultures of microorganisms ranging from 8% to 35% of dry cell weight. Adsorption, precipitation, and organic binding are considered to be major mechanism responsible for removal of cadmium in biological samples [49]. Under alkaline conditions it is biologically available [50]. Conventional chemical treatment methods which comprise precipitation, filtration, ion exchange, oxidation reduction, electrochemical recovery, membrane separation and other techniques may be ineffective or uneconomical when heavy metals concentration in polluted environment is above the threshold level (Figure 3).

Potential Agents of Remediation

In 2015 Paranthaman and Karthikeyan had studied the remediation of heavy metals by isolating *Pseudomonas* spp. from paper mill sewage. The effect of pH and temperature on the biosorption capacity was examined. The optimal pH and temperature were pH 7-9 and 25-35°C for the *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. Previously remediation the content of the metals like Fe²⁺, Zn²⁺, Pb²⁺, Mn²⁺ and Cu²⁺ was around 100 mg/L, after that the metal elimination percentage was 86 and 74. Samples taken at predetermined intervals were centrifuged and supernatants were analyzed. The examines of Fe²⁺, Zn²⁺, Pb²⁺, Mn²⁺, Cu²⁺ ion was carried out by atomic adsorption spectrophotometer (Perkin-Elmer) at 0.01 ppm sensitivity level after dilution of the samples.

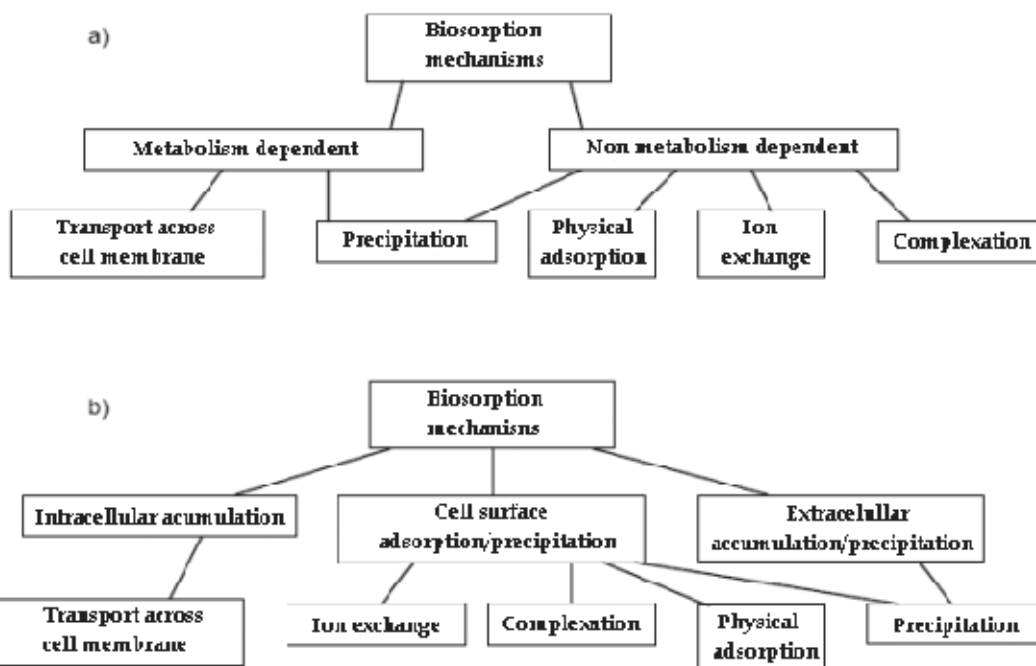


Figure 2: Mechanisms of biosorption, a) classification according to dependence on cell metabolism, b) classification according to the location within the cell and the metal removed.

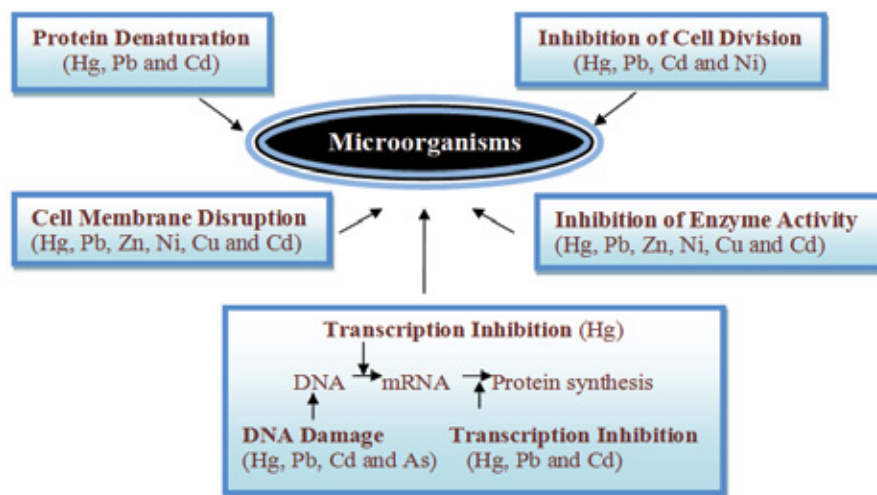
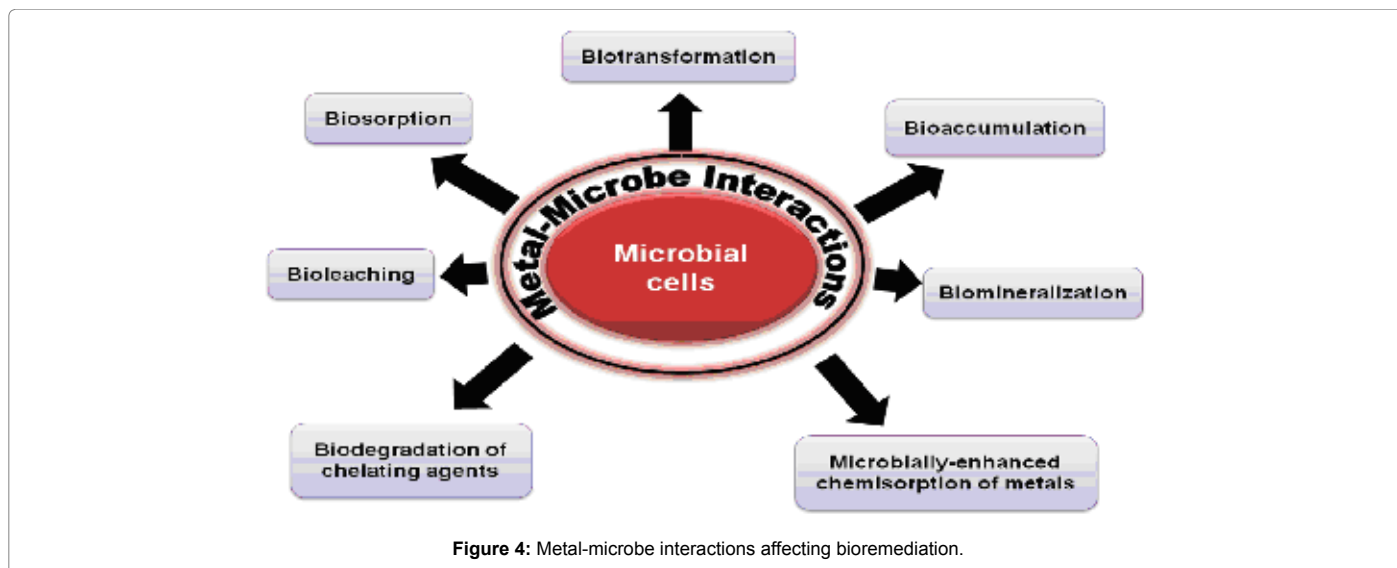


Figure 3: Heavy metal-toxicity mechanisms to microbes.

Pena-Montenegro et al. in 2015 reported *Lysinibacillus sphaericus* CBAM5 a heavy metal tolerant strain from the Easter Planes of Colombia. This strain had possible in bioremediation of heavy-metal polluted environments and biological control of *Culex quinquefasciatus*. Biomass of *L. sphaericus* has been functional in the bioremediation of heavy metals, such as cobalt, copper, chromium and lead [51] with specific metal binding in the cell surface [52]. Native Colombian isolates *L. sphaericus* OT4b.31 and IV (4)10 showed heavy metal biosorption in living and dead biomass, both strains conveying the S-layer proteins [53]. *L. sphaericus* strain CBAM5, along with other 24 native isolates, shown effective growth in arsenate, hexavalent chromium and/or lead [54,55]. Phylogenetic analysis of 16S rRNA gene sequences revealed the

strain as *Lysinibacillus sphaericus* which belongs to taxonomic group 1 that comprises mosquito pathogenic strains (Figure 4).

KCR et al. in 2015 had reported *Streptomyces flavomacrosporus* a multi metal tolerant strain from paddy fields irrigated with industrial sewages. Negligible Glucose yeast Extract Agar media was used for culturing inoculated with 0.3 mM mercury. Cultures were tested for their tolerance to mercury chloride (0.3, 0.4, 0.5 and 0.6 mM) and lead nitrate (0.5, 1.0 and 1.5 mM) in liquid minimal media. By means of Molecular characterization and computational technique their study exposed that strain had a great possible towards precipitation of mercury and also revealed tolerance to multi metals.



Microbacterium profundum strain Shh49^T was isolated from a polymetallic nodule area located in the East Pacific Ocean by Wu et al. in 2015. This strain is thought to comprise genes related to the reduction/oxidation of metals. Strain Shh49^T may have possible capability to oxidize iron from ferrous to ferric iron on the basis of the detection of two ferroxidases. Obtainability of four multi-copper oxidases (MCOs), a family of enzymes known to be involved in Fe [56], Cu [57,58], and Mn oxidation [59], were also noticed. Strain Shh49^T and its genome was sequenced and analysed by using Solexa paired-end sequencing technology (HiSeq 2000 system; Illumina, Inc., USA) [60] by a whole-genome shotgun (WGS) strategy, with a 500-bp paired-end library (333 Mb available reads, 100-fold genome coverage) and a 2,000-bp paired-end library (140 Mb available reads, 42-fold genome coverage).

A heavy metal tolerant fungal strain was reported by Soleimani et al. in 2015 from cadmium polluted sites in Zanjan province, Iran. Cadmium tolerance and bioremediation capacity of seven isolates including *Aspergillus versicolor*, *A. fumigatus*, *Paecilomyces* sp., *Paecilomyces* sp., *Terichoderma* sp., *Microsporium* sp., *Cladosporium* sp. were resolved. Minimum inhibitory concentration values among 1,000-4,000 mg l⁻¹ proved that isolated strains had the capability to survive in cadmium polluted environments. The utmost tolerant fungi, *Aspergillus versicolor*, showed tolerance index of 0.8 in 100 mg l⁻¹ cadmium agar media. Fungal resistance against cadmium is dependent unswervingly on strain's biological function.

Mirlahiji and Eisazadeh in 2014 described the bioremediation of Uranium by *Geobacter* spp. In situ reduction of Fe (III) oxide stimulated by *Geobacter* bacteria lead to the elimination of U (VI) from groundwater [61]. The machinery used was the expression of conductive pili. Pili expression increased the value and growth of uranium reduction for each cell and concludes in the fixation of dissolvable hexavalent uranium, U (VI) beside the pili as mononuclear tetravalent uranium U (IV). It was also described that the lack of pili strains, reduction the uranium in the periplasm and had lowered aerobic activities and applicability which the conductive pili work as the first mechanism for Uranium reducing and cellular protection in it.

Infante et al. reported the use of biomass of *Saccharomyces cerevisiae* to eliminate lead, mercury and nickel in the form of ions dissolved in water. Synthetic solutions were equipped comprising the three heavy

metals, which were put in contact with viable microorganisms at diverse conditions of pH, temperature, aeration and agitation [62]. It was detected that the biomass had distant a higher percentage of lead (86.4%) as associated to mercury and nickel (69.7 and 47.8% respectively). When the pH was set at a value of 5 the effect was positive for all three metals. Bio sorption of lead was significantly influenced by optimizing the pH conditions. The affinity of the heavy metals for the biomass followed the order Pb>Hg>Ni.

Marques et al., studied the reduction of mobility, availability and toxicity found in soil contaminated with lead (Pb) and cadmium (Cd) from Santo Amaro Municipality, Bahia, Brazil. Proposed machinery was the mixture of two methods (metal mobilization with phosphates and phytoextraction) [63]. The strategy applied was the treatment with two sources of phosphates (separately and mixed) followed by phytoremediation with vetiver grass (*Vetiveria zizanioides* (L)). The treatments applied (in triplicates) were: T¹-potassium dihydrogen phosphate (KH₂PO₄); T²-reactive natural phosphate fertilizer (NRP) and; T³-a mixture 1:1 of KH₂PO₄ and NRP. After this step, untreated and treated soils were planted with vetiver grass. The extraction processes and assays useful to contaminated soil before and after the treatments included metal mobility test (TCLP); consecutive extraction with BCR method; toxicity assays with *Eisenia andrei*. The soil-to-plant transfer factors (TF) for Pb and Cd were estimated in all cases. All treatments with phosphates followed by phytoremediation condensed the mobility and availability of Pb and Cd, being KH₂PO₄ (T1) plus phytoremediation the most effective one. Soil toxicity however, remained high after all treatments.

Priyalaxmi et al. in 2014 reported a marine bacterium *Bacillus safensis* (JX126862) from mangrove sediments. Two bacterial strains (PB-5 and RSA-4) were described to be cadmium resistant but in the end it was revealed that RSA-4 strain was the finest for remediation [64]. Cadmium reduction was evaluated at various pH levels with two diverse cadmium concentrations (40 and 60 ppm) and the results presented that the cadmium reduction and absorption was to a maximum of 83.5, 39% and 98.10, 92% for 40 and 60 ppm of cadmium, respectively at pH 7. The potent strain RSA-4 (accession no. JX126862) was identified as *Bacillus safensis* by phylogenetic analysis.

In 2014 Jain and Bhatt have isolated the two cadmium resistant strains from contaminated soils of Semera mines, Palamau,

Jharkhand, India [65] (Table 2). Strains *Pseudomonas putida* SB32 and *Pseudomonas monteilli* SB35 were further processed to disclose the mechanism behind the cadmium resistance by isolating the plasmid DNA and subjected to amplification of *czc* gene which is responsible for the efflux of metal ions. The mechanism is plasmid mediated established by atomic absorption spectroscopy and transmission electron microscopy.

Genetics Involved in Biotransformation of Heavy Metals

Many genetic systems are known in bacteria for maintaining intracellular homeostasis of vital metal ions and for acquiring resistance against toxic metals [66]. The usage of microorganisms to sequester, precipitate, or alter the oxidation state of numerous heavy metals has been widely studied [67]. Expression of metallothionein or metalloproteins was also used to upsurge the affinity and a biosorptive ability of bacterial cells for heavy metals is a promising technology for the expansion of bacterium-based biosorbents [68]. Metallothioneins are small, cysteine-rich proteins synthesized under heavy metal stress circumstances that have been found in both prokaryotes and eukaryotes [69-71]. The only known bacterial metallothionein locus, designated *smt*, that has been cloned and structurally characterized in *Synechococcus* strain PCC 6301 and in *Synechococcus* strain PCC 7942 [72,73]. The *smt* locus contains two divergently transcribed genes, *smtA* and *smtB* which mediate resistance to zinc and cadmium in *Synechococcus* strains [74,75].

Gomes et al. in 2013 reported the isolation of 178 *Escherichia coli* strains from residential, industrial, agricultural, and hospital wastewaters and recreational waters at Rio de Janeiro city [76]. Strain stood reported to harbour a genetic mercury resistance marker which marks it a capable alternative for bioremediation processes. The effort was done to investigate the phenotypic and genetic characteristics associated to diversity and mercury resistance. RAPD data discovered a high degree of polymorphism among *E. coli* mercury resistant strains and exhibited reproducibility and good discriminative effects. Random amplification of polymorphic DNA (RAPD-PCR) and denaturing gradient gel electrophoresis (DGGE) were used to investigate genetic variability. DGGE typing detected diversity within the *merA* gene fragment. This study discovered that there is a development in epidemiological studies of HgR *E. coli* and upkeep the evidence of non-clonal environment of mercury resistant *E. coli* strains circulating in rural and urban aquatic systems in Rio de Janeiro city.

Naik et al. in 2012 explored the role of bacterial metallothionein in Lead-resistant bacterial isolates *Salmonella choleraesuis* strain 4A, *Proteus penneri* strain GM10, *Bacillus subtilis* strain GM02, *Pseudomonas aeruginosa* strain 4EA, *Proteus penneri* strain GM03 and *Providentia rettgeri* strain GM04 which were quarantined from soil polluted with car battery waste from Goa, India [77]. All the isolates except *Pseudomonas aeruginosa* strain 4EA displayed the occurrence of plasmids. Polymerase chain reaction amplification of *smtAB* genes encoding bacterial metallothionein and intracellular bioaccumulation of 19 and 22 mg lead per gram dry weight in *Salmonella choleraesuis* strain 4A and *Proteus penneri* strain GM10 correspondingly discovered occurrence of metal-binding metallothionein (*SmtA*) and accountable for the resistance towards lead.

Ruiz et al. in 2011 had conveyed a mercury remediation which was preferably sequestered by metal-scavenging agents inside transgenic bacteria for subsequent retrieval. Expression of polyphosphate kinase and metallothionein in transgenic bacteria as long as high resistance to mercury, up to 80 μM and 120 μM , correspondingly [78]. Study also

exhibited that metallothionein can be efficiently expressed in bacteria without being attached to a carrier protein. The amount of mercury remediation was such that the polluted media remediated by the mt-1 transgenic bacteria sustained the growth of untransformed bacteria. Cell aggregation, precipitation and colour variations were visually observed in mt-1 transgenic bacteria when these cells were full-grown in high mercury concentrations. Cold vapour atomic absorption spectrometry analyzes revealed that the mt-1 transgenic bacteria hoarded up to 100.2 \pm 17.6 μM of mercury from media containing 120 μM Hg.

Phytoremediation

Phytoremediation also mentioned as botanical bioremediation [79], which contains the use of green plants to decontaminate soils, water and air. It is an emergent technology that can be applied to both organic and inorganic contaminants existent in the soil, water or air [80]. Nevertheless, the capability to hoard heavy metals varies suggestively between species, as diverse mechanisms of ion uptake are effective based on their genetic, morphological, physiological and anatomical features. There are diverse groups of phytoremediation, including phytoextraction, phytoremediation, phytostabilization, phytovolatilization and phytodegradation, reliant on the mechanisms of remediation. Phytoextraction contains the use of plants to eliminate contaminants from soil. The metal ion hoarded in the aerial parts that can be detached to dispose or burnt to recover metals. Phytoremediation comprises the plant roots or seedling for elimination of metals from aqueous wastes. In phytostabilization, the plant roots absorb the contaminants from the soil and keep them in the rhizosphere, rendering them innocuous by stopping them from leaching. Phytovolatilization includes the use of plants to volatilize pollutants from their foliage such as Se and Hg. Phytodegradation means the use of plants and related microorganisms to destroy organic pollutants [81]. Some plants may have one function while others can include two or more functions of phytoremediation.

Within the past 30 years extensive research has gone into finding species that not only thrive in toxic environments, but that can aid in the remediation of those environments. Species range from grasses, agricultural crops, and wild plants to microorganisms and mushrooms. Motivation for the idea of Phytoextraction happened with the discovery of a diversity of wild plants, often endemic in naturally mineralized soils that checked high levels of heavy metals in their foliage. Baker recommended this was due to the plants evolving within these toxic

Organisms	Genus/species	Reference
Bacteria	Arthrobacter	Roanne and Pepper [61]
	<i>Bacillus</i> sp.	Gupta et al, Kim et al [30,60]
	Citrobacter	Renninger et al. [71]
	<i>Cupriavidus metallidurans</i>	Roanne and Pepper, Grass et al. [58,61]
	Cyanobacteria	Gupta et al. [30]
	<i>Enterobacter cloacae</i>	Hernandes et al., Gupta et al., [25,30]
	<i>Pseudomonas aeruginosa</i>	Dias et al., Zhang et al. [39,42]
	<i>Streptomyces</i> sp.	Dias et al. [39]
Fungi	<i>Zoogloea ramigera</i>	Gupta et al. [30]
	<i>Aspergillus terreus</i>	Kumar et al. [32]
	<i>Penicillium chrysogenum</i>	Dias et al. [39]
Yeast	<i>Candida utilis</i>	Kujan et al. [33]
	<i>Hansenula anomala</i>	Breierová et al. [22]
	<i>Rhodotorula mucilaginosa</i>	Dias et al. [39]
	<i>Rhodotorula rubra</i> GV45	Ghosh et al. [61]
	<i>Saccharomyces cerevisiae</i>	Gupta et al., Dias et al., Ghosh et al., [30,39,61]

Table 2: Microorganisms used for bioremediation of heavy metals.

Plant material	Metal ion	Result	References
Carica papaya wood	Hg (II)	96%	Basha et al. [80]
Sawdust(<i>Acacia arabica</i>)	Pb(II), Hg (II), Cr (VI), Cu(II)	Pb>Cr>Cu and Hg	Meena et al. [66]
<i>Oryza sativa</i> husk	Pb(II)	98%	Zulkali et al. [41]
<i>Ricinus communis</i> L. (Castor) leaves	Hg (II)	80%	Rmalli et al. [55]
Water hyacinth	Pb (II), Cu (II), Co (II), Zn (II)	70–80%	Kamble and Patil [73]

Table 3: Selective detoxification of heavy metals using plant material.

environments to be able to tolerate previously toxic amounts of non-essential metals within their systems [82]. Phytoremediation can be showed in many ways. Phytoaccumulation/Phytoextraction is the elimination of metals from polluted soils whereby the metal is extracted from the soil, and then translocated to, and concentrated in, the harvestable parts of the plants [83-85]. Many of these species of plants are proficient of hoarding non-essential heavy metals, including As and Pb, into the plant roots, but fewer can mass the metals into the aerial/harvestable parts for the plant. The metals are frequently hoarded through the plant roots but can hoard them from their aerial surfaces as well. Plants that are proficient of attaining a shoot to root metal concentration ratio greater than 1 are known as hyper accumulators [86,87]. The amassing of metals in hyper accumulators often reaches 1–5% of the dry weight [88]. Another type of bioremediation is mycoremediation which uses fungal mycelium to decontaminate or filter the toxic waste from contaminated area. The fungal mycelia secrete numerous extracellular enzymes and acids that break down the lignin and cellulose. The key to mycoremediation is to govern the right fungal species to target a specific pollutant. Fungi (Ligninolytic fungi) such as the white rot fungus *Phanaerochaete chrysosporium* and *Polyporus* sp. are capable candidates for bioremediation, as it shows the capability to degrade an enormously varied range of persistent or toxic environmental pollutants such as petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), explosives, polychlorinated biphenyls (PCBs) and organochlorine pesticides [80,81].

In current years, the expansion of efficient green chemistry methods for detoxification of metal poisoning has become a major focus of researchers. Kumar et al. investigated an eco-friendly and recyclable technique for the elimination of heavy metal (Pb²⁺, Hg²⁺) contamination from the natural resources. Bio sorption by plants comprises complex mechanisms, mainly ion exchange, chelation, adsorption by physical forces and ion entrapment in inter and intra fibrillar capillaries and spaces of the structural polysaccharide cell wall network (Table 3).

Concept of Nanotechnology

Environmental contamination with heavy metals is a communal problem in many countries. Great hard work has been made in the last two decades to reduce pollution sources and remedy the polluted soil and water resources. A field study was conducted in a dried waste pool of a Lead mine in Zanjan (Iran) to find the native accumulator plant(s). Absorptions of heavy metals were resolute both in the soil and the plants that were full-grown in a dried waste pool by spending flame atomic absorption method. The concentration of total Cu, Zn, Pb and Ni were found to be higher than that natural soil and the toxic levels. The consequences displayed that six dominant vegetation namely *Centaurea virgata*, *Gundelia tournefortii*, *Scariola orientalis*, *Reseda lutea*, *Noaea mucronata* and *Eleagnum angustifolia* accumulated heavy metals.

Noaea mucronata belonging to Chenopodiaceae is the best Pb accumulator and also a good accumulator for Zn, Cu and Ni, but the best Fe accumulator is *Reseda lutea* and the best one for Cd is

Marrobium vulgare. The bioaccumulation capability of nano-particles equipped from *N. mucronata* was evaluated in experimental water containers by Mohsenzadeh and Rad. Nano-particles of the powder were composed by passing through a mesh with pores of 0.2-2 µm and used for heavy metals eliminating from watery metal-polluted media.

Conclusion

Bioremediation is measured to be very safe and obliging technology as it depend on microbes that occur naturally in the soil and pose no hazard to environment and the people living in that area. The procedure of bioremediation can be simply carried out on site without initiating a major disruption of normal actions and threats to human and environment during transportation. Bioremediation is less affluent than other technologies that are used for clean-up of dangerous waste. Even still numerous sources of bioremediation for instance bacteria, archaeobacteria, yeasts, fungi, algae and plants are accessible, but, the biological treatment alone is not adequate enough to treat the pollutants or contaminated sites. Each biological forms has a dissimilar growth requirements (temperature, pH and nutrients) so we necessity to isolate those forms, which can cultured easily in the lab, with minimal prerequisite and can be useful in treating diversity of pollutants. A comprehensive study of area wise and pollutant type data base is much desirable to finalize the priority area and the need for the operative elimination of the contaminant from the contaminated sites. As regular resources are major assets to humans their adulteration resulted in long term effects of pollution (noise and radiation), global warming, ozone depletion and greenhouse gases. The sanitization of these natural resources is important for the preservation of nature and environment using bioremediation process. Thus, there is a vital need to study the consequence of numerous microorganisms in combination against various pollutants for the preservation of natural resources and environment management. Bacteria is one of the greatest vital microbial candidate which needs to be widely explored for the bioremediative ability and though, a few studies have been carried out in the said area, more inclusive and complete studies need to be conceded out for extracting the best out of bacterial systems as “heavy-metal contamination alleviators”.

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