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Microbial remediation of heavy metals

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Abstract

Bioremediation harnesses the naturally occurring biological processes to degrade contaminants or alleviate their toxicity in soils, sediments, and groundwater. As an alternative to conventional pollutants remediation technologies and sometimes by supplementing common techniques, bioremediation holds promise of green technology with eco-friendly and cost-effective approach. Bioremediation aims to reduce total energy use in cleaning up degraded site/polluted site through increasing renewable energy use with reducing greenhouse gas emissions also. Bioremediation holds great promise in recycling precious freshwater resources which are enormously being fed with great number of chemical pollutants, heavy metals and xenobiotics. It aims to reduce negative impacts on water resources and help enhancing land management practices. Overall ecosystem protection, ecosystem restoration and biodiversity restoration are possible with this promising technology through its mechanisms of restoring natural state of ecosystem by acting as buffering agent. Bioremediation technology which helps in handling common organic pollutants in water and agricultural ecosystems also holds promise in remediating heavy metals pollution which is discussed in this article.

Keywords: Bioremediation, microorganisms, heavy metals, contaminants, environment, organic matter, biosorption

1. Introduction

The rapid increase in population and rapid industrialization to meet human needs have created problems such as overutilization of accessible resources and increased pollution taking place in the land, air, and water environment. Anthropogenic activities such as the unmanaged use of agro-chemicals, fossil fuel burning and dumping of industrial waste and sewage-sludge have caused soils and waterways to be severely contaminated with contaminants and heavy metals (Adams *et al.*, 2015) [1]. Production of a variety of compounds and chemicals followed by increased consumption makes many unwanted pollutants with serious problems and risks to enter the environment. Heavy metals are defined based on three different criteria, including density, atomic number, and their chemical properties. Heavy metal is any metal or metalloid element that has a relatively high density ranging from 3.5 to 7 g/cm^3 and is toxic or poisonous at low concentrations. Heavy metals have a relatively high density compared to water (which has density of 0.99 g/cm^3). Heavy metals include mercury (Hg, 13.6 g/cm^3), cadmium (Cd, 8.65 g/cm^3), arsenic (As, 5.72 g/cm^3), chromium (Cr, 7.19 g/cm^3), thallium (Tl, 11.85 g/cm^3), zinc (Zn, 7.13 g/cm^3), nickel (Ni, 8.9 g/cm^3), copper (Cu, 8.96 g/cm^3) and lead (Pb, 11.35 g/cm^3) which are potentially hazardous in combined or elemental forms. The level of toxicity of some selected metals for humans follows the sequence $Co < Al < Cr < Pb < Ni < Zn < Cu < Cd < Hg$. Heavy metals are highly soluble in the aquatic environments and therefore they can be absorbed easily by living organisms. Once the heavy metals enter the food chain, they bioaccumulate in tissues and are biomagnified along with the trophic levels that may end up accumulating in the human body. Heavy metals above allowable limits will often lead to disadvantageous effects in humans, other organisms, and the environment at large. Since most heavy metals are widely applied in industries they are of economic significance in industrial use and currently become a significant environmental problem throughout the whole world. Heavy metals are non-biodegradable and persist in the environment causing pollution which is a serious concern due to hazardous impacts at even very small concentrations. Heavy metal pollution is a major environmental problem which reduces crop production and food quality due to excessive application of agricultural inputs like fertilizers, pesticides, and mulch have resulted in the heavy metal contamination of soils.

Most of the pesticides are organic compounds, and a few are inorganic compounds or pure minerals, and some pesticides contain Hg, As, Cu, Zn, and other heavy metals. Unlike organic contaminants, metals are not degradable and thus remain in the environment for long periods of time; when present at high concentrations, metals can negatively affect plant metabolism. Hence, remediation is required to avoid heavy metal leaching or entry into food chain/environmental segments and to facilitate their extraction. There is a need for innovative treatment technologies for the removal of heavy metal ions from soil, waterbodies, and wastewater. Different microbes have been proposed to be efficient and economical alternatives for the removal of heavy metals from soil and water.

2. Heavy metals sources

Heavy metals enter environment through pedogenic (natural geochemical weathering of soil and rocks) and anthropogenic sources. Weathering of geological bedrock and volcanic eruptions discharge heavy metals into the surrounding environment. The type of heavy metals released from the rock substratum depends on its composition and other factors such as the inherent chemistry of the bedrock/soil, climate, nature, and composition of the soil. Subsequent releases and the entry of heavy metals into the food chain depends on their concentration and uptake by the local flora and fauna. Atmospheric deposition has also been reported to be one of the major causes of deposits in urban and sub-urban areas. Through anthropogenic activities, heavy metal pollution occurs directly from mining wastes, landfill leaches, industrial wastewaters (like electroplating, electronic industries, metal-finishing industries, tanneries, dyeing industries), agricultural fields, municipal wastewater, urban runoff, sewage sludge and waste treatment plants. Untreated industrial wastewater is often colored, frothy, and contains hazardous chemicals including heavy metals, toxic dyes, acids, alkalis, and other toxic chemicals. Long-term use of untreated wastewater from industrial sources can adversely affect water quality, making it unfit for human consumption or as surface/irrigation water. According to the report ‘Status of trace and toxic metals in Indian rivers’, out of 414 river water quality stations across various rivers in India, 57 stations have been found to contain two or more heavy metals beyond permissible limits. Use of heavy metals in the technology, domestic, industrial and agricultural sectors have increased the chances of their contamination. Heavy metals are used extensively in electronics, machines and the artifacts of everyday life and high-tech gadgets. Application of heavy metal-contaminated water in agricultural fields has led to their bioaccumulation in crops and associated food chains. The heavy metals are among the most common pollutants found in wastewater. Heavy metal concentrations have the characteristic of having long biological half-lives as well as resistance to degradable processes and exhibit their chemical toxicity by solubilising in water. When the concentration of heavy metals enters the human body through absorption, these metal ions can bind various biomolecules, such as proteins, nucleic acids, and interfere with their functions.

3. Major heavy metals

3.1 Mercury: The toxicity of mercury has been recognized worldwide, such as in Minamata Bay incident of Japan. Anthropogenic mercury comes from the extensive use of the metal in industrial applications, its mining and processing, applications in batteries and mercury vapor lamps. Methyl mercury is more toxic than any other species of mercury. The adverse health effects that are associated with Hg and mercuric compounds in humans includes possible carcinogens, damage to the brain, lungs and kidneys, affect developing foetuses, high blood pressure or heart rate, vomiting and diarrhoea, skin rashes and eye irritation. The US EPA’s regulatory limit of Hg in drinking water is 2 parts per billion (ppb). The WHO recommended safe limits of Hg in wastewater and soils for agriculture are 0.001 and 0.05 ppm respectively (Wang *et al.*, 2012) [77].

3.2 Cadmium: Cadmium is used widely in electroplating industries, solders, batteries, television sets, ceramics, photography, insecticides, electronics, metal-finishing industries and metallurgical activities. It can be introduced into the environment by metal-ore refining, cadmium containing pigments, alloys and electronic compounds, cadmium containing phosphate fertilizers, detergents and refined petroleum products. Rechargeable batteries with nickel–cadmium compounds are also sources of cadmium. Cadmium exposure causes renal dysfunction, bone degeneration, liver and blood damage. It has been reported that there is sufficient evidence for the carcinogenicity of cadmium. A study carried out in Iran reported that the level of Cd was higher than the maximum permissible limit (MPL) in canned fish samples, and this was due to discharge of heavy metal rich pollutants into aquatic ecosystems. The US EPA’s regulatory limit of Cd in drinking water is 5 ppb or 0.005 parts per million (ppm). The WHO recommended safe limits of Cd in both wastewater and soils for agriculture is 0.003 ppm.

3.3 Nickel: Nickel is a silver-colored metal used in making stainless steel, electronics and coins. Nickel and its salts are used in several industrial applications such as in electroplating, automobile and aircraft parts, batteries, coins, spark plugs, cosmetics and stainless steel, and is used extensively in the production of nickel–cadmium batteries on an industrial scale. The water-soluble salts of nickel are the major problems of contamination in aquatic systems. Paint formulation and enamelling industries discharge nickel containing effluents to the nearby water bodies. Globally, the release of Ni to the environment is estimated to vary from 150,000 to 180,000 metric tons per year. Exposure of Ni to humans is through food, air and water. Previous study has shown that ingestion of dust contaminated with Nickel was the main exposure pathway and upon exposure individual show increased levels of Ni in body tissues and urine. The effects of nickel on human health include dermatitis, allergy, organ diseases, and cancer of the respiratory system. The recommended safe limits by WHO for Ni in wastewater and agricultural soils are 0.02 and 0.05 ppm respectively. Ni has potential of clastogenic, toxic, and carcinogenic effects. The different solubilities of nickel compounds have different carcinogenic potentials.

3.4 Arsenic: Arsenic enters the environment through mining and smelting processes, pesticide use and coal combustion. The toxicity of arsenic because of the contamination of groundwater bodies and surface waters is of great concern. Arsenic exists as arsenate, As(V) and arsenite, As(III), in most of the groundwater. Adsorption and solution pH commonly controls the mobility of arsenic in the aqueous environment. Metal oxides of Fe, Al and Mn play a role in the adsorption of arsenic in aquatic bodies. Arsenic is found naturally at high concentration in groundwater in countries such as India, Bangladesh, Taiwan, Brazil and Chile. Its high concentration in drinking water causes toxic effects on humans and animals. Inorganic arsenic compounds are readily absorbed and interfere with cellular reactions compared to the organic forms due to their poor cellular absorption (Akhtar *et al.*, 2013; Yamamura and Amachi, 2014) [3, 81].

3.5 Lead: Lead is released from various industries and lead contributes significantly towards pollution of both lentic and lotic water bodies. Exposure to Pb can occur through inhalation of contaminated dust particles and aerosols or by ingesting contaminated food and water. Lead is extremely toxic and its poisoning in humans damages the kidneys, liver, heart, brain, skeleton, nervous system and reproductive system. Exposure to lead causes irreversible brain damage and encephalopathic symptoms. Initial symptoms of poisoning associated with exposure to Lead may include headache, dullness, memory loss and being irritable. Lead poisoning may cause disturbance of hemoglobin synthesis and anemia. In children, chronic exposure to low levels of Lead may decrease their intelligence capacity. According to the International Agency for Research on Cancer (IARC), Lead is a possible carcinogenic substance in humans. The regulatory limit of Pb in drinking water according to US EPA is 15 ppb. The WHO recommended safe limits of Pb in wastewater and soils used for agriculture are 0.01 and 0.1 ppm respectively.

3.6 Chromium: Anthropogenic contributions of chromium come from the burning of fossil fuels, production of chromates, plastic manufacturing, electroplating of metals and extensive use in the leather and tannery industries. Chromium is widely used in metallurgy, electroplating, and in the manufacturing of paints, pigments, preservatives, pulp and papers among others. The introduction of Chromium into the environment is often through sewage and fertilizers. Hexavalent chromium is more toxic than trivalent chromium. Hexavalent Chromium compounds including chromates of Ca, Zn, Sr, and Pb are highly soluble in water, toxic and carcinogenic. Compounds of Chromium have been associated with slow healing ulcers and Chromate compounds can destroy DNA in cells. The WHO recommended safe limits for Cr (hexavalent) in wastewater and soils used for agriculture are 0.05 and 0.1 ppm respectively (Zhang and Li, 2011) [86].

3.7 Thallium: Thallium is a soft, tasteless, odorless white blue metal in its pure form and it oxidizes to thallium oxide when exposed to air. Sources of Tl include electronics, optical glasses, semi-conductors, mercury lamp among others. Humans become exposed to Tl through ingestion, inhalation and dermal exposure. Thallium is highly toxic with a lethal dose of 6 to 40 ppm. Thallium poisoning is associated with anorexia, vomiting, gastrointestinal bleeding, abdominal pain, polyneuropathy, alopecia, renal failure, skin erythema, seizures, emotional changes, autonomic dysfunction, cardio toxicity, and coma among others. Recommended safe limit of thallium in drinking water is 0.0001 ppm.

3.8 Zinc: Mining and metallurgical processing of zinc ores and its industrial application are the major sources of zinc in the air, soil and water. It also comes from the burning of coal. Zinc is one of the major metals that can be found in effluents discharged from industries (electroplating, manufacture of batteries, galvanization, and metallurgical industries). Basically, zinc in metallic form does not cause any harm to the environment and it has limited bioavailability. However, the presence of other chemicals, such as acids and oxygen can react with zinc to form a potentially toxic compound that can cause severe damage to biological systems.

3.9 Copper: The pollution of Cu in the environment may have been caused by animal manure. Cu is used as a supplement for the inhibition of parasites. Industries such as the paint factory, electronic and electrical factories, landfills, mine sites, combustion of fossil fuels, and domestic wastewaters are the anthropogenic sources of a copper. Copper contamination can cause nose, eyes and mouth irritation, may cause nausea and diarrhea. Continuous exposure or high concentrations may lead to kidney and liver damage and even death. Even though copper plays important activities in several enzymes for the production of hemoglobin, it has detrimental effects on some facts of the organisms. Cu is essential for living organisms as it acts as an antioxidant, participating in the electron transport chain as well as in collagen and elastin. This micronutrient is only required in certain amounts that mostly accumulate inside human tissues but at higher concentrations it shows harmful effects on the human body.

4. Conventional techniques for removing heavy metal contamination

Numerous clean-up techniques are practiced for removal of heavy metals from contaminated waters or polluted areas using chemical, physical, and biological methods. The conventional technologies are precipitation, membrane filtration, electrocoagulation, adsorption, heterogeneous photocatalysts, electro-dialysis, ion-exchange, electrolytic technologies, chemical extraction (using clays/layered double hydroxides-LDHs), leaching, hydrolysis, polymer microencapsulation, magnetic nanoparticles/nanosorbents and the most commonly practiced excavation and landfilling. All of these chemical methods pose a serious health and ecological threats due to their toxicity and mutagenicity while handling. Vapor extraction, stabilization, solidification, and membrane technology have been previously used to remove the heavy metal ions from the pollutant areas. However, most of these techniques are expensive for implementation in large scale and difficult to monitor/control constantly and sometimes they cannot completely remove the heavy metals contaminated.

4.1 Disadvantages of conventional methods

The process of chemical precipitation involves adding anions for precipitating metals as suspended particles, which are then removed. The process is not specific and cannot remove heavy metals at low concentrations. Through the ion exchange process, heavy metals can be removed to the level of parts per billion. However, it's a non-specific, pH-sensitive and expensive method. The method of reverse osmosis makes use of membranes. The conventional techniques have drawbacks such as slow and inefficient removal, generation of contaminated sludge requiring careful disposal, high cost and energy involved in the processes, and blockage of membranes. There is a need for a cheap and effective technology to remove heavy metals with an eco-friendly approach. There has been increasing interest in the use of biological agents for heavy metal removal as an alternative to these methods. Due to high cost of technologies, industrialists do not implement clean-up processes. The use of conventional chemicals for treating heavy metal pollution can be economically feasible, when dealing with low metal ion concentrations. Conventional methods are expensive for handling large amounts of water, and wastewater contains heavy metals at low concentrations. Innovative treatment technologies for the removal of heavy metal ions from wastewater and polluted sites are required. Possibilities of employing the technology using biological treatments or bioremediation techniques as alternative methods for removing heavy metal ions from contaminated soils or waters thus holds promise.

5. Bioremediation: microbe mediated clean up system

According to US Environmental Protection Agency, bioremediation is an engineered technology that modifies environmental conditions (physical, chemical, biochemical, or microbiological) to encourage microorganisms to destroy or detoxify organic and inorganic contaminants in the environment. The process can be applied above ground in land farms, tanks, biopiles, or other treatment systems (referred to as ex situ) or below ground in the soil or groundwater, referred to as in situ. Bioremediation is a process in which the microbes are used to degrade, break down, or transform hazardous contaminants into less toxic or nontoxic forms, thereby remedying or removing and eliminating contaminants from environment. Bioremediation is a technique for removing/converting harmful contaminants like heavy metals into less harmful substances; and/or removing toxic elements from the contaminated environment; or degrading organic substances and ultimate mineralization of organic substances into carbon dioxide, water, nitrogen gas, etc., employing dead or alive biomass. In bioremediation technological process biological systems like plants, animals and microorganisms are harnessed to effect the cleanup of pollutants from environmental matrices. During the past few years, microbe-assisted bioremediations have been widely applied for heavy metals and metalloids contamination. Microorganisms are ubiquitously present in nature and play a crucial role in elemental biogeochemical cycles of metal transformations between soluble and insoluble species. Metal-microbe interactions can have beneficial or harmful consequences. Microbe-assisted bioremediation and phytoremediation of heavy metals are cost-effective technologies. Bioremediation employs microorganisms for removing heavy metals and environmental pollutants. Microorganisms have adopted different mechanisms for bioremediation. These mechanisms are unique in their specific requirements, advantages, and disadvantages, the success of which depends chiefly upon the kind of organisms and the contaminants involved in the process. Bioremediation uses relatively low-cost techniques which generally have a high public acceptance and can often be carried out on site. The microorganisms may be isolated from an indigenous contaminated area or elsewhere and applied to the contaminated site. Contaminant materials are transformed by living organisms through reactions that take place as part of their metabolic processes. The principles of bioremediation can be divided into several techniques, including biofilters, bioventing, biosorption, composting, bioaugmentation, bioreactor, land farming, and biostimulation. Bioremediation process optimization factors include the presence of a microbial population proficient for degrading pollutants, the availability of contaminants to the microbial population, and environmental factors as like as soil type, temperature, pH, the presence of oxygen or other electron acceptors, and nutrients. Bioremediation is a unique method for cleaning polluted environments from the atmosphere (industrial emissions and soil vent gases), solids (soils, sediments, and sludge), liquids (groundwater, industrial effluents), raw materials from industrial processing.

Some microbes can tolerate heavy metals and either they are able to remove them from the environment or break them down to less toxic forms to utilize in their metabolic processes for growth. Microbial resistance/tolerance to pollutants, particularly heavy metals, is vital in the bioremediation processes. The process of pollutant removal depends primarily on the nature of the pollutant, which may include agrochemicals, chlorinated compounds, dyes, greenhouse gases, heavy metals, hydrocarbons, nuclear waste, plastics, and sewage. Bioremediation techniques try to effectively restore polluted environments in an eco-friendly approach at a very low cost. Researchers have developed different bioremediation techniques; however, owing to the nature and/or type of pollutant, there is no single bioremediation technique that serves as a silver bullet to restore polluted environments. Autochthonous (indigenous) microorganisms present in polluted environments hold the key to solving most of the challenges associated with biodegradation and bioremediation of polluting substances, provided that environmental conditions are suitable for their growth and metabolism. The term biodegradation is used interchangeably with bioremediation, but the former is a term that applies to a process under the latter. Bioremediation is the microbe-mediated process for clearance or immobilization of the contaminants, including all possible toxins like hydrocarbons, agrochemicals and other organic toxicants. Remediation of environment niches such as soil, sediments and water contaminated with heavy metals can be achieved through biologically induced changes in the oxidation state. For inorganic toxic compounds such as heavy metals, microbes are unable to simplify them into harmless compounds, and thus should be used according to their specialization for the type of contaminants. Hence bioremediation strategy for heavy metals depends on the active metabolizing capabilities of microorganisms. Several microorganisms are known to require varying amounts of heavy metals as essential micronutrients for growth and development. For example, Fe^{3+} is essentially required by all bacteria while Fe^{2+} is important for anaerobic bacteria. However, the adsorption capacity depends on microbial total biomass and geochemistry of the system. Microorganisms as metal accumulators possess an inherent novel remediation property for toxic metals in the soil. Stimulation of indigenous microbial flora (intrinsic) or introduction of microorganisms (engineered) for bioremediation is in-situ method. In-situ treatment generally involves pumping oxygen/nutrients (bioventing/biostimulation) into the soil. In ex-situ treatment, contaminated soil and water is moved from contaminated area to another site for further treatment. It may be classified as a solid-phase technique (for land treatment), slurry-phase and pile techniques (for a mixed medium containing solid and liquid phases in bioreactors). It uses techniques like bioreactors, biopiles, and land farming. For the slurry-phase technique, contaminated soil is mixed with water along with other additives in a bioreactor. However, the efficiency of the bioreactor depends on biosorbent (live/dead), optimal conditions required for microbial growth and adaptability of biomass to the configuration of the bioreactor.

6. Mechanisms of microbial heavy metal detoxification

Microorganisms adopt different mechanisms to interact and survive in the presence of inorganic metals. In bioremediation processes, microorganisms mineralize the organic contaminants to end-products such as carbon dioxide and water, or to metabolic intermediates which are used as primary substrates for cell growth and/or as an energy source through their metabolic processes. Different mechanisms of bioremediation are biosorption, metal-microbe interactions, bioaccumulation, biomineralisation, biotransformation, bioleaching, extrusion, use of enzymes, electrostatic interaction, ion exchange, precipitation, extracellular chemical precipitation, redox process, valence conversion, surface complexation, metal oxidation, methylation, enzymatic decrease, metal-organic complexion, metal ligand degradation, metal efflux pumps, demethylation, volatilization, intracellular and extracellular metal sequestration, exclusion by permeability barrier, and production of metal chelators like metallothioneins, production of exopolysaccharide (EPS) and bio surfactants (Azubuike *et al.*, 2016) [5].

Microorganisms remove the heavy metals from soil by using chemicals for their growth and development. They are capable of dissolving metals and reducing or oxidizing transition metals. Microorganisms have negative charge on their cell surface because of the presence of anionic structures that help microbes to bind to metal cations. The negatively charged sites of microbes involved in adsorption of metal are the amino, hydroxyl, alcohol, phosphoryl, amine, carboxyl, ester, sulfhydryl, sulfonate, thioether, and sulfo groups. Extra-cellular polymeric substances present on the biomass cell wall can attach to heavy metals by mechanisms like proton exchange (potential ion exchange sites) or micro-precipitation of metals (metal sinks).

6.1 Bioremediation by adsorption or biosorption and bioaccumulation

Biosorption is the process by which alive or dead biomass removes heavy metals or other pollutants from solutions. The uptake of heavy metals by microbial cells through biosorption mechanisms can be classified into metabolism-independent biosorption, which mostly occurs on the cells exterior and metabolism-dependent bioaccumulation, which comprises sequestration, redox reaction, and species-transformation methods. Heavy metals are biosorbed by microbes at binding sites present in cellular structure without the involvement of energy. Microbial biosorption occurs by surface adsorption thru gathering of metals on the cell surface and linking them with extracellular polymers or because of properties of outer cell shield. Among the various reactive compounds associated with bacterial cell walls, the extracellular polymeric substances are of particular importance and have significant effects on acid-base properties and metal adsorption. Studies on the metal binding behavior of extracellular polymeric substances (EPS) revealed a great ability to complex heavy metals through various mechanisms, which include proton exchange and micro-precipitation of metals. Metals are linked by active groups of compounds occurring in this EPS layer. Most often, this is the reaction of ion transfer between metal cations and active groups present in EPS having negative potential. Bio sorption can be carried out by dead biomass or living cells as passive uptake through surface complexation onto the cell wall and surface layers (Comte *et al.*, 2008; Dixit *et al.*, 2015) [14, 15].

Bioaccumulation involves heavy metal uptake by living biomass (metabolism dependent/active uptake) and is characterized by the uptake of contaminants by living biomass/cells which comprises sequestration, redox reaction and species-transformation methods. Employing living biomass for remediation may not be a viable option owing to highly toxic metals which can accumulate in cells and interrupt metabolic activities resulting in cell death. Bioaccumulation relies on metal infiltration to inside of the cell (intracellular accumulation). Bioaccumulation depends on a variety of chemical, physical and biological intracellular and extracellular processes. Bioaccumulation can be intracellular sequestration by concentration of metal ions within the microbial cells. It involves complexation of heavy metal ions due to surface interactions and their subsequent transport into the cell (Doyle *et al.*, 1980) [16]. Extra-cellular sequestration comprises a concentration of metal ions in the periplasm or their complexation as insoluble precipitates. In bioaccumulation active metabolic transport takes place whereas biosorption involves a passive process in which interaction between sorbent and sorbate occurs. Biosorption is advantageous compared to active uptake/bioaccumulation, as it is metabolism independent and hence microbial biomasses of fungi, algae or yeast have been utilized for bioremediation for in-situ processes (Ahluwalia and Goyal, 2007; Dusengemungu *et al.*, 2020) [2, 17]. Heavy metal bioremediation in the form of metallic nanoparticles with the help of bacteria and the use of genetically modified microorganisms as a part of the bioremediation process have also been reported. Bioaccumulation takes place when the absorption rate of the contaminant is higher than the rate of losing it. Thus, the contaminant remains contained and accumulates inside the organism. Bioaccumulation is a toxicokinetic process that affects the sensitivity of living organisms to chemicals. Organisms can normally resist concentrations of chemicals up to certain levels, beyond which these chemicals become toxic and endanger the organism. The sensitivity of organisms to chemicals is highly variable depending on the types of organisms and chemicals involved. Bioaccumulation may demonstrate superior biotransformational capabilities, changing the toxic chemical to a nontoxic form that enables the organism to reduce the toxicity of the contaminant while keeping it contained. The bioaccumulation of metals may be practical and economically beneficial if they lead to a high concentration of metals (Gadd, 2000 & 2004) [22].

Intracellular sequestration is the complexation of metal ions by various compounds in the cell cytoplasm. The concentration of metals within microbial cells can result from interaction with surface ligands followed by slow transport into the cell. The ability of bacterial cells to accumulate metals intracellular has been exploited in practices, predominantly in the treatment of effluent treatment. Cadmium-tolerant *Pseudomonas putida* strain possessed the ability of intracellular sequestration of copper, cadmium, and zinc ions with the help of cysteine-rich low molecular weight proteins. Also, intracellular sequestration of cadmium ions by glutathione was revealed in *Rhizobium leguminosarum* cells. The rigid cell wall of fungi is made up of chitin, mineral ions, lipids, nitrogen-containing polysaccharide, polyphosphates, and proteins. They can decontaminate metal ions by energetic uptake, extracellular and intracellular precipitation, and valence conversion, with several fungi accumulating metals to their mycelium and spores. The exterior of the cell wall of fungi behaves like a ligand used for labelling metal ions and brings about the elimination of inorganic metals. Peptidoglycan, polysaccharide, and lipid are components of cell wall that are rich in metal-binding ligands (e.g., -OH, -COOH, -HPO42−, SO42− -RCOO−, R2OSO3−, -NH2, and -

SH). Amine can be more active in metal uptake among these functional groups, as it binds to anionic metal species via electrostatic interaction and cationic metal species through surface complexation. Extracellular sequestration is the accumulation of metal ions by cellular components in the periplasm or complexation of metal ions as insoluble compounds (Gadd, 2010) [24]. Copper-resistant *Pseudomonas syringae* strains produced copper-inducible proteins CopA, CopB (periplasmic proteins), and CopC (outer membrane protein) which bind copper ions and microbial colonies. Bacteria can eject metal ions from the cytoplasm to sequester the metal within the periplasm. Zinc ions can cross from the cytoplasm by efflux system where they are accumulated in the periplasm of *Synechocystis* PCC 6803 strain. Metal precipitation is an extracellular sequestration. Iron reducing bacterium such as *Geobacter* spp. and sulfur reducing bacterium like *Desulfuromonas* spp. can reduce harmful metals to less or nontoxic metals. *G. metallireducens*, a strict anaerobe, can reduce manganese (Mn), from lethal Mn (IV) to Mn (II), and uranium (U), from poisonous U (VI) to U (IV). *G. sulfurreducens* and *G. metallireducens* can decrease chromium (Cr) from the very lethal Cr (VI) to less toxic Cr (III). Sulfate-reducing bacteria generate large amounts of hydrogen sulfide that causes precipitation of metal cations. Immobilized biosorption of heavy metal can be done by the use of encapsulated biomass which enhances biosorption performance and increases its physical and chemical stability. Immobilizations of microbial biomass in polymeric matrixes confer rigidity and heat resistivity with optimum porosity for practical applications. *Agrobacterium* biomass was encapsulated in alginate with iron oxide nanoparticles and showed an adsorption capacity of 197.02 mg/g for Pb and was effective for five consecutive cycles.

Biosorption and bioaccumulation are attractive options to substitute conventional methods for heavy metal remediation. The biosorbents can be biomass of microorganisms (the secondary product in the sewage or pharmaceutical industry and in sewage treatment processes), microorganisms from cultured and proliferated on a special base indicating the ability to efficiently metals and sorbents of vegetable or animal origin (as nutshells, crust-rich tannins, sea plants, humus, moss peat, agro-wastes like wheat/rice straw, tea/coffee/yeast waste, cotton waste and similar materials). Microorganisms (bacteria, fungi, yeast or algae) sourced from their natural habitats can be excellent biosorbents. Biosorbent material should be selected based on its efficiency which should be highly selective and economical too. Also, biosorbent material should be easily available or should demonstrate quick growth. Biosorbents absorb heavy metals at very low concentrations. Dead biomass (biosorption) remains unaffected by toxicity, does not require any growth/nutritional medium and is flexible to environmental conditions. Heavy metals are adsorbed on the surface in a passive mode without involving energy expenditure (independent of metabolism) until equilibrium is achieved. The practical application of biosorption to the removal or the recovery of heavy metals is mainly the result of the reversibility of this process. Desorption allows the recovery of metals (which is profitable in the case of more valuable heavy metals like gold, copper, and zinc) or their removal. During the desorption process of metals linked by microorganisms, solutions of weak mineral acid solutions (like 0,1 M HCl) or chelating compounds (like 10 mM EDTA) are applied. In the pH of range 5–7, metal ions like $Cu^{2+,}$ Cr^{3+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Cd^{2+}, and Co^{2+} are strongly linked to the microbial biomass. Lowering the pH to the value of 2 causes the liberation of metals from biosorbents. However, metal ions like Au^{2+} and Ag^{2+} stay at this pH in biosorbents. Bagasse based biosorbents were used for removal of Cr(VI) and Ni(II) from aqueous solution. The bagasse was chemically treated with 0.1 N NaOH followed by 0.1 N CH_3COOH. The materials adsorption capacity in order of selectivity for Cr(VI) and Ni(II) was powdered activated carbon >bagasse > fly ash and powdered activated carbon > fly ash > bagasse, respectively. A lower pH of 6.0 favours the uptake of Cr(VI) and pH 8.0 was suitable for Ni(II) ions removal. However, an increase in pH values of the solution reduces the Cr(VI) adsorption because of the abundance of OH^- ions, causing hindrance to the diffusion of dichromate. However, the adsorption capacity of bagasse is very low and their application for industrial effluent treatment cannot be justified. Pectin-rich fruit wastes act as biosorbents for heavy metal ion removal. Biosorption of cadmium by pectin-rich fruit materials and citrus peels were found to be most suitable. It has also been observed that the metal uptake decreased with decreasing pH, indicating competition of protons for binding to acidic sites. Biosorbent materials like *Parthenium hysterophorous* weed, coconut copra meal, fennel biomass (*Foeniculum vulgari*), pectin for the removal and recovery of Cd ions, rice husk ash for the removal of Zn(II) and Se(IV) ions, *Carica papaya* for the removal of mercury, sulfuric acid treated almond husk based activated carbon for the sorption of Ni(II) ions from water showed positive results. Among microbial cell biomass biosorbents studies reported the adsorption of heavy metals not only in the form of metallic ions but also organometallic compounds. Biosorbents from the biomass of *Phanerochaete chrysosporium* adsorbed inorganic mercury and alkylmercury species with an affinity to CH_3HgCl_4, $C_2H_5HgCl_4$ and Hg_{21} with maximum sorption capacities of 79, 67 and 61 mg/g, respectively. Cadmium precipitation has been reported in *Pseudomonas aeruginosa* and *Klebsiella planticola*. Encapsulated *Agrobacterium* sp. in alginate with nano-particles of Fe has shown an excellent adsorption capacity for continuously five cycles. *Klebsiella planticola* strain generates hydrogen sulfide from thiosulfate under anaerobic conditions and precipitates cadmium ions as insoluble sulfides. *P. aeruginosa* strain precipitate cadmium under aerobic conditions. *Vibrio harveyi* strain precipitate soluble divalent lead as complex lead phosphate salt. Biosorption process involves higher affinity of a biosorbent towards sorbate (metal ions), continued until equilibrium is established between the two components. *Saccharomyces cerevisiae* acts as a biosorbent for the removal of Zn (II) and Cd (II) through the ion exchange mechanism. *Cunninghamella elegans* is a promising sorbent against heavy metals released by textile wastewater.

6.2 Biotransformation

Microbiological transformations of heavy metals are reactions of oxidation, reduction, methylation, and demethylation. The enzymatic systems of microorganisms take part in reactions. Practically useful may be reactions of significantly toxic or valuable metal reduction, like bacteria Gram- positive isolated from tannery sewers, caused the reduction of highly toxic chromium (VI) to less toxic chromium (III), which may be removed from the environment. Any bacteria, microscopic fungi, may conduct a reduction of metal ions (particularly valuable as gold or silver) to metallic form. This reaction may occur in vacuoles, on the cell surface, and in the extracellular environment, which is important from the point of view of this metal recovery. Methylation of metals increases metal toxicity as a result of increased lipophilicity and thus increased permeation across cell membranes. Microbial methylation plays a significant function in metal remediation (Gupta *et al.*, 2002) [27]. Methylated compounds are regularly explosive; for instance, Hg (II) can be bio methylated by some bacteria such as *Bacillus* spp., *Escherichia* spp., *Clostridium* spp., *and Pseudomonas* spp. to gaseous methyl mercury. Bio methylation of selenium (Se) to volatile dimethyl selenide and arsenic (As) to gaseous arsines as well as lead (Pb) to dimethyl lead happen in polluted top soil. Reduction of heavy metal ions by microbial cell from one oxidation state to another reduce their harmfulness. Bacteria use metals and metalloids as electron donors or acceptors for energy generation. Metals in the oxidized form could serve as terminal acceptors of electrons during anaerobic respiration of bacteria. Reduction of metal ions through enzymatic activity could result in formation of less toxic form of mercury and chromium. Bioprecipitation and biocrystallization occur by microbes which causes the transformation of metal into sparingly soluble form which lowers their toxicity. Some precipitation and biocrystallization processes take part in biogeochemical cycles, like forming microfossils, deposition of iron and manganese, and mineralization of silver and manganese. Precipitation of metals on the surface or inside of the cell may be the result of not only the direct activity of enzymes but also by secondary metabolites (Boriová *et al.*, 2014) [10].

Heavy metal degradation involves energy for the cell metabolic cycle. Fungi have emerged as potential biocatalysts to access heavy metals and transform 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. Pb (II) contaminated soils can be biodegraded by fungal species like *Aspergillus parasitica* and *Cephalosporium aphidicola* with biosorption process. Hg resistant fungi (*Hymenoscyphus ericae*, *Neocosmospora vasinfecta* and *Verticillum terrestre*) were able to biotransform a Hg (II) state to a nontoxic state. Many of the contaminants are hydrophobic, and these substances appear to be taken up by microbes through the secretion of some biosurfactant and direct cell-contaminant association. Biosurfactants form stronger ionic bonds with metals and form complexes before being desorbed from soil matrix to water phase due to low interfacial tension. Bioremediation also involve aerobic or anaerobic microbial activities. Aerobic degradation often involves introduction of oxygen atoms into the reactions mediated by monooxygenases, dioxygenases, hydroxylases, oxidative dehalogenases, or chemically reactive oxygen atoms generated by enzymes such as ligninases or peroxidases. Anaerobic degradations of contaminants involve initial activation reactions followed by oxidative catabolism mediated by anoxic electron acceptors. The technique used to reduce the mobilization of heavy metals from contaminated sites by changing the physical or chemical state of the toxic metals is called immobilization. Solidification treatment involves mixing of chemical agents at the contaminated sites or precipitation of hydroxides. Microbes mobilize the heavy metals from the contaminated sites by leaching, chelation, methylation and redox tansformation of toxic metals. Heavy metals can never be destroyed completely, but the process transforms their oxidation state or organic complex, so that they become water-soluble, less toxic and precipitated. Microorganisms remove heavy metals through the mechanisms which they employ to derive energy from metals redox reactions, to deal with toxic metal through enzymatic and non-enzymatic processes (Murray *et al.*, 2007; Nwaehiri *et al.*, 2020) [43, 44].

Microorganisms may initiate metal mobilization/ immobilization by redox reactions; and hence, impact bioremediation processes. Heavy metals like Fe, As, Cr, and Hg undergo oxidation and reduction cycles. Bioremediation is facilitated by converting an element from its insoluble and stationary form in sediments into its mobile and soluble phase. Mobilization can also have deleterious impacts when toxic metal ions are redistributed and released from their solid phase from sediments into the solution phase. This increases their bioavailability and heavy metals can reach microbial metabolic systems. The bacteria reduces Hg(II) to the elemental and more volatile form of Hg(0). Microbial reduction can also enhance the solubility of ions like Fe(III) and As(V) by reducing them to Fe(II) and As(III), respectively, and can facilitate leaching from soil. Studies have reported bacteria from different natural aquifers which can transform arsenic. Heavy metal biomethylation is an important process in soil and water and may modify toxicity, volatility, and mobility of heavy metals. It also serves as an important means of detoxification as volatile methylated species can be removed from cells. Dimethylmercury and alkyl arsines, the methylated products of Hg and As, respectively, are volatile and evaporate and are lost from soil. The organic matter fraction of soil serves as the methyl donor. Yet another indirect mechanism of metal mobilization involves the microbial decomposition of organic matter, which accelerates the release of these ions. *Schizophyllum commune* has been found to release heavy metals along with dissolved organic matter. Excretion of metabolites like carboxylic acids and amino acids by microbes is an important mechanism of chelating metal ions.

6.3 Bioleaching of metals

Bioleaching is done using bacteria and fungi their metabolic products to transfer the metal contained in mineral to solution. In case of sulfide materials it has become a known industrial technology. The process is based on the transformation of compounds of metals present in the environment in the form of sparingly soluble substances (most often sulfides) into forms easily soluble, where removal of metals is an easy task. The ability of fungi to bioleach, mobilize metals from ores and industrial waste is connected mainly with two processes: the secretion of various organic acids in the living environment (citric acid, gluconic acid, oxalic acid) and secretion of complexion agents (Rohwerder *et al.*, 2003) [54]. Some fungi capable of bioleaching ores are *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Mucor* sp., *Alternaria* sp., and *Cladosporium* sp. because of their biochemical abilities and relatively high resistance to factors such as pH and temperature. Bacterial leaching done with an application of *A. ferrooxidans* and *A. thiooxidans*. Biological methods of leaching are mainly used in biohydrometallurgy commonly in sulfide and oxide minerals. There is a possibility to recover metals such as antimony, bismuth, zinc, cobalt, gold, lead, copper, molybdenum, nickel, vanadium, and uranium by biohydrometallurgical methods. Industrial application of this method in the world is limited mainly to copper, gold, and uranium leaching (Bosecker, 1997; Jing *et al.*, 2018) [11, 31].

6.4 Nano-biotechnology in bioremediation

The remediation of heavy metals and trace elements can be achieved by an emerging technology, nanotechnology. Various nanoparticles or nanomaterials have been found to be very effective for the removal of a wide range of toxic metals from the environment as compared to conventional methods. Using nanoparticles to enhance microbial activity in removing toxic pollutants is called nano-bioremediation. Nano-based technologies not only reduce the costs of cleaning up contaminated sites at a large scale, but also reduce the process time as well. Bio-nanotechnology or nanotechnology through biotechnology is the bio-fabrication of nano-objects or bifunctional macromolecules used as tools to construct or manipulate nano-objects. Wide physiological diversity, small size, genetic manipulability and controlled culturability make microbial cells ideal producers of nanostructures ranging from natural products, such as polymers and magnetosomes, to engineered proteins or protein constructs, such as virus-like proteins (VLP) and tailored metal particles. *Deinococcus radiodurans*, a radioactive-resistant organism, has the ability to withstand radiation well beyond the naturally occurring levels, thus its application in radioactive waste clean-up initiatives funded by US Department of Energy (DOE). Metal chelating polymers require toxic solvents for their synthesis and ultrafiltration for their separation, and this can be solved by developing metal binding materials that can be recovered by changing the environment surround them like pH, temperature etc. One such material is nanoscale modified biopolymers, which are manufactured by genetic and protein engineering of microorganisms, and their size can be controlled at the molecular level. This innovative technique would be a promising tool to address the escalating problem of heavy metal as well as organic contaminants in the environment.

7. Types of bioremediation

7.1 Phytoremediation

Phytoremediation basically refers to the use of plants and associated microorganisms to partially or completely remediate selected contaminants from soil, sludge, sediments, wastewater and ground water. It can be used for removal of radionuclides, organic pollutants as well as heavy metals. Phytoremediation utilizes a variety of plant processes and the physical characteristics of plants to aid in remediation of contaminated sites. Phytoremediation can be exploited for remediation of heavy metal polluted soils, groundwater and wastewater as it is a cost-effective, efficient and eco-friendly in situ remediation technology driven by solar energy. The technique of phytoremediation includes several different processes such as phytoextraction, phytofiltration, phytostabilization, phytovolatilization, phytodegradation and rhizoremediation. The initial step of phytoremediation is phytoextraction, the uptake of contaminants from soil or water by plant roots and their translocation to and accumulation in biomass, mainly shoots. Translocation of metals to shoots is an important biochemical process and is desirable in an effective phytoextraction. The next important process of phytoremediation is phytofiltration, which includes rhizofiltration (use of plant roots), blastofiltration (use of seedlings) or caulofiltration (use of excised plant shoots). In this, the metals are absorbed or adsorbed and thus their movement in underground water is minimized. In addition to the above process, phytostabilization or phytoimmobilization occurs, which reduces the mobility and bioavailability of metals in the environment and thus prevents their migration into groundwater or the food chain. Plants perform the immobilization of heavy metals in soils by sorption through roots, precipitation, complex formation or metal valence reduction in the rhizosphere. Organic pollutants taken by plants are metabolized by enzymes such as dehalogenase and oxygenase, which are not dependent on rhizospheric microorganisms. However, several heavy metals absorbed by plants get converted into volatile forms and subsequently released into the atmosphere by the process called phytovolatilization. This process has been used for removal of some volatile heavy metals like Hg and Se from polluted soils. However, this is limited by the fact that it does not remove the metals completely but rather transfers them from one medium (soil or water) to another (atmosphere) from which they can re-enter soil and water (Ali *et al.*, 2013) [4].

Aquatic plants, especially wetland ecosystems, have unique properties to sequester heavy metals and metalloids. Wetland ecosystems are much superior in comparison with other conventional methods, for example because of the low cost, frequent growth of microorganisms, easy handling and low maintenance cost. The rhizospheres in wetlands provide an enhanced nutrients supply to the microbial ecosystems of plants, which actively transform and sequester heavy metals in their biological functions. Constructed wetlands have been actively used for the treatment of heavy metals from agricultural runoff, mine drainage and municipal wastes. Many aquatic plants (macrophytes) such as Phragmites, Lemna, Eichchornia, Azolla and Typha have been used for the treatment of wastewater containing heavy metals. Even though it is well known that metals are toxic to many plants, they have developed some internal mechanisms that allow the uptake, tolerance and accumulation of high concentrations of metals that would be toxic to other organisms. In phytoremediation plants like grasses, herbs, forbs and woody species are mainly used. In contrast to organic compounds the heavy metals and metalloids cannot be metabolized but accumulate in the plant biomass. The biomass generated by phytoremediation remains very limited in amount and persists, whereas all the biomass can be utilized in the form of fertilizer, mulch or for the production of bio-gas. In general, two strategies of phytoextraction have been developed: (1) normal phytoremediation of heavy metals from aquatic bodies through the plants in their entire growth cycle and (2) chemically induced phytoextraction techniques to cleanup contaminated water by using metal-tolerant plants to remove heavy metals and metalloids. The efficiency of phytoextraction can be increased by using more biomass producing plant species and with the application of suitable chelates. Hyperaccumulators or hyper accumulating plants can accumulate large amounts of heavy metals and metalloids, including Ni, As, Zn, Cd and Pb, in their aboveground tissues without any toxic symptoms. Recently, removal of heavy metals through phytoremediation, especially hyperaccumulators to degrade and detoxify contaminants, has received wide attention due to its efficacy and cost efficiency. The criteria used for hyperaccumulation varies according the metal, ranging from 100 mg/kg dry mass for Cd to 1000 mg/kg for Cu, Co, Cr and Pb. These values exhibit a shoot-to-soil ratio of metal concentration and the factor for bioaccumulation is higher than 1.

Hyperaccumulators have been found to exhibit higher heavy metal tolerance and accumulating abilities compared to other plants. Some plants have certain detoxification mechanism within their tissue, which allow the plant to accumulate high amounts of metals. Some hyperaccumulator plants and the metals they accumulate are: *Pteris vittata* L. and *Thlaspi caerulescens* hyperaccumulate As, *Minuartia verna* for Pb, *Aellanthus biformifolius* for Co and Cu, *Berkheya coddi* for Ni, *Macadamia neurophylla* for Mn, *Thlaspi caerulescens* for Zn, *Arabidopsis helleri* and *Solanum nigrum* for phytoremediation of cadmium. However, phytoremediation on a commercial scale is limited because of its low biomass production, limited growth rate and time consumption (Miransari, 2011) [41]. In order to compensate for the low metal accumulation, much research has been conducted using synthetic chelators or ligands such as ethylenediaminetetraacetic acid (EDTA); S,S-ethylenediaminedisuccinic acid (S,S-EDDS); nitrilotriacetate (NTA) and naturally occurring low molecular weight organic acids to enhance the availability of heavy metals and increase phytoextraction efficiency. Common crop plants with a high biomass can be triggered to accumulate large amounts of low bioavailability metals when phytochelates are applied to enhance accumulation. In such chemically enhanced phytoextractions, chelating agents are used almost exclusively as the mobilizing agents. However, EDTA was the most efficient chelate to increase metal uptake by plants of Pb, but the slow degradation of chelating compounds in the root zone limits its application on an industrial scale. Nevertheless, more biodegradable chelates, such as NTA, (S,S-EDDS) and other chelates are also recognized for metals removal. Application of these chelating agents with plants for the uptake of metal ions is gaining more popularity. The full-scale application for treating wastewater on an industrial scale should be based on optimization of several parameters such as solubilization of metals, chelates stability, plant roots and the capacity of metal transport through the shoots of plants (Eapen and D'souza, 2005) [18].

The disadvantages that limit the use of hyperaccumulators include difficulty in finding heavy metal hyperaccumulators, slow growth and lower biomass yield. This makes the process quite time-consuming and therefore not feasible for rapidly contaminated sites or sewage treatments. However, different rhizospheric microorganisms that may play important roles in plant growth and/or metal tolerance via different mechanisms are known, and these can be beneficial for the design of a phytoremediation plan to select appropriate multifunctional microbial combinations from the rhizosphere, which may include arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria. It is suggested that the remediation role of rhizosphere is the main part of phytoremediation and one of the main basic theories for removing contaminants by the combined activity of plants and microorganisms. The main reason for the enhanced removal of metals in the rhizosphere is likely the increase in the number and metabolic activities of microorganisms (Ojuederie *et al.*, 2017) [45]. In the rhizospheric degradation process, the metal toxicity to plants can be reduced by the use of plant growth-promoting bacteria, free-living soil microorganisms that exert beneficial effects on plant growth. In this process, plants can stimulate microbial activity about 10–100 times by the secretion of exudates which contain carbohydrates, amino acids, flavonoids etc. In return, the rhizosphere bacteria that contain ACC deaminase may act to ensure that the ethylene level does not impair root development and to facilitate the generation of larger roots which enhance seedling survival. It is reported that nickel-resistant soil bacterium *Kluyvera ascorbata* SUD 165 promoted the growth of *Brassica campestris* in the presence of high concentration of nickel due to its ability to lower the level of ethylene stress in the seedlings. Among the fast-growing and high-biomass-yielding plants, poplar is the most commonly studied because of its rapid growth rate and potential to produce high biomass within a short period of time (5–8 years). Many of the poplar hybrid varieties have been genetically modified with microbial catabolic genes and specific transporters for increased remediation. For example, mercuric reductase and γ-glutamylsysteine synthetase genes showed increased resistance to Hg and Cd and Cu, respectively, through accumulation of higher concentrations of these metals. Manipulation of desired plant species with multiple genes will facilitate complete degradation of pollutants to ensure that the harvested biomass can be utilized completely for additional benefits.

7.2 Bacterial bioremediation

Biosorption by bacteria is an inexpensive and efficient technique to remove pollutants, including non-biodegradable elements, like heavy metals, from contaminated site and polluted waters. Bacterial biomass can be living or non-living cells. Several bacterial species have adapted and developed mechanisms for metals ions resistance and remediation for their survival. Bacterial biomass accomplishes the rapid removal of metals such as Cu, Zn, Pb, Cd, and Cr. Biosorption efficiency depends on heavy metal ions and bacterial species. Cell wall structures (like peptidoglycans, N-acetylmuramic acid and poly-N-acetylglucosamine) make bacterial cell wall as the primary physical contact linking metal ions and the bacterial biomass. The overall negative charge due to anionic functional groups (like amine, hydroxyl, carboxyl, sulphate, phosphate) present in Gram-positive bacteria (in peptidoglycan, teichoic acids, and teichuronic acids) and in Gram-negative bacteria (in peptidoglycan, lipopolysaccharides, and phospholipids) imparts metal-binding capacity on or within the cell wall. The heavy metal removal by dead biomass cells is extracellular. Functional groups, including carboxyl, phosphonate, amine and hydroxyl groups on the cell wall are responsible for these interactions. For example, carboxyl groups can bind Cd on the surface by complexation. The amino groups have displayed efficient removal of Cr by chelation and electrostatic interactions. In case of living biomass, bacterial species need to be exposed to the contaminants for enzymatic induction before using them for bioremediation. There is a minimum requirement of contaminant concentration to initiate enzymatic expression necessary for the process. Species like *Pseudomonas, Desulfovibrio*, *Bacillus*, and *Geobacter* have been used for bioremediation (Fomina and Gadd, 2014) [20].

Apart from individual cell-metal interactions, heavy metals removal also occurs due to bacterial/microbial biofilms. Several reports indicate the application of biofilms for the removal of heavy metals. Biofilm acts as a proficient bioremediation tool as well as biological stabilization agent. Biofilms have a very high tolerance against toxic inorganic elements even at concentrations that are lethal. It was revealed in a study conducted on *Rhodotorula mucilaginosa* that metal removal efficiency was from 4.79% to 10.25% for planktonic cells and from 91.71% to 95.39% for biofilm cells. *Pseudomonas aeruginosa* biofilm cells show higher resistance to ions of copper, lead, and zinc than planktonic cells, while cells located at the periphery of the biofilm were killed. Extracellular polymers of biofilm accumulated metal ions and then protect bacterial cells inside the biofilm. Biofilms mechanisms of bioremediation could either be via biosorbent or by exopolymeric substances present in biofilms that contain molecules with surfactant or emulsifier properties (Edwards and Kjellerup, 2013) [19].

Micrococcus luteus was used to remove a large quantity of Pb from a synthetic medium and the elimination ability was 1965 mg/g under ideal conditions. Mercury-resistant bacterial strains comprised of *Alcaligenes faecalis, Bacillus pumilus, Pseudomonas aeruginosa,* and *Brevibacterium iodinium* were tested for the removal of cadmium (Cd) and lead (Pb). *P. aeruginosa* and *A. faecalis* remove around 70% and 75% cadmium (Cd) with reduction of 1000 mg/L to 17.4 mg/L of cadmium (Cd) by *P. aeruginosa* and to 19.2 mg/L by *A. faecalis* in about 72hrs. *Brevibacterium iodinium* and *Bacillus pumilus* remove greater than 87% and 88% of lead (Pb) with a reduction of 1000 mg/L to 1.8 mg/L in 96 hours. In another study, uses indigenous facultative anaerobic *Bacillus cereus* to detoxify hexavalent chromium. *Bacillus cereus* has an excellent capacity of 72% Cr (VI) removal at 1000 μg/mL chromate concentration. The bacteria were capable of reducing Cr (VI) under a wide range of temperatures (25 to 40°C) and pH (6 to 10) with optimum at 37°C and initial pH 8.0. Biosorption of Pb, Cr, and Cd in tannery effluent using *Bacillus subtilis, B. megaterium, Aspergillus niger*, and *Penicillium* sp. *B. megaterium* recorded the highest Pb reduction (2.13 to 0.03 mg/L), followed by *B. subtilis* (2.13–0.04 mg/L). *A. niger* showed the highest ability to reduce the concentration of Cr (1.38–0.08 mg/L) followed by *Penicillium* sp. (1.38–0.13 mg/L), while *B. subtilis* exhibited the highest ability to reduce the concentration of Cd (0.4–0.03 mg/L) followed by *B. megaterium* (0.04–0.06 mg/L) after 20 days. Kim *et al.* (2015) designed a batch system using zeolite-immobilized *Desulfovibrio desulfuricans* for the removal of Cr^{6+}, Cu, and Ni with removal efficiencies of 99.8%, 98.2%, and 90.1%, respectively. Efficient removal of chromium, zinc, cadmium, lead, copper, and cobalt by bacterial consortia at approximately 75% to 85% in less than 2 h of contact duration (Joerger *et al.*, 2001; Kapahi and Sachdeva, 2019) [33, 34].

7.3 Fungal bioremediation (myco-remediation)

Fungi withstand and survive stress conditions of moisture, nutrients, pH, etc. Myco-remediation involves use of fungus (live or dead) for the removal of contaminants from different environments. Myco-remediation is a cost-effective process and does not leave harmful waste products. Hence, it poses a complete solution because of the full mineralization of the pollutants in nature. The success of myco-remediation depends on the identification and usage of a suitable fungal species for the target heavy metal or other contaminants. A variety of mechanisms occur in fungal cells for the removal of heavy metals from the solution, which depend on intracellular metal accumulation or extracellular precipitation of metals. Metal uptake by living fungi can be divided into two main phases: (i) uptake by dead cells, and (ii) energy dependent intracellular metal uptake across the cell membrane. In growing cells either or both phases of uptake may be observed by additional aspects of metabolism such as extracellular products, which may complex or precipitate metals outside the cells. Three main types of adsorption involve electrical attraction, Vander-wall attraction, and chemical attraction of the solute to the adsorbent. First type is related to ion-exchange and is called *exchange adsorption*, occurring widely in fungal biomass, and adsorption has been defined as the attraction of positively charged ions to negatively charged ligands in cell materials. A variety of ligands are involved in metal binding, including carboxylamine, hydroxyl phosphate, and sulfhydril groups. Biosorption can, therefore, be affected by the composition of the biomass and by other factors. Fungi are widely used as biosorbents for the removal of toxic metals and active/lifeless fungal cells play a significant role in the adhesion of inorganic chemicals (Karunasagar *et al.*, 2003) [35].

Fungi have the ability to accumulate heavy metals in their fruiting bodies in an efficient manner, making them unavailable or decreasing their concentration in the media/soil. The future availability of heavy metals and other contaminants in the media/soil depend upon the life of the fungi and behaviour of the elements. *Saccharomyces cerevisiae* has been reported to sequester up to 65–79% of Pb and Cd from polluted soil. The process of biosorption involves fungal cell walls (having chitin, proteins, glucans, lipids, pigments, polysaccharides) and functional groups like hydroxyl, carboxyl, amino, sulphate, or phosphate and is mediated through interactions like adsorption, ion-exchange and complexation. *Aspergillus* sp. have been reported to remove Cr from tannery wastewater (85% of chromium was removed at pH 6 in a bioreactor system from the synthetic medium, compared to 65% removal from the tannery effluent). Dead fungal biomass of *A. niger, Rhizopus oryzae, Saccharomyces cerevisiae,* and *Penicillium chrysogenum* could be used to convert toxic Cr (VI) to less toxic or nontoxic Cr (III). The phylum basidiomycetes includes wood-decaying species (white- and brown-rot fungi), mushrooms and other fungi. Different species of white-rot fungi, including *Pleurotus ostreatus* and *Termitomyces clypeatus* have been reported to degrade persistent pollutants. Mushrooms besides their use as food, are also used for myco-remediation due to their potential for heavy metal uptake. Metal uptake in mushrooms is affected by contact time, age of mycelia and fructification. Some edible wild varieties of mushrooms can accumulate heavy metals over their background concentrations. Heavy metals scatter disproportionately in the mushroom fruiting body. *Coprinopsis atramentaria* was studied for its ability to bioaccumulate 76% of Cd^{2+} at a concentration of 1 mg/L of Cd^{2+}, and 94.7% of Pb^{2+}, at a concentration of 800 mg/L of Pb^{2+}. It is an effective accumulator of heavy metal ions for myco-remediation. Biosurfactants have gained interest in recent years owing to their low toxicity, biodegradability nature, and diversity. Surfactins like rhamnolipid and sophorolipid for the removal of heavy metals (Cu and Zn) showed that a single wash with 0.5% rhamnolipid removed 65% of Cu and 18% of Zn, whereas 4% sophorolipid removed 25% of the Cu and 60% of Zn. Several strains of yeast such as *Hansenula polymorpha, S. cerevisiae, Yarrowia lipolytica, Rhodotorula pilimanae, Pichia guilliermondii*, and *Rhodotorula mucilage* have been used to bio convert Cr (VI) to Cr (III). *Candida sphaerica* produce biosurfactants with removal efficiencies of 95%, 90%, and 79% for Fe, Zn, and Pb, respectively. These surfactants could form complexes with metal ions and interact directly with heavy metals before detachment from the soil. *Candida* spp. accumulate substantial quantity of nickel Ni (57–71%) and copper Cu (52– 68%), but the process was affected by initial metal ion concentration and pH (optimum 3–5). Some microfungi show positive response towards heavy metals, as they grow very well along heavy metal gradient. Some of the common fungi, found to grow in iron-ore tailings, can accumulate iron. The mixed cultures *of Aspergillus niger* oxidize sulphides of heavy metals like copper, lead and zinc to their respective sulphates. Except cadmium sulphide, the sulphide particles in the medium were adsorbed on to the surface of the mycelium. Various other fungi, such as *Candida humicola, Saccharomyces cerevisiae, Penicillium digitatum, Trichonderma cutaneum,* and *Poxillus filamentous,* can remove cations, including toxic and radioactive metal ions from aqueous solutions by bioabsorption. *Penicillium digitatum,* uptake of high amounts of zinc, and cadmium along with nickel by its mycelium has been reported, which is sensitive to inhibition by acidic pH. The potential of using fungal biomass especially that of *Rhizopus* sp. is particularly effective and looks promising for practical exploitation for removal of metal ions.

7.4 Algal bioremediation (phyco-remediation)

Algae are present in freshwater, marine ecosystems and as well as terrestrial ecosystems. Algae comprise of both eukaryotic algae and cyanobacteria (Blue green algae, prokaryotes) which are autotrophic organisms, have low nutritional requirements and generate vast biomass. Phyco-remediation is the use of various types of algae and cyanobacteria for the remediation of heavy metals by either removal or degradation of toxicants. Among the three algal groups brown, green and red algae (Phaeophyta, Rhodophyta and Chlorophyta), brown algae have been reported to possess better biosorption capacity (Gupta *et al.*, 2001) [28]. Metal ion biosorption varies with the kind and structure of the algal biomass, charge and chemical constitution of the heavy metal ion. Different algae, in live or dead forms, have been used, as single or in combination, in batch or column, for in-situ remediation. Algae have various chemical moieties on their surface, such as amine, hydroxyl, carboxyl, sulphate, and phosphate are potential metal-binding sites in algal proteins, which operate by complex formation methods during heavy metal remediation. Calcium, magnesium, and sodium ions present in the cell wall get replaced by heavy metal ions via ion exchange. The intrinsic ability of various micro- and macrophytes to sequester and concentrate metals and other chemicals from the water bodies has long been recognized. Various green and blue-green algae possess high metal uptake capacities and, thereby, they decontaminate the water from pollutants. However, only a few studies have been made to overcome metal toxicity by aquatic macrophytes such as *Eichhornia crassipes, Pistia stratiotes,* and *Hydrilla verticillate* (Henriques *et al.*, 2015) [29].

Dead cells of freshwater alga, *Chlorella vulgaris* removed Cd^{2+}, Cu^{2+}, and Pb^{2+} ions from aqueous solutions under various conditions of pH, biosorbent dosage, and contact time. The biomass of *C. vulgaris* is an extremely efficient biosorbent for the removal of Cd^{2+}, Cu^{2+}, and Pb^{2+} at 95.5%, 97.7%, and 99.4%, respectively as reported in one study, from a mixed solution of a 50 mg/dm^3 of each metal ion. The essential trace metallic element, zinc, and the non-essential metal, cadmium, are accumulated by the *Chlorella vulgaris,* which also show high resistance to arsenic. *Chlorella vulgaris* has a capability to tolerate and accumulate arsenic to the extent of 5,000 As mg/l and 50,000 mg As/kg of dry cell weight, respectively and has the ability to biotransform toxic inorganic arsenic to less toxic methylated arsenic compounds (Yewalkar *et al.*, 2007; Yang *et al.*, 2015) [83, 82]. Zinc and cadmium are accumulate in C. *vulgaris* due to the physico-chemical adsorption by cell components, while arsenic accumulate by metabolic processes. Biosorption of Cd^{2+} generally comprises: (i) binding of cations to negatively charged groups on the cell surface, and (ii) energy-dependent cellular Cd 2+ uptake. *Scenedesmus acutiformis* var. *alternans*, can accumulate copper and nickel metals to a substantial degree from its environment. Algae isolated from the lakes are able to grow in media containing copper upto 1.0 ppm and nickel upto 3.0 ppm. Macrophytes accumulate heavy metals in aquatic conditions. For example, *Ceratophyllum demersum* has been demonstrated as Cd^{2+} hyperaccumulator species under field conditions. *Hydrilla verticillate* and *Pistia stratiotes* are the other aquatic plants, which have been reported to be hyperaccumulators of Cd, and have demonstrated the ability to remove toxic metals, including Cd from wastewater. It is hypothesized that cadmium hyper accumulating ability of the macrophyte is associated with induction of the metal chelating peptides, the phytochelatins (PCs), to cope up with high cellular Cd levels. Chromium and Cadmium bioaccumulation and toxicity studies in *H. verticillata* and *Chara corallina* during single and mixed treatment revealed that Cd uptake was enhanced in combined metal treatments, and the combined metal toxicity was more pronounced as compared to that of individual ones. Wastewater treatability potential of aquatic macrophytes (removal of heavy metals from metal contaminated pond water) was indicated for *C. demersum, Hydrilla reticulatum* and *Sporodela polyrrhiza* for removal of chromium. Both free (*Hydrilla verticillata*) and rooted (*Vallisneria spiralis*) macrophytes showed high potential to accumulate mercury, maximum being in the roots of *V. spiralis.* Mercury stress induced the synthesis of various species of *phytochelatins* which bind to Hg (II) and detoxify it. Phytochelatin induction was accompanied by a decline in the levels of cellular glutathione, although this decline was only observed at high concentrations and long duration of mercury exposure, indicating the involvement of glutathione in PC synthesis. Root showed less concentration of PCs than leaves. Lead has been found to be accumulated by a large number of aquatic plants. Recently, substantial accumulation and high tolerance of Pb has been reported in *H. verticillata*. Reasonably high concentration of Pb (1000 laM) has been found to induce synthesis of phytochelatins (PC2, PC3, and PC 4) in the cell suspension culture of *Rauwolfia serpentina* in flowing water contaminated with Pb (Romera *et al.*, 2007) [55].

8. Biotechnological interventions and genetic engineering in bioremediation

Bioremediation of heavy metals has been extensively studied and the performance of several bio remediating microbial mechanisms were reported. Genomics is being explored in many aspects of agriculture like stress biology, genetically modified organisms, gene expression studies, pest/disease resistant varieties development, microbial genetics etc. Similarly, genomic tools are now being employed for bioremediation also. Knowledge of genomics in of the context of understanding microbe-mediated remediation provides a view of genes related to the sensitivity of microbes towards toxic metals in the soil. Application of genomics in bioremediation makes it possible to analyse the microorganism based on not only biochemical parameters but also molecular levels related to mechanism. Many natural biosorbents of microbial origins have been identified with efficient biosorption characteristics. Recent surface modifications on these bioremediators have helped to ameliorate their metal-binding properties and increase the overall cost of the process. These biosorbents showed effective metal removal over a wide range of temperature, pH, and solution conditions (Gadd and White, 1993; Mosa *et al.*, 2016) [25, 42].

Genetically engineered microorganisms (GEM) whose genetic material has been altered using recombinant DNA technology to generate a character-specific efficient strain for bioremediation of soil, water and activated sludge by exhibiting enhanced degrading capabilities against a wide range of chemical contaminants is new potential research and applied area in bioremediation. It offers the advantage of constructing microbial strains which can withstand adverse stressful situations and can be used as a bioremediators under various and complex environmental conditions. Genetic engineering has led to the development of microbial biosensors to measure the degree of contamination in contaminated sites quickly and accurately. Various biosensors have been designed to evaluate heavy metal concentrations like mercury (Hg), cadmium (Cd), nickel (Ni), copper (Cu) and arsenic (As). Genetic engineering of endophytes and rhizospheric bacteria for plant-associated degradation of pollutants in soil is considered to be one of the most promising new technologies for remediation of metal contaminated sites. Bacteria like *Escherichia coli* and *Moreaxella* sp. expressing phytochelatin on the cell surface have been shown to accumulate 25 times more Cd or Hg than the wild-type strains. However, one major obstacle for utilizing these GEMs in hostile field conditions is sustaining the recombinant bacteria population in soil, with various environmental conditions and competition from native bacterial populations. Further, the molecular approaches have been applied to only limited bacterial strains like *Escherichia coli*, *Pseudomonas putida*, *Bacillus subtilis* etc. This means other microorganisms need to be explored for their application in heavy metal bioremediation through molecular intervention. With the advancement in genetic engineering, microbes are engineered with desired characteristics such as ability to tolerate metal stress, overexpression of metal-chelating proteins and peptides, and ability of metal accumulation. For example, Frederick *et al.* engineered microorganisms to produce trehalose and to reduce Cr (VI) to Cr (III). Engineered *Chlamydomonas reinhardtii* showed significant increase in tolerance to Cd toxicity and its accumulation. Genetically engineered microbes for heavy metal remediation involve the use of *Escherichia coli* (ArsR) to target As(III) and *Saccharomyces cerevisiae* (CP2 HP3) to target Cd^{2+} and Zn^{2+}. *Corynebacterium glutamicum* was genetically modified using overexpression of *ars* operons (*ars*1 and *ars*2) to decontaminate As-contaminated sites. In a genetically engineered bacterium *Deinococcus geothemalis*, Hg reduction has been reported at high temperatures due to the expression of mer operon from *E. coli* coded for Hg^{2+} reduction. Mercury resistant bacteria *Cupriavidus metallidurans* strain MSR33 was modified genetically by introducing a pTP6 plasmid that provided genes (merB and merG) regulating Hg biodegradation along with the synthesis of organomercurial lyase protein (MerB) and mercuric reductase (MerA). Modification of *Pseudomonas* strain with the pMR68 plasmid with novel genes (mer) made that strain resistant to mercury. Two different mechanisms for Hg degradation by bacteria (Klebsiella pneumonia M426) are mercury volatilization by reduction of Hg (II) to Hg (0) and mercury precipitation as insoluble Hg due to volatile thiol (H_2S). Genetic engineering of *Deinococcus radiodurans* (radiation resistant bacterium) which naturally reduces Cr (IV) to Cr (III) has been done for complete toluene (fuel hydrocarbon) degradation by cloned genes of tod and xyl operons of *Pseudomonas putida*. Microbial metabolites like metal bound coenzymes and siderophores mainly involved the degradation pathway (Kostal *et al.*, 2004; Kotrba *et al.*, 2009) [37, 38].

Recent advances in omics technologies such as genomics, proteomics, transcriptomics and metabolomics play important roles in identifying traits that maximize the benefits of field phytoremediation technologies. The main constraint of phytoremediation technology is the accumulation of pollutants or their metabolites in plant tissues, which shortens plant life and releases contaminants into the atmosphere via volatilization. This problem can be minimized by manipulation of metal tolerance, accumulation and degradation potential of plants against various inorganic pollutants (Ruiz *et al.*, 2011) [56]. Using the designer plant approach, the bacterial genes responsible for metal degradation can be introduced in plant tissues to allow degradation of metals within the plant tissues. Application of genetically engineered plant-based bioremediation for various heavy metals pollutants is in the forefront due to its eco-friendliness and reduced health hazards compared to physico-chemical based strategies, which are less eco-friendly and more hazardous to human health. Various microbial genes can be harnessed in the transgenic plant for detoxification and accumulation of inorganic contaminants. The metal-detoxifying chelators such as metallothineins and phytochetains can confer resistance to the plant by enhancing uptake, transport and accumulation of various heavy metals. Similarly, transgenic plants carrying bacterial reductase can enhance the volatilization of Hg and Se while accumulating the arsenic in plant shoots. Fast-growing as well as high-biomass-yielding plants like poplar, willow and Jatropa could be used for both phytoremediation and energy production. However, transfer of the metals from soil or water does not solve the problem, and burning metal-contaminated plant material for energy production will release the metals into the atmosphere, moving the problem from soil or water to the air. Thus, such metal-accumulating biomasses should be stored or disposed of appropriately so that they do not pose any hazard to the environment.

9. Heavy metals toxicity and bioremediation capacity of microorganisms

Heavy metals cause detrimental effects on microorganisms depending on the bioavailability of heavy metals and the absorbed dose. The propensity of heavy metals to be stimulatory or inhibitory to microorganisms is determined by the total metal ion concentrations, chemical forms of the metals, and related factors such as redox potential. Environmental factors like temperature, pH, low molecular weight organic acids, and humic acids can alter the transformation, transportation, valance state of heavy metals, and the bioavailability of heavy metals towards microorganisms. Heavy metals tend to form free ionic species at acidic pH levels, with more protons available to saturate metal-binding sites. At higher hydrogen ion concentrations, the adsorbent surface is more positively charged, hence reducing the attraction between adsorbent and metal cations, thereby increasing its toxicity. Temperature plays a significant role in the adsorption of heavy metals. An increase in temperature increases the rate of adsorbate diffusion across the external boundary layer. The solubility of heavy metals increases with an increase in temperature, which improves the bioavailability of heavy metals. However, the actions of microorganisms increase with a rise in temperature within a suitable range, and it enhances microbial metabolism and enzyme activity, which will accelerate bioremediation. The stability of the microbes-metal complex depends on the sorption sites, microbial cell wall configuration, and ionization of chemical moieties on the cell wall. The outcome of the degradation process depends on the substrate and the range of environmental factors (Boopathy, 2000; Choudhary *et al.*, 2017) [9, 13].

Mechanisms or reasons for heavy metal toxicity include: fatal enzymatic functions, production of reactive oxygen species (ROS), destructing ion regulation, and affecting the formation of DNA as well as protein. The physiological and biochemical properties of microorganisms can be altered by the presence of heavy metals. Cr and Cd induce oxidative damage and nucleic acid denaturation in microbes. Chromium (Cr III) change the structure and activity of enzymes by reacting with their carboxyl and thiol groups and it complexes electrostatically with phosphate groups of DNA, which could affect transcription, replication, and cause mutagenesis. Heavy metals catalyse the production of ROS via Fenton and Haber–Weiss reactions. ROS formed cause severe injury to cytoplasmic molecules, DNA, lipids, and other proteins. Aluminum (Al) can stabilize superoxide radicals, which are responsible for DNA damage (Booth *et al.*, 2015). Heavy metals can stop vital enzymatic functions by competitive or non-competitive interactions with substrates, which will cause configurational changes in enzymes. Heavy metals cause ion imbalance by adhering to the cell surface and entering through ion channels or transmembrane carriers. For example, Cd and Pb damage cell membranes and destroy the structure of DNA causing deleterious effects on microbes. Metal binding sites or ligand interactions are used for bringing cell membrane destabilization. Some common microbial toxic heavy metals are mercury, lead, cadmium, arsenic and silver and because of this toxicity these heavy metals are used in surface cleansing agents or disinfectants.

Different defence systems (exclusion, compartmentalization, complex formation and synthesis of binding protein and peptides) reduce the stress developed by toxic metals. Heavy metal accumulation by microorganisms can be studied by the expression of metal binding protein and peptides (phytochelatins and metallothionein). To survive under metal-stressed conditions, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions like biosorption to the cell walls and entrapment in extracellular capsules, precipitation, the efflux of metal ions outside the cell, reduction of heavy metal ions to a less toxic state accumulation, and complexation of metal ions inside the cell. In bacteria, many metal resistance systems are associated with plasmids apart from bacterial chromosomes that contain genes for resistance to many of the same heavy metals' cations and oxyanions as do plasmids. Metal binding protein transcription factors are known to mediate in hormone and redox signalling process in the context of toxic metal (Cd, Zn, Hg, Cu, Au, Ag, Co, Ni and Bi) exposure. *Synechococcus* sp. (cynobacterial strains) has been reported with the expression of the smtA gene and production of metal-binding protein. Expression of different proteins and peptides by the *Escherichia coli* regulates the range of accumulation of cadmium. Co-expression of precursor glutathione (GSH) along with phytochelatins (PC) resulted in the 10-fold increase in PC that finally increased cadmium accumulation twofold. Natural resistant pathways for heavy metals (Hg and Ar) in microorganisms have been regulated by metalloregulatory protein. Two main mechanisms for development of resistance in bacteria are detoxification (transformation of the toxic metal state and making it unavailable) and active efflux pumping of the toxic metal from cells. The basic redox (oxidation and reduction) reaction takes place in the soil between toxic metals and microorganisms. Microbes act as an oxidizing agent for heavy metals and cause them to lose electrons, which are accepted by alternative electron acceptors (nitrate, sulphate and ferric oxides). In aerobic conditions, oxygen acts as an electron acceptor, while in anaerobic conditions microbes oxidize organic contaminants by reducing electron acceptors. The microorganism takes energy for growth by oxidizing the organic compound with Fe (III) or Mn (IV) as an electron acceptor. Anaerobic degradation of organic contamination is stimulated with the higher availability of Fe (III) for microbial reduction. Metals being used as terminal electron acceptors is called dissimilatory metal reduction. Microorganisms reduce the state of metals and change their solubility, as seen in the *Geobaccter* species that reduce the Uranium soluble state (U^{6+}) to insoluble state (U^{4+}).

Microbial biomass has different biosorptive abilities, which also varies significantly among microbes. However, the biosorption ability of each microbial cell depends on its pretreatment and the experimental conditions. Microbial cell must adapt to alteration of physical, chemical and bioreactor configuration to enhance biosorption. Bacteria are important biosorbents due to their ubiquity, size, and ability to grow under controlled conditions and resilience to environmental conditions. Several heavy metals have been tested using bacterial species like *Flavobacterium*, *Pseudomonas*, *Enterobacter*, *Bacillus*, and *Micrococcus* sp. Their great biosorption ability is due to their high surface-to-volume ratios and potential active chemisorption sites (teichoic acid) on the cell wall. Bacteria are more stable and survive better when they are in mixed cultures. Therefore, consortia of cultures are metabolically superior for the biosorption of metals and are more appropriate for field applications. De *et al.* (2008) reported a 78% reduction of chromium (Cr) using a bacterial consortium of *Acinetobacter* sp. and *Arthrobacter* sp. at 16 mg/L metal ion concentration. Microbes perform metal immobilization and act as sinks for metals by adopting different mechanisms (ex- or in-situ) like biosorption, bioaccumulation, bioconversion and/or inter/intracellular precipitation (as oxalates of Zn, Cu, Co, Cd, Ni) operating in different ways. By immobilization, an element can be easily removed from its aqueous phase in groundwater or wastewater. Bacterial oxidation of As(III) to As(V) makes them immobilized and retained by the sediments. *Methanothermobacter thermautotrophicus* has been employed to reduce Cr(VI) to Cr(III) and immobilize it in hydroxide-/oxide-forms. Bacterial reduction and immobilization for Cr(VI) has also been reported for *Bacillus cereus* and *Shewanella* sp. Cellular structures like the cell wall and plasma membrane act as barriers and check the entry of metal ions into cells. Removal of metal ions from inside cells is also achieved by efflux systems. For instance, plasmid-encoded and energy-dependent metal efflux systems involving ATPases and chemiosmotic ion/proton pumps are reported for As, Cr and Cd resistance in many bacteria.

10. Conclusions

Rapid industrialization and technology development have adverse side effects like soil contamination and degrading soil health. The unregulated discharge of industrial effluents in agricultural fields or water bodies increases their chances of entering the food chain through crops and aquatic animals and subsequent bioaccumulation. Due to the complexity and cost issues involved in the conventional methods of remediation of soil, the role of microbes has been recognised and microbial remediation is now being employed to clean up the contaminated systems. Various in-situ and ex-situ methods of bioremediation suitable to different environmental conditions have been investigated and recommended. However, bioremediation technology has limitations as microbes cannot break toxic metals into harmlessss metabolites, and heavy metals have inhibitory effects on microbial activity. Also, few factors prevent the widespread application of this technology like difficulty in obtaining a reliable and inexpensive biomass of metal accumulating/transforming microbes and negative effects of coexisting metal ions on biosorption capacity among others. Hence, the design, development, and application of bioremediation techniques require careful selection of biological agents. Extensive research is being carried out using specific strains of microorganisms for bioremediation. The process of heavy metal bioremediation is more efficient using different microbial strains concurrently instead of only a single species. Appropriate and adapted microorganisms/appropriate biomass waste and/or mixtures of different kinds of microbial biomass with conventional technologies can be used for remediation of heavy metal contaminated sites through ex- or in-situ methods.

11. Future outlook

Keeping in focus the inhibitions of bioremediation technology, the future prospect looks promising on microbial genetic technologies and the development of increased specificity using biofilms which could be achieved by optimization process and immobilization techniques. Advances in genetic engineering and optimization techniques suggest that the future of these technologies is promising. Genetically modified microorganisms may have a better bioremediation potential for various contaminants. Hence, more effort should be made in genetically modified microbes and biofilms mediated bioremediation in the bioremediation of heavy metals in the ecosystem. In addition, the potential of agricultural and industrial waste biomass as bioremediators on a lab/commercial scale is currently being tested. Few examples are sugarcane bagasse, coconut shell waste, rice husk, and beer waste yeast. The biosorption capacity of various biosorbents is improved after various physical and chemical modifications and further research is needed to use these biosorbents on a commercial scale across industries. The bioremediation approach requires a holistic and inclusive method for systematic, feasible and sustainable strategies which can be easily customized for each scenario.

Interest in understanding processes involving heavy metal uptake by microorganisms has increased considerably in recent years and also their practical application especially in metal recovery scenarios and site restoration purposes. Biotechnological potential of microorganisms in removing and/or recovering metals is also getting attention. Conventional methods, such as synthetic ion exchangers are considered as mature technologies. In contrast, bioremediation is yet to replace conventional methods despite being eco-friendly and cost effective. The factors inhibiting widespread use of microbial technologies for heavy metal bioremediation are to be understood and addressed in order to make this potential technology as common remedy measure to cleanup metal contaminated systems. Exploring novel species that have great potential for heavy metal remediation is also needed. The cleaning up of air, water, soil (especially agricultural soil) is important for the preservation of nature and the environment and to stop xenobiotics entering into food chain of animals and humans.

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