

## Book Chapter

# An Overview of Bioremediation: An Ecofriendly Approach for Heavy Metals Removal

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## Abstract

Global industrialization and rising unsuitable anthropogenic activity have greatly harmed the ecosystem. For general health issues, effective treatment is essential. The level of pollution may vary depending on the properties of the contaminants. The development of effective methods for treating wastewater containing pollutants that are also economical, ecologically friendly, and efficient is essential. As it has several benefits over traditional treatment methods, bioremediation is an innovative method of handling contaminants. A method called bioremediation employs biological activity that occurs naturally to remove or render harmless a variety of contaminants. Emerging contamination issues are also thought to be solved via bioremediation. Based on the location of the polluted site as well as the characteristics, type, and strength of the pollutants, bioremediation is carried out either ex-situ (i.e, bioventing, biosparging, bioaugmentation, bio attenuation, and bio stimulation) or in-situ (land farming, composting, bio pile, and bio filter). Microbes can be highly useful in cleaning up contaminated environments. Numerous naturally occurring microorganisms, including the bacteria *Alcanivorax*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, algae, *Anabaena spiroides*, *Microalgae*, *Cladophora*, and *Navicula pupula* fungus, as well as *Phanerochaete chrysosporium*, *Lentinula edodes*, and *Phanerochaete chrysosporium*, for the ingestion of contaminants, hydrophytes like *Eichhornia*

*crassipes*, *bulrush*, *Hornworth weed*, yeast, and enzymes lipases, hydrolases are used in the bioremediation process. Using plants to remove pollutants from soil and water is a new technology called phytoremediation. The development of microorganisms and the effectiveness of bioremediation are substantially accelerated by the presence of macro-and micronutrients and by parameters including pH, temperature, humidity, soil structure, water solubility, site features, and oxygen content. Generally speaking, bioremediation is a very efficient and environmentally responsible approach for removing dangerous contaminants from the environment. Because of this, it is less invasive and can help with environmental impact remediation without endangering sensitive ecosystems. To identify additional biological solutions for the bioremediation of pollutants from various environmental systems, more research is therefore required to create bioremediation technologies.

## Keywords

Bioremediation; Bacteria; Algae; Fungi; Hydrophytes; Heavy Metals; Waste Water Treatment

## Introduction

Huge quantities of inorganic and organic compounds are discharged into the environment each year as a result of anthropogenic activities [1]. In a number of cases, the discharge is well regulated and deliberated such as industrial emissions while in some cases they are accidental such as oil or chemical spills [2]. Many of these compounds released in aquatic and terrestrial environments are both persistent and toxic. The pollution of groundwater, surface and soil is due to the accumulation of toxic compounds exceeding permissible levels [3]. Pollution of the topsoil, freshwater and environment has evolved from global urbanization and industrialization. Human activities results in deterioration of water quality, such as release of metal effluents from electricity generation, battery companies, steel mills and mining, posing major environmental issues. Industrial effluents contain pollutants such as trace metals, polythenes and petroleum harm the environment.

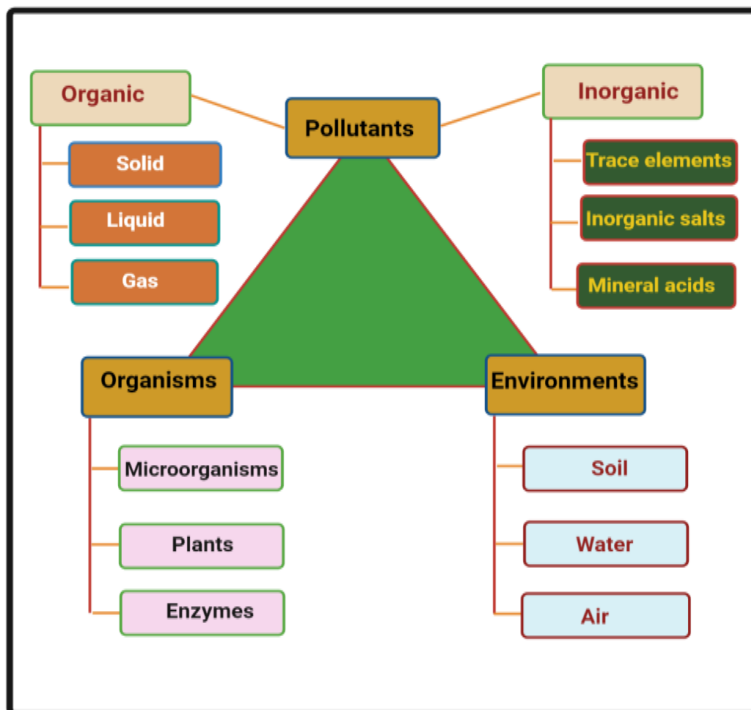
Heavy metals are contaminants that exist naturally in the Earth's crust and are hard to decompose [4]. "Heavy metals" is a term in general referred to metalloids and metals with density exceeding  $5 \text{ g/cm}^3$  including zinc (Zn), silver (Ag), mercury (Hg), lead (Pb), iron (Fe), copper (Cu), chromium (Cr), cadmium (Cd), arsenic (As), and others [5]. They are extensively involved in anthropogenic activities like fertilizers, pigments and dye manufacturing, electroplating, mining, fossil fuel combustion and other industrial processes, which are then discharged in large quantities into the environment via wastewater or other pathways. Due to their non-biodegradable nature, heavy metals lean to persist in nature, leading to accumulation in food chain that results severe health and environmental issues [6]. Many of these metal contaminants are mutagenic to humans and their surroundings. The heavy metals accumulate in the kidney, liver and brain. Other effects on animals consist of stunted growth, nervous system damage, cancer and even death [7]. Heavy metals present in soil decrease food quantity and quality by inhibiting plant growth, nutrient absorption and metabolic processes.

The global utilization of organic compounds is growing due to technological expansions, agricultural practices and industrialization. Unluckily, this is accompanied by increasing organic pollution and toxic waste discharge into the environment [8]. The organic contaminants can be categorized based on molecular structures, and chemical functional groups (esters, ethers, amino, ketonic, hydroxyl and others). The production of materials like paints, gasoline, adhesives and plastics contributes to release of toxic organic compounds [9] that are severely affecting human health, aquatic life and land quality. The noteworthy environmental damage caused by organic contaminants has led to significant efforts to decrease their introduction into environment (by wastewater treatment) and their presence in contaminated sites (by remediation of the soil and contaminated groundwater). Chlorinated hydrocarbons such as carbon tetrachloride, polychlorinated biphenyls, chloroform, perchloroethylene, and trichloro-ethylene are the most polluting

organic contaminants present in huge quantity in environment, resulting from industrial effluents, herbicides and insecticides [10]. A chief category of organic pollutants results from pharmaceutical industry which discharges huge quantities of wastewater [11]. The textile industry is an additional key source of contaminants as dyes are synthesized from various toxic chemicals. The organic pollutants cause severe health issues such as birth-related defects, cancer and reproductive problems [12]. Hence, it is decisive to remediate the heavy metals in wastewater before releasing them into environment.

The overall quality of the environment is intrinsically tied to the quality of human life on our planet. Environmental contamination is a long-term problem posing harmful effects on human health. Although, numerous ways have been used to document and address this problem, as it continues to be a troubling issue for the environment and humans [13]. Many modern physical and chemical remediation techniques such as chemical precipitation, ion exchange, membrane filtration, floatation, coagulation, flocculation, and other traditional pollution removal procedures are used for wastewater treatment [14]. However, they are insufficient to clean up the environment. Bioremediation is one of the strategies that should be developed to protect the environment and humans from the negative effects of pollution. Bioremediation is a term used to describe a process that uses biological entities to reduce (degrade, detoxify, mineralize, or transform) pollution concentration to a safe level. Recent improvements in bioremediation techniques have occurred in the last two decades, with the ultimate goal of effectively restoring damaged areas in an environmentally acceptable and cost-effective manner. Compared to chemical and physical methods of treatment, bioremediation has many advantages, including being environmentally benign and cost-effective. Bioremediation employs microbes, algae, fungus, green plants, or their enzymes to restore the natural environment to its pre-contamination state. Bioremediation uses natural biological activity to render harmless or eliminate a variety of pollutants. As a result, bioremediation employs low-cost, low-tech procedures that are often well-received by the public and may often be performed on-site [15]. Instead of employing

complete biomass of microorganisms for bioremediation, microbial enzymes isolated from their cells have been used in recent years that are a simple, rapid, environmentally friendly, and socially acceptable method of remediation. Although, owing to fact degradation of pollutants by microorganisms is a long process, bioremediation is not in practice generally [16].



**Figure 1:** Bioremediation is a triple corner process.

*Alcaligenes, Arthrobacter, Aspergillus, Bacillus, Burkholderia, Mucor, Penicillium, Pseudomonas, Stenotrophomonas, Talaromyces, and Trichoderma* are just a few of the microbial species that have been implicated in bioremediation. *Archaea* from severe habitats, such as *Natrialba* and *Haloferax*, have also been found to be useful bioresources for biological remediation [17-18].

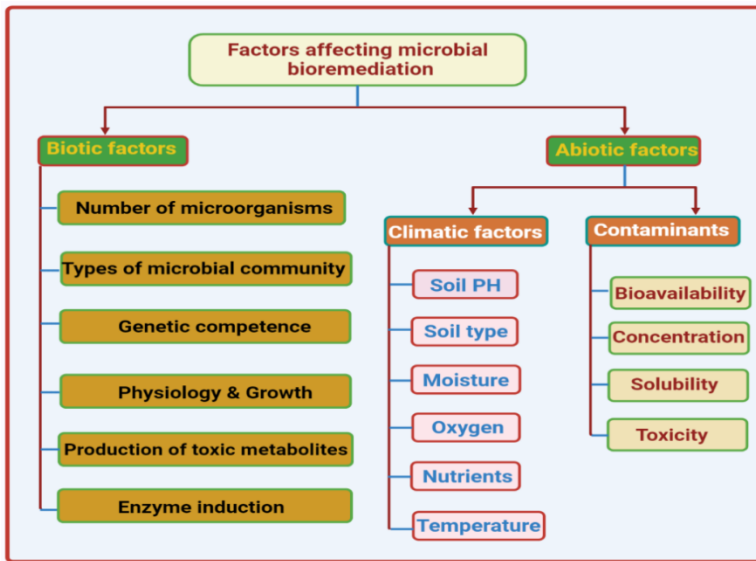


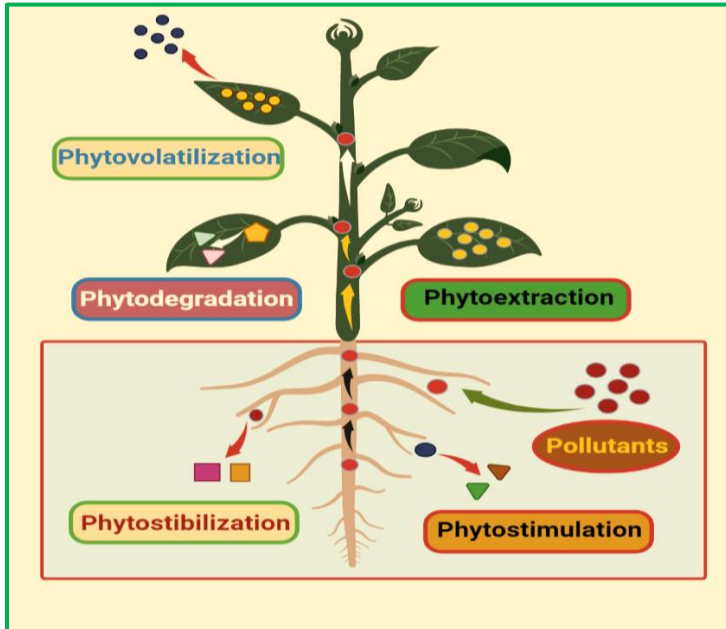
Figure 2: Factors affecting bioremediation.

## Types of bioremediation

### In Situ Bioremediation

In-situ bioremediation is the application of bioremediation in the subsoil, as compared to ex-situ bioremediation. In-situ bioremediation can be used in the unsaturated zone (for example, bioventing) or in saturated soils and groundwater [19]. **Bioventing** is the most frequent in-situ remedy, which includes providing air and nutrients to contaminated soil via wells to activate the local bacteria. Low air flow rates are used in bioventing, which delivers only the oxygen required for biodegradation while limiting volatilization and pollutants released into the atmosphere. Bioventing is effective for simple hydrocarbons removal and can be used in situations where pollution is deep below the surface [20]. **Bioaugmentation** is the injection of pollutant-degrading microorganisms (natural, foreign, or manufactured) to boost the bio-degradative capacity of indigenous microbial communities in contaminated areas [21].

**Biosparging** is injecting high-pressure air beneath the water table to increase groundwater oxygen levels and speed up bacterial bioremediation of pollutants. Despite unfavorable conditions, bioventing and biosparging procedures were used simultaneously to ensure the efficient removal of soil pollutants [22]. **Bio stimulation** involves increasing bacterial growth to begin the bioremediation process. First, filthy soil is mixed with enriched nutrients and important chemicals to boost microbial activity, allowing pollutants or harmful compounds to be rapidly degraded into carbon, nitrogen, and phosphorous sources [23]. **Phytoremediation** is regarded as an effective, aesthetically pleasing, cost-effective, and environmentally benign method for removing potentially hazardous metals from the environment. In phytoremediation, plants collect toxins through their roots and then translocate them to the aerial parts of their bodies. Phytoremediation employs various methods including phytoextraction, phytostabilization, phytovolatilization, and rhizofiltration, for heavy metal uptake and accumulation in plants [24].



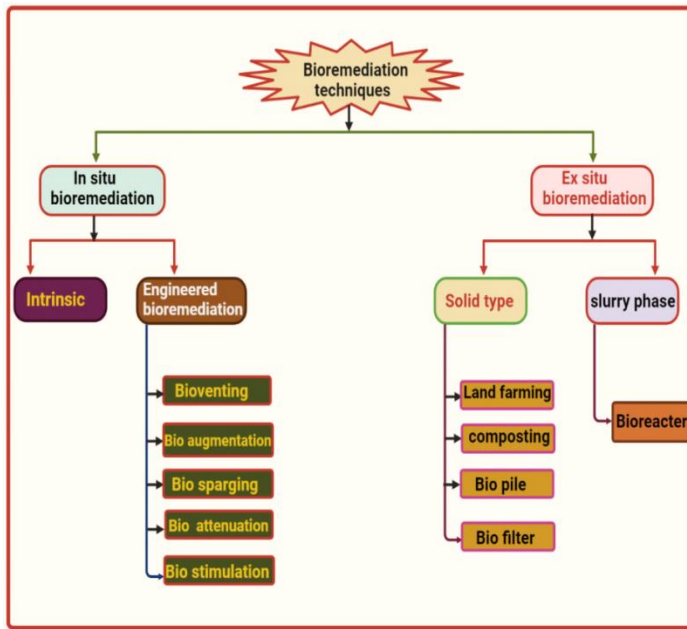
**Figure 3:** Phytoremediation.



## Ex Situ Bioremediation

Ex-situ bioremediation is the collection of contaminated materials from its original location and treating it elsewhere [25]. Excavation or removal of contaminated soil from the ground is included in these approaches [26]. **Land farming** is a relatively easy soil remediation technique that entails excavating polluted soils and placing them in lined beds. The main advantage of Land farming is that it uses simple equipment and is easy to operate, making it a cost-effective method [27]. **Composting** is a long-standing process for treating agricultural and municipal solid waste and sewage sludge, with its usage in soil bioremediation being relatively new. The basic idea is to mix contaminated soil with non-hazardous organic amendments, such as other solid wastes (such as manure or agricultural wastes) that may be composted to promote the growth of bacterial communities that can digest contaminants in the soil through metabolic pathways [28].

Biocells, bioheaps, biomounds, and compost Cells are all terms for **Biopiles**, which have been widely used to remediate various petrochemical pollutants in soils and sediments. The bio pile approach is hybrid of land farming and composting that creates a favorable environment for native aerobic and anaerobic microorganisms while also limiting physical contamination losses through leaching and volatilization [29]. **Bioreactor** approach is an ex-situ biochemical processing system that uses microbes to remove contaminants from wastewater or pumped groundwater. It can also be used to treat contaminated soils in both solid and liquid (slurry) phases. The idea is the same regardless of the medium undergoing bioremediation; only the biodegradation equipment differs. Under aerobic, anoxic, and anaerobic circumstances, slurry bioreactor technology can be used in batch, continuous, and semi-continuous versions [30].



**Figure 4:** Types of Bioremediation.

## Role of Algae in Bioremediation

Several marine organisms and human-beings may be at risk due to the abundance of organic pollutants in aquatic habitats. Therefore, it must first be remediated before being discharged into rivers. Algae are one of humanity's most potentially valuable organisms in various ways and represent a significant potential resource in various applications, including food and feed, fine chemicals, pharmaceuticals, biofertilizers, and biofuel. They also contribute to the degradation of organic pollutants and the accumulation of metals, polycyclic aromatic hydrocarbons (PAHs), phenanthrene, and fluoranthene, as well as the biodegradation of xenobiotic compounds and polythene [31]. Algae cells can store radioactive materials, hazardous organic and inorganic chemicals, or both and are essential to self-purifying wastewater from industries, farms, and municipalities. Algae are excellent decontaminating agents because they are cheap, easy to use, non-polluting, and relatively quick at recovering metal contaminants for recycling and do not generate

secondary waste [32]. Therefore, algae are crucial to the biological treatment of wastewater, known as "**Phycoremediation.**" [33].

Phycoremediation technique that uses algae to remove various organic toxins is realistic and has a lot of potentials. As this technique is environmentally friendly and don't produce secondary pollutants, phycoremediation is gaining popularity [34]. *Microalgae* and *Cyanobacteria* being independent of nitrogen and carbon trophic, represent a potential new option for distillery wastewater bioremediation. To prevent light blocking, coloured effluents must be diluted before treatment since this is light-dependent reaction [35].

### Phycoremediation

The buildup of organic contaminants in the environment has the potential to be extremely harmful, significantly affecting the stability of many aquatic ecosystems as well as the environment and human health. Human activities such as farming, burning fossil fuels, industrial discharges, home effluents, and agricultural runoff all result in introducing organic contaminants into aquatic environments. Persistent organic pollutants are hazardous to both the environment and human health. To eliminate, decompose, or render harmless organic contaminants in aquatic systems, there has recently been an increase in interest in employing bacteria, fungi, plants, and algae. The management and biomonitoring of organic contaminants in aquatic habitats are essential functions of algae [42].

A new bioremediation technique that uses algae to remove various organic toxins is realistic and has a lot of potentials. Several marine animals' health and well-being may be at risk due to the abundance of organic pollutants in aquatic habitats. It must first be cleansed before being discharged into rivers. Because they are environmentally friendly and don't produce secondary pollutants, algae-based wastewater treatment technologies are gaining popularity [34]. *Microalgae* and *cyanobacteria*, nitrogen and carbon trophic independent represent a potential new option for distillery wastewater bioremediation. To prevent light

blocking, coloured effluents must be diluted before treatment since they are light-dependent reactions [35].

## Bioremediation of Polythene Using Algae

Algae are known to degrade polythene. Crystallinity, molecular weight, shape, and the functional groups on the polythene surface are among the characteristics that change after colonization of algae. Algae have been observed to grow quickly, widely available in nature, relatively simple to isolate and colonise on polythene surfaces using polymeric carbon. *Scenedesmus dimorphus*, *Anabaena spiroides*, and *Navicula pupulawere* proven efficient at breaking down polythene. On polythene sheets, a significant amount of algal growth was seen [36]. Because of their extraordinary ability to sorb metals, algae hold great promise for wastewater treatment. It is generally recognized that *Oscillatoria quadripunctulata*, *O. tenuis*, *Spirogyra hatillensis*, *Spirogyra hyaline*, *Cladophora glomerata*, *Oedogonium rivulare*, *C. vulgaris* and *Spirulina maxima* are capable of removing heavy metals from the environment. According to previous research on the possible application of *Cyanobacteria* species in the phycoremediation of municipal wastewater, *O. limosa* and *Nostoc commune* were observed to be very effective for removing NO<sub>3</sub>, PO<sub>4</sub>, SO<sub>4</sub>, and Cl<sub>2</sub>, and lowering electrical conductivity (EC) values [37].

## Role of Algae in Bioremediation of Heavy Metals

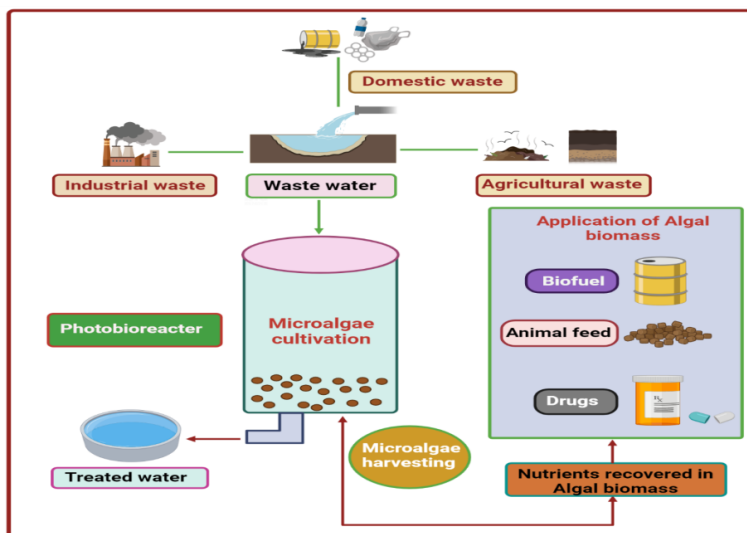
The algae showed potential for both the hyperaccumulation of heavy metals and the breakdown of xenobiotics. Microalgae have a key role in carbon dioxide fixation, which has sparked a lot of interest in their usage in the bioremediation of coloured wastewater in recent years. Additionally, the biomass of algae produced has enormous potential as a feedstock source for biofuels generation. Microalgae's bioremediation abilities are beneficial for environmental sustainability [38]. *Cyanobacteria* have lately gained attention as experimental organisms in environmental biotechnology programs due to their function in soil fertility, their potential to remove heavy metals from polluted places, and their active involvement in soil reclamation.

*Cyanobacteria* play a vital function in suppressing weeds, degradation, or transformation in addition to enriching the soil, turning heavy metals into less harmful or non-toxic forms. Natural hydrocarbons can also be broken down by *Cyanobacteria*[39].

In recent years, the increased global transportation of dangerous chemicals has resulted in accidental chemical spills into the environment. One chemical frequently found in unintentional spills is phenol [40] that are harmful, carcinogenic, and poisonous to humans and animals even in small amounts. It is preferred to use inexpensive phycoremediation technique to remove organic pollutants like phenol. The microalgal enzymes increase effectiveness in the degradation process and involved in phenol's metabolic breakdown [41]. Phycoremediation technique is now successfully replacing physiochemical methods in the remediation of the environment due to the special properties of algae in digesting different harmful contaminants, including aromatic hydrocarbons, phenols, heavy metals, and organochlorine. Currently, *Chlorella*, *Phytoplankton*, *Spirulina*, *Scenedesmus*, and *Chlamydomonas* are the major non-pathogenic *microalgae* that have been used in phenolic chemical phycoremediation [32].

## Microalgae-based Wastewater Bioremediation

Microalgae also referred as "wonder organisms," may easily carry out bioremediation through the use of biosorption and bioassimilation. They can grow as "algal blooms" and absorb different chemicals in polluted water. After harvesting and extraction of lipids and proteins, the algal biomass can be employed as a powerful biosorbent [35]. According to previous research, green algae can interact with heterotrophic bacteria and may break down various chemicals, including prometryne and a variety of complex pesticides. Microalgae also reduce the need for external aeration and promote the growth of various degraders by raising the oxygen concentration in the water [42].



**Figure 5:** Microalgae based wastewater bioremediation.

The production of microalgae involves four primary steps: (i) site selection; (ii) growing; (iii) harvesting; and (iv) extraction. Light, carbon dioxide, water, and inorganic salts are required for algae cultivation, and the growth medium should be rich in inorganic components such as nutrients, phosphorous, nitrogen, iron, and silicon. It's crucial to pre-and post-treat contaminated locations to control microbial development. The pre-treatment, which is the container's colour during cultivation, impacts the growth and photosynthetic activity of microalgae. The primary objective of post-treatment is to raise effluent quality. However, pre- and post-treatment demand extra money and effort [43]. Additionally, microalgae frequently utilize solar energy and convert it to biomass. A significant portion of the feedstock used to make various products is microbial biomass. As a result, there are several applications for microalgae-based technologies in product development and the environment [44].

**Table 1:** Potential of Algae in bioremediation of organic pollutants.

S.NO	Algae species	Organic pollutants	Reference
1	<i>Cladophora glomerata</i> , <i>Vaucheria debaryana</i>	Cadmium and lead	[7]
2	<i>Rhodophyta</i>	Hexachlorocyclohexane	[31]
3.	<i>Phytoplankton</i>	Chlorinated hydrocarbons	[32]
4	<i>Chlamydomonas reinhardtii</i>	Herbicide, cupric ions	[32,41]
5	<i>Selenastrum capricornutum</i>	Benezene, pyrene	[33]
6	<i>Monoraphidium braunii</i>	Bisphenol	[45]
7	<i>Navicula pupula</i>	Polycaprolactone, Plastics	[37, 42]
8	<i>Scenedesmus acuminatus</i>	Phenol	[34]
9	<i>Anabaena spiroides</i>	Polythene	[36]
10	<i>Chlorella miniata</i>	Chromium, Nickel	[37]
11	<i>Spirogyra neglecta</i> , <i>Pithophora oedogonia</i>	Cadmium, nickel	[39]
12	<i>Cladophora</i>	AMD	[40]
13	<i>Spirulina maxima</i>	Phenol	[40]
14	<i>Scenedesmus obliquus</i> GH2	Crude-oil	[46]
15	<i>Green algae</i>	Tannins	[47]
16	<i>Microalgae</i>	Organophosphorus pesticides	[48]
17	<i>Zygnema pectinatum</i> , <i>spirogyra spp</i>	Chromium and Zinc	[49]

The origin and ongoing buildup of industrial micro pollutants in the environment, such as dyes, heavy metals, organic debris, and pharmaceutically active compounds (PhACs), pose a grave threat to health of the world's flora and fauna. Living algae have recently been recognized and widely used as promising agents in the bioremediation of micropollutants to provide environmentally acceptable solutions. Removing dyes and heavy metals is typically more suited for non-living algae, whereas removing organic matter and PhACs is better suited for living algae [45]. Acid mine drainage (AMD) is a major global environmental hazard. It is extremely acidic and includes large concentrations of heavy metals that harm the ecosystem. For

AMD, traditional therapeutic approaches might not work and for the effective removal of heavy metals from AMD, cost-effective solutions must be used. A few algae strains that have demonstrated the ability to remove a significant amount of heavy metals from AMD are *Spirulina sp*, *Chlorella*, *Scenedesmus*, *Cladophora*, *Oscillatoria*, *Anabaena*, and *Phaeodactylumtricornutum*. They have high selectivity for different elements and function as "hyper-accumulators" and "hyper-adsorbents." Furthermore, they produce a lot of alkalinity, which is necessary to precipitate heavy metals during treatment [46].

### **Bioremediation of Tannins using Phytoplankton Species**

Tannins are unique plant metabolites used in leather production that acts as pollutants. These chemicals can potentially burst cells and are poisonous to aquatic biota. The removal of wastewater from tanneries is challenging due to these negative impacts. The community components of phytoplankton species like cyanobacteria, silica-encased diatoms, *Cinoflagellates*, green algae, and chalk-coated *Coccolithophores*, are infrequently considered in the biodegradation of organic compounds. However, these organisms can enhance the biodegradation of contaminants in diverse ways when interact with bacteria. Tannic acid (TA) is biodegraded using non-axenic cultures of *Synechococcus* sp. with *Rhizobium rosettiformans*, *Sphingomonaskoreensis* and *Chlorella vulgaris* containing *Lactobacillus casei* [47].

### **Role of Fungi in Bioremediation**

The most powerful natural decomposers are fungi, a vital part of the soil food web and a source of food for another soil-dwelling biota [50]. Fungi are among Nature's most active decomposers of waste material. The majority of fungi can grow in soils under various climatic circumstances, even in quite harsh conditions. They can spread by dispersing spores in the air and keeping the environment balanced [51]. Fungi can be used in various biotechnological applications, including bioremediation.



Compared to other conventional methods for remediating contaminated soil, using fungi and their consortia is an efficient, cost-effective, and economical strategy [52]. Fungal bioremediation is the most secure and environmentally friendly technique for cleaning up polluted sites [53]. Additionally, it has been claimed that they can endure in effluent treatment plants (ETPs) that process different waste fluids [54, 55]. Fungi are prospective candidates for bioremediation in a variety of sites due to their capacity to secrete a wide variety of enzymes and can survive in a variety of habitats. Numerous fungi may degrade organic contaminants that are resistant to degradation in various environmental situations. [56].

## **Mycoremediation**

The mycoremediation involves the total breakdown of harmful pollutants into non-toxic substances like CO<sub>2</sub>, H<sub>2</sub>O, N<sub>2</sub>, and HCl with the assistance of various fungus species. Mycoremediation is a process that actively cleans up contaminated environments in a sustainable and environmentally beneficial manner. Fungi are considered significant biological agents for the bioremediation of pollutants because of their distinctive shape and metabolic capacity. On the other hand, the use of fungi to improve environmental biotechnology and remove contaminants was already being discussed in the 1980s [57]. Since then, several studies have been conducted on the fundamental and applied aspects of mycoremediation in both soil and water. Due to their ubiquity, the majority of fungus species have been used in a variety of fields all over the world. By dispersing their spores and secreting various enzymes, fungi may thrive and grow well in harsh environmental conditions. Additionally, they employ mycelium to expel poisons from hazardous locations like industrial effluent treatment facilities. Mycoremediation is promoted as a safe method for removing contaminants from soil since fungus may break down organic chemicals without extraction, reducing the likelihood of pollution transmission and bioaccumulation into the ecological food chain. Compared to other organisms, fungi are more appropriate and have better capabilities for soil bioremediation than other microorganisms such as bacteria and yeasts [58].

Fungi are among the feasible options that produce a variety of hydrolyzing enzymes that are essential for the breakdown of waste products. Therefore, to achieve successful mycoremediation, it is necessary to identify the fungal species that target certain pollutants [19]. It has been demonstrated that fungi are important players in the bioremediation of contaminants such as pesticides, polycyclic aromatic hydrocarbons (PAHs), textile dyes, coal, paper leather tanning effects, persistent organic pollutants (POPs), and textile dyes. Numerous reports have discussed the bioremediation and decolorization of textile dyes, sugar industry effluent, chemicals used in kraft pulp mills, and leather tanning effluent using fungi from various groups, including *Aspergillus*, *Penicillium*, and alkalophilic white-red fungi, demonstrating the wide range of substrate choices made by these fungi [59].

### **Fungal Enzymes for Bioremediation**

Many enzymes of fungi have low specificity, allowing the fungal strain to metabolize multiple pollutants, even those with different chemical structures. A common crust fungus called *Phanerochaete chrysosporium* degrades a wide range of harmful chemicals, including benzene, ethylbenzene, xylene, toluene, organochlorines, and N-heterocyclic explosives. To break down organic substances fungi release various extracellular oxidoreductase enzymes such as laccases, tyrosinases, lignin peroxidases, manganese peroxidases. Basidiomycetes and Ascomycetes primarily make the most well-known copper-containing laccases. They are employed in decolorizing and detoxifying textiles, the bleaching of ink in paper manufacturing, the degradation and detoxification of recalcitrant compounds in wastewater, and the degradation of hazardous compounds produced during coal processing, and the decolorization of soil pollutants. Tyrosinase, a different copper-containing enzyme, also oxidizes phenols and chlorinated phenolic compounds. Such chlorinated chemicals could potentially be eliminated from the environment by fungal enzymes [51].

Additionally, cytochrome P450s are crucial for the intracellular breakdown of pollutants. They have a role in the fungal

metabolism of several medicines, including diphenyl ether herbicides, anti-inflammatory pharmaceuticals, lipid regulators, anti-epileptic and anti-analgesic drugs, and drugs that regulate lipids. The contaminants subject to microbial bioremediation are broken down by microbial metabolism until they are mineralized. Transferases, which transfer the functional groups and transform hazardous pollutants into nonhazardous compounds, mostly comprise aromatic nitroreductases, quinone reductases, etc. By turning the contaminants with hydroxyl groups into conjugates, transferase enzymes destroy them. The conjugates are kept inactive by being fixed or secreted into the environment [60]. Cellulolytic fungi like *Aspergillus*, *Penicillium*, *Chaetomium*, *Trichoderma*, *Fusarium*, *Stachybotrys*, and *Cladosporium* have all been studied previously for bioremediation. Numerous fungal strains have produced proteases from the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, and *Trichoderma reesei* [61].

### Bioremediation of Heavy Metals using Fungi

Heavy metals are another group of toxins of environmental concern with a possible solution arising from fungal treatments. The wastewater, especially from industrial sources, has a high concentration of heavy metals that get into people and animals through the food chain. Heavy metals are found in extremely high concentrations in wastewater, primarily from the electroplating, paint, leather, metal, and tanning industries [62]. According to reports, fungi and other microorganisms can remove heavy metals from wastewater through bioaccumulation and biosorption in an inexpensive and environmentally benign manner. *Trichoderma viride*, *Aspergillus awamori*, *Phanerochaete chrysosporium*, and *Aspergillus awamori* have been utilized to remove Pb, Cd, Cr, and Ni heavy metals from wastewater [63].

The most extensively researched and understood ligninolytic fungi are the white rot fungi. These fungi grow and break down lignocellulosic materials (often tree wood) in nature, which is how they cause white rot in the wood. The white rot fungus including *Phanerochaete chrysosporium*, *Bjerkandera adusta*,

and *Pleurotus* secretes various ligninolytic enzymes, such as laccases and peroxidases. A variety of organic environmental pollutants have been demonstrated to be metabolized by *Chrysosporium*. Later, *Pleurotostreatus* and *Trametes versicolor* were among the other white rot fungi for which the enzyme secreting capability was observed. [64]. *Aspergillus niger* and its hydrolytic enzymes cellulolytic, hemicellulolytic, pectinolytic, and amylolytic could be used to transform complex polymeric compound to simple ones, acting as mediators for biogas production. Marine fungi produce efficient secondary metabolites, biosurfactants, enzymes, polysaccharides, and fatty acids used in the bioremediation of hydrocarbons and heavy metals [65].

The mycelium of *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Pleurotostreatus*, *Cladosporium sphaerospermum*, *Deuteromycete* fungi were found to be capable of breaking down high molecular weight Polycyclic Aromatic Hydrocarbons (PAHs) in non-sterile soils (on average 23 percent). Due to their ability to remove metal ions from aqueous solutions, fungi have been studied as potential biosorbents. Filamentous fungi can create large quantities of extracellular enzymes during hyphal colonization of soil and are also more resistant to environmental stress, which results in faster rates of bioremediation of xenobiotics [66]. It has been discovered that ligninolytic fungi are more effective than bacteria at degrading PAHs. Taxonomically heterogeneous higher fungi known as *ligninolytic* fungi are distinguished by their ability to depolymerize lignin [61].

## Genetically Engineered Fungi for Mycoremediation

Developing engineered better fungus and enzymes for mycoremediation is being pushed forward by recent developments in molecular biology, biotechnology, and enzymology. To achieve the goals of mycoremediation, fungal genes can be cloned. The production of fungal mutants that secrete particular enzymes, allow for the design and scaling up of numerous systems for the treatment of wastes and wastewater [67]. The genome sequences of the filamentous fungi *Aspergillus*

*nidulans*, *Aspergillus fumigatus*, *Neurospora crassa*, *Coprinus cinereus*, and the yeasts *Schizosaccharomyces pombe* and *Candida albicans*, have been made public through a number of programs. The *S. pombe* comprises 13.8 million base pair genome and 4,940 protein-coding genes, including the mitochondrial genome.

The 40-Mb genome of the first filamentous fungus, *N. crassa*, is anticipated to include 10,000 genes. The genome of the first basidiomycete has 30 million base pairs. The genome has been shown to contain genes for oxidases, peroxidases, and hydrolytic enzymes [68]. Genetic engineering has opened up new avenues for research in the field of mycoremediation and the biodegradation of lignin and other organic pollutants. A binding affinity for cellulose has recently been bred into yeast. In contrast to bacteria, fungal role in biodegradation is poorly recognized relatively in terms of biotechnological advancements. *White rot fungi* have been developed through biotechnology, and they are now being used to treat a variety of refractory wastes and clean up contaminated soils [69].

**Table 2:** Bioremediation of pollutants using fungi.

S.NO	Fungal species	Pollutants	References
1	<i>Phanerochaete chrysosporium</i>	Cadmium, Cobalt	[63]
2	<i>Bjerkandera adusta</i>	Lignin, Lead	[61]
3	<i>Cladosporium sphaerospermum</i>	PAHs	[66]
4	<i>Dichomitussqualens</i>	Textile dyes	[59]
5	<i>Pleurotussp. pulmonarius</i>	Crude oil	[50]
6	<i>Phlebia sp.</i>	TNT, AmDNT, coal	[50, 63]
7	<i>Lentinula edodes</i>	2,4-Dichlorophenol	[52]
8	<i>Calocybe indica</i>	Copper, zinc, iron	[58]
9	<i>Pleurotussajorcaju</i>	Heavy metal, Zn	[56]
10	<i>Gongronella sp. and R. stolonifer</i>	Gasoline	[51]
11	<i>Penicillium sp.,</i>	Pesticides	[55]
12	<i>Trichoderma sp.</i>	Organic pollutants	[70]
13	<i>Rhizopus sp.,</i>	Lead, Arsenic	[67]
14	<i>Fusarium ssp</i>	Thallium	[71]
15	<i>Trametes versicolor</i>	Lignin, Zinc	[65]

## Factors Affecting Mycoremediation

The physical and chemical characteristics of soil, such as temperature, pH, moisture content, chemical make-up, redox potential, soil type, and presence of macro- and micronutrients greatly affect the development of microorganisms and the efficiency of bioremediation [71]. Pollutant degradation is mainly influenced by various parameters, including the types of microorganisms present, their genetic make-up, metabolic potential, fungal species, their life cycles, surface-active and chelating chemicals, and extracellular and intracellular enzymatic systems. In mycoremediation, filamentous fungi with metabolic pathways, such as *Aspergillus* and *Penicillium* spp., hasten the production of mycotoxins to eliminate various contaminants, including medicinal chemicals. Low oxygen levels are one of the environmental variables that fungi can withstand for effective mycoremediation [67].

The concentration of pollutants has an impact on the activity of microbes, which in turn has an impact on the mycoremediation process. By allowing access for oxygen supply, enzyme production, fungal development and water availability in soil influences the efficiency of pollutant biodegradation. The impact of temperature and pH are critical element for microbial development and efficient bioremediation of contaminants. It has been observed that the ideal temperature for the mycoremediation process is 25-30°C with high soil pH [71]. When treating contaminated soils with mycoremediation, the relative humidity is typically kept above 60%. Soil nutrient content is a significant component in speeding up bioremediation. The growth, activity, and functionality of microorganisms that move water and nutrients in and out of cells depend on the soil's moisture [70].

## Role of Bacteria in Bioremediation

Bioremediation is a biological process that relies on microorganisms, plants and/or their derivatives (enzymes or used biomass) to degrade or modify environmental pollutants while performing normal living functions [71-73]. The nutritional

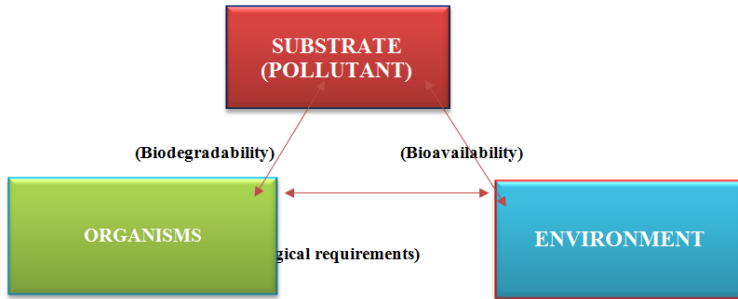
diversity of microorganisms can also be used to biodegrade pollutants [74]. Microorganisms are important for removing pollutants from soil, water and sediments. Microbes restore the original natural environment and prevent further pollution [75]. The use of microbial bioreactors for treatment is very attractive in that it provides a controlled environment that is important for optimizing the microbial bioreactor process. Another advantage is the flexibility in designing the bioreactor (size and configuration) for the intended purpose of the application or reactor [8,76-78].

## **Factors Affecting Bacterial Bioremediation**

Bioremediation involves the decomposition, removal, alteration, immobilization, or detoxification of various chemicals and physical wastes from the environment by the action of bacteria, fungi, and plants. Microorganisms engage through their enzymatic pathways, act as biocatalysts, and promote biochemical reactions that break down desired contaminants. Microorganisms can only neutralize contaminants if they have access to several substance compounds that help produce the energy and nutrients to build more cells. The efficiency of bioremediation depends on many factors [79].

### **Factors**

- Biological factors
- Environmental factors
- Nutrients
- Temperature
- Concentration of oxygen
- Moisture content
- PH



**Figure 6:** Bioremediation process.

## Biological Factors

Biological factors affect the degradation of organic compounds through competition between microorganisms for limited carbon sources, antagonistic interactions between microorganisms, or predation of microorganisms by protozoa and bacteriophage. The decomposition rate of pollutants often depends on the concentration of pollutants and the amount of "catalyst" present. In this context, the amount of "catalyst" represents the number of organisms capable of metabolizing contaminants and the amount of enzyme produced by each cell. The expression of certain enzymes by cells can increase or decrease the rate at which contaminants are broken down. The most important biological factors included are mutations, horizontal gene transfer, enzyme activity, interactions (competition, inheritance, and predation), self-growth to reach critical biomass, population size and composition [80-81].

## Environmental Factors

The metabolic properties of microorganisms and the physicochemical properties of target pollutants determine possible interactions during the process. The environmental factors such as pH, temperature, humidity, soil structure, water solubility, nutrients, site characteristics, redox potential and oxygen content determine the dynamics of decomposition. Biodegradation occurs over a wide pH range. However, generally the optimum pH is 6.5-8.5 for biodegradation in most aquatic and terrestrial systems [80,82].



## Nutrient

For microorganisms to survive and continue their activity, they need various nutrients such as carbon, nitrogen, and phosphorus. The addition of nutrients regulates the balance of nutrients essential for microbial growth and reproduction, affecting the rate and effectiveness of biodegradation. Nutritional balance, especially intake of essential nutrients such as N and P, can improve biodegradation efficiency by optimizing the bacterial C: N: P ratio [83-84].

## Temperature

The process of decomposing certain compounds requires a certain temperature. Temperature also strongly influences the physiological properties of microorganisms, thus accelerating or slowing down the bioremediation process. The activity of microorganisms increases with temperature and reaches a maximum at the optimum (25-30°C) temperature [71].

## Bacteria and Pollutants

**Table 3:** Bacteria and organic compound interaction.

S.No	BACTERIA	COMPOUND (be made up of)	REFERENCE
1	<i>Mycobacterium spp</i>	Aromatic hydrocarbons	[86]
2	<i>Pseudomonas putida</i>	Benzene and xylene	[87,88]
3	<i>Acinetobacter spp</i>	Aromatic hydrocarbons	[86]
4	<i>Bacillus subtilius</i>	Phenol	[ 89]
5	<i>Cyanobacteria,</i>	Naphthalene	[90,91]

**Table 4:** Bacteria involved in oil degradation.

S.NO	BACTERIA	COMPOUND (oil related)	REFERENCES
1	<i>Corynebacterium propinquum</i>	Oil (fuel)	[92]
2	<i>B. brevis</i>	Crude oil (rock oil)	[93]
3	<i>P. aeruginosa</i>	Diesel oil (petro diesel )	[94]
4	<i>Bacillus cereus A</i>	Diesel oil(petro diesel )	[95]
5	<i>Citrobacter koseri,</i> <i>Bacillus coagulans</i>	Diesel oil(petro diesel ), crude oil(rock oil)	[96]

**Table 5:** Ideal examples of bacteria in dyes biotreatment (bioremediation).

S. NO	BACTERIA	COMPOUND (dyes)	REFERENCE
1	<i>B. subtilis strain</i> (NAP1, NAP2, NAP3)	Oil paints	[97]
2	<i>Myrothecium roridum</i> IM 6482	Textile dyes(colors)	[98-100]
3	<i>Micrococcus lutents</i>	Textile dyes (azo dyes)	[101]
4	<i>Bacillus firmus,</i> <i>Staphylococcus aureus</i>	Vat dyes, Textile effluents	[102]

**Table 6:** Bacteria responsible for the degradation of high-density metals.

S. NO	BACTERIA	COMPOUND (high-density metals)	REFERENCES
1	<i>Aerococcus spp</i>	Chromium (Cr),	[104-105]
2	<i>P. fluorescens and P. aeruginosa</i>	Ferrous ion, zinc ion, copper ion	[106]
3	<i>Microsporium spp</i>	Cadmium ( Cd)	[107]
4	<i>Aeromonas spp</i>	Uranium (U), nickel (Ni)	[108]
5	<i>Geobacter spp</i>	Iron metal and uranium (radioactive metal)	[109]

## Role of Hydrophytes in Bioremediation

Hydrophytes are also known as aquatic macrophytes. The hydrophytes can grow in different types of water system such as a lake, marshes, rivers, ditches, springs etc., The plants are fixed in the bed of water bodies, and can grow up to 25 feet in length in horizontal form. Hydrophytes can tolerate different conditions, such as high and low nutrient conditions and salinity of about 7%. The hydrophytes can treat domestic and textile wastewater and remove heavy metals from textile industry effluents. Due to its potential to absorb heavy metals in its body tissue, it is used as a bioremediation agent. For the removal of metal ions from water samples hydrophytes are very effective. Thus, the method is environmentally friendly and is best to stop the pollution of metal ions in different water bodies. Pollutants from air, soil, water are absorbed that collected in the tissues of

plants without causing any harm to them and play an efficient role in environmental pollution treatment in the sites of their growth [108-109].

### **Hydrophytes Involve in Heavy Metals Extraction**

Bioaccumulation can be defined as the quantity of chemical compounds including pesticides and heavy metals within the organism's body [110-112]. When there is an increase in level of heavy metals within the plant's tissue, these heavy metals are accumulated by plants in special sites in the stem or roots and convert it to non-toxic form for again usage and distribution in various metabolic processes [113]. For removing heavy metals, Hydrilla is more effective [114]. *Hydrilla verticillata* was most effective for accumulating copper ions. *Salvinia natans* may also be involved in the treatment of heavy metals and oil spills from aquatic environments. Water hyacinth is also involved in the removal of a high range of contaminants like heavy metals, hardness, Biological oxygen demand, Chemical oxygen demand, dissolved solid, Total suspended solids and many other contaminants related to wastewater [115].

### **Effect of Heavy Metals on Other Organisms**

Due to growing domestic and industrial activities, heavy metals concentration in water bodies is increased, especially in ponds and rivers that, cause harm to fauna and aquatic flora. In the tissues of aquatic plants, bioaccumulation may cause harm to wild life in the different water systems [116]. Excessive concentration of copper is toxic to living organisms such as humans and other living organisms especially fish [117]. Due to persistent nature of heavy metals, they gather into the food chain through bio magnification process and caused serious threats to aquatic and human health [118-119]. The heavy metals, through the aquatic food chain, enter the human body and cause harmful effects. Blood constituents are affected and cause toxicity to the kidney, liver, lungs, and other important organs lead to cancer [120-121]. Heavy metals lead to the damage of aquatic environments and, as a result, oxygen depletion, pollution and oxidative stress take place.

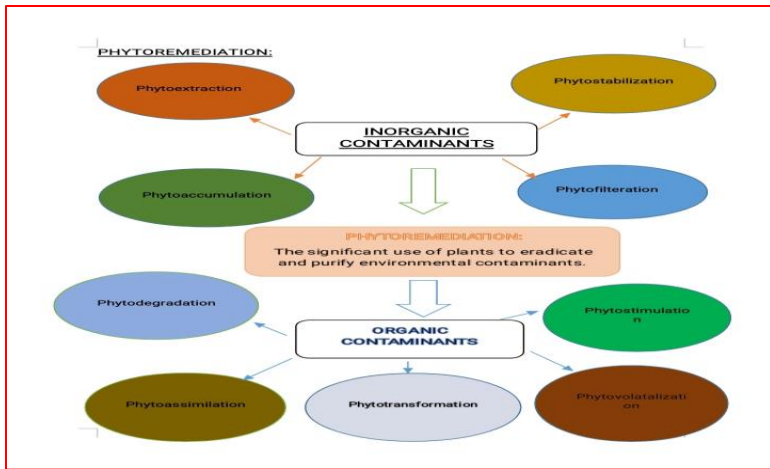


Figure 7: Phytoremediation.

## Significance of Hydrophytes for Phytoremediation of Waste Water

For the treatment of heavy metals, phytoremediation is considered significant due to cost-effectiveness [122-123]. Earlier researchers revealed that from different kinds of wastewater, heavy metals can be eliminated by aquatic plants / Hydrophytes [124-127]. Hydrophytes are involved in removing heavy metal either through absorption or through surface adsorption and merging them into their tissues (128-129). The different types of aquatic plants such as water lettuce, water hyacinth, *Salvinia*, and giant duckweeds show greater phytoremediation ability of different kinds of wastewater [130-131].

### The Function of Hydrophytes Plant

The most important function of hydrophytes is the consumption of dissolved nutrients (phosphorus and nitrogen) from water [132]. For the removal of excess amounts of phosphorus and nitrogen from polluted water, hydrophytes are widely used in constructed wetlands around the world [133]. Different hydrophytes plants are involved in wastewater treatment and

provide habitat to wild life [134]. Hydrophytes are involved in reducing velocities of water flow and play an important role in suspended solids deposition [135].

## Factors

The heavy metals are accumulated inside plant bodies depends on several factors such as light intensity, temperature, salinity, ions, pH, amount of oxygen humic substances and organic matter called plant claw [136].

**Table 7:** Hydrophytes involve in degradation of heavy metals.

S.NO	Hydrophytes	Degradation of compound(metalloid/metals)	References
1	Water hyacinth( <i>Eichhornia crassipes</i> )	Lead(pb), mercury(Hg),Zinc(Zn), nickel(Ni)	[139-140]
2	Water lettuce( <i>Pistia stratiotes</i> )	Iron(Fe), Magnesium ( Mn), Zinc (Zn), Chromium(Cr)	[141-142]
3	Water fern( <i>Salvinia herzogii</i> )	Cadmium(Cd), Chromium (Cr)	[143-144]
4	Duckweed( <i>Lemna minor</i> )	Nickel(Ni),lead(pb),copper (Cu), Chromium(Cr).	[145-147]
5	Bulrush ( <i>Scirpus</i> )	Iron (Fe), Cadmium (Cd),Aluminium (Al)	[148]
6	<i>Hydrilla verticillata</i> (water thyme)	Lead (pb),chromium(Cr), Mercury (Hg), cadmium(Cd).	[149]
7	Duck weed( <i>polygonum hydropiperoids</i> )	Lead (Pb), Cupper( Cu ), Zinc (Zn)	[150]
8	Hornworth( <i>Ceratophyllum Demersum</i> )	Lead (Pb), Cadmium( Cd ), Arsenic (As), Chromium(Cr)	[151,152]
9	Water spangles( <i>Salvinia minima</i> )	Chromium(Cr), Nickel (Ni), Cadmium(Cd), Aresnic ( As)	[153,154]
10	Duck weeds( <i>Spirodela intermedia</i> )	Iron (Fe), Cupper(Cu), Magnesium (Mn), lead (pb), Zinc(Zn), Chromium(Cr)	[155,156]

11	Water Cress( <i>Nasturtium officinale</i> )	Nickel (Ni), Chromium( Cr), Cupper(Cu), Zinc(Zn)	[157,158]
12	Parrot feathers( <i>myriophylluspicatum</i> )	Cadmium(Cd), lead(pb), Cupper(Cu), Iron (Fe)	[159,160]
13	Hornwort( <i>Cerato phyllum Demersum</i> )	Chromium (Cr), Arsenic (As), lead (pb), Cadmium(Cd)	[161,162]
14	Pondweed( <i>Potamogeton Crispus</i> )	Iron(Fe), Cupper(Cu), Zinc(Zn), Nickel (Ni), Magnesium (Mn)	[163]
15	American pondweed( <i>potamogeton pectinatus</i> )	Lead(Pb), Cadmium( Cd), Zinc(Zn), Cupper(Cu)	[164,165]
16	Common Cattail( <i>Typhalatifolia</i> )	Magnesium (Mn), Nickel (Ni), Zinc(Zn), Cupper(Cu), Iron(Fe),Lead (Pb)	[166-168]
17	Water mint( <i>Metha aquatics</i> )	Iron (Fe), lead(pb), Cupper (Cu),Cadmium(Cd)	[169,170]
18	Tape grass( <i>vallisneria spiralis</i> )	Argon(Ar)	[171]
19	Cordgrass( <i>Spartina alterniflora</i> )	Zinc(Zn), Cupper(Cu), Nickel (Ni),Chromium(Cr), Cadmium(Cd), lead(pb), Magnesium (Mg)	[172,173]
20	Smart weed( <i>Polygonum hydropiperoids</i> )	Zinc (Zn), Cupper(Cu), Lead (Pb)	[174]

## Genetically Modified Microorganisms in Bioremediation

Bioremediation is most commonly done with bacteria, but it has also been done with fungi, algae, and plants [172]. Microorganisms are a promising solution for treating pollution in the environment. Several genetic techniques have been developed and employed to enhance enzymes, metabolic pathways, and organisms relevant to bioremediation [173]. Organisms can be incorporated with supplementary genetic characters for biodegradation of particular contaminants if naturally existing organisms are not capable to do

bioremediation job quickly or appropriately. Genetically engineered microorganisms have shown potential in bioremediation applications in activated sludge environments, groundwater and soil due to the improved degradative abilities of a wide range of pollutants (Table.8)[174]. Recent developments in molecular biology have opened up novel perspectives to advancement in engineering organisms with the aim of performing bioremediation.

Bacteria have high potential for degradation of environmental contaminants. Microorganisms that can biodegrade different contaminants have been isolated with the ultimate goal of exploiting their metabolic ability for the bioremediation of polluted areas[175]. Though, a number of toxic and recalcitrant xenobiotic compounds including highly halogenated and nitrated aromatic compounds, and some explosives and pesticides, are generally stable, inert chemically under, and not known to be efficiently degraded by several microorganisms [176]. Moreover, the toxicity of the organic contaminants to the naturally occurring microbial populations, together with complications caused by pollutants mixture, is a chief hindrance to thriving biodegradation by microorganisms. These limitations to bioremediation have paved the road for the development of genetically engineered microorganisms that have artificially designed catabolic pathways [177].

*Alcanivorax* is a genus of marine bacteria that degrades alkanes. Oil leakage is one of the most devastating calamities that can happen in marine environment. A shift in the organization of the early bacterial population was discovered using 16S rDNA research. In petroleum-contaminated sea water that had been reported to be nitrogen and phosphorus-rich, bacteria closely related to the species *Alcanivorax* formed the dominating bacterial group. This implied that these bacteria were crucial in the bioremediation of oil-contaminated maritime ecosystems [178].

The isolated *Pseudomonas putida* strain APRRJVITS11 (51.89% efficiency) shows great promise in removing chromium pollution from waste effluents. Depending on the size of the affected area,

effluent volume, and site contamination clearance time, it can be employed on-site and in a bioreactor. *Bacillus thuringiensis* strain APRRVITS15 also demonstrated high potential in chromium removal from effluent wastes, albeit with a slightly lower effectiveness (50.07%) [179]. **Microbial fuel cells** are a new platform for treating wastewater and generating power simultaneously. The refinery effluent could be treated while generating an electric current in an air-cathode chamber using *Pseudomonas putida* (BCRC 1059), a wild-type bacterium, across four batch cycles for 63 days [180]. *P. putida*, *P. aeruginosa*, and *P. stutzeri* can potentially adsorb metals such as Cu and Zn from wastewater.

*Pseudomonas putida* and *Pseudomonas stutzeri* can degrade phenol up to 80.5% and cyanide up to 80.6%, as well as reducing the pH of effluent from alkaline to near neutral. Furthermore, it possessed the capacity to minimize biological and chemical oxygen demand. According to the study, such prospective bacterial strains could be used to remove phenol and cyanide-containing industrial wastewater before it is released into the environment [181]. In recent years, microbial breakdown of pollutants has been identified as a long-term solution for cleaning up a polluted environment. Microbial degradation is the most important and effective natural method for removing petroleum hydrocarbon contaminants and dispersants from the environment. Microorganisms have enzyme systems that can breakdown and use various hydrocarbons as a source of carbon and energy [182].

The potential of *Pseudomonas aeruginosa* KX828570 to decompose crude oil in an oil-polluted soil location suggests that it could be used to remediate other oil wastes such as oil spill dispersant polluted terrestrial soil, marshland, and water. In the oil spill dispersant-damaged soil environment, it is a good development to carry out bioremediation employing the bio-augmenting bacterium *Pseudomonas aeruginosa* KX828570. **Bio mineralization** is the process of organisms producing minerals, which results in complex compounds, including minerals and organic components. Many microorganisms revealed the



potential to precipitate a variety of minerals such as carbonates, phosphates, sulfides, oxides, and oxalates.

The ease of scaling-up, transportation and genetic engineering makes fungus the organisms of choice in bioremediation [183]. Several genetic engineering approaches have been developed and proven favorable in adding the desired characters in enzymes or metabolic pathways. Alterations of specific gene can be controlled and designed by means of metabolic engineering. Genes of fungi can be cloned to meet the mycoremediation objectives. Fungal mutants that secrete particular enzymes can be formed, and different methods using such mutants may be scaled up and designed in the remediation of wastewaters and wastes. Protoplasts of fungi can be exploited to improve mycoremediation. Recently, efforts to boost flux via specific pathways have met with partial success. The future of metabolic engineering is bright potentially, but there is still a long way to go to comprehend this area of the metabolic network before the introduction of bioengineered fungi or yeast in mycoremediation. Recent development in biotechnology can pave the way for the designing genes responsible for mineralization of polychlorinated biphenyls by fungi. Genes that encodes Lignin peroxidase in about 30 fungi have been screened that may help in the degradation of polychlorinated biphenyls [184]. *Neurospora crassa*, a urease-positive fungus, was studied for its ability to biomineralize calcium carbonate and its potential use in metal biorecovery and/or bioremediation [185]. *Nigrosporasphaerica*, *Penicillium discolor*, *Penicillium digitatum*, *Neurospora crassa*, *Alternariuminfectoria*, *Trichophyton verrucosum*, and *Mucor mucedo*, isolated from rhizosphere soils, were also employed for heavy metal bioremediation [186].

**Table 8:** Genetically engineered microorganisms.

S.NO	Microorganism	Pollutant	Reference
1.	<i>Burkholderiapseudomallei</i>	Naphthalene	[15]
2	<i>Neptunomonasnaphthovorans</i>	Polyaromatic hydrocarbon	[15,17]
3	<i>Alcaligenesspecies</i>	2,4Dichlorophenoxy acetic acid	[16, 17]
4.	<i>Pseudomonasaeruginosa</i>	Crude oil	[172, 181]
5	<i>Enterobacter cloacae</i>	hexavalent chromium	[181, 173]
6	<i>Alcanivorax</i>	Alkane	[178]
7	<i>Deinococcusradiodurans</i>	Toluene and Chlorobenzene	[180]
8	<i>Pseudomonas putida</i>	Mono-and dichloro aromatic compounds	[182]
9	<i>Acinetobacter species</i>	4- Chlorobenzene	[185]
10	<i>Comamonastestosteroni</i>	2- and 4-chlorobiphenyl	[187]
11	<i>Saccharomyces cerevisiae</i> (CP2 HP3)	Cd and Zn	[188]
12	<i>Phanerochaetechrysosporium</i>	polycyclic aromatic hydrocarbons	[189]

## Future Prospects for Bioremediation

The process of bioremediation is a very effective and environmentally friendly method for removing harmful substances from water systems/water bodies. Fungi are thought of as naturally occurring decomposers that can greatly reduce and destroy persistent and extremely harmful pollutants. The specific lifestyle and metabolic characteristics of fungi highlight their significance in biotechnological implications for converting hazardous compounds to nonhazardous ones. Using microorganisms, different toxic and dangerous substances can be removed and converted into harmless substances. Different

Hydrophytes plants and algal species are involved in the removal of different types of toxic, chemical and many other substances that cause harm to the environment. Various microbes are investigated and identified from different places to reduce waste and produce useful substances.

Environmental contamination is society's most significant issue in the twenty-first century, and research communities are paying close attention to it. Microbes are an effective tool for bioremediation because they speed up the natural biodegradation processes and swiftly adjust to toxic and changing surroundings. A complete understanding of the microbial communities and how they behave in the presence of pollutants and in the natural environment are essential for creating innovative, potentially effective bioremediation methods that are ecologically stable. The microbial community is still regarded as a "black box" even though there is currently limited information of the biological contribution to the effect of bioremediation and its impact on the ecosystem. The molecular microbiological methods outlined will spur an investigation into these problems. Bioremediation will become a more dependable and secure technology due to the modern molecular microbiological techniques' new understanding of process optimization, validation, and influence on the ecosystem.

There are still gaps in our knowledge of the ecology, gene expression, and metabolism of the bacteria participating in bioremediation, which might be filled by expanding the scope of the studies to include multi-omics. Key metabolic genes from several microorganisms are available for incorporation into other organisms. Microbes that have been genetically altered and have improved pollution-degrading abilities will undoubtedly play a significant role in the future of this subject. Field trials and expanding our understanding of microbial genetics to improve the capacity to breakdown contaminants will undoubtedly lead to advancements in this area. It would also be intriguing if bioremediation-related products were created for widespread use.

Bioremediation combines the methods used by numerous fields. Opportunities for novel bioremediation methods will exist as each profession develops and as there are new cleanup needs. Improvements in our understanding of microbes may also increase the efficiency of bioremediation in achieving cleanup standards. Bioremediation is being advanced through creative engineering methods for supplying ingredients that promote microorganisms. For instance, the recently developed technique of gas sparging has greatly increased the potential for aerobically destroying petroleum hydrocarbons. Engineering advancements that, if effectively used, would increase the availability of pollutants to microbes are now the subject of research. These advancements would boost the effectiveness of bioremediation. High-pressure matrix fracture, heat-induced solubilization of pollutants (through steam, hot water, or hot air), and maybe surfactant addition are new methods for enhancing contamination delivery to organisms. Improved techniques for distributing the microbes may increase microbial contact with the pollutants and result in more successful bioremediation. The need for reliable approaches for evaluating bioremediation will grow as new bioremediation methods are introduced from the lab into industrial practice. Future bioremediation evaluation practices will become more common as the search for better cleanup solutions picks up speed. The simplicity of assessing bioremediation would be significantly impacted by quick, trustworthy, and affordable site characterization tools.

It is necessary to take measures to inform all facets of society about what bioremediation is and what it can and cannot accomplish. The persons who hold positions of direct decision-making need to be better educated, especially. Practitioners and regulators who are already dealing with complex bioremediation application must receive training in technical fields outside of their areas of competence. A sustainable and ideal agricultural system preserves the environment, maintains and improves human health, benefits consumers and producers spiritually and economically, and produces enough food to feed a growing population. One of the biggest obstacles facing Pakistani agriculture is salt-affected soils. Salinity is affecting a large area of Pakistan. Soil salinity is a disease or cancer of soil. The

country's economy is dependent on the agricultural sector. Soil is the backbone of the economy of an agricultural country. The indiscriminate use of brackish water can harm the ecosystem and the soil. Researchers and practitioners have attempted to either change the plant to fit the saltwater environment better or modify the environment to suit the plant better. Plant breeding and genetic engineering have produced salt-tolerant plant varieties that aid in stabilizing these extremely saline soils. Phytoremediation is the use of plants such as *Prosopis pallida* (mesquite), *Acacia nilotica* (gum arabic tree), and *Acacia ampliceps* were the species (Symbol) the most effective eucalyptus species for bioremediation. Approximately 400 ha of salt-affected soils have been successfully restored to productive use in the Faisalabad area of the Punjab province using saltbushes (*Atriplex lentiformis* and *Atriplex amnicola*) and the River Red Gum tree, *E. camaldulensis*. In salt-affected soils, there are several processes for phytoremediation.

## Conclusion

Bioremediation stands as a promising and ecofriendly approach for the removal of heavy metals from contaminated environments. Through the utilization of various microorganisms, plants, and their metabolic processes, bioremediation offers a cost-effective and sustainable alternative to traditional remediation methods. This review article has highlighted the diverse strategies employed in bioremediation, including biosorption, bioaccumulation, and microbial transformations, showcasing their potential to mitigate the detrimental effects of heavy metal pollution on ecosystems and human health. However, it is important to acknowledge that successful implementation of bioremediation requires careful consideration of factors such as site-specific conditions, microbial activity optimization, and regulatory compliance. Continued research and innovation in this field hold the key to harnessing the full potential of bioremediation as an efficient and environmentally friendly solution to heavy metal contamination, paving the way for a cleaner and healthier future for our planet.

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