
10. Bioremediation: A Remedy for Contaminated Soil for Sustainability and Environmental Stability

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Abstract:

An increasing trend has been seen in environmental pollution due to developing human activity on energy resources, intensive farming practices, and rapid industrialization during last few years. The release of contaminants such as pesticides, nuclear wastes, heavy metals, hydrocarbons, and greenhouse gases by different process in the environment causing toxic effect on human and environment health. Bioremediation has proven to be an efficient and widely used solution for removing pollutants from soil and water. Bioremediation is sustainable and cost-effective approach for soil decontamination by degrading or transforming contaminants, thereby restoring soil quality. Bioremediation process uses plant and microbes to degrade or convert harmful contaminants to fewer toxic contaminants using different mechanism and method. There are different in-situ and ex-situ techniques based on the location and appropriate method of treatment for the decontamination of soil. This chapter includes various bioremediation techniques and elaborates on phytoremediation and microorganism remediation technique for contaminated soil.

Keywords:

Bioremediation, contaminated soil, heavy metal, phytoremediation

10.1 Introduction:

Soil contamination caused by industrial activity, intensive cultivation, and inadequate landfill disposal methods is a matter of concern for long-term negative effects on the environment. Soil contaminants were classified into two types: inorganic and organic. Heavy metals *viz.* mercury, arsenic, lead, and cadmium are examples of inorganic pollutants, whereas organic pollutants *viz.* phenolic compounds, hydrocarbons, petroleum, herbicides, fertilizers, and pesticides. Harmful organic and inorganic compounds are principal causes of environmental pollution and constitute a serious human health hazard (Dhanam, 2017). The limited availability of pollution-free land and the need to safeguard the human health and ecosystem have heightened importance of remediation and rejuvenation of polluted land. Contaminated soil remediation involves the elimination or breakdown of pollutants into non-harmful substances. This can be achieved through various approaches such as biological degradation, heat processes (such as incineration), or

chemical-physical processes (such as chemical oxidation, base catalysed dechlorination, or UV oxidation). Bioremediation, specifically, utilizes natural processes to reduce the concentration of contaminants to a safe and harmless state. It involves the use of biological agents, such as microorganisms or enzymes, to degrade, detoxify, mineralize, or modify the contaminants (Wang *et al.*, 2021). The increasing need for sustainable techniques to address environmental pollution has led to a growing interest in utilizing biological organisms and plants for the decontamination of polluted environments.

These organisms have the unique ability to absorb and/or convert inorganic contaminants, making them valuable tools in bioremediation processes. Bioremediation offers various advantages, including the opportunity for on-site application, affordability, and minimal technology requirements, which simplify the implementation process.

Moreover, bioremediation can be synergistically combined with other chemical or physical methods to enhance its efficiency and broaden its applicability. In this chapter, our objective is to provide a comprehensive understanding of bioremediation and explore the potential strategies underlying various bioremediation techniques specially focusing on phytoremediation and microbial remediation techniques.

10.2 Concept of Bioremediation:

As part of the SARA (Superfund Amendments and Reauthorization Act) of 1986, the U.S. Environmental Protection Agency (U.S. EPA) endorsed the use of biological remediation as an alternative treatment method for contaminated soil.

This approach, considered an effective and long-lasting clean-up solution, harnesses microbes to remove and breakdown harmful substances in contaminated soil. By utilizing bioremediation, the primary objective is to maintain human wellness and ensure environment sustainability by breaking down and neutralizing harmful components present at contaminated sites (Wang *et al.*, 2021).

Biodegradation and bioremediation share a common reliance on microorganisms to convert or metabolize chemicals. Both processes involve the microbial transformation of contaminants.

However, the key differentiation lies in their nature and application. Biodegradation is a natural process that occurs spontaneously in the environment, where microorganisms naturally break down chemicals over time. In contrast, bioremediation is a purposeful method that harnesses the power of microbes to address and mitigate environmental contamination.

Bioremediation is a technique that utilizes bacteria, fungi, and plants to modify or break down contaminants to a minimum level through different metabolic processes of these organisms have the ability to utilize chemical contaminants as a source of energy, allowing them to transform contaminants (Singh *et al.*, 2008). It provides a long term in-situ solution instead of merely transferring the problem. This approach involves the degradation of specific organic compounds, leading to the complete mineralization of contaminants into

harmless byproducts. Bioremediation can also utilize plants and microorganisms to absorb and detoxify inorganic pollutants. Various contaminants such as chlorinated compounds, agrochemicals, heavy metals, and more can be effectively remediated using bioremediation techniques. The choice of technique depends on factors such as the nature and severity of the pollutant, environmental conditions, cost, and regulatory considerations.

Although complete degradation may not always occur, pollutants can be transformed into intermediate products that may be less or more hazardous and have varying mobility in the environment (Khalid *et al.*, 2017)

10.3 Bioremediation Techniques:

Depending on the soil to be handled and the application location, bioremediation techniques can be divided into two major categories: in-situ and ex-situ procedures.

10.3.1 Ex-Situ Bioremediation Techniques:

Ex-situ bioremediation methods involve the removal of pollutants from contaminated areas and transporting them to different locations for treatment. When choosing an ex-situ bioremediation technique, several factors are considered, such as the treatment cost, extent and form of contamination, pollution magnitude, site location, and geology.

Performance parameters are defined to guide the selection of appropriate ex-situ strategies. Ex-situ technologies offer competitive advantages, primarily the ability to better control the remediation process compared to in-situ methods. Even basic solutions like biopiles provide a contained environment that is easier to predict and manage.

However, ex-situ bioremediation has drawbacks, including higher expenses, the risk of pollution dispersal during excavation and transport, and associated additional costs. Consequently, this method is deemed undesirable (Azubuike *et al.*, 2016).

10.3.2 In-Situ Bioremediation Techniques:

In-situ bioremediation methods involve treating contaminated materials directly at the site of pollution, without the need for excavation. This approach minimizes disruption to the soil structure. As there are no additional costs for excavation, in-situ techniques are more economical than ex-situ methods. However, a significant challenge lies in the expense of designing and implementing sophisticated tools on-site to enhance microbial activity during bioremediation. Nonetheless, this method is relatively simple to implement and offers the crucial advantage of preventing contamination spread that may occur during transportation.

In-situ bioremediation involves the introduction of air, nutrients, and/or microorganisms (either native or foreign) directly into the polluted soil, requiring minimal technological tools. One of the main limitations is its slow kinetics, necessitating extended treatment periods to complete the biodegradation process.

Additionally, the diffusion of oxygen in the soil is typically limited to the upper layer (around 30 cm from the surface), imposing constraints.

In-situ bioremediation approaches have proven effective in treating various types of pollution, including chlorinated solvents, dyes, heavy metals, and hydrocarbon-contaminated sites (Roy *et al.*, 2015).

Table 10.1: Different Bioremediation Techniques, Their Principle and Uses

Methods	Principle	Uses
In-situ bioremediation techniques		
Biosparging	Injects air/oxygen into the groundwater to enhance aerobic biodegradation of contaminants dissolved in the groundwater.	Petroleum hydrocarbons spill sites (Kao <i>et al.</i> , 2008).
Bioventing	Enhances microbial activity for degradation of organic compounds using injected air or oxygen.	Light spilled petroleum products (Ho'hener and Ponsin, 2014).
Bioslurping	Simultaneously extracts free-phase contaminants (liquid) and enhances aerobic biodegradation through the injection of air/oxygen into the groundwater.	Petroleum hydrocarbons, contaminated sites (Kim <i>et al.</i> , 2014).
Biostimulation	Provides nutrients and conditions to enhance microbial growth and degradation capabilities.	Polychlorinated biphenyls (PCBs) (Di Gregorio <i>et al.</i> , 2013).
Bioaugmentation	Introduces specific microbial strains or consortia to enhance degradation capabilities.	Crude oil-polluted sediments (Fodelianakis <i>et al.</i> , 2015).
Natural attenuation	Relies on natural processes (e.g., microbial degradation, dilution, sorption) to reduce contaminant concentrations without external interventions.	Ground water contaminated with trichloroethene (TCE) (Adetutu <i>et al.</i> , 2015).
Ex-situ bioremediation techniques		
Slurry phase bioremediation		
Bioreactor	Enclosed systems that optimize environmental conditions for microbial degradation of contaminants.	Crude oil-polluted soil (Chikere <i>et al.</i> , 2012).
Solid phase bioremediation		
Biopiling	Contaminated soil is stacked into piles or windrows, and microbial degradation is facilitated through periodic aeration, nutrient addition, and moisture control.	Hydrocarbons contaminated soil (Smith <i>et al.</i> , 2015).
Land farming	Enclosed systems that optimize environmental conditions for microbial degradation of contaminants.	Oil-polluted soil (Silva-Castro <i>et al.</i> , 2012).

Methods	Principle	Uses
Composting	Promotes degradation of organic contaminants through microbial activity under controlled conditions, often with organic waste.	Petroleum contaminates soil (Wang <i>et al.</i> , 2011).
Biofilter	Uses a packed bed of organic or inorganic media to support microbial growth and degrade volatile contaminants in air or gas streams.	Water contamination by copper and cadmium (Loutseti <i>et al.</i> , 2009).

10.4 Phytoremediation:

The word "phytoremediation" originates from the combination of the Greek word "phyto" which stands for plant, and the Latin word "remedium" which signifies the ability to heal or restore (Vamerali *et al.*, 2010). This method uses physical, chemical, biological, biochemical, and microbiological interactions between plants in contaminated areas to reduce the toxicity of contaminants. This process of removing hazardous material from contaminated locations using living plants associated with numerous mechanisms, including accumulation or phytoextraction, phytofiltration, phytostabilization, phytovolatilization and phytodegradation. The removal of toxic heavy metals and radionuclides involves extraction, transformation, and sequestration processes. On the other hand, organic pollutants such as hydrocarbons and chlorinated compounds are primarily eliminated through degradation, filtration, stabilization, and volatilization. In some cases, mineralization can occur when specific plants like willow and alfalfa are utilized (Haq *et al.*, 2020).

10.4.1 Phytoextraction:

Phytoextraction, also described as phytoaccumulation, phytoabsorption, or phytosequestration, is a process that involves the uptake and accumulation of contaminants from soil/water through roots of plants, followed by their transfer and storage in the plant biomass or shoots, later harvested (Muthusarayanan *et al.*, 2018). The phytoextraction process can be divided into five main stages: mobilization of pollutants in the rhizosphere, uptake of pollutants by plant roots, translocation from roots to aboveground plant parts, and the accumulation of pollutants in plant tissues. Certain plant species, referred to as hyperaccumulators, have the unique capacity to accumulate high levels of certain contaminants in their tissues. The selection of hyperaccumulator plants is based on their tolerance to the target contaminants and their efficient metal uptake and accumulation capacity. Hyperaccumulator plants are defined as those capable of accumulating 1000 ppm of As, Pb, Co, Ni and Cu; 100 ppm of Cd; or >10,000 ppm of Zn and Mn. Notable families of plants like Brassicaceae, Asteraceae, Fabaceae, Euphorbiaceae, Flacourtiaceae, and Violaceae have been reported to extract heavy metals in significant amount (Kumar *et al.*, 1995). Brassicaceae in particular have high potential for phytoextraction due to their ability to maximum heavy metal extraction. Two main types of phytoextraction methods exist: induced phytoextraction or chelate-assisted phytoextraction, which includes enhancing the mobility of heavy metal ions by using synthetic chelates for plant uptake and continuous phytoextraction (Ayyappan *et al.*, 2016).

10.4.2 Phytofiltration:

Phytofiltration also called rhizofiltration, is a technique that relies on the capacity of plant root systems to absorb, concentrate, and precipitate contaminants found in the soil. Through this process, contaminants are transported from the soil into the roots and accumulate in the harvestable portions of the plants. Phytofiltration leverages the natural abilities of plants and the microbes related with their roots to uptake and transform pollutants, with a specific focus on treating soil and addressing soil pollution issues. Due to the fact that some plants may synthesis certain chemicals like phytochelatins within roots which may boost the binding of pollutants like metal ions to roots (Verma *et al.*, 2006). The rhizosphere environment differs from the bulk soil, providing unique conditions that can enhance contaminant degradation. Factors such as increased oxygen availability, pH changes, and root exudates create a more favorable environment for microbial activity and contaminant breakdown (Haq *et al.*, 2020). Plant roots release various organic compounds (sugars, amino acids, and organic acids) into the rhizosphere. These compounds serve as a food source for soil microorganisms, attracting them to the root zone. Some of these microorganisms possess specific enzymes that can break down contaminants. The terrestrial and aquatic plants can use for in-situ or ex-situ remediation process and the toxins are not passed to shoots are the two main advantages of rhizofiltration. Previous studies have indicated that terrestrial plants (sunflower and Indian mustard) possessing deep and fibrous root systems are well-suited for phytofiltration due to their enhanced ability to absorb metals. Indian mustard, as noted by Raskin and Ensley (2000), has been observed to effectively eliminate a wide range of lead concentrations (4-500 mg l⁻¹). These terrestrial plants have also demonstrated the capability to remove metals such as Cd, Zn, Cu, Ni, and Cr from hydroponic solutions (Dushenkov *et al.*, 1995).

10.4.3 Phytostabilization:

Phytostabilization or phytoimmobilization type of phytoremediation technique used to stabilize or immobilize contaminants in soil using plants. The process of phytostabilization involves planting these selected plants in contaminated areas, where they grow and establish a root system. The plant roots have crucial role in stabilizing contaminants by physically binding or absorbing them and by forming insoluble compounds or metal complexes with organic substance in root zone. They can minimize their movement and ultimately bioavailability of contaminants in the environment reduces, also preventing pollutant discharge into groundwater or their uptake by other organisms. Root exudates play a significant role in phytostabilization by complexation or chelation, process which reduce the mobility and bioavailability of metals by forming less soluble (Cheraghi *et al.*, 2011).

The majority of microbial populations in polluted soil are supported by plants which speed up plant growth and lead to the stabilization of harmful metals. The extensive root system and a low transfer of metals from roots to shoot are prerequisites in plant species to be utilized for phytostabilization (Mendez and Maier, 2008). Phytostabilization is often employed in areas where the complete removal or extraction of contaminants is technically or economically impractical. It can be used in various settings, including abandoned mines, industrial sites, and landfills. The main goal of phytostabilization is to stabilize pollutants rather than their removal, therefore reduce the risk posed by the contaminants and create a more stable and environmentally sustainable ecosystem.

According to the research conducted by Leung *et al.* (2007) which suggests that *Cynodon dactylon* (commonly known as Bermuda grass) has a high capacity for accumulating arsenic and could be a promising candidate for phytostabilization of arsenic-contaminated sites.

10.4.4 Phytovolatilization:

Phytovolatilization is a process of uptake contaminants, convert them into volatile compounds, and then release into the environment. This process is mediated by plant metabolic activities and transpiration pull (Khan *et al.*, 2019). As the contaminants are transformed into volatile compounds, they can be released into the atmosphere through different pathways, one of the main routes of release is through the stomata on the leaf surfaces. These transformations can occur through various enzymatic reactions, microbial interactions, or metabolic processes such as oxidation, reduction, methylation, or other chemical reactions within the plant (Muthusaravanan *et al.*, 2018). As a result, the volatile compounds released during phytovolatilization may be in the same form as the original contaminants or in altered forms due to the plant's metabolic activities. Several studies showed that *Brassica juncea* (Indian mustard), *Populus spp.* (poplar trees), *Salix spp.* (willow trees), maize etc have been studied and utilized for phytovolatilization to remove pollutants from soil. rice, azolla, rabbit foot grass, and pickle weed are some aquatic plants that are the finest volatilizers. Sunflowers (*Helianthus annuus*) possess the capability to volatilize selenium (Se) into the atmosphere. This is achieved through the process of assimilating selenium as organic seleno-amino acids from soil, particularly selenocysteine and selenomethionine, which are then released as volatile Se compounds (Terry *et al.*, 1992).

10.4.5 Phytodegradation:

Phytodegradation is a process through which plants can break down or degrade contaminants in the environment. Phytodegradation or phytotransformation, refers to the process by which plants capture pollutants from the surrounding and then undergo chemical modifications within their tissues as a result of metabolic processes and enzymatic reactions. This transformation can also occur through the actions of microorganisms associated with the plant's root system. Ultimately, this process leads to the inactivation, degradation, or immobility of pollutants, either in the roots or shoot of plants (Da Conceição Gomes *et al.*, 2016). Plants can possess enzymes capable of breaking down specific pollutants like cytochrome P450 monooxygenases and peroxidases enzymes are involved in the metabolism and detoxification of organic compounds. Additionally, plants can also release exudates and enzymes into the rhizosphere, which can enhance microbial activity and promote the degradation of contaminants in the soil (Mahar *et al.*, 2016).

10.5 Microorganism Remediation:

Microorganism remediation is a method which utilizes microorganisms to degrade or transform contaminants in soil, resulting in their remediation. Microorganisms such as bacteria, fungi, and archaea possess metabolic capabilities that enable them to break down various pollutants, including organic compounds and certain heavy metals though immobilizing and reducing the bioavailability of contaminants in polluted soil.

Heavy metals are an example of an inorganic contaminant that cannot be broken down by microbes but can be changed into another form because they have different physical and chemical characteristics (Ashraf et al., 2019). Microorganisms can utilize contaminants as a carbon or energy source, metabolizing them and converting them into less harmful substances. Organic pollutants are broken down by microorganisms either through respiration in aerobic condition or through denitrification, methanogenesis, and sulfidogenesis in anaerobic condition. Bacteria are known for their ability to degrade hydrocarbons, pesticides, and other organic pollutants. Fungi, on the other hand, are adept at breaking down complex organic compounds i.e., polycyclic aromatic hydrocarbons (PAHs) and dioxins. Microbial remediation mechanisms include oxidation-reduction reactions, intracellular storage, extracellular complexation, and precipitation of contaminants from the soil and reducing their bioavailability (Yang et al., 2018).

Among these processes, bioaccumulation and biosorption are crucial because they allow microorganisms, or biomass, to bind to and concentrate environmental pollutants. The initial stage involves quick sorption, similar to biosorption, where microbial population and metabolites of microbes bind to contaminants. The second stage is steadier and entails the physiological transport of the sorbate into the interior of cells through an actively metabolically transport system (Chojnacka, 2010).

Understanding and harnessing bioaccumulation and biosorption processes can contribute to the development of efficient microbial remediation strategies for contaminated soil and water environments. Microbes utilize functional groups present in their polysaccharide slime layers to adsorb pollutants. These functional groups have affinity for various contaminants, allowing microbes to bind and immobilize them. In addition to the polysaccharide slime layers, extracellular polymeric substances (EPS) produced by microbes also contribute to the adsorption of pollutants. EPS are composed of nucleic acids, proteins, lipids, and complex carbohydrates. They form a matrix around microbial cells and provide a sticky, gel-like structure. This matrix enhances the adsorption capacity of microorganisms, allowing them to capture and retain pollutants (Gupta and Diwan, 2017). Field testing of promising microorganisms identified in laboratory or greenhouse studies is essential, as environmental variables in the field can lead to different outcomes. The effective utilization of microbes is necessary for successful implementation of microbe-assisted phytoremediation. Various methods such as seed treatment, foliar sprays, and direct inoculation of soils can be employed to introduce inoculants into contaminated soils. A study by Boricha and Fulekar (2009) identified *Pseudomonas plecoglossicida* as a novel organism suitable for the bioremediation of cypermethrin pesticide. Also, they found that *Pseudomonasaeruginosa*, *Bacillus* sp., *Streptomyces* sp., and *Pseudomonas fluorescens* were used for remediation against chromium heavy metal. Some bacteria such as *Bacillus* spp. and *P. aeruginosa* showed positive result for remediation of lead contaminated soil (Akhtar et al., 2013).

10.6 Conclusion:

Bioremediation offers a promising solution for the remediation of polluted soils, providing an eco-friendly and sustainable approach to restore soil quality and ecosystem health. This process offers effective, cutting-edge treatments for a wide range of pollutants. Among the several known bioremediation techniques phytoremediation, rhizoremediation, and

bioremediation by bacteria are potentially effective methods to decontaminate soil from pollutant. Continued research and technological advancements are needed to optimize bioremediation strategies, improve efficiency, and expand their applicability to a wide range of contaminants and soil types.

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