

AN OVERVIEW OF BIOREMEDIATION

Abstract

Bioremediation is a process that employs bacteria, fungi, green plants, or enzymes derived from bacteria to remediate the environment affected by diverse pollutants. It harnesses natural environmental processes, operating without human interventions, to diminish the mass, toxicity, mobility, volume, or concentration of contaminants at various sites. These pollutants stem from industrial activities and the human-induced consequences of the industrial revolution, resulting in the substantial release of chemicals into the environment over time. In comparison to conventional methods such as incineration and chemical treatment, bioremediation offers numerous advantages. Notably, it can be efficiently utilized to generate a renewable source of energy by converting plant biomass. Key microorganisms involved in bioremediation encompass aerobic bacteria, actinomycetes, cyanobacteria, anaerobic bacteria, fungi, and yeast. The use of Genetically Modified Organisms (GMOs) represents a recent approach with significant advantages. It is imperative to focus on various factors that enhance the degradation rate of pollutants by organisms or plants.

Keywords: Pollutants, Bioremediation, GMOs, Natural processes.

Authors

Dr. Darshana Patil

Associate Professor
Department of Botany,
Smt. Chandibai Himathmal Mansukhani
College,
Ulhasnagar, Thane, Maharashtra, India.
drdarshanapatil@rediffmail.com

Dr. Kishori Tarfe

Assistant Professor
Department of Biotechnology,
Smt. Chandibai Himathmal Mansukhani
College,
Ulhasnagar, Thane, Maharashtra, India.
drkishoristarfe@gmail.com

Human-accessible resources, including land and water, exhibit varying degrees of contamination attributed to industrial activities and the disposal of hazardous waste. The contamination of these resources poses a potential threat to human, animal, and plant life. Given the associated risks of these contaminants, it is essential to explore diverse approaches aimed at either removing or minimizing the entry of these contaminants into the environment.

The traditional remediation approaches involve high-temperature incineration and various chemical decomposition methods, such as base-catalyzed dechlorination and UV oxidation. Some techniques include landfilling or employing caps to contain and isolate contaminated sites. In the case of landfilling, contaminants are relocated, posing significant risks during excavation, handling, and transport of hazardous materials. Identifying new landfill sites for the final disposal of such materials becomes challenging and costly. The cap and contain method retains contamination on-site, necessitating ongoing monitoring and maintenance of isolation barriers, leading to associated costs and potential liabilities. While these techniques effectively reduce levels of various contaminants, they have drawbacks such as technological complexity, high costs for small-scale applications, and a lack of public acceptance. Moreover, individuals handling these methods are at risk of exposure to contaminants, posing potential harm to both human health and the environment.¹

Bioremediation entails employing biological agents, primarily microorganisms such as yeast, fungi, or bacteria, to break down environmental pollutants and remediate contaminated soil and water. This technology facilitates the removal of pollutants from the environment within controlled conditions, ultimately restoring the original natural surroundings and preventing further pollution. Specific microorganisms play a crucial role in converting, modifying, and utilizing toxic pollutants, transforming them into less toxic or non-toxic elemental and compound forms. This process serves the dual purpose of obtaining energy and promoting biomass production.^{1,2}

Various life-forms, including plants, can be employed in bioremediation, but microorganisms, particularly bacteria and fungi, exhibit the highest potential. Microorganisms possess a natural recycling capacity, enabling them to transform both natural and synthetic chemicals into sources of energy and raw materials for their growth. This inherent ability allows for the replacement of costly chemical or physical remediation processes with more cost-effective and environmentally friendly biological processes.^{2,3}

Hence, microorganisms offer substantial potential as a reservoir for innovative environmental biotechnologies. The potential of microorganisms in the realm of bioremediation is being substantiated through extensive research. *Geobacter metallireducens*, a bacterium identified for its ability to extract radioactive waste like uranium from polluted groundwater and drainage waters in mining operations, exemplifies the efficacy of microorganisms in this field. Additionally, even deceased microbial cells can contribute to the effectiveness of bioremediation processes.²

Microorganisms find extensive application in addressing pollution caused by hydrocarbons, including petroleum and its byproducts, oils, and petroleum itself when introduced into the environment. Particularly worrisome scenarios involve the occurrence of oil slicks resulting from oil tanker incidents and the release of gasoline into the marine environment.²

Oil can serve as a nutrient source for various microorganisms, and several of these microorganisms produce potent surface-active chemicals capable of emulsifying oil in water, facilitating its removal. The microbial emulsifier is non-toxic and biodegradable, distinguishing it from chemical surfactants. Microorganisms with the capacity to degrade petroleum include certain yeasts, mycobacteria, several corynebacteria, and pseudomonads.

In addition to hydrocarbon decomposition, microorganisms possess the capability to remediate industrial waste and transform the hazardous cations of heavy metals, such as selenium, into substantially less harmful soluble forms. For example, locoweed and various other plants play a significant role in eliminating a considerable amount of the hazardous element selenium. These plants retain selenium in their tissues, rendering it safe for consumption until the plant is ingested. Numerous bacteria and algae release substances that attract highly toxic metals, effectively removing these metals from the food chain due to their attachment to the secretions. Specific fungi and anaerobic bacteria also contribute to color degradation.²

Pesticides are employed to address the increasing demand for food from the expanding global population. The extensive use of these synthetic agents has led to the accumulation of xenobiotics, a class of artificially complex substances. One approach to addressing this issue is the introduction of genetically modified bacteria, which can effectively break down these xenobiotics.²

The primary pollutants responsible for causing pollution are organic pollutants, characterized by a diverse range of functional groups and affiliations with various families. These compounds include petroleum hydrocarbons, encompassing mixtures of n-alkanes, other aliphatics, mono-, di-, and polyaromatic compounds, heterocyclic aromatics, and other minor constituents. Additionally, surfactants, biocides (such as chlorinated phenols), and various other compounds specific to particular industries (e.g., nitroaromatics from munitions) fall within this category.⁴

Various industries, including manufacturing and ore extraction, release metals into the environment. Metals are commonly employed in catalysis processes. Metals can be categorized into two groups: those with exclusive toxicity, such as mercury and arsenic, and those serving biological functions, such as copper, zinc, and cobalt. Microbes cannot destroy metals, but they can uptake and convert many of them into less hazardous oxidation forms. Contaminated areas often exhibit a combination of organic and metallic contaminants, as numerous industries produce both types in their waste streams.⁴

I. MECHANISM OF BIOREMEDIATION

Microorganisms possess enzymes that facilitate the breakdown of environmental pollutants, enabling them to transport and neutralize toxins. The objective of bioremediation is to supply the appropriate nutrients and essential elements for microbial metabolism, facilitating the decomposition and detoxification of harmful pollutants detrimental to the environment and living organisms. Enzymes mediate all metabolic reactions and are categorized as hydrolases, lyases, transferases, isomerases, ligases, oxidoreductases, and hydrolases. Certain enzymes exhibit a notably broad capacity for degradation, owing to their

dual affinity for both selective and nonspecific substrates. For the bioremediation process to be effective, microorganisms must enzymatically attack contaminants, transforming them into safe products.^{3,4}

For bioremediation to be effective, the environmental conditions must support microbial growth and activity. It is common to modify environmental conditions during its implementation to enhance the development and expedite the microbial degradation process.^{3,4}

The involvement of living organisms and fertilizers supports the inherent bioremediation process. Biodegradation serves as the foundational principle of the bioremediation technique, involving the complete conversion of organic hazardous contaminants into benign or naturally occurring substances such as carbon dioxide, water, and inorganic compounds that are safe for utilization by aquatic, terrestrial, and animal life. Numerous processes and pathways have been identified for the biodegradation of a diverse array of organic molecules.³

The target of bioremediation is to completely destroy the pollutants if possible or at least to transform them to nontoxic or less toxic form.

Table 1: Microbial population involved in Bioremediation⁴

Microorganism	Compound degraded	Compound used as
<i>Agaricus bisporus</i>	4-carboxy-4'-sulphoazobenzene	Dye
<i>Alcaligenes eutrophus</i>	2,4-Dichlorophenoxyacetic acid (2,4-D)	Systemic herbicide
<i>Amorphotheca resinae</i>	Kerosene alkanes	Jet fuel
<i>Arthrobacter aurescens</i>	o,o-Diethyl-o-p-nitrophenyl phosphorothionate (Parathion)	Organophosphorus insecticide
<i>Aspergillus niger</i>	Phenanthrene	Common Polyaromatic Hydrocarbon (PAH)
<i>Burkholderia cepacia</i>	Phthalate, Pentachlorophenol, Toluene	Plasticizer
<i>Clostridium butylicum</i>	2,4,6-Trinitrotoluene	Wood preservative
<i>Phanerochate shryosporium</i>	Phenanthrene	Solvent
<i>Pseudomonas oleovorans</i>	n-Octane	High explosive
<i>Pseudomonas putida</i>	Methyl tertiary butyl ether (MTBE)	Alkane in petrol
<i>Rhodococcus rhodochronus</i>	Styrene	Monomer polystyrene

II. FACTORS AFFECTING BIOREMEDIATION PROCESS ARE AS FOLLOWS

1. Microbial populations are pivotal to the bioremediation process. These microorganisms are universally present in nature, exhibiting the capacity to flourish in a broad spectrum of environments, ranging from subzero temperatures and extreme heat to arid deserts, aquatic habitats, oxygen-rich surroundings, and anaerobic conditions containing hazardous compounds or various waste streams. Their remarkable adaptability enables them to thrive in such diverse settings. Employing microorganisms as agents for the removal of pollutants from soil, water, sediments, and other environmental substrates offers several advantages. These microorganisms efficiently mitigate pollution and contribute to the restoration of the original natural environment, thus preventing further contamination.

Microorganisms necessitate an energy source and a carbon source. Given their ability to adapt to other microbes and biological systems, they can be employed for the degradation or remediation of hazardous pollutants.

These Microorganisms can be Subdivided into the Following Groups:

- **Aerobic Microorganisms:** They flourish in environments abundant in oxygen. Notable examples of aerobic bacteria renowned for their degradation capabilities comprise *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*. These bacteria are commonly acknowledged for their proficiency in breaking down pesticides and diverse hydrocarbons, encompassing alkanes and polyaromatic compounds. A significant trait observed in many of these bacteria is their capacity to utilize contaminants as their sole source of carbon and energy.
- **Anaerobic Microorganisms:** They flourish in oxygen-deprived environments. Anaerobic bacteria, though less frequently utilized compared to aerobic bacteria, are gaining increasing attention for bioremediation applications. This includes tasks such as eliminating polychlorinated biphenyls (PCBs) from river sediments, dechlorinating the solvent trichloroethylene (TCE), and degrading chloroform.
- **Ligninolytic Fungi:** Fungi, as demonstrated by the white rot fungus *Phanaerochaete chrysosporium*, have the capacity to decompose a broad range of persistent or hazardous environmental contaminants. Commonly employed substrates for this degradation process include materials such as straw, sawdust, or corn cobs.
- **Methylophils:** Aerobic bacteria exhibit growth by utilizing methane as a source of carbon and energy. The primary enzyme in the aerobic degradation pathway, methane monooxygenase, demonstrates a broad substrate range and is effective against a diverse array of compounds, including chlorinated aliphatics such as trichloroethylene and 1,2-dichloroethane.

For degradation to occur, it is crucial for bacteria to make contact with contaminants. Achieving this contact can be challenging since both microorganisms and contaminants are not evenly distributed in the soil. Some bacteria exhibit mobility and chemotactic responses, sensing contaminants and actively moving towards them.

In contrast, certain microbes, such as fungi, adopt a filamentous growth pattern directed towards the contaminants. Enhancing the mobilization of contaminants can be achieved using specific surfactants, such as sodium dodecyl sulfate (SDS).

2. Environmental Factors Affecting Bioremediation^{1,3,4}

- **Nutrients:** Although microorganisms may be present in contaminated soil, their numbers might not be sufficient for the effective bioremediation of the site. To stimulate their growth and activity, biostimulation typically involves introducing nutrients and oxygen to support indigenous microorganisms. These nutrients serve as the fundamental building blocks of life, enabling microbes to produce the necessary enzymes for breaking down contaminants. All microorganisms require nitrogen, phosphorus, and carbon for this purpose. Among these elements, carbon is the most essential element in living organisms and is needed in larger quantities than others. Alongside hydrogen, oxygen, and nitrogen, carbon constitutes approximately 95% of a cell's weight. Phosphorus and sulfur make up the remaining 5%. The ideal nutritional ratio of carbon to nitrogen is 10:1, while the carbon to phosphorus ratio is 30:1.
- **Temperature:** One of the key physical parameters affecting both hydrocarbon content and the survival of microbes is temperature. Biochemical reaction rates are temperature-dependent, with many doubling for every 10 °C increase. Cells perish when the temperature surpasses a specific point. Extremely low temperatures in water lead to the closure of transport channels in microbial cells, potentially causing the cytoplasm to freeze and oleophilic bacteria to become biologically dormant. There exists a temperature range within which biological enzymes can actively participate in the degradation pathway. Different compounds degrade at different temperatures, resulting in varying metabolic turnovers. As temperature influences the physiological characteristics of microorganisms, it can either accelerate or decelerate the bioremediation process.

Access to water is essential for all living organisms, and irrigation is necessary to achieve the optimal moisture content. Microbial activity reaches its peak at an ideal temperature; however, deviations from this temperature, either above or below, result in a decrease in activity, ultimately ceasing after reaching a specific temperature.

- **Concentration of Oxygen:** Various microorganisms have distinct oxygen requirements, and the existence of either an aerobic or anaerobic system is contingent on the available oxygen levels. Some organisms necessitate oxygen, while others operate in anaerobic conditions, with each species having specific oxygen requirements. The biodegradation process can be accelerated based on the amount of oxygen required by these microorganisms. Since oxygen is a gas essential for most living organisms, biological deterioration occurs in both aerobic and anaerobic conditions. Oxygen often enhances hydrocarbon metabolism. While chlorate compounds can only be degraded in anaerobic environments, hydrocarbons can undergo rapid breakdown in aerobic settings. Introducing more oxygen to the environment can be achieved through soil tillage or air sparging. Occasionally, magnesium or hydrogen peroxide may be introduced into the environment.

- **Moisture Content:** Microbes require an adequate supply of water for growth, and the moisture content of the soil negatively affects the biodegradation agents.
- **pH:** The impact of pH on microbial metabolism is not entirely elucidated. Soil pH serves as an indicator of the feasibility of microbiological growth, and even minor pH fluctuations can exert a notable influence on metabolic processes. Both high and low pH levels can have adverse effects. The pH of the soil can be modified by adding lime if it is excessively acidic.
- **Metal ions:** Bacteria and fungi require small quantities of metals, but elevated concentrations inhibit the metabolic processes of their cells. The presence of metal compounds directly or indirectly influences the rate of deterioration.
- **Toxic Compounds:** The presence of hazardous chemicals can impede the breakdown process. The degree and mechanisms of toxicity vary depending on the specific toxic components, their concentration, and the exposed microorganisms. Certain inorganic and organic substances pose harm to specific types of life.

III. FOLLOWING ARE OPTIMUM ENVIRONMENTAL CONDITIONS FOR THE DEGRADATION OF CONTAMINANTS

Temperature, moisture, and pH directly influence the growth and activity of microbes. Despite microorganisms being isolated in challenging environments, they typically thrive within a specific range, necessitating the creation of ideal conditions. The pH of acidic soil can be modified by adding lime. The rates of biological reactions are impacted by temperature, with many reactions doubling for every 10 °C increase in temperature.

Nevertheless, cells face mortality at elevated temperatures. Late spring, summer, and fall present opportune periods for employing plastic covering to enhance solar warming. Access to water is indispensable for all living organisms, necessitating irrigation to achieve the optimal moisture content. The existence of either an aerobic or anaerobic system is contingent on the available oxygen levels.

While chlorurate compounds exclusively undergo degradation in anaerobic environments, hydrocarbons can be readily broken down in aerobic settings. Enhancing soil oxygenation can be accomplished through soil tillage or air sparging. Occasionally, magnesium or hydrogen peroxide may be introduced into the environment. The efficiency of nutrient, water, and air delivery relies on the soil structure. The addition of materials like gypsum or organic matter can improve the soil's structure. Low soil permeability can hinder the movement of water, nutrients, and oxygen through the soil; consequently, soils with low permeability may not be suitable for *in situ* cleanup methods.

The availability of nutrients limits biodegradation in aquatic environments. Oil-eating bacteria require nutrients for optimum growth and development, just like other organisms do. The amount of nutrients present in the natural environment is limited.

Table 2: Environmental Conditions affecting Bioremediation⁶

Parameters	Condition required for microbial action
Soil moisture	Water holding capacity 5-28%
Soil pH	5.5-8.8
Oxygen content	Aerobic, minimum air-filled pore space of 10%
Nutrient content	Nitrogen and Phosphorus for microbial growth
Temperature (°C)	15-45%
Contaminants	Not too toxic
Heavy metals	Total content 2000 ppm
Type of soil	Low clay or silt content

The effectiveness of the interaction hinges on the prevailing conditions at the interaction site. The growth and activity of microorganisms are influenced by various factors, encompassing pH, temperature, moisture, soil structure, water solubility, nutrients, site characteristics, redox potential, and oxygen concentration.

Apart from the scarcity of skilled labor in this domain, the physico-chemical bioavailability of pollutants (including pollutant concentration, type, solubility, chemical structure, and toxicity) also contributes to the scenario. These factors determine the kinetics of degradation. In most aquatic and terrestrial environments, the optimal pH range for biodegradation is 6.5 to 8.5, although it can occur at much lower or higher pH levels. The type and quantity of soluble elements present, along with the pH and osmotic pressure of terrestrial and aquatic systems, collectively impact the rate of contaminant metabolism, which is further influenced by moisture.

1. Site Characterization and Selection: A sufficient amount of remedial investigation work must be conducted to precisely assess the extent and magnitude of contamination before proposing a bioremediation plan. Key elements that must be considered include the degree of contamination in both horizontal and vertical dimensions, a compilation of parameters and sample sites with an elucidation of the selection process, and a delineation of the procedures for sample collection and analysis.

IV. Types of Bioremediation^{2,4}

On the basis of removal and transportation of wastes for treatment there are basically two methods:

- ***In situ* Bioremediation**

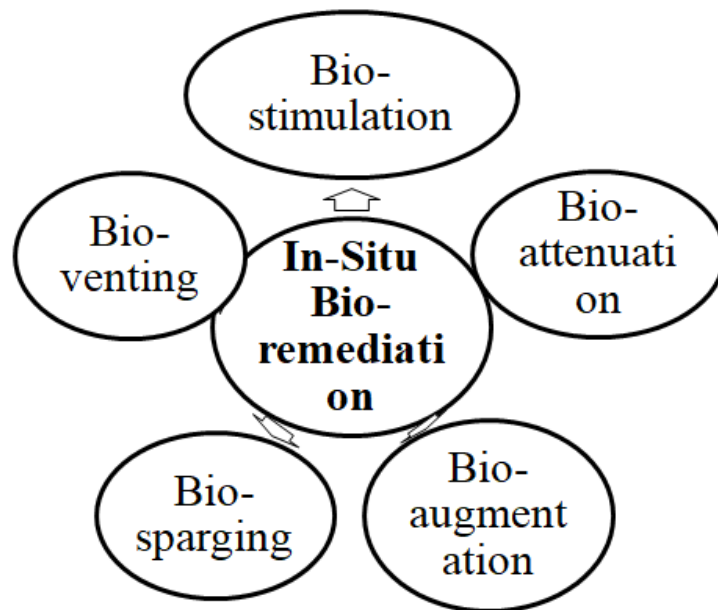


Figure 1: Types of *In-situ* Bioremediation

- ***Ex Situ* Bioremediation**

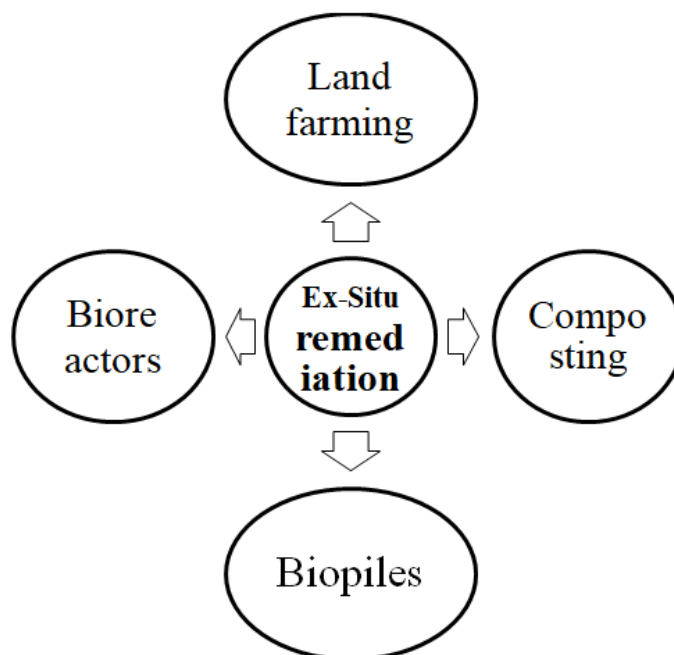


Figure 1: Types of *Ex-situ* Bioremediation

Through "*in situ*" bioremediation, the remediation process can be accomplished without the necessity of removing water or soil through excavation. *In situ* bioremediation is predominantly employed for the breakdown of pollutants in saturated soils and groundwater. It proves to be a more efficient method for cleansing contaminated environments as it utilizes

safe microorganisms to degrade contaminants, rendering it a cost-effective approach. Understanding the crucial role of microbial organisms in infiltrating toxin-laden areas through chemotaxis is essential for comprehending in-situ bioremediation. Consequently, enhancing the chemotactic capabilities of cells will make in-situ bioremediation a safer technique for breaking down hazardous substances.

In situ bioremediation methods offer numerous potential advantages, as they eliminate the need for excavating the contaminated soil, proving to be cost-effective. This approach minimizes site disruption, resulting in reduced dust generation, and enables simultaneous treatment of both soil and groundwater.

Additionally, there are certain drawbacks to *in situ* bioremediation, including a longer process than other remedial techniques, seasonal variations in microbial activity as a result of direct exposure to uncontrollably changing environmental factors, and challenging treatment additive application. Microorganisms can only function properly when their waste products enable them to generate nutrients and energy for the growth of new cells. Their ability to deteriorate is lessened when these circumstances are unfavorable. In certain *situations*, it is necessary to employ genetically modified microbes, however it is ideal to encourage naturally occurring bacteria.

Types of *In Situ* Bioremediation

- **Intrinsic Bioremediation:** This method involves providing nutrients and oxygen to native or naturally occurring microbial communities in order to stimulate their metabolism.
- **Engineered *In Situ* Bioremediation:** The second strategy entails introducing certain microbes to the contaminated area. It is necessary to add designed systems to a place when the conditions there are unsuitable.

Engineered In Situ By promoting the growth of microorganisms through improved physico-chemical conditions, bioremediation quickens the degradation process. Microbial development is aided by oxygen, electron acceptors, and nutrients like phosphorus and nitrogen.

1. **Bioventing:** The predominant *in situ* treatment method, known as "bioventing," involves the introduction of nutrients and air into contaminated soil through wells to stimulate the growth of indigenous microorganisms. This technique releases oxygen into the soil, supporting the proliferation of naturally occurring or intentionally introduced bacteria and fungi by providing oxygen to existing soil microorganisms. Bioventing specifically targets aerobically degradable substances, utilizing low air flow rates to supply sufficient oxygen for maintaining microbiological activity. The widely employed approach for oxygenating soil containing contaminants is direct air injection through wells. When vapors slowly traverse biologically active soil, both volatile chemicals and adsorbed fuel residues undergo biodegradation. Numerous studies have substantiated the efficacy of bioventing in the bioremediation of petroleum-contaminated soil.

2. **Biostimulation:** In biostimulation, the activity of indigenous microorganisms is enhanced by introducing specific nutrients into the soil or groundwater at the site, with a focus on promoting the local, naturally occurring bacterial and fungal communities. The initial step involves supplying fertilizers, growth supplements, and trace minerals. Subsequently, environmental requirements such as pH, temperature, and oxygen are provided to expedite the metabolic rate and pathway of these microorganisms. Interestingly, even a minute amount of pollution can serve as a stimulant by activating the operons that regulate the production of bioremediation enzymes. Typically, this process involves adding nutrients and oxygen to support natural bacteria. These nutrients, essential components of life, enable bacteria to produce vital elements including energy, cell biomass, and enzymes that facilitate the breakdown of pollutants—all of which necessitate carbon, phosphorus, and nitrogen.²
3. **Bioattenuation:** The process of reducing pollutant concentrations in the surrounding environment is termed "bioattenuation" or "natural attenuation." This process encompasses not only plant and animal absorption but also various chemical events such as ion exchange, complexation, and abiotic transformation, along with physical phenomena including advection, dispersion, dilution, diffusion, volatilization, and sorption/desorption. The broader concept of natural attenuation includes terms like intrinsic remediation and biotransformation.

When there is chemical pollution in the environment, nature can work in four ways to clean up:

- Microscopic organisms residing in soil and groundwater utilize specific chemicals as a source of nutrition. When they fully metabolize these chemicals, they can transform them into water and harmless gases.
 - As pollutants migrate through soil and groundwater, they may intermingle with clean water, leading to a reduction or dilution of the contamination.
 - Chemical substances have the capacity to adhere or bind to soil particles, anchoring them in place. While this process doesn't eliminate the chemicals, it can prevent them from contaminating groundwater and escaping the site.
 - Certain chemicals, such as oils and solvents, can undergo evaporation, transitioning from liquid to gaseous states within the soil. If these gases are released into the atmosphere at the soil's surface, exposure to sunlight may result in their degradation. If natural attenuation is not rapid or comprehensive enough, bioremediation can be augmented through methods like biostimulation or bioaugmentation.
4. **Bioaugmentation:** It is one of the mechanisms of biodegradation. Bioaugmentation refers to the introduction of pollutant-degrading microorganisms—whether natural, exotic, or engineered—into a contaminated area to enhance the capability of native microbial populations to break down pollutants. This aims to facilitate degradation that selectively targets the pollutants on the site and rapidly accelerates the population growth of natural microorganisms. This involves the introduction of either indigenous or foreign microbes into the contaminated areas.

Two factors limit the use of added microbial cultures in a land treatment unit:

- Seldom can non-native cultures rival an indigenous population sufficiently to support and grow to useful population sizes.
- If the land treatment unit is properly managed, most soils that have been exposed to biodegradable waste for an extended period of time have native microbes that digest the waste efficiently. The microorganisms are extracted from the remediation site, cultivated separately, altered genetically, and then put back. When soil and groundwater are contaminated with chlorinated ethylene compounds, such as trichloroethylene and tetrachloroethylene, all necessary microorganisms are present.

This is done to ensure that the *in situ* microorganisms are able to completely eliminate and transform these pollutants into non-toxic forms like ethylene and chloride. Adding synthetic microbes to a system that function as bioremediators is known as "bioaugmentation," and it aims to completely and rapidly remove complex contaminants.

2.

It is a part of the biodegradation system. The process known as "bioaugmentation" involves the creation of toxin-degrading microorganisms (trademark /uncommon/intended) to extend the biodegradative reach of native microbial populations on the contaminated zone. In order to enhance debasement and quickly expand the signature microorganism humans that infrequently eat on the toxic location. Microorganisms are assembled from the remediation site, refined on their own, modified genetically, and then released back into the environment. To be convincing, every basic microbe can be discovered in the areas where groundwater and soil have been contaminated by chlorinated ethers, such as trichloroethylene and tetrachloroethylene. Its purpose is to guarantee that the in-situ microorganisms are able to totally eliminate these contaminants and convert them into non-destructive forms such as ethylene and chloride.

The process of retaining designed microorganisms for a system that functions as a bioremediator to swiftly and completely remove complicated harmful chemicals is known as bioaugmentation. Additionally, microbes with altered genetic makeup are emerging and demonstrating the potential to degrade a variety of distinct poisons to a greater level.⁵

5. **Biosparging:** Utilizing a technique termed "biosparging," it becomes feasible to enhance groundwater oxygen concentrations and expedite the natural bacterial degradation of pollutants by injecting pressurized air beneath the water table. This process, by augmenting mixing in the saturated zone, enhances the interaction between groundwater and soil. The system design and construction offer significant flexibility, as the installation of small-diameter air injection stations is cost-effective and straightforward.¹

Ex Situ Bioremediation processes require excavation of contaminated soil or pumping of groundwater to facilitate microbial degradation. This technique has more disadvantages than advantages.²

6. **Landfarming** is a simple approach that entails excavating contaminated soil, spreading it over a prepared bed, and periodically tilling it to facilitate the breakdown of contaminants. The objective is to promote the aerobic degradation of pollutants by indigenous biodegradative bacteria. Typically, this process is limited to treating the upper 10 to 35 cm of soil. Landfarming has garnered considerable interest as a disposal option

due to its potential to reduce monitoring and maintenance costs, as well as cleanup liabilities.¹

7. **Composting** is a method that combines organic materials that aren't toxic, such as manure or agricultural waste, with contaminated soil. These organic ingredients are necessary for the formation of the rich microbial population and high temperature that composting requires.¹
8. **Biopiles:** "Biopiles" serve as a method for treating excavated soil containing hydrocarbons amenable to aerobic remediation. The combination of composting and land farming techniques forms the basis of biopiles. Essentially constructed like composting piles with the incorporation of engineered cells to facilitate aeration, biopiles—also known as compost piles, biocells, bioheaps, and biomounds—are employed in the biodegradation process to reduce concentrations of petroleum pollutants in excavated soils. During this operation, air is introduced into the pile either under positive pressure or drawn through it under negative pressure via a network of pipes and pumps, supplying air to the biopile system. This enhances microbial activity through microbial respiration, resulting in a high rate of destruction of adsorbed petroleum pollution. Biopiles create an optimal habitat for native aerobic and anaerobic bacteria.

An good remediable hydrocarbon-spoiled soil technique is the use of biopiles. Biopiles, also known as biocells, bioheaps, biomounds, and manure piles, are utilized to reduce oil poisoning groups in exposed soils during the duration of the biodegradation hour. In this cycle, air is supplied to the biopile system during a plan of diversion, and siphons are used to either suck air through the stack under negative pressure or control air into the pile under specific strain. Microbial breath enhances microbial activity, which leads to a high level of degraded adsorbed oil pollution..^{3,5}

9. **Bioreactors** are employed for the *ex situ* treatment of polluted soil and water extracted from a contaminated plume, utilizing slurry reactors or aqueous reactors. Reactor bioremediation involves directing polluted water or solid materials (such as soil, sediment, sludge) through a specially designed containment system. A slurry bioreactor, as a containment vessel and apparatus, is utilized to create a three-phase mixing condition (solid, liquid, and gas) by using a water slurry of contaminated soil and biomass (typically native microorganisms) capable of degrading the target contaminants. Due to the more controlled and predictable confined environment of a bioreactor system compared to *in situ* or solid-phase systems, the speed and extent of biodegradation are often increased. Despite their advantages, reactor systems have certain drawbacks. Contaminated soil must undergo pretreatment (such as excavation) or be physically extracted (e.g., vacuum extraction) or washed to remove contamination before being introduced into a bioreactor.

V. METHODS INVOLVED IN TREATMENT OF WASTE MATERIALS²

Depending on the state of the contaminant to be removed, **ex situ** bioremediation is classified as;

1. Solid phase system (including land treatment and soil piles)
2. Slurry phase systems (including solid- liquid suspensions in bioreactors)

1. **Solid Phase Treatment:** It encompasses organic wastes (e.g., leaves, animal manures, and agricultural wastes) as well as problematic wastes (e.g., domestic and industrial wastes, sewage sludge, and municipal solid wastes). Solid-phase soil treatment methods consist of landfarming, soil biopiles, and composting.
2. **Slurry-Phase Bioremediation:** In comparison to alternative treatment approaches, slurry phase bioremediation is a relatively expedited process. In this method, contaminated soil is combined with water and supplementary additives in a substantial tank, referred to as a bioreactor, to sustain the existing microorganisms in contact with the toxins. To create an optimal environment for microbial degradation of pollutants, nutrients and oxygen are introduced, and the bioreactor's conditions are meticulously controlled. Subsequent to the treatment, water is separated from the solids, which are either disposed of or, if contaminants persist, subjected to further treatment.

VI. MICROORGANISMS AND THEIR INTERACTION WITH HEAVY METALS^{3, 8, 9, 10}

The biological destruction of heavy metals is not possible as no degradation changes occur in the nuclear structure of the element. These are only transformed from one oxidation state or organic complex to another.

In the bioremediation of heavy metals, bacteria demonstrate effectiveness. Various processes, encompassing methylation, adsorption, absorption, oxidation, and reduction, contribute to microorganisms' defense against the toxicity of heavy metals. Microorganisms possess the ability to passively absorb heavy metals through adsorption or actively through bioaccumulation. The microbial methylation of heavy metals is a crucial step in bioremediation, especially as methylated substances are often volatile. For instance, diverse bacterial species, including *Alcaligenes faecalis*, *Bacillus pumilus*, *Bacillus sp.*, *P. aeruginosa*, and *Brevibacterium iodinium*, exhibit the capability to biomethylate mercury (Hg II), resulting in the formation of gaseous methyl mercury.

Table 3: Microorganisms used in heavy metals¹⁰

Class of microorganisms	Examples	Heavy metals removed
Bacteria	<i>Bacillus cereus</i> strain XMCr-6	Cr (VI)
	<i>Kocuria flava</i>	Cu
	<i>Bacillus cereus</i>	Cr (VI)
	<i>Sporosarcina ginsengisoli</i>	As (III)
	<i>Pseudomonas veronii</i>	Cu, Zn, Cu
	<i>Pseudomonas putida</i>	Cr (VI)
	<i>Enterobacter cloacae</i> B2-DHA	Cr (VI)
	<i>Bacillus subtilis</i>	Cr (VI)
Fungi	<i>Aspergillus versicolor</i>	Ni, Cu
	<i>Aspergillus fumigatus</i>	Pb
	<i>Gleophyllum sepiarium</i>	Cr (VI)

	<i>Rhizopus oryzae (MPRO)</i>	Cr (VI)
Yeast	<i>Saccharomyes cerevisiae</i>	Pb, Cd
Algae	<i>Spirogyra spp. And Cladophora</i>	Pb (II), Cu (II)
	<i>Spirogyra spp.and Spirullina spp.</i>	Cr, Cu, Fe, Mn. Zn
	<i>Hydrodictylon, Oedogonium and Rhizoclonium spp.</i>	As

1. Bioremediation of Heavy Metals: Due to diverse human activities such as metalliferous mining and smelting, agriculture, waste disposal, and industrial discharge, a spectrum of metals, including silver (Ag), arsenic (As), gold (Au), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), selenium (Se), and zinc (Zn), can be released into the environment. When these metals accumulate in quantities surpassing an organism's processing capacity, they can exert detrimental effects on human health. Some metals are essential for plant growth and performance but are only required in trace amounts. Nevertheless, the industrial revolution has led to a concerning escalation in the concentrations of several metals in soil and water, posing significant risks to both human life and aquatic ecosystems.

Conventional methods for managing sites contaminated with heavy metals typically encompass excavation and solidification/stabilization. While these technologies adeptly control contamination, they lack a lasting solution for heavy metal removal. Furthermore, they present certain drawbacks such as cost-effectiveness limitations, the production of hazardous by-products, and inefficiency. In contrast, biological approaches hold promise in addressing these shortcomings. They are easily deployable, avoid the generation of secondary pollution, and are particularly effective in dealing with heavy metals, which, despite their high density, can be toxic at low concentrations.

Microorganisms and plants are commonly utilized for heavy metal removal, a process known as bioremediation. This natural method is gaining recognition for its significance in preserving biodiversity, both above and below the ground. While all metals exhibit some level of toxicity, some can be beneficial in low concentrations. Elevated toxicity levels of these metals can lead to severe health issues and even mortality. It has been observed that the introduction of organic nutrients into the soil, such as manure, compost, and biosolids, enhances the bioavailability of metals. These organic additives condition the soil, improving its fertility and facilitating the remediation of metal-contaminated ecosystems.

To create a safer environment for human habitation, it is essential to remediate contaminated water bodies and land, eliminating heavy metals and trace elements. Numerous methods exist for the removal of these heavy metals, encompassing chemical precipitation, oxidation or reduction, filtration, ion-exchange, reverse osmosis, membrane technology, evaporation, and electrochemical treatment. These techniques are instrumental in the purification of affected areas from the presence of harmful heavy metals and trace elements.

The majority of heavy metal salts readily dissolve in water, rendering them unsuitable for separation through physical means. Furthermore, physico-chemical methods are either inefficient or costly when dealing with low concentrations of heavy metals. In contrast, biological techniques, such as biosorption and/or bioaccumulation, offer a promising alternative for the removal of heavy metals. Leveraging microorganisms and plants in the remediation process presents a viable solution to heavy metal pollution. This approach embraces sustainable remediation technologies aimed at restoring and reinstating the natural state of contaminated soil.

However, introduction of heavy metals into the soil causes considerable modification of the microbial community, despite their vital importance for the growth of microorganisms at relatively low concentrations.

Microorganisms actively engage in the uptake (bioaccumulation) and/or passive uptake (adsorption) of heavy metals. The microbial cell walls, primarily composed of polysaccharides, lipids, and proteins, possess multiple functional groups capable of binding heavy metal ions. These functional groups include carboxylate, hydroxyl, amino, and phosphate groups. Among various microbe-mediated methods, the biosorption process appears to be more practical for large-scale implementation compared to the bioaccumulation process. This preference arises from the fact that the active uptake of heavy metals by microbes requires the addition of nutrients, subsequently increasing the biological oxygen demand or chemical oxygen demand in the waste. Furthermore, maintaining a robust microbial population proves challenging due to factors such as heavy metal toxicity and other environmental influences.⁸

- 2. Treatment of Heavy Metals:** Heavy metals typically refer to metals with atomic numbers ranging from 22 to 92, encompassing all groups within periods 3 to 7 of the periodic table. Several of these metals, including Cu, Zn, Cd, Pb, Fe, Cr, Co, Ni, Mn, Mo, V, and Se, are vital in trace amounts for the overall well-being of living organisms. However, an excess of these metals can have lethal effects. Environmental pollutants of significant concern are heavy metals such as cadmium, copper, lead, chromium, and mercury. Even in trace quantities, their presence in soil and water can pose serious challenges to all forms of life.

The accumulation of heavy metals in soils is a significant issue in agricultural production, as it has adverse effects on food quality (affecting safety and marketability), crop growth (due to phytotoxicity), and environmental well-being. In recent times, there has been growing concern over the contamination of extensive land areas by heavy metals. This contamination primarily arises from various sources, including municipal waste incineration, vehicle emissions, residues from metalliferous mining and the metallurgical industry, as well as the application of urban compost, pesticides, fertilizers, sludge, and sewage. Sewage composition is highly complex and diverse, varying depending on its source and production process. Notably, urban sewage that includes industrial effluents has been observed to contain relatively high concentrations of heavy metals.⁸

3. Treatment of Heavy Metals-Containing Wastes: Biotechnological approaches have demonstrated efficacy in the treatment of liquid and solid wastes containing heavy metals. Specific enzymes present in microorganisms can facilitate reduction or oxidation processes for certain metals. Microbial metabolism results in the production of substances such as hydrogen, oxygen, and H₂O₂, which can be utilized for the oxidation or reduction of metals. This process often leads to the solubilization or precipitation of metals. Moreover, microbial metabolites can play a role in mediating the solubilization or precipitation of metals. For example, during fermentation, microorganisms can generate organic acids, while in aerobic oxidation, inorganic acids like nitric and sulfuric acids are produced. These acids contribute to the formation of dissolved chelates involving metals.

Microbial activity leading to the production of phosphate, hydrogen sulfide (H₂S), and carbon dioxide (CO₂) can effectively trigger the precipitation of non-dissolved compounds such as phosphates, carbonates, and sulfides. This process is particularly advantageous in the removal of heavy metals such as arsenic, cadmium, chromium, copper, lead, mercury, and nickel. Sulfate-reducing bacteria play a pivotal role in generating H₂S, which is especially beneficial for eliminating heavy metals and radionuclides found in sulfate-containing drainage waters from mining operations, liquid waste from nuclear facilities, and drainage from the tailing ponds of hydrometallurgical plants. The use of materials like wood straw or sawdust is common in this context. Additionally, organic acids produced during the anaerobic fermentation of cellulose can serve as a source of reduced carbon, facilitating sulfate reduction and the subsequent precipitation of metals.

Microbial cell surfaces exhibit the presence of negatively charged carboxylic and phosphate groups, along with positively charged amino groups. As a result, heavy metals can be significantly adsorbed onto the microbial surface under varying pH conditions. This phenomenon, referred to as biosorption, is efficiently utilized in processes such as the utilization of fungal fermentation residues to accumulate uranium and other radionuclides from waste streams.^{8,9}

VII. GENETICALLY ENGINEERED MICROORGANISMS (GEMs) 3, 11

A microorganism is labeled as genetically modified when its genetic composition has been intentionally modified using genetic engineering techniques, inspired by either spontaneous or artificial genetic exchange among bacteria. This process is known as recombinant DNA technology. The application of genetic engineering has improved the handling and disposal of hazardous waste in laboratory settings through the development of genetically modified organisms.

Recombinant DNA procedures or spontaneous genetic material exchange between organisms are the two ways to create recombinant living beings.

It is feasible to insert the right gene to produce a specific enzyme that can break down different types of contaminants. In soil, groundwater, and activated sludge environments, genetically modified microbes (GEMs) have demonstrated potential for bioremediation applications due to their improved ability to degrade a broad variety of chemical pollutants.

There exist several prospects for enhancing degrading performance through the utilization of genetic engineering techniques. For the degradation of previously resistant substances, for instance, entirely new metabolic pathways can be introduced into bacterial strains or rate-limiting steps in established metabolic pathways might be genetically altered to produce higher degradation rates.

In GEMs the following four activities/strategies are required:

- Modification of enzyme specificity and affinity
- Pathway construction and regulation
- Bioprocess development, monitoring and control
- Bioaffinity bioreporter sensor applications for chemical sensing, toxicity reduction, and end point analysis.

Bacteria's essential genes are situated on a singular chromosome, although certain plasmids may encompass genes that dictate the enzymes necessary for the breakdown of specific non-standard substrates. Plasmids have been associated with the catabolic processes. Consequently, GEMs (Genetically Engineered Microorganisms) can be effectively utilized for biodegradation purposes, showcasing a noteworthy research frontier with extensive implications for the future.²

VIII. BIOREMEDIATION USING ADVANCED MOLECULAR TECHNIQUES AND GENETICALLY ENGINEERED ORGANISMS

Microorganisms are employed in bioremediation due to their ability to degrade environmental pollutants through biochemical pathways closely tied to their metabolic activity and growth. Utilizing co-metabolism, microorganisms can convert hazardous substances in contaminated environments into benign end products. While relying on indigenous microorganisms for remediating polluted areas, particularly for heavy metals like Hg, has shown limited efficacy, the integration of recombinant DNA technology is instrumental in enhancing bioremediation processes. The advent of recombinant DNA technology in the 1970s, marked by the identification of restriction enzymes and DNA ligases, facilitated the modification of the genetic makeup of living organisms. Subsequently, research has concentrated on investigating the metabolic capabilities of microorganisms and genetically modifying them for specific remediation objectives.

The aim of genetic engineering in bioremediation is to modify plants, microorganisms, and enzymes, rendering them valuable tools for the breakdown of harmful substances. Genetic engineering has facilitated the modification of bacteria for the removal of heavy metals such as As, Cd, Cu, Fe, Hg, and Ni. However, the degradation rate depends on the catalytic efficiency of enzymes within the cells or those induced for specific substrates.

Genetically engineered microorganisms (GEMs) are microorganisms with genes from another organism, whether of the same or a different species, inserted into their genome using recombinant DNA technology. These modified microbes have been employed to create proficient strains for the bioremediation of polluted environments, as they possess an improved capacity to degrade various contaminants.

Most approaches involved the identification and incorporation of metal-uptake-related genes into plants and microorganisms proficient in metal absorption. Genes such as phenol catabolic genes (pheA, pheB, pheC, pheD, and pheR), ArsM gene for As removal from contaminated soils, and merA gene for Hg uptake have been widely employed in these processes.

The utilization of Genetically Engineered Microorganisms (GEMs) plays a pivotal role in addressing environmental contaminants. To utilize GEMs for bioremediation, it is essential to secure the stability of these microbes before their deployment in the field, as the catabolic activity of released GEMs is intricately linked to the stability of the introduced recombinant plasmid. This leads to the development of novel metabolic pathways that empower engineered bacteria to transform toxic heavy metals into less harmful forms, thereby improving bioremediation processes.

Common techniques involve the construction of novel pathways, the replacement of existing gene sequences, and the introduction of single genes or operons into the microorganism. Recent advancements in genetic engineering techniques, such as site-directed mutagenesis and rational design, have been used to genetically modify microorganisms and their enzymes to degrade organic pollutants in the environment.

New recombinant DNA techniques include the use of innovative vectors to introduce gene fragments into potential host organisms, the development of novel methods for gene expression regulation, and the application of targeted and random mutagenesis to enhance the activity of biodegrading enzymes.

Genetic engineering has also been employed to develop microbial biosensors, which play a crucial role in rapidly and accurately quantifying pollutants in contaminated sites. For instance, Dixit et al. reported the use of biosensors to assess the levels of heavy metals such as Cd, Ni, Hg, Cu, and As in contaminated sites. However, biosensors have limitations, including varying response times, detection thresholds, sensitivity, signal relaxation lengths, and stability.

The utilization of genetic engineering offers greater potential for obtaining highly effective pollutant-degrading microorganisms compared to indigenous microbes. To successfully employ GEMs for bioremediation in challenging environmental conditions, it's imperative to preserve the recombinant bacterial population in the soil under suitable environmental conditions and ensure their resistance to competition from indigenous bacterial populations. Therefore, exploring novel molecular techniques for screening and isolating microorganisms for heavy metal bioremediation is essential.

Recent progress in genetic and omics technologies, including genomics, proteomics, and metabolomics, has empowered scientists to investigate the breakdown of organic pollutants by microorganisms. This advancement has facilitated a comprehensive understanding of the physiology, ecology, and biochemistry of microorganisms involved in the degradation of polycyclic aromatic hydrocarbons (PAHs). With the availability of whole genome sequencing, it is now feasible to delve deeply into the physiology of microorganisms associated with the removal of contaminants from the environment.

The identification of genes responsible for bacterial inorganic transformations has paved the way for employing molecular genetics to enhance metal tolerance. Through genetic engineering, it becomes feasible to generate transgenic plants with improved bioremediation capabilities. This is accomplished by introducing or amplifying specific genes within the plant's DNA, thereby effectively enhancing its potential for phytoremediation. Genetic engineering also entails utilizing molecular detoxification mechanisms to equip these plants with the ability to efficiently metabolize pollutants and degrade xenobiotics. In regions affected by metal contamination, genetic modification of endophytes and PGPR (Plant Growth-Promoting Rhizobacteria) can be applied for the degradation of soil pollutants, contributing to effective remediation.

The augmentation of metal accumulation capability and tolerance is achieved through the overexpression of genes responsible for antioxidant enzymes or enzymes engaged in the synthesis of glutathione and other phytochelators. Transgenic plants, such as *Nicotiana tabacum*, *Brassica juncea*, *Brassica oleracea* var. *botrytis*, and *Lycopersicon esculentum*, have been utilized in the field of pollutant bioremediation.

One high-throughput tool that has addressed the drawbacks of previous culture-independent methods is DNA microarrays, which can identify several genes in a single test.

The most popular gene array method for examining gene function is the GeoChip array. It targets a large number of genes involved in the several geochemical cycles of sulfur, phosphorus, nitrogen, and carbon as well as metal resistance, pollution reduction, and degradation.

The deployment of Genetically Engineered Microorganisms (GEMs) for bioremediation is frequently postponed due to safety and legal considerations, as well as public concerns regarding potential risks associated with GEMs. Consequently, strict regulations have been established by various biosafety regulatory bodies, including the United Nations Environmental Programme (UNEP) and the United States Environmental Protection Agency (EPA), responsible for overseeing the governance of genetically modified organisms (GMOs) and living modified organisms. Recombinant DNA technology plays a pivotal role in the bioremediation process, enabling researchers to analyze, monitor, and assess its implementation. However, it is crucial to exercise caution and adhere to biosafety regulations when employing this technology¹¹

- 1. Advantage of GEMs in Bioremediation:** GEMs' primary roles in bioremediation are to accelerate the cleanup of waste-polluted sites, improve substrate degradation, exhibit a high catalytic or utilization capacity with a small amount of cell mass, and produce safe, sterile environmental conditions by neutralizing or decontaminating any hazardous materials.
- 2. Disadvantage of GEMs in Bioremediation:** Occasionally, cells die, which presents a problem when they are released into the environment. At a certain point, it has been noted that microbial activity is directly and indirectly impacted by growth and substrate degradation delays, seasonal variations, and other abiotic factor fluctuations. The presence and composition of the native structural and functional microbe population are

adversely affected in an unmeasurable and unreacted manner when a foreign modified strain is introduced into the system.³

IX. PHYTOREMEDIATION^{1,2, 2,13}

Phytoremediation entails the remediation of organic pollutants and heavy metal contaminants through the utilization of plants and rhizospheric microorganisms. It provides a cost-effective, environmentally friendly, and efficient approach to restoring polluted environments, particularly those impacted by heavy metals. Nevertheless, the efficacy of phytoremediation at a specific polluted site hinges on factors such as the degree of soil contamination, the concentration of metal contaminants in the soil, and the plants' ability to effectively absorb metals from the soil. Plants utilized in phytoremediation can be classified into hyperaccumulators, characterized by a notable capacity for heavy metal accumulation but lower biomass, and non-hyperaccumulators, which exhibit a lower extraction capability than hyperaccumulators but possess significantly higher total biomass yield and rapid growth rates.⁸

Several processes are used to remove heavy metals from contaminated soils by some plants as illustrated in Figure 3.

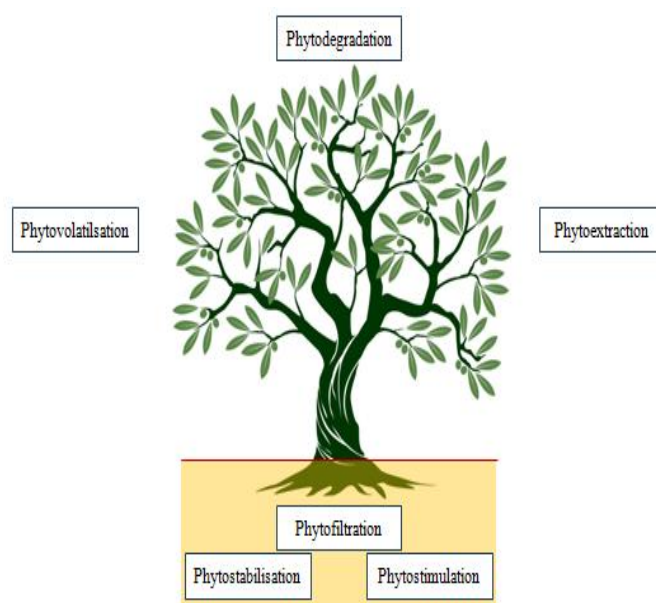


Figure 3: Processes used by several plants for removal of contaminants from soil.

- 1. Phytoextraction/Phytoaccumulation:** By employing the hyperaccumulation technique, phytoextraction involves the absorption and transportation of metal contaminants in the soil through plant roots into above-ground plant components. Hyperaccumulator plants prove to be promising candidates for phytoremediation, as they absorb substantial quantities of metals from contaminated soils, transport and accumulate them in above-ground organs at concentrations 100–1000 times higher than in non-hyperaccumulating species, all without exhibiting any signs of phytotoxicity.

These plants yield a large amount of easily harvested biomass and are typically seen growing in regions where soil pollution from persistent metals has occurred over time. Based on metal concentrations in dried leaves (Cd 100, Co, Cu, Cr 300, Pb, Ni 1000, Zn 3000, and Mn (µg/g, respectively), Van der Ent provided the following criteria for hyperaccumulator plants. Around 500 taxa have been identified as hyperaccumulators of certain metals based on these criteria; Table 4 shows which of these families' members are most common. These families include Brassicaceae, Caryophyllaceae, Violaceae, Fabaceae, Euphorbiaceae, Lamiaceae, Asteraceae, Cyperaceae, Poaceae, Cunouniaceae, and Flacourtiaceae.

These plants are unique because of the following characteristics:

- an increased capability for absorbing heavy metals from the soil;
- improved transfer of metal ions from roots to shoots;
- an enhanced capacity to detoxify and store substantial quantities of heavy metals in the above-ground portions;
- rapid growth potential; and
- a dense and extensive root system.

Due to its capability to uptake heavy metals from the environment, there has been considerable interest in utilizing sunflower (*Helianthus annuus*) for phytoremediation of organic pollutants and heavy metals in recent decades. Different plant parts exhibit varying patterns of heavy metal accumulation. While some sources emphasize predominant accumulation in the roots of sunflower plants with minimal migration to the above-ground mass, others observe significant movement in the opposite direction. Recent research by Angelova et al. indicates that, for lead (Pb), approximately 59% accumulates in the leaves and as little as 1% in the seeds, highlighting the metal-specific distribution in sunflower organs. Similar patterns were observed for zinc and copper, with accumulation rates of 47% and 79%, respectively, in sunflower leaves.

Hyperaccumulator plants such as certain species within the *Brassica genus* (*Brassica napus*, *Brassica juncea* and *Brassica rapa*) are fast growers with high biomass. Some hyperaccumulator plants are listed in table 4.

Table 4: Some hyperaccumulators¹⁰

Family	Species	Heavy metals
Asteraceae	<i>Berkheya coddi</i>	Ni
Asteraceae	<i>Helianthus annuus</i>	Pb, Cd, Zn
Brassicaceae	<i>Alyssum bertolonii</i>	Ni
Brassicaceae	<i>Alyssum murale</i>	Ni
Brassicaceae	<i>Arabisopsis halleri</i>	Zn, Cd
Brassicaceae	<i>Arabisopsis halleri</i>	Cd, cd
Caryophyllaceae	<i>Minuartia verna</i>	Zn, Cd, Pb
Crassulaceae	<i>Sedum alfredii</i>	Pb
Euphorbiaceae	<i>Euphorbia cheiradenia</i>	Cu, Fe, Pb, Zn
Fabaceae	<i>Astragalus racemosus</i>	Se

Fabaceae	<i>Medicago sativa</i>	Pb
Poaceae	<i>Spartina argentinensis</i>	Cr
Pteridaceae	<i>Pteris vittata</i>	As
Pteridaceae	<i>Pteris vittata</i>	Hg
Violaceae	<i>Viola boashanensis</i>	Pb, Zn, Cd

Factors Affecting Efficiency of Phytoextraction :

- the choice of plant used,
- the degree of plant tolerance to higher concentrations of heavy metals and
- the capacity of plants to drastically take up heavy metals and move them from the roots to exposed surfaces which are essential for the phytoextraction process .

Phytoextraction has the potential for commercial viability, as it not only eliminates heavy metals from the soil but also generates valuable biomass. Among the various methods employed by plants for the remediation of polluted environments, phytoextraction stands out as the most favored, especially when facilitated by plant growth-promoting rhizobacteria (PGPR) residing in close proximity to the plant roots.¹⁰

2. **Phytofiltration:** Phytofiltration comprises three main modalities: rhizofiltration (using plant roots), blastofiltration (employing seedlings), and caulofiltration (utilizing excised plant shoots). It pertains to the remediation of contaminated environments by utilizing plant roots or seedlings to treat aqueous waste. To enhance the efficacy of phytofiltration as a phytoremediation method, additional research is required to identify the plant components that are most adept at accumulating metal contaminants. This understanding is crucial for the effective implementation of this technique in bioremediation.¹⁰
3. **Phytostimulation:** Phytostimulation involves enhancing microbial activity to facilitate the breakdown of organic contaminants, achieved through exudates released from plant roots. Ethylene, when present in low concentrations, stimulates root elongation. However, elevated levels of ethylene can impede cell division and DNA synthesis. Nevertheless, this challenge can be addressed by reducing ethylene levels within plants, a process facilitated by the enzyme 1-aminocyclopropane-1-carboxylase deaminase. This enzyme, produced by plant growth-promoting rhizobacteria (PGPR) associated with plant roots, helps regulate plant ethylene production, thus mitigating abiotic stress and maintaining a balance in plant ethylene levels. PGPR utilize exudates released by plants as sources of carbon and energy to degrade metal contaminants.¹⁰
4. **Phytostabilization:** This process involves utilizing plant roots to uptake pollutants from the soil, confining them within the rhizosphere, and facilitating their separation and stabilization. This effectively renders the pollutants harmless and prevents their dispersion into the environment. The accessibility and mobility of heavy metals in the environment are restricted through processes like precipitation within the vicinity of plant roots, root sorption, reduction of metal valence, and complexation. The effectiveness of the phytostabilization process relies on the quantity of metal available for uptake in the rhizosphere soil, determining how efficiently metals are transported within the plant. Plants selected for phytostabilization should possess extensive root systems and limited metal mobility from roots to shoots. Augmenting the pH and organic matter content by

adding substances such as biochar or compost can enhance the phytostabilization capacity of a plant, promoting increased plant yield while immobilizing metals. Phytostabilization stands out as a preferable approach for sequestering metals *in situ* because it prevents the uptake of pollutants into plant tissues and their release into the environment. This method primarily focuses on the sequestration of heavy metals exclusively within the rhizosphere.

5. **Phytovolatilization:** This process entails plants removing soil contaminants that can readily transform into vapor and subsequently be released into the atmosphere. For example, tobacco plants can accumulate the highly toxic methyl mercury from mercury-contaminated areas and convert it into a less toxic elemental form of mercury in a volatile state. This volatile form is then released through the leaves into the atmosphere. The conversion of contaminants into volatile forms during phytovolatilization is attributed to the metabolic capabilities of plants in conjunction with the microorganisms residing within the rhizosphere.¹⁰
6. **Phytodegradation:** Phytodegradation involves the enzymatic breakdown of organic contaminants by plants into non-hazardous forms. Plants employ specific enzymes like nitroreductases and dehalogenases to facilitate the degradation of organic contaminants. For efficient degradation, these enzymes must operate under optimal conditions of temperature and pH. Additionally, the presence of rhizospheric microorganisms can enhance the degradation of organic pollutants in the soil through a process known as rhizodegradation. This occurs because the rhizospheric region surrounding the plant's roots contains elevated levels of nutrients released from the roots, attracting a greater population of bacteria that aid in the degradation of contaminants compared to the bulk soil, which contains fewer organic compounds and thus fewer microbes. However, it's important to note that this process is limited to the removal of organic pollutants, as heavy metals are nonbiodegradable.¹⁰
7. **Rhizofiltration:** Rhizofiltration is a process designed to eliminate toxic substances or pollutants from groundwater through filtration using plant roots. It relies on the mechanism of rhizospheric accumulation by plants. Terrestrial plants exhibit greater effectiveness in rhizofiltration compared to aquatic plants because they utilize natural solar-driven pumps to extract specific elements from the environment. Plants with the ability to absorb and tolerate high concentrations of toxic metals, such as hyperaccumulators, are well-suited for rhizofiltration. Introducing plant growth-promoting rhizobacteria (PGPR) to a contaminated site can reduce metal toxicity in plants, decreasing the bioavailability of these metals. This, in turn, enhances the plants' capability to eliminate heavy metal contaminants and shield themselves from environmental stress. However, it's essential to acknowledge that phytoremediation technology has certain limitations, including a reduced remediation rate that may be insufficient when dealing with numerous pollutants at a contaminated site, as well as the accumulation and storage of pollutants in plant materials.¹⁰

X. ENHANCEMENT OF BIOTECHNOLOGICAL TREATMENT OF WASTES

The objective of bioremediation is to eliminate pollution until it reaches undetectable concentrations. Achieving success in the application of biotechnology for the treatment of hazardous wastes depends on several crucial factors. Bioremediation is employed to

remediate contaminated soils, groundwater, sewage, and sludge. According to Kołwzan et al. (2006), when utilizing bioremediation to neutralize pollution, the following conditions must be satisfied:

- The environment undergoing bioremediation must host microorganisms with well-defined catabolic processes.
- Microorganisms employed in the bioremediation process should possess the capability to efficiently convert chemical compounds, reducing their concentration to levels compliant with regulations.
- Metabolites generated during biodegradation must not exhibit toxic, mutagenic, or carcinogenic properties.
- The local conditions where the process takes place should be conducive to the growth and activity of microorganisms, including adequate nutrient availability, suitable pH levels, the presence of oxygen or other electron acceptors, and favorable redox conditions, along with appropriate moisture levels. Factors such as temperature, contaminant toxicity, or mass transfer limitations may also influence the rate of biodegradation.⁸

1. The Advantage of Bioremediation

- It can be completed on site with very little effort and frequently without seriously interfering with regular operations. Consequently, there is less need to move large amounts of waste off site, and any possible risks to the environment or public health that may arise during the transportation process are also reduced.
- This method is natural, quick, and suitable for treating waste made from polluted materials like soil. When a pollutant is present, microbes can both grow and break down the contaminant. The biodegradative population decreases as the pollutant degrades. The byproducts of the therapy are harmless substances like cell biomass and water carbon dioxide.
- All pollutants are entirely destroyed, and a large number of dangerous substances can be converted into safe products. This also removes the possibility of future legal responsibility arising from the handling and disposal of contaminated material.
- It is an economical procedure because it is less expensive than other traditional techniques for hazardous waste cleanup. This is a crucial technique for treating locations affected by oil.
- No hazardous substances are utilized. Fertilizers in particular are added along with nutrients to stimulate and trigger the microbial development. These are frequently utilized in gardens and lawns. Through the process of bioremediation, toxic compounds are entirely destroyed and transformed into innocuous gases and water.
- Eco-friendly and sustainable.
- Because of their innate place in the environment, they are simpler, less labor-intensive, and less expensive.
- Elimination of pollutants rather than merely their transfer to alternative environmental media.
- Non-intrusive, which might permit continuous site usage.
- It's not too difficult to implement.

- An efficient and environmentally beneficial method of cleaning up various pollutants from the natural habitat.
- Target pollutants can be completely destroyed, as opposed to being transferred from one environmental medium to another, such as from land to water or air.³

2. The Disadvantage of Bioremediation

Some of the drawbacks of bioremediation have been enlisted below:

- The possibility that the byproducts of biodegradation will be more hazardous or persistent than the parent substance is one of the worries.
- Only biodegradable substances can be processed using this method.
- Not every compound is prone to total and quick breakdown.
- It is challenging to transition from bench and pilot-scale research to large-scale field operations.
- A lot of biological processes are very specialized. The availability of metabolically competent microbial populations, ideal environmental growth conditions, and the right amounts of pollutants and nutrients are critical site requirements for success.
- A great deal of study is required to develop bioremediation systems suitable for locations with complex combinations of pollutants that are not evenly distributed in the environment. There could be gases, liquids, or solids that contain contaminants.
- Regulations are still unclear as to what acceptable performance standards for bioremediation are. There is no agreed-upon definition of "clean," it is challenging to assess bioremediation's effectiveness, and there are no recognized benchmarks for bioremediation procedures.
- Compared to alternative treatment methods like soil excavation and removal or incineration, this procedure is more time-consuming.³

- 3. Role of Environmental Biotechnology in Pollution Management:** Biotechnology can be utilized to create biodegradable products from renewable sources, convert pollutants into benign chemicals, evaluate the health of ecosystems, and create environmentally safe production and disposal methods.

In order to decrease the environmental load of harmful compounds, environmental biotechnology uses genetic engineering to increase cost and efficiency, two important aspects for the widespread use of microorganisms in the future.²

XI. BIOMONITORING OF BIOREMEDIATION: BIOSENSORS

A critical application of environmental biotechnology is biomonitoring, which involves evaluating biodegradability, toxicity, mutagenicity, concentrations of hazardous substances, and monitoring concentrations of microorganisms and pathogenicity in both waste and the environment. Biodegradability tests, whether conducted manually or through automated off-line or on-line methods, can be performed by quantifying the production of gases like CO₂ or CH₄, as well as O₂ consumption. Biosensors, functioning as analytical instruments, harness biological specificity for the detection of target molecules.

Biosensors offer the flexibility to utilize either whole bacterial cells or enzymes for the detection of specific molecules of hazardous substances. The tracking of toxicity can be accurately performed using whole cell sensors, where the presence of a hazardous substance may impede bioluminescence. A prevalent approach involves employing cells with an introduced luminescent reporter gene to evaluate changes in the metabolic status of the cells in response to intoxication. Nitrifying bacteria, with intricately folded cell membranes, are sensitive to various membrane-disrupting substances like organic solvents, surfactants, heavy metals, and oxidants. As a result, respirometric sensors measuring the respiration rates of these bacteria can serve as a means to monitor toxicity in wastewater treatment.

Biosensors developed for the quantification of concentrations of hazardous substances often utilize the measurement of bioluminescence. A specific example of a toxicity sensor is a bioluminescent toxicity bioreporter designed for the treatment of hazardous wastewater. Its development entails the incorporation of bioluminescence genes into a microorganism. These whole-cell toxicity sensors demonstrate remarkable sensitivity and can be utilized in real-time to monitor and improve the biodegradation of hazardous soluble substances. Similar types of sensors can also be applied to assess the concentration of specific pollutants.

The fusion of a bioluminescence gene with bacterial genes associated with pollutant metabolism results in the emission of light as the pollutant undergoes breakdown. The extent of biodegradation and bioluminescence is dependent on the pollutant concentration, and real-time measurement using fiber optics is employed. Utilizing arrays of biosensors collectively enables the assessment of both the concentration and toxicity of various hazardous substances.⁸

Mutant bacterial strains have been utilized to evaluate the mutagenicity of both synthetic and natural chemicals. One widely used test, originally introduced by Ames in 1971, involves inducing back mutations in auxotrophic bacterial strains that lack the ability to produce specific essential nutrients. These auxotrophic cells typically do not grow when plated on a minimal medium lacking the required nutrients. However, cells exposed to a tested chemical that induces a reversion mutation can grow in such a minimal medium. The frequency of mutations identified in the test is directly proportional to the potential mutagenicity and carcinogenicity of the tested chemical. Microbial mutagenicity tests are extensively applied in contemporary research.⁸

XII. FUTURE PROSPECTS FOR BIOREMEDIATION

Further investigations are warranted to enhance the viability of transgenic microbes upon release into the environment for bioremediation. Despite their substantial potential in improving detoxification and degrading xenobiotics and heavy metal contaminants, the existing survivability of these microbes is suboptimal. Environmental factors that are challenging to regulate, including low nutrient concentrations and temperature, may impede the efficacy of the bioremediation process.

While attempts have been made to stop suicidal and anti-sense RNA from spreading from engineered microbes to native microorganisms, antibiotic genes should no longer be used as selectable markers; instead, other selectable markers should be used to prevent antibiotic resistance genes from accidentally spreading to other soil microorganisms.

Furthermore, in order to determine the efficacy and potential negative impacts of transgenic plants and bacteria employed in bioremediation, further study is necessary to completely comprehend their metabolic pathways.

The identification and enhancement of hyperaccumulator plants with high biomass production through genetic engineering is necessary to efficiently extract heavy metals from the environment using the phytoextraction process, which has been shown to be a successful phytoremediation method. The success of bioremediation depends on the microorganisms' capacity to compete with the indigenous microbial population.^{10, 14}

XIII. CONCLUSION

Bioremediation is a suitable technique for cleaning up pollution by enhancing biodegradation activities of microorganisms and plants that occur naturally. For this the development of understanding microbial communities and their response to the natural environment and pollutants is necessary. This knowledge can be expanded to modify the genetics of the microbes to increase capabilities to degrade pollutants.

It is necessary to understand the various ways by which living forms degrade pollutants, mechanisms involved, factors affecting degradation rates, optimum environmental conditions required so that pollutants can be successfully converted to less toxic or non-toxic forms.

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