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Bioremediation of Heavy Metals: A brand New Methodology to Sustainable Agriculture

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ABSTRACT: Heavy metal contamination is a global concern with the natural environment since it is indestructible and, at high concentrations, most of it is toxic to living organisms. Many heavy metals aren't even biodegradable, so once released into the environment, they continue to circulate. When the concentration of these heavy metals exceeds this threshold, they can cause chronic and fatal illnesses in people, as well as disrupt animal and plant metabolism. Several conventional technologies have already been used for removal, but most are cost efficient and are not able to obtain optimal results. Bioremediation is a more environmentally friendly, safer, and cost-effective method of cleaning up polluted areas. Bioremediation's primary goal is to degrade and convert pollutants into less toxic forms. Ex-situ and in-situ bioremediation are both viable options, depending on a variety of factors such as cost, site characteristics, contaminant nature, and concentration. As a result, the most effective bioremediation method was chosen. Because each bioremediation process has its own specialised application, the benefits and drawbacks of each are unique. This paper provides an overview of heavy metal pollution issues, Sources and effect of heavy metal on plants and human health as well as details about the various mechanisms utilized by microbes for remediation of heavy metal. Furthermore, bioremediation techniques use a variety of agents to treat heavy metal, organic, and inorganic compounds in the atmosphere, including bacteria, fungi, yeasts, algae, plants and genetically engineered microbes. It also provides clear information on the role of PGPR in plant growth promotion, phytoremediation acceleration, and toxic metal clearance.

KEYWORDS: Pollution, Heavy metal, Microorganism, Bioremediation, Phytoremediation.

I. INTRODUCTION

Heavy metal contamination is becoming a major global concern as a result of its toxicity and danger to human life and the environment as a result of urbanisation and industrialization [74]. Industrialization and the destruction of the environment have resulted in major pollution and degradation of the atmosphere. One of the big issues facing the world today is the contamination of soils, groundwater, sediments, surface water, and air with toxic heavy metals and dangerous chemicals. Because of their ability to reach the food chain, the need to remediate these natural resources (soil, water, and air) has led to the development of new technologies that focus on the destruction of contaminants rather than the conventional method of disposal [9]. Heavy metal pollution in the natural environment is a global issue since these metals are indestructible and most of them have harmful effects on living organisms when concentrations are increased [36]. Industries discharge large amounts of effluents (developed during the production of raw materials)

into water reservoirs, contaminating them strongly. If left untreated and released into the atmosphere, effluents have been linked to contamination in both water sources and soil [76].

Metals can cause severe health issues by interfering with our normal functions while they are found in our bodies. Some of these metals, such as arsenic, copper, iron, nickel, and others, are beneficial for health in low concentrations but harmful in high concentrations [85]. Heavy metals can enter the human body by inhaling dust, soil intake directly and the production of food plants grown in highly toxic metal soil causing major health hazards [13, 25,44].

Table 1. Global emissions of atmospheric trace metals (1000 tonnes a year) [116].

Metal	Natural Source	Anthropogenic Source
Copper	28.0	35.0
Cadmium	1.3	7.6
Arsenic	12.0	18.0
Zinc	45.0	132.0
Lead	12.0	332.0
Nickel	30.0	56.0

II. ENVIRONMENTAL CONTAMINATION

A polluted or contaminated environment can exist. However, contaminants could be pollutants and have harmful impacts on the environment. From research, pollution is defined as the introduction of substances or energy directly or indirectly into the environment that leads to harmful effects such as damage to living standards, harm to human health, harm to the environment, and a reduction in environmental quality. Environmental pollution is defined as any undesirable and unwelcome change in the physical, chemical, and biological properties of air, water, and soil that is harmful to living organisms (both animal and plant). Pollution can be in the form of chemicals or energy, like noise, heat, or light [112].

Pollutants are either foreign substances/energies or naturally occurring chemicals that cause pollution.

2.1. Types of pollutants.

Pollutants in the environment continue to be a global problem and one of the greatest problems facing humanity. Pollutants are naturally occurring compounds or foreign materials that cause negative changes in the atmosphere when they come into contact with it. Inorganic, organic, and biological contaminants are the three forms of pollutants. Regardless of which category contaminants fall under, they all attract a lot of coverage because of the negative effects they have on the ecosystem. The correlation between environmental emissions and global population has become an unarguable equal relationship, as the volume of potentially toxic compounds emitted into the atmosphere increases in parallel with the significant increase in global population. As a result of this epidemic, pollution has become a major environmental issue [70].

2.1.1. Inorganic pollutants

Industrial, farming, and household waste both lead to environmental degradation, which affects human and animal welfare. Inorganic compounds are emitted from such sources. Inorganic contaminants are usually mineral-derived compounds, such as metals, salts, and rocks [113]. According to studies, inorganic toxins are naturally occurring materials that have been altered by human production to maximise their amount in the atmosphere. Inorganic compounds enter the atmosphere from a variety of anthropogenic operations, including mine runoff, smelting, metallurgical and chemical processes, and natural processes. Because of their presence in food chains, these contaminants are harmful [86].

2.1.2. Organic pollutants

Bio-degradable pollutants in an atmosphere may be described briefly as organic contamination. These emission sources are naturally identified and generated by the atmosphere but also by anthropogenic activities to satisfy human requirements. The most frequent chemical contaminants which have been identified as being of particular concern include waste from human products, agricultural waste is polychlorinated biphenyls (PCBs), diphenyl polybrominated ethers (PBDEs), polycyclic aromatic hydrocarbons (PAH), pesticides, oil, and organochlorine (OCPs) [27].

Organic contaminants have been seen to be a significant environmental issue. Chemical contaminants have recently been declared consistent due to their properties, including high lipid solubility, stability, lipophilicity, and hydrophobicity. These properties allow organic contaminants to quickly bioaccumulate and cause toxicological effects in various spheres of the ecosystem [62, 108].

2.1.3. Biological pollutants

Biological pollutants are pollutants that occur as a result of human activity which have an effect on the condition of the marine and terrestrial ecosystem. Bacteria, viruses, moulds, mildew, cat-saliva, house-dust, mites, cockroaches, and pollen are among other toxins. Different causes of toxins have also been identified, including plant-based pollens, human and animal infection, living organisms, animal-borne bacteria, and plant and soil particles [26].

II. HEAVY METALS

Heavy metals are naturally present elements with atomic weights and densities that are higher than those in water [50]. Although some of these critical metals are currently desired by ecosystems at low concentrations, the term "Heavy Metal" refers to a metal or metalloid with a density greater than 5gm/ cm³ and is usually associated with contamination and toxicity. Anthropogenic emissions, including industrial and residential effluents, urban storms, river water, landfill sludge, air springs, and sewage dumping, can cause greater metal concentrations in comparison with standard background values [4]. Heavy metals are commonly found in trace quantities in natural waters, but all of them are toxic even at extremely low concentrations [86]. Arsenic, lead, cadmium, nickel, mercury, chromium, cobalt, copper, and selenium are also extremely toxic metals, also in trace amounts. The increasing number of heavy metals in our resources is causing further concern, particularly because many factories are discharging their metal-containing pollutants into freshwater without proper treatment [47].

When heavy metals are not metabolised and accumulated in the softened tissues, they become poisonous. When they come into contact with humans in agricultural, manufacturing, pharmacy, commercial, or residential environments, they may reach the human body through food, water, air, or absorption through the skin. A typical route of exposure for adults is industrial exposure. In infants, the most popular route of exposure is ingestion. Natural and human actions pollute the atmosphere and deplete its energy by releasing more than the environment can manage (Figure 1.1) [70]. On the basis of their position in living environments, heavy metals can be classified into two categories: essential and non-essential.

- 1) The living organisms are required for their growth, production, and physiological functions by essential heavy metals, such as Mn, Fe, Ni, Zn.
- 2) Non-essential heavy metals such as Cd, Pb, Hg, and which do not need any physiological activity in living organisms [39].

Any metals are essential for life and play critical roles in the function of body organs as sources of vitamins and minerals. Metals are used by all living organisms in various quantities, but at higher concentrations, they become toxic [75]. Heavy metals are distinguished from other toxic toxins by their non-biodegradability and proclivity to accumulate in living materials [80]. Bacterial development has been shown to be inhibited by heavy metals, as seen by an increase in growth rate and generation time [67].

3.1. Sources of heavy metals

Heavy metals can be derived both from natural and anthropogenic sources and lead to different environmental areas (soil, water, air and their interface).

3.1.1. Natural processes

Various natural forms of heavy metals have been identified in several studies. Natural heavy metal emissions occur under various and specific environmental conditions. Volcanic activity, sea-salt sprays, forest fires, rock weathering,

biogenic causes, and wind-borne soil particles are examples of such contaminants. Metals may be released from their endemic spheres into various environment compartments as a result of natural weathering cycles. Hydroxides, oxides, sulphides, sulphates, phosphates, silicates, and organic compounds are all forms of heavy metals. Lead (Pb), nickel (Ni), chromium (Cr), cadmium (Cd), arsenic (As), mercury (Hg), zinc (Zn), and copper (Cu) are the other widely known heavy metals [47].

3.1.2. Anthropogenic processes

Pollutants are often released into various environmental compartments by industries, agriculture, drainage, mining and metallurgical activities, and runoffs. Heavy metal anthropogenic mechanisms have been observed to go beyond normal fluxes for certain metals. The majority of metals found in wind-blown dusts come from manufacturing regions. Automobile exhaust, which releases lead; smelting, which releases arsenic, copper, and zinc; insecticides, which release arsenic; and the combustion of fossil fuels, which releases nickel, vanadium, mercury, selenium, and tin, are all essential anthropogenic causes that contribute greatly to heavy metal pollution in the setting. Due to the daily production of products to satisfy the needs of a huge population, human activities have been found to add much to environmental degradation [46].

Table 2. Industrial source of common heavy metals [87]

Metals	Industry
Chromium (Cr)	Mining, chrome plating, coolants in industry, As catalyst in tanning of leather, road runoff and making alloys.
Lead (Pb)	Mining, road runoff, bangle industry, Lead batteries, ceramics, electronic-waste, ship Building, smelting and in paints industry.
Mercury (Hg)	Chlor-alkali plants, fluorescent lights, fluorescent lamps, dental amalgams, thermometers, barometer, electrical appliances, thermal power plants.
Arsenic (As)	Burning of fuel, Alloys, as dopant, arsenical pesticides, herbicide, in electronic industry, thermal power plants and preservatives of wood.
Zinc (Zn)	Smelting, road runoff, electroplating.
Cadmium (Cd)	Ni/Cd batteries, incinerations and fuel combustion, paint sludge, e-waste, road runoff.
Copper (Cu)	Mining, electronic industry, copper plating and smelting operations.
Nickel (Ni)	Metal plating, thermal power plants, combustion of fossil fuel, electroplating, battery industry, smelting process, road runoff.

3.3. Harmful Effect of heavy metal pollution

Heavy metal has a variety of impacts on living organisms, including humans, insects, and animals [17]. Heavy metals can harm living organisms through a variety of mechanisms, including influencing cell membranes, affecting enzyme specificity, disturbing cellular functions, and causing DNA damage [81]. Heavy metals are present in textile effluents and create major environmental issues by absorbing light in receiving water sources such as streams, rivers, and reservoirs, and eventually interfering with aquatic biological processes [73]. Heavy metals like Cd, Pb, and Zn are obtained in various plants which cause different clinical problems, including damage to the hepatic and renal systems, delay in the mind, and degradations of the basal brain ganglia [77, 28]. Excess chemicals in the water and soils will affect people's health in the region [100]. In many tanneries in our country untreated waste is literally released into the atmosphere due to the lack of treatment and environmental protection systems and to serious environmental and public health problems in particular in areas where it is being recorded [69]. The presence of heavy metals as a contaminant, poor solubility and carcinogenic and mutagenic potential is a major concern for human health and environmental problems [7, 23].

(i) Effects on plants

The consumption and resulting deposition of heavy metals in the food chain is a possible risk for animal and human health [96]. Heavy metal concentration in plants depending on plant types, and plant absorption, as well as soil, is analysed for the performance of various plants in absorbing metals [57]. High heavy metal in soils may lower fertility of soils and a low heavy metal concentration may prevent some of the essential processes of the plant, such as photosynthesis, mitosis, and absorption of water with toxic symptoms of dark green leaves, wilding of old leaves stunted foliage and small brown roots [12]. Heavy metals are potentially harmful and phytotoxic to plant growth, low

plant growth, depression in production and can also be combined with a decreasing intake of nutrients, plant metabolism disorders, and decreased molecular nitrogen-fixing capacity in legume plants [41].

(ii) Effects on Human health

Considering the food-chain effects, plant absorption of heavy metals from soils at high concentrations can pose a significant health danger. One of the main routes of human contamination is the use of food crops polluted with heavy metals. Food plants with an extensive and continuous irrigation method have the ability to remove elements from soils. The culture of such plants in polluted land poses a potential risk because heavy metals will build up in plant tissues [54]. When not metabolised by the body and built up in soft tissue, heavy metals become toxic [95]. Chronic ingestion of radioactive metals has harmful effects on humans, and the resulting adverse effects become apparent only after many years of exposure [57].

Table 3. Effect of some heavy metal on human health [116]

Heavy Metal	EPA Regulatory Limit, ppm	Toxic Effect on Plant	Toxic Effect on Human
Copper	1.3	Plant growth is inhibited, cellular processes such as photosynthesis and respiration are disrupted, biomass is reduced, and chlorotic symptoms appear.	Brain and kidney damage, anemia stomach and intestine, irritation elevated levels result in liver cirrhosis and chronic.
Cadmium	5.0	Mineral uptake is altered, the population of bacteria in the soil is reduced, iron deficiency, phosphorous deficiency, or Mn transport is reduced, and nitrate levels are reduced. absorption and transportation of nutrients from the roots to the shoots.	Carcinogenic, mutagenic, endocrine Disruptor, lung damage and fragile bones, affects calcium regulation in biological systems
Lead	15	Reduces germination percentage, root and shoot length and dry mass, mineral nutrition is disrupted, and cell division is reduced.	Excess exposure in children causes impaired development, reduced intelligence, short-term memory loss, disabilities in learning and coordination problems, risk of cardiovascular disease
Nickel	0.2 (WHO Permissible limit)	Reduces yield by inhibiting growth and development, causing leaf chlorosis, necrosis, and wilting, as well as disrupting photosynthesis.	An allergic skin disease, hair, such as itching, cancer through continuous inhalation, immunotoxin, neurotoxic, genotoxic, affects fertility.
Zinc	0.5	Inhibits metabolic processes, resulting in slowed development and ageing, as well as limiting root and shoot growth and causing chlorosis.	fatigue, Dizziness, etc.
Mercury	2.0	Interferes with mitochondrial activity and causes oxidative stress by causing the production of reactive oxidative species (ROS), which disrupts bio membrane lipids and cellular metabolism.	Autoimmune diseases, hair loss, loss of memory, insomnia, depression, drowsiness, fatigue, restlessness, disturbance of vision, tremors, temper outbursts, brain damage, lung and kidney failure
Chromium	0.1	Reduces germination and bud sprouting, as well as root, shoot, and leaf development, yield, and dry matter production.	Breathing problems, such as asthma, cough, shortness of breath, or wheezing and hair loss, Nose ulcers, runny nose.

(iii) Effects on Aquatic Environment

Heavy metals are extremely stable, poisonous in trace concentrations, and can cause significant oxidative stress in marine species. Heavy metal toxicity can affect plant cell structure by blocking cytoplasmic enzymes. Heavy metal contamination in a river may have disastrous consequences for the marine ecosystem's ecological equilibrium [10]. Heavy metals introduced into aquatic ecosystems are mostly bound to particulate matter, which settles down and is

dissolved into sediments over time. This of the following is the most critical metal and another pollutant reservoir in the marine ecosystem. Trace metals added into the marine ecosystem gradually become correlated with the bottom sediments; environmental contamination of metals can occur in environments where water quality thresholds are not violated, but species in or near the sediments are negatively impacted [43].

High levels of micropollutants, especially metals, which are commonly found in rivers, may have an impact on diatom group structure [20]. Contaminants in marine environments, such as heavy metals, promote the development of reactive oxygen species (ROS), which can damage fish and other aquatic life [114].

III. BIOREMEDIATION

In today's scenario, there is a clear need to recognize the tri-partite relationship between emissions, public health, and the environment, given the elevated pollution levels. If left untreated and released into the atmosphere, effluents have been linked to contamination in both water sources and soil. These effluents are hazardous to human health and aquatic life as they contain several toxins and hazardous substances, including heavy metals like cadmium (Cd), lead (Pb) & Nickel (Ni), and many more. When they are consumed in quantities that the body cannot absorb, they will have harmful effects on human health. However, as a result of industrialization, the concentrations of several metals in soil and water have increased; represent a major threat to human life and aquatic biota. Excavation and solidification/stabilization are two traditional approaches for remediating heavy metals contaminated sites.

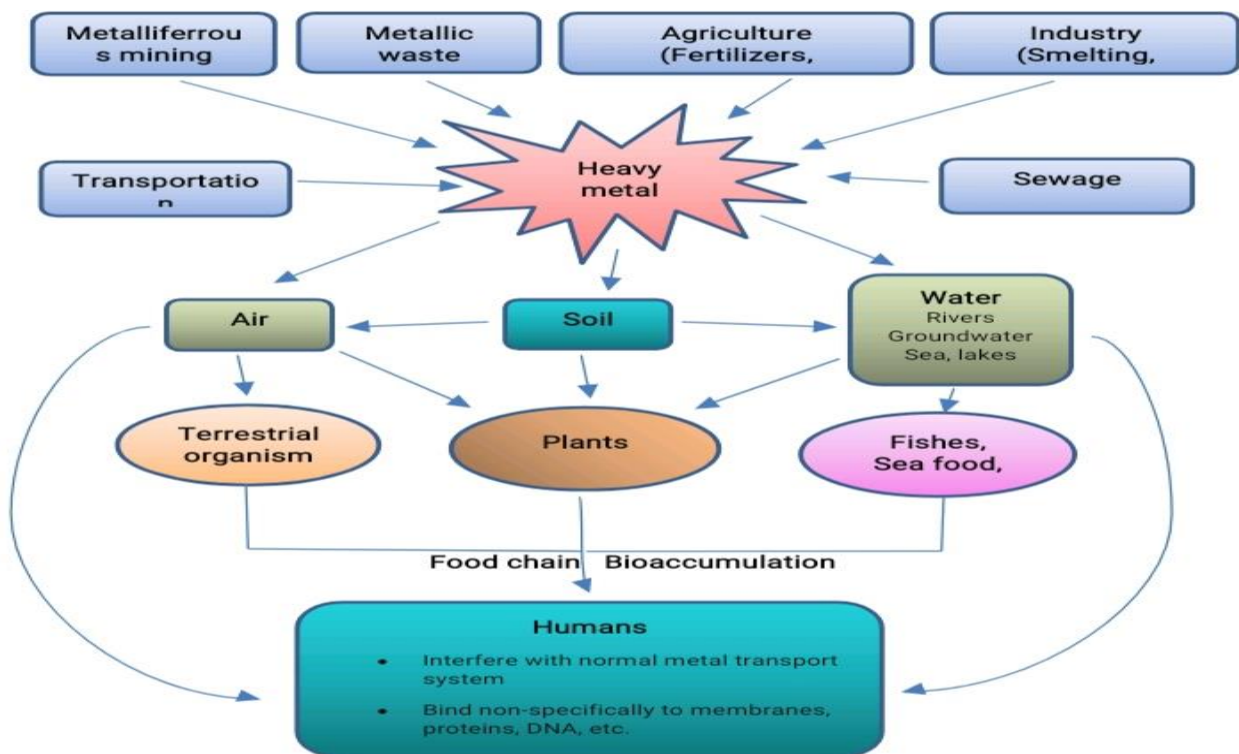


Figure 1 Sources of heavy metal pollution and impact on the environment and organisms [31]

Several physico-chemical methods such as precipitation, ion exchange, reverse osmosis, membrane technology, evaporation, electrolytic technologies, oxidation or reduction, chemical extraction, leaching, hydrolysis, polymer micro-encapsulation, landfilling, and other Physico-chemical methods have not found to be effective enough for heavy metal removal. Many methods have been attempted in the past to eliminate these contaminants, including vapor extraction, stabilisation, solidification, soil washing and flushing, vitrification, incineration, and thermal desorption. Again, on a large scale, these are very costly and necessitate continuous monitoring and supervision, with the pollutants occasionally not being entirely removed. Other methods, such as using chemicals to treat effluents, have been reported to be financially unfeasible, especially when dealing with low metal concentrations and high contaminant effects. However, biological treatment, also known as bioremediation (as biosorption and/or bioaccumulation), has been

proposed as a better alternative option for treating polluted waters and soils as compared with conventional method [55, 37].

Biological processes, on the other hand, maybe able to overcome these drawbacks since they are easy to use and do not produce secondary emissions [92]. Bioremediation is a natural process involving the involvement of microorganisms to minimise pollutant concentrations. The importance of biodiversity (above or below ground) is increasingly being considered for the clean-up of metal-contaminated and polluted biodiversity. Metal toxicity does have a high mortality rate and causes a lot of morbidities [85]. Furthermore, Jin et al., 2011 [83] recorded that adding organic nutrients to the soil, such as manure, compost, and biosolids, can boost bioavailability by conditioning the soil and increasing its fertility. To degrade and transform environmental pollutants into harmless or less harmful forms, bioremediation largely relies on microorganisms or microbial processes [34]. Heavy metals are actively (bioaccumulation) and/or passively absorbed by microorganisms (adsorption) [51].

4.1. Principles of Bioremediation

Recent research in molecular biology and ecology has shown a variety of opportunities for enhancing biological processes [60]. Bioremediation is a mechanism in which hazardous substances are converted to nontoxic compounds using naturally occurring microorganisms [9]. It degrades or detoxifies components that are toxic to human health and the environment using naturally occurring bacteria, fungi, or plants. The microorganisms could be native to the polluted region, or they could have been isolated elsewhere and transported to the contaminated site [60]. Microorganisms also metabolise chemicals to generate carbon dioxide or methane, as well as water and biomass. Microorganisms must enzymatically attack contaminants and transform them to harmless products for bioremediation to be efficient. The existence of the contaminants, ii) the soil structure, pH, moisture content, and hydrogeology, iii) the nutritional condition, microbial diversity of the site, and iv) temperature and oxidation-reduction (redox-potential) are the most critical parameters for bioremediation. Microorganisms use contaminants as nutrient or energy sources in bioremediation [9].

4.2. Types of bioremediation

According to the Environmental Protection Agency (EPA,2003) [29], there are essentially two approaches for removing and transporting wastes for treatment. There are examples of in-situ and ex-situ bioremediation.

4.2.1. In-situ Bioremediation

In-situ bioremediation eliminates the need to excavate or extract soils or water from the remediation process. Since pollution is removed either the source or at the stage of contamination, it can be less costly, produce less dust, and allow for the treatment of a large amount of soil with less contamination release. It's ideal for soil and groundwater [110, 30]. In-situ bioremediation is most often used to degrade toxins in saturated soils and groundwater. It is a suitable strategy for cleaning polluted areas because it is less expensive and uses healthy microbial species to degrade the pollutants. It is also a safer method for degrading harmful compounds. There are two forms of in-situ bioremediation. Intrinsic bioremediation and engineered in-situ bioremediation are the two options. The in-situ bioremediation approach involves feeding nutrients and oxygen to indigenous or naturally occurring microbial communities to increase their metabolic activity, whereas the engineered in-situ bioremediation approach involves the introduction of specific microorganisms to the site of contamination. Engineered systems must be applied to a specific site when the conditions are not suitable. Designed in situ bioremediation speeds up the decomposition process by improving the physicochemical conditions that promote microorganism growth. Microbial development is supported by oxygen, electron acceptors, and nutrients (nitrogen and phosphorus) [30].

There are two types of in-situ bioremediation: intrinsic and engineered in-situ bioremediation.

a) Intrinsic bioremediation

Intrinsic bioremediation, also known as natural reduction, is an in-situ bioremediation technique that involves the passive remediation of polluted sites without the use of any outside force (human intervention). The activation of an indigenous or naturally occurring microbial community is the aim of this operation. The biodegradation of polluting constituents, including those that are recalcitrant, is dependent on both microbial aerobic and anaerobic processes. Since there is no external force, the procedure is less costly than most in-situ techniques [89].

b) Engineered in-situ bioremediation

The second method includes introducing a specific microorganism to the contaminated area. Genetically engineered microorganisms used in in-situ bioremediation speed up the degradation process by improving the physicochemical conditions that promote microorganism development [89].

Advantage and Disadvantage of in- situ Bioremediation

This approach has several potential benefits, including the fact that it does not necessitate the excavation of polluted soil, which saves money; there is minimal site disturbance, which reduces dust production; and simultaneous treatment of soil and groundwater is feasible. It also has some drawbacks, such as being time-intensive compared to other remedial approaches, seasonal variation in microbial activity due to direct exposure to unmanageable environmental factors, and inconsistent application of treatment additives [29]. Microorganisms can only function normally when the waste materials they encounter cause them to generate nutrients and energy for the growth of new cells. Their ability to degrade is reduced when these conditions are unfavourable. In such situations, genetically modified microorganisms must be used, even though it is preferable to stimulate indigenous microorganisms [29].

4.2.2. Ex- situ Bioremediation

To promote microbial degradation, this method requires the excavation of contaminated soil or the draining of groundwater. This technique has more drawbacks than benefits. Ex-situ bioremediation entails excavating or removing polluted soil from the contaminated site. Ex-situ bioremediation is known as a solid phase method or a slurry phase system depending on the state of the contaminant to be removed [37].

There are various other bioremediation methods are as per below

4.2.3 Solid Phase treatment

Chemical wastes such as leaves and animal manures are handled in the solid process. Domestic and industrial wastes, sewage sludge, and urban solid wastes, as well as farm wastes, Land farming, soil bio piles, and composting, are examples of solid-phase soil treatment methods [37].

a) Land Farming

Land farming is a simple technique that involves excavating polluted soil, spreading it over a prepared bed, and tilling it regularly until contaminants have degraded. The aim is to stimulate indigenous biodegradative microorganisms and make it easier for them to degrade pollutants aerobically. Since land farming has the potential to minimise monitoring and maintenance costs as well as clean up liabilities, it is becoming increasingly popular [29].

b) Composting

Composting is a process that involves mixing polluted soil with non-hazardous organic fertilizer such as compost. Agricultural wastes or manure [19].

c) Biopiles

Land farming and composting are combined in biopiles. Engineered cells are essentially aerated composted piles with aeration. They are a modified version of land farming that aims to monitor physical losses of pollutants by leaching and volatilization. They are typically used for the treatment of surface pollution of petroleum hydrocarbons [29].

4.2.4 Slurry-phase bioremediation

In comparison to other treatment methods, slurry phase bioremediation is a faster procedure. In a big tank called a bioreactor, contaminated soil is mixed with water and other chemicals to keep the microorganisms already present in the soil in touch with the pollutants in the soil. Nutrients and oxygen are applied, and the bioreactor's conditions are carefully monitored to provide the best conditions for the microorganisms to degrade the pollutants. When the treatment is over, the water is separated from the solids, which are either disposed of or treated further if contaminants remain [19].

I. Bioreactors

A bioreactor is a storage vessel and apparatus used to produce a three-phase mixing state of solid, liquid, and gas to increase the bioremediation rate of soil-bound and water-soluble pollutants as a water slurry of polluted soil and biomass capable of degrading target contaminants as a water slurry of contaminated soil and biomass capable of degrading target contaminants. Since the enclosed environment is more manageable and therefore more controllable and predictable, the rate and scale of biodegradation in a bioreactor system are greater than in-situ or solid-phase systems. Despite the benefits of reactor systems, they have some drawbacks. Before being placed in a bioreactor, the

contaminated soil must also be pre-treated, or the contaminant can be extracted from the soil by soil washing or physical extraction [112, 29].

II. Bioventing

Bioventing strategies include regulated airflow enhancement by supplying oxygen to the unsaturated region in order to increase the activities of indigenous microbes for bioremediation. To maximise bioremediation, bioventing changes are made by adding nutrients and moisture. As a result, toxins can be microbially transformed into a harmless environment. Among other in-situ bioremediation methods, this one has gained attention [49].

III. Biosparging

This method is similar to bioventing in that air is pumped into the subsurface of the soil to increase microbial activity, which stimulates pollutant elimination from contaminated areas. Bioventing, on the other hand, involves injecting air into the saturated zone and will aid in the upward migration of volatile organic compounds to the unsaturated zone, thus accelerating the biodegradation phase. Biosparging performance is determined by two main factors: soil permeability and pollutant biodegradability. Biosparging is a closely related technique in bioventing and soil vapor extraction (SVE) known as in-situ air sparging (IAS), which relies on high air-flow thresholds for pollutant volatilization, while biosparging induces biodegradation. Biosparging has been widely used to treat polluted aquifers with gasoline and kerosene [89].

IV. Bioaugmentation

It is one of the biodegradation mechanisms. Bioaugmentation is the incorporation of pollutant-degrading microorganisms (natural, exotic, or engineered) to augment the biodegradative ability of endogenous microbial species in polluted areas. In order to accelerate the development of natural microorganism populations and improve depletion, which preferentially feeds on contaminant sites. Microbes are taken from the remediation site, cultured individually, genetically modified, and then recalled. For example, when soil and groundwater are polluted with chlorinated ethenes, such as tetrachloroethylene and trichloroethylene, all-important microorganisms are discovered. It is used to ensure that in situ microorganisms can completely remove and convert these toxins to non-toxic ethylene and chloride [78].

4.2.4 Plant growth promoting rhizobacteria assist phytoremediation

Phytoremediation (Phyto – plant, remediation – clean) is the process of removing toxins from a polluted area using green plants. Phytoremediation has a lot of benefits, including being an autotrophic device with massive biomass that requires relatively little nutrient input, being easy to maintain, and being widely accepted by society due to its visual attractiveness and environmental sustainability [66]. Many plant species are capable of accumulating toxic metals in both their vegetative and reproductive portions. Plants for phytoremediation should be native to the region, have a rapid growth rate with high biomass yield, a large root system, high resistance, adaptability, and the ability to absorb contaminants. Plants can be classified into three groups based on their metal resistance and metal accumulating efficiency: (i) excluders (metal sensitive plants), (ii) indicators (poor metal uptake and transport), and (iii) accumulators (higher uptake and accumulated) [56]. Plants that are hyperaccumulators can tolerate higher heavy metal concentrations and retain a higher amount of toxic heavy metals in their tissues [68]. However, the performance of the hyperaccumulator has been influenced by a number of edaphic factors that may affect or restrict the plant's productivity, biomass, and metal uptake. Metal bioavailability, redox capability, cation exchange capacity (CEC), pH, extent aeration, organic matter, temperature, and water content are the most important edaphic variables [8]. Furthermore, many other biotic factors influence soil nutrient status, reducing the effectiveness of phytoremediation by reducing growth and metal uptake [53].

Phytoremediation limitation

It is the most effective method for removing heavy metals from polluted soil, but it has several disadvantages [18, 61, 106].

- **Time-consuming-** It takes a long time to remove heavy metals from polluted soil.
- **Efficiency is less-** due to the fact that some hyperaccumulators have a poor growth rate and produce less biomass.
- **Less mobilization-** as a result of certain metal ions that are closely bound.
- **Risk in the food chain-** Contamination of the food chain occurs as a result of poor management and treatment [22].

Plant growth-promoting rhizobacteria (PGPR) are soil-borne bacteria that are associated with plants and have the ability to promote plant growth through a variety of mechanisms. Kloepper 1994 [59] classified PGPR based on their inherent characteristics, such as (i) root colonisation, (ii) plant growth promotion, and (iii) the ability to adapt, thrive,

reproduce, and compete with other microbial flora in order to express their plant growth promotion/protection activities. Furthermore, PGPR can classify objects based on their functional properties. Furthermore, PGPR can be classified according to their functional properties as (i) biofertilizers (increasing soil nutrient availability), (ii) phytoestimators (stimulation of plant growth via the production of phytohormones), (iii) rhizoremediators (modulating pollutant availability through metal solubilization), and (iv) biopesticides (controlling plant pathogens and diseases via the secretion of (Fig. 2) [5]. PGPR strains with the ability to promote plant growth through specific and non-specific mechanisms. PGPR strains promote plant growth by various pathways such as increasing soil nutrient supply, promoting root growth, cell division, elongation, and metabolic stabilisation. However, in non-specific pathways, PGPR strains improve plant growth by suppressing heavy metal-induced toxicity and plant pathogens and enhancing systemic resistance induction. Plant-growth promoting pathways of the PGPR strains are discussed in detail in the following sections [68].

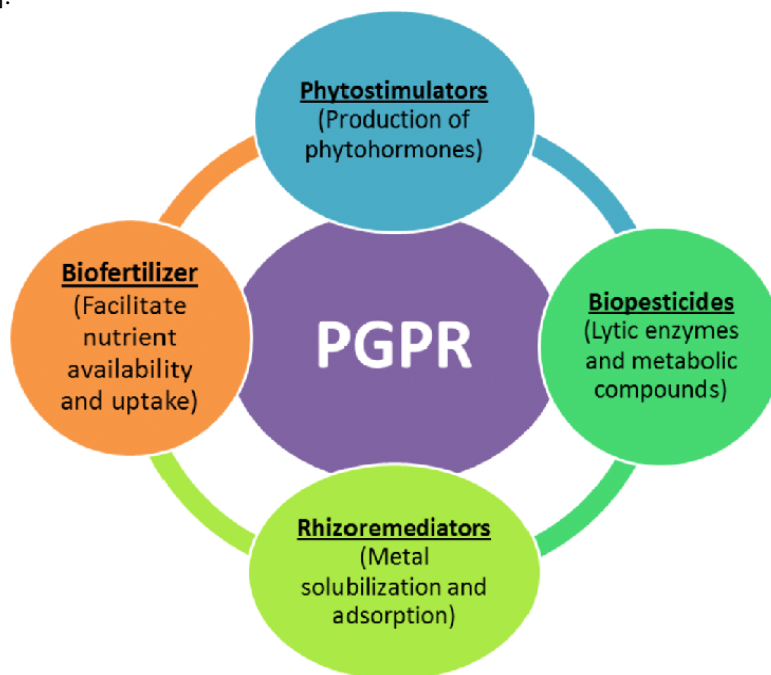


Figure 2. Classification of PGPR based on their functional properties [68]

S. R. Manoj et al., 2020 [68] reported that PGPR are the best candidates for increasing the plant's metal removal production. Reclamation of metal-contaminated soils through a combination of potential PGPR and plants is a sustainable, low-cost, and evolving solution that has made significant progress in in-situ remediation. PGPR alters the nature of heavy metals through a variety of metabolism-dependent and/or independent activities that help plant growth and phytoremediation capacity. Furthermore, PGPR strains regulate metal chelation, absorption, and compartmentalization by modulating the expression pattern of major metal transporters and their related genes. As a result, the use of appropriate PGPR – plant combinations and the optimization of agronomic methods would be beneficial in resolving heavy metal pollution on a wide scale.

V. MECHANISM USED BY MICROORGANISM FOR REMEDIATION OF HEAVY METALS

In the presence of inorganic metals, microorganisms use a variety of mechanisms to associate and function. Microbes use a number of mechanisms to survive metal toxicity, including biotransformation, extrusion, enzyme use, and exopolysaccharides [EPS] development and metallothionein synthesis [24]. Microorganisms have evolved ingenious metal tolerance and detoxification mechanisms in response to metals in the ecosystem. Electrostatic interaction, ion exchange, precipitation, redox mechanism, and surface complexation are some of the processes involved in the mechanism [117]. Metal oxidation, methylation, enzymatic decrease, metal-organic complexation, metal decrease, metal-ligand degradation, metal efflux pumps, demethylation, intracellular and extracellular metal sequestration, exclusion by permeability barrier, and development of metal chelators like metallothioneins, exopolysaccharides, and biosurfactants

are the main mechanical means through which microorganisms resist heavy metals. Metal contamination may be removed by microorganisms by valence conversion, volatilization, or extracellular chemical precipitation [84]. Because of the existence of anionic structures that allow microbes to attach to metal cations, microorganisms have a negative charge on their cell surface [35]. Heavy metals are known to dislodge essential components in biological molecules, hindering their activities and altering the structure or function of enzymes, proteins, or membrane transporters, making them toxic to plants [101]. Coagulation, chemical precipitation, electrodialysis, evaporative recovery, floatation, flocculation, ion exchange, microfiltration, reverse osmosis, ultrafiltration, as well as other methods for heavy metal degradation have been used [42]. Extraction, stabilisation, immobilisation, soil cleaning, and other physicochemical techniques Apart from the development of secondary noxious end-products, these approaches, even if successful, are generally costly due to high energy and chemical reagent requirements [88].

5.1. Biosorption

Bioremediation of heavy metals by microbial cells can be divided into metabolism-independent biosorption, which occurs mainly on the cell's exterior, and metabolism-dependent bioaccumulation, which includes sequestration, redox reaction, and species-transformation methods [111]. Bio sorption is a passive absorption process carried out by dead biomass or living cells by surface complexation on the cell wall and surface layers [32]. Bioaccumulation is based on a number of chemical, physical, and biological mechanisms (Figure 3), both intracellular and extracellular processes, with biosorption playing a minor and ill-defined role [32].

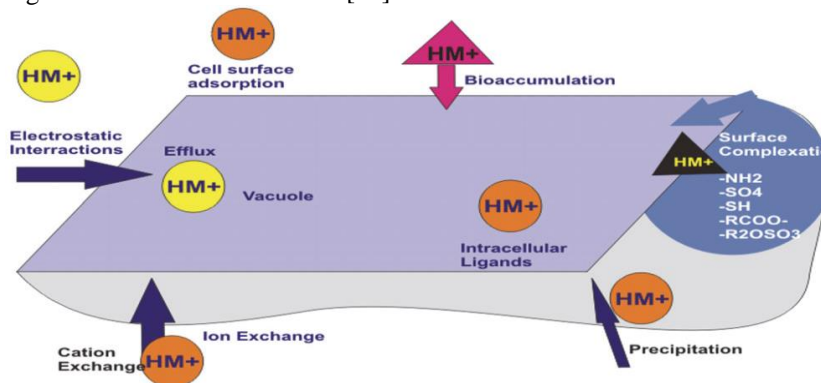


Figure 3. Mechanisms of heavy metal uptake by microorganisms [117]

5.2. Adsorption

When an adsorbate adheres to the surface of an adsorbent, it is called adsorption. Adsorption is regarded as the most efficient and economically viable option for removing metals from an aqueous solution due to its reversibility and desorption capabilities. Adsorption is ineffective with highly concentrated solutions because the adsorbent rapidly becomes saturated with the adsorbate. It is only effective for very dilute solutions, is time - consuming process due to frequent regeneration, and is not selective in terms of metal attenuation. As a result, adsorption is not used in large-scale metal remediation [71].

5.2.1. Process of Sequestering

1. Intra-Cellular Sequestering

Metal ions are complexed by different compounds in the cell cytoplasm, resulting in intracellular sequestration. Interaction with surface ligands, followed by slow transport into the cell, can result in a high concentration of metals within microbial cells. The ability of bacteria to absorb metals intracellularly has been used in a variety of applications, most notably in effluent treatment. With the aid of cysteine-rich low molecular weight proteins, a cadmium-tolerant *P. putida* strain was able to sequester copper, cadmium, and zinc ions intracellularly [48]. In *Rhizobium leguminosarum* cells, glutathione was also found to be involved in the intracellular sequestration of cadmium ions [63]. Chitin, mineral ions, lipids, nitrogen-containing polysaccharide, polyphosphates, and other substances make up the solid cell wall of fungi as well as proteins with many fungi accumulating metals to their mycelium and spores, they can decontaminate metal ions by energetic absorption, extracellular and intracellular precipitation, and valence conversion. The exterior of the cell wall of fungi acts as a ligand for labeling metal ions, causing inorganic metals to be eliminated [115]. Metal-binding ligands (e.g., -OH, -COOH, -HPO₄²⁻, SO₄²⁻, -RCOO, R₂OSO₃, -NH₂, and -SH) are abundant in peptidoglycan, polysaccharide, and lipid components of the cell wall. Amine, which binds to anionic metal species via electrostatic

interaction and cationic metal species via surface complexation, may be more involved in metal uptake among these functional groups [93].

2. Extra-Cellular Sequestering

The accumulation of metal ions in the periplasm by cellular components or the complexation of metal ions as insoluble compounds is both examples of extracellular sequestration. Copper-inducible proteins CopA, CopB (periplasmic proteins), and CopC (outer membrane protein) are formed by copper-resistant *Pseudomonas syringae* strains and bind copper ions and microbial colonies [15]. Metal ions can be ejected from the cytoplasm by bacteria and sequestered in the periplasm. Zinc ions will cross from the cytoplasm into the periplasm of *Synechocystis* PCC 6803 strain through the efflux mechanism [104]. Extracellular sequestration of metals is known as metal precipitation. Iron-reducing bacteria, such as *Geobacter spp.*, and sulfur-reducing bacteria, such as *Desulfuromonas spp.*, can reduce toxic metals to less or nontoxic metals. *G. metallireducens*, a strict anaerobe, may reduce manganese (Mn) from lethal Mn (IV) to Mn (II), as well as uranium (U) from poisonous U (VI) to U (II) (IV) [35].

G. sulfurreducens and *G. metallireducens* can reduce chromium (Cr) from the extremely toxic Cr (VI) to the less toxic Cr (III) [14]. Sulfate-reducing bacteria produce large amounts of hydrogen sulphide, that allows metal cations to precipitate [64]. Under anaerobic conditions, the *Klebsiella planticola* strain produces hydrogen sulphide from thiosulfate and precipitates cadmium ions as insoluble sulphides [90]. Under aerobic conditions, cadmium was also precipitated by the *P. aeruginosa* strain. The soluble divalent lead was precipitated as a complex lead phosphate salt by the *Vibrio harveyi* strain.

Metal ions could be prevented from entering the cell by the microbial plasma membrane, cell wall, or capsule. Metal ions can be adsorbed by bacteria using ionizable cell wall groups (amino, carboxyl, phosphate, and hydroxyl groups) [82]. For nonviable cells of *Pseudomonas putida*, *Brevibacterium sp.*, and *Bacillus sp.*, observed a high degree of passive biosorption of heavy metal ions [40]. Biofilm cells of *Pseudomonas aeruginosa* are more resistant to copper, lead, and zinc ions than planktonic cells, whereas cells at the biofilm's periphery are destroyed. Metal ions were accumulated by biofilm extracellular polymers, which then protected bacterial cells within the biofilm [103].

5.3. Ion Exchange

The exchange of ions between two or more electrolyte solutions is known as ion exchange. It may also apply to the ion exchange between a solid substrate and a soil solution. For the uptake of metals from aqueous solutions, high cation exchange potential clay and resins are widely used. However, this process is time-consuming and only works for some metal concentrations in the solution. This system is also sensitive to temperature and pH. Metals have been removed and attenuated from wastewater using natural and synthetic clays, zeolites, and synthetic resins [79].

5.4. Methylation of heavy metal

As a result of increased lipophilicity and thus increased permeation through cell membranes, methylation increases metal toxicity. In metal remediation, microbial methylation plays an important role. Methylated compounds are very often explosive; for example, Hg (II) can be bio methylated to gaseous methyl mercury by bacteria such as *Bacillus spp.*, *Escherichia spp.*, *Clostridium spp.*, and *Pseudomonas spp.* In contaminated topsoil, bio methylation of selenium (Se) to volatile dimethyl selenide, arsenic (As) to gaseous arsines, and lead (Pb) to dimethyl lead was observed [84].

VI. BIOREMEDIATION OF HEAVY METAL USING ORGANISMS

Microorganisms absorb heavy metals by bioaccumulation, which is an active process and/or adsorption, which is a passive process. To clean up heavy metal polluted areas, microorganisms such as bacteria, fungi, and algae have been used [58]. Metal-resistant strains have been used to remediate heavy metals in single, consortium, and immobilised forms, with the immobilised type possibly having more chemisorption sites to biosorb heavy metals [52].

6.1. Bacterial bioremediation

Microbial biomass has a variety of biosorptive abilities that differ greatly between microbes. However, each microbial cell's biosorption potential is influenced by its pretreatment and experimental circumstances. To improve biosorption, microbial cells must respond to changes in physical, chemical, and bioreactor configuration [11]. Bacteria are essential biosorbents because of their widespread distribution, scale, ability to thrive in regulated environments, and resistance to environmental conditions [99].

Abioye and his coworkers [3] reported *Bacillus subtilis*, *Bacillus megaterium*, *Aspergillus niger*, and *Penicillium sp.* to investigate the biosorption of Pb, Cr, and Cd in tannery effluent. Pb reduction was greatest in *B. megaterium* (2.13 to

0.03 mg/L), followed by *B. subtilis* (2.13–0.04 mg/L). After 20 days, *A. niger* had the best ability to reduce Cr concentrations (1.38–0.08 mg/L), accompanied by *Penicillium sp.* (1.38–0.13 mg/L), and *B. subtilis* had the best ability to reduce Cd concentrations (0.4–0.03 mg/L), followed by *B. megaterium* (0.04–0.06 mg/L). Kim and his co-authors [58] developed a batch method that removed Cr^{6+} , Cu, and Ni with removal efficiencies of 99.8%, 98.2%, and 90.1%, respectively, using zeolite-immobilized *Desulfovibrio desulfuricans*. Abbas et al., 2014 [58] found that bacterial consortia efficiently removed chromium, zinc, cadmium, lead, copper, and cobalt at a rate of 75 to 85% in less than 2 hours of contact time.

6.2. Fungal bioremediation

Fungi are commonly used as biosorbents for the removal of radioactive metals because of their high metal absorption and recovery capacities [33]. The majority of research found that both active and dormant fungal cells play a part in the adhesion of inorganic chemicals [105]. Srivastava and Thakur, 2006 [98] have investigated the efficacy of *Aspergillus sp.* for chromium reduction in tannery wastewater. At pH 6, 85% of the chromium in the synthetic medium was extracted in a bioreactor device, compared to 65% in the tannery effluent. This may be due to the presence of organic contaminants, which stifle the organism's development. The capacity of *Coprinopsis atramentaria* to bioaccumulate 76% of Cd^{2+} at activities of microorganisms increase with a rise in temperature within a suitable range, and it enhances microbial metabolism and enzyme activity, which will significantly improve bioremediation, was investigated. The sorption sites, microbial cell wall configuration, and ionized particles of chemical moieties on the cell wall all influence the stability of the microbe-metal complex. The substrate and a variety of environmental conditions influence the outcome of the degradation process [102].

6.3. Algal bioremediation

In comparison to other microbial biosorbents, algae are autotrophic, meaning they need few nutrients and generate a large amount of biomass. These biosorbents have also been used to remove heavy metals from the environment due to their high sorption potential [2]. Adsorption or incorporation of algae biomass into cells is used for bioremediation of heavy metal contaminated effluent. Phycoremediation is the use of different species of algae and cyanobacteria to remove or degrade toxic substances in order to remediate heavy metals [16]. Algae have chemical moieties on their surface that serve as metal-binding sites, such as hydroxyl, carboxyl, phosphate, and amide [45]. Dead *Chlorella vulgaris* cells were used to extract cadmium (Cd^{2+}), copper (Cu^{2+}), and lead (Pb^{2+}) ions from aqueous solution under different pH, biosorbent dosage, and contact time conditions and the findings indicated that the biomass of *C. vulgaris* is an extremely effective biosorbent for the removal of cadmium (Cd^{2+}), copper (Cu^{2+}), and lead (Pb^{2+}) from mixed solutions containing 50 mg dm³ of each metal ion (95.5%, 97.7%, and 99.4%, respectively) [38].

6.4 Bioremediation of heavy metal by Genetic engineering microbes

Genetic engineering techniques known as recombinant DNA technology, which require gene exchange between microbes, can be used to modify the genetic material of microorganisms. GMMs (genetically modified microorganisms) or GEMs (genetically engineered microorganisms) are such microorganisms (GEM) have been shown to be involved in bioremediation of soil, activated sludge, and groundwater. In the production of GEM for bioremediation, four main approaches are being considered: (1) Chemical sensing, end point analysis, and toxicity reduction applications of bio affinity, bio reporter, sensors (2) Bioprocess growth, monitoring, and control; (3) affinity and enzyme specificity enhancement; (4) pathway construction and regulation [109].

- The PCB catabolic genes are found on the chromosomes of *R. eutropha* A5, *A. denitrificans* JB1, and *Achromobacter sp.* LBS1C1 was naturally conjugated to the heavy metal resistant strain *R. eutropha* CH34 to remove polychlorinated biphenyls [107].
- For heavy metals, the *Arabidopsis thaliana* gene for phytochelatin synthase (PCS; PCSAt) was inserted into *Mesorhizobium huakuii* subsp. *Rengeli* strain B3, resulting in a symbiosis between *M. huakuii* subsp. *Rengeli* strain B3 and *Astragalus sinicus*. [97].
- Genes were expressed to accumulate Cd^{2+} and release phytochelatins in the presence of a bacteroid-specific promoter (the *nif H* gene) [109].
- Heavy metal degradation using genetically engineered microorganisms has piqued interest, as shown by the use of *Alcaligenes eutrophus* AE104 for chromium degradation from industrial wastewater and the construction of a recombinant photosynthetic bacterium with simultaneous expression of the Hg transport system and metallothionein for Hg degradation from heavy metal and wastewater *Rhodospseudomonas palustris* [109].

VII. THE ADVANTAGES AND DISADVANTAGES OF BIOREMEDIATION

Advantages

- It is a natural process that takes some time, but it is an acceptable waste disposal process for polluted materials like dirt. Microbes will degrade the contaminant and multiply in numbers while it's there. The biodegradative population decreases as the contaminant is degraded. The treatment residues are usually innocuous products such as water, carbon dioxide, and cell biomass [1].
- It takes considerably less time and can also be done on-site without creating any damage to daily operations. This also reduces the need to ship large amounts of waste off-site, as well as the possible health and environmental risks that can occur during transportation [1].
- It is used in a cost-effective process because it loses less than other traditional approaches (technologies) for hazardous waste cleanup. Important treatment system for oil-contaminated sites [72].
- It does not have any potentially harmful chemicals. Fertilizers and other nutrients are applied to promote active and rapid microbial development. Usually used in lawns and gardens. The toxic contaminants are totally dissolved as a result of bioremediation, which converts them into the water and harmless gases [91].
- Eco-friendly and long-lasting[21].
- Implementation is relatively simple [60].
- Environmentally-friendly solutions for remediating natural ecosystems from a variety of contaminants [94].

Disadvantages

- Some chlorinated compounds, heavy metals, and radionuclides are examples of compounds that are not amenable to complete degradation. A metabolically capable microbial population, an adequate amount of nutrients and pollutants, and appropriate environmental growth conditions are all needed for biological processes to take place [6].
- Bioremediation systems that are suitable for sites with diverse mixtures of chemicals that are not equally distributed in the atmosphere need further research. Contaminants may exist in the form of solids, liquids, or gases [1].
- Biological systems are often very specialized. The existence of metabolically active microbial species, adequate environmental growth conditions, and sufficient levels of nutrients and pollutants are all important site variables [1].
- Extrapolating from the bench and pilot-scale experiments to full-scale field operations is difficult [1].

VIII. CONCLUSION

This review highlighted the source, environmental impacts of heavy metal contamination caused by some human activities, potential health risks, and the multiple mechanisms and enzymatic reactions utilized by plants and bacteria to efficiently remediate contaminated environments. Heavy metal's toxic effects on the biosphere have sparked international interest in finding a viable solution for eliminating these pollutants from the environment. Heavy metals are removed from the soil, air, and wastewater effluents via a range of biological treatment techniques, the most popular of which are microbial bioremediation and phytoremediation. Microbial bioremediation appears to be a relatively cost-effective and efficient approach. The site description is the first step toward successful bioremediation, as it aids in the development of the most appropriate and promising bioremediation technology (ex-situ or in-situ). Because of the excavation and removal from the archaeological site, ex-situ bioremediation procedures are more expensive. Bioremediation could be aided by C and N sources like glucose and peptone. Many species of bacteria, fungi, and plants have acquired great resistance to well-known toxic metals that allow them to remove large amounts of these metals from contaminated media. Bioremediation technologies have been effectively used in the field, and their importance is growing as the acceptability of environmentally friendly remediation treatments grows. In most cases, the benefits outweigh the disadvantages. Phytoremediation is a new cleanup approach that describes using plants to clean or stabilise polluted environments. The major candidate for enhancing the plant's metal removal efficiency is PGPR. Metal contaminated soil remediation using a combination of prospective PGPR and plant is a sustainable, low-cost, and emerging technique that has made significant success in in-situ remediation



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