

Bioremediation, a sustainable approach to alleviate soil contaminants

Abstract

Dangerous contaminants generating from industry, agricultural and human activity are causing the degradation of soil health, along with detrimental effects on human and the environment. It is imperative to safeguard the soil from these dangerous pollutants by using soil remediation techniques that may be effective breakdown these dangerous toxins. A sustainable approach to remediate the soil from different contaminants is bioremediation. Bioremediation is a method where microbes are used to alleviate soil pollution effectively. Natural microorganisms like fungus, bacteria, and algae are employed in the bioremediation process to break down heavy metal (lead, arsenic, chromium etc.) or organic based. chemical contaminants. This study examines the use of microorganisms and various bioremediation methods, including genetic engineering, nanotechnology, and electro bioremediation, for recovering polluted soil. This investigation clarified the challenges associated with applying these bioremediation technologies and microorganisms, as well as their effects on the ecosystem and inherent soil microbial population. The goal of this current study is to illustrate the various technologies which are more effective in this remediation process.

Key words- Contaminants, soil pollution, bioremediation, soil microbes, breakdown

Introduction

The soil is contaminated with toxins that damage living organisms which generated due to the rapid rise of industry and human population. Pollutants from different industrial activities are important sources of pollution to the soil and aquatic habitats (Sekhar et al., 2024). Soil and sediments are contaminated by a range of hazardous organic and inorganic contaminants that are released into water bodies. One of the most harmful chemicals released by industry into the ecosystem is heavy metal pollution (Gautam et al., 2016). Currently, 557 locations in India, covering an approximate area of 175 million hectares, have been classified as polluted with heavy metal. A significant amount is still unknown. UP, Punjab, Gujarat, AP, MP, National Capital Territory and Rajasthan are home to the majority of these locations that have been

recorded (Jain et al.,2017).Industrial wastewaters are frequently found to contain heavy metal like cadmium, zinc, copper, nickel, lead, mercury, and chromium. These wastewaters come from mining operations, battery manufacturing, tanneries, paint and pigment manufacturing, and photography(Parmar et al.,2013). The use of fertilizers, pesticides, and herbicides by the agricultural industry releases a number of contaminants into the soil environment, including lead, arsenic, copper, zinc, nickel, and aluminium(Sekhar et al.,2024).The health of humans can be negatively impacted by heavy metals. Toxic metal contamination of air and water is a major environmental issue that affects hundreds of millions of people worldwide (Mood et al.,2021). Even at lower exposure levels, metals are recognised systemic toxicants that can cause harm to various organs. Generally speaking, the most hazardous metals to which humans are exposed are lead (Pb), cadmium (Cd), methylmercury (MeHg), and arsenic (As). These metals affect vital organs such as the kidney, liver, and brain and can cause nephrotoxicity, hepatotoxicity, and neurotoxicity (Karri et al.,2016).

Bioremediation is one of the most efficient, cost-effective, and ecologically safe processes available(Sekhar et al.,2024). Microorganisms, enzymes, genetic engineering, and plants may be employed in biological remediation however, as microbes are easier to work with and take longer to develop than plants, they are favoured. Moreover, microorganisms minimize the impacts of heavy metals, improve plant growth, and improve soil fertility(Saha et al.,2021). Using this method, microbes break down both harmful and non-hazard elements and transform them into compound form. Notable bioremediators include bacteria, fungi, and archaea (Abatenh et al.,2017). By storing pollutants in their tissues or metabolising them into less harmful forms, plants can also aid in the bioremediation of toxins through their roots. Because some plant species naturally collect large amounts of particular pollutants, they can be used to remove pollutants from soil (Yaashikaa.,2022). Genetic engineering is method of bioremediation to alter the genetic composition of microorganisms in order to improve their capacity to break down contaminants.The finest examples of it are transgenic organisms and genetically modified bacterial species(Kumar et al.,2020). This paper highlights the value of bioremediation and provides an overview of the factors influencing bioremediation technology. This review also covered the emergence of genetically modified microorganisms, which have powerful bioremediation capabilities and enormous promise for eliminating dangerous contaminants (Saravanan et al.,2023).

Sources of contaminants

Pollutants are growing as a result of increased industrialization, contaminating the soil, water, and air. These pollutants are becoming more prevalent, endangering people, marine life, and the ecosystem. Contamination is caused by substances such as hydrocarbons, pesticides, herbicides, naturally occurring rock disintegration, and hazardous metals found in the environment. Both anthropogenic and geogenic processes are the sources of contamination (Li et al., 2021). Figure 1 contains different types of contaminants sources.

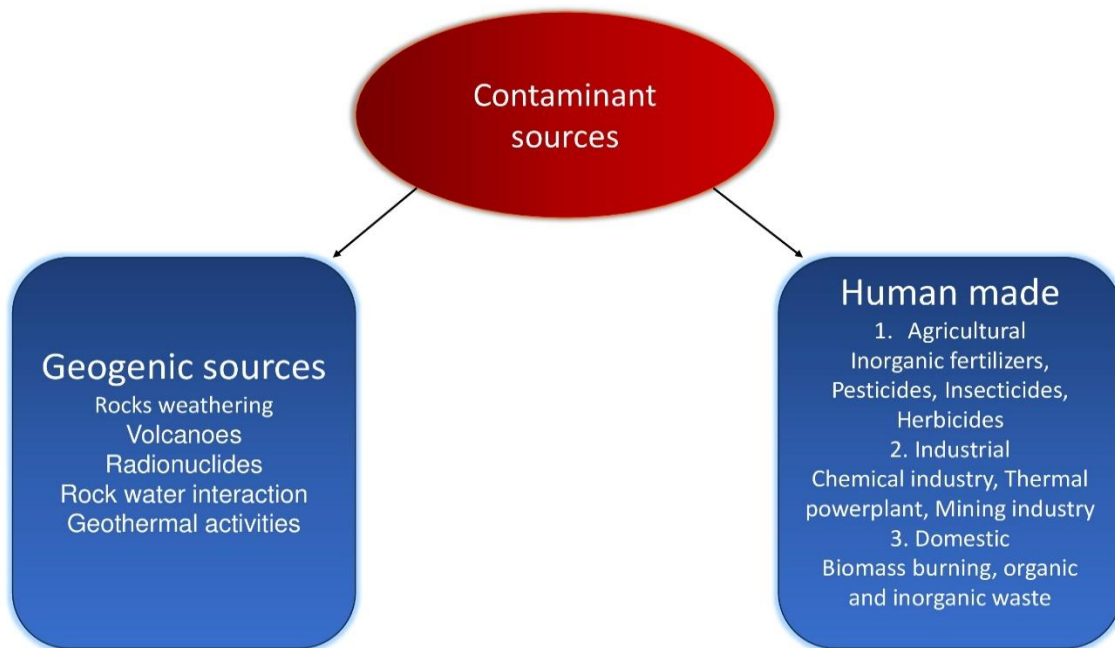


Figure 1. Different sources of contaminants.

1. Geogenic Sources

Geogenic sources denote natural sources as the source of contamination. The reason of the contamination might be attributed to several factors such as weathering of rocks, radionucleotides, or volcanic activity (Bundschuh et al., 2017). Rocks that are good for weathering include ultramafic (olivine), dunite, and basalt. Dunite has a higher concentration of nickel and chromium as a result of rock weathering. Soil becomes poisonous when these components are present in high concentrations (Suhrhoff et al., 2022). The elevated fluoride content of granite rock due to the leaching of sedimentary and igneous rock particles, creating a cracked zone in close proximity to groundwater and contaminating it (Nakayama et al., 2022). Like rock weathering, volcanoes contaminate soil and water sources. When volcanic eruption occurs, the lava is released and combines with basaltic and rhyolitic rocks to create toxicity in aquatic environments (Flavia et al., 2023). Rock weathering and volcanic eruption are often influenced by environmental factors or natural disturbances (Pickarski et al., 2023).

2. Anthropogenic Sources

Anthropogenic sources, on the other hand, indicate contamination caused by human activity and could stem from residential, industrial, or agricultural practices (Ismanto et al., 2022).

A. Agricultural sources

Agriculture based contamination is due to several contaminants coming from organic and inorganic fertilizers, pesticides, insecticides, herbicides.

i) Organic manures and inorganic fertilizers

Nutrient sources include both organic manures and inorganic fertilizers; however, excessive usage of inorganic fertilizers combined with improper management and storage of manures might contaminate soil (Rashmi et al., 2020). Overuse of fertilizers high in phosphorus and nitrogen degrades soil quality and contaminates water sources through runoff and excessive erosion (Srivastav et al., 2020). The utilization of hazardous components in feeds or as growth promoters in organic manures, such as chicken manure, poses health risks (Muhammad et al., 2020).

ii) Pesticides

Pesticide exposure poses serious health risks to humans, and when combined with heavy metals, it results in more contamination than either alone (Zhang et al., 2020). The pesticides in the organochlorine class, such as dichloro-diphenyl-trichloroethane (DDT) and hexachlorocyclohexane (HCH), are more persistent in soil and can be hazardous to the environment and human health (Kafaei et al., 2020).

iii) Insecticide

When pesticides are used on plants to eradicate hazardous insects, they also kill beneficial insects that are not intended targets, leading to contamination (Butu et al., 2020). For instance, some significant insects, like whiteflies, ingest honeydew that has been contaminated by insecticides applied on the plant. When beneficial insects consume this contaminated honeydew, it might kill them (Calvo et al., 2021). Certain man-made pesticides, including neonicotinoid, pollute soil and water sources (Zhang et al., 2020). The most popular insecticides are neonicotinoid pesticides, which account for 30% of the global market based on consumer use (Zhang et al., 2021).

iv) Herbicides

Both the soil and the groundwater are contaminated by herbicides. Because atrazine and bromacil are used excessively and are contaminating groundwater, it is critical to degrade these herbicides because groundwater is becoming scarcer (Gawel et al., 2020). Additionally, herbicides contaminate coastal areas, infecting marine life. The breakdown of the herbicide triazine is crucial since it seriously contaminates water bodies (Dzikowitzky et al., 2020).

B.Industrial sources

Urban soil contamination is caused by human activity, such as the disposal of industrial waste. The causes of industry contamination include mining, power plants, and hazardous chemicals (Long et al., 2021). This involves the contamination of heavy metals including lead, arsenic, mercury, cadmium, etc. These metals combine with the soil's organic matter to increase soil toxicity (Mohammadi et al., 2020). The mining sector seriously harms the environment, soil, and human health. Coal, copper, zinc, and other mines are examples of mining. The concentration of heavy metals (cd, pb, cr, and zn) in the mines is higher than typical, which has a negative effect on the local population (Zerizghi et al., 2022). One major risk associated with brownfields (industrial production sites) is the pollution of industrial waste. Chemicals from industrial processes, including those involving iron, nonferrous metals, and chemical manufacture, are released into wastewater, which contaminates water, soil, and human health (Peng et al., 2022). The disposal of wastewater into water bodies poses a threat to aquatic and human life, as the presence of hazardous substances can disrupt the food chain (Sonone et al., 2021). Aquaculture is a rapidly expanding industry that produces 43% of sea food and 50% of fish for human consumption and it important to remediate the water bodies and soil (Emenike et al., 2022).

C.Domestic Sources

The majority of water bodies and the land are contaminated by domestic trash. Waterborne enteric viruses that can cause outbreaks and pose a major threat to life are originated by human wastewater (Farkas et al., 2020). Waste water from the home can be utilized for a variety of things, including irrigation in farmland. Domestic waste cannot be used for various reasons because of the presence of organic micropollutants, oil, and hazardous organic pollutants in this waste water (Yacouba et al., 2021). Due to their intricate structure, these wastewaters in soil and aquafarm cannot be neutralized. They have a long-term effect on soil biota and affect marine life (Mushtaq et al., 2019). Nowadays, the quality of fresh water is deteriorating due to the disposal of sewage and domestic wastewater in water bodies without

first eliminating the metals that cause contamination. (Shukla et al., 2021). In coastal locations, antibiotic-resistant genes (ARGs) can be used to analyze contaminants found in household wastewater. The most accurate way to determine the level of contamination in water bodies is through these genes (Zhang et al., 2020).

Types of bioremediations

There are two types of bioremediation techniques: ex-situ and in situ, which are utilized to remove contaminants. ex-situ bioremediation is taking contaminated material out of its original habitat and treating it somewhere else (Nwankwegu et al., 2022). On the other hand, In situ treatment includes dealing with pollutants at their original location and turning them into non-hazardous forms without removing them (Ossai et al., 2020). Figure 2 type of bioremediation

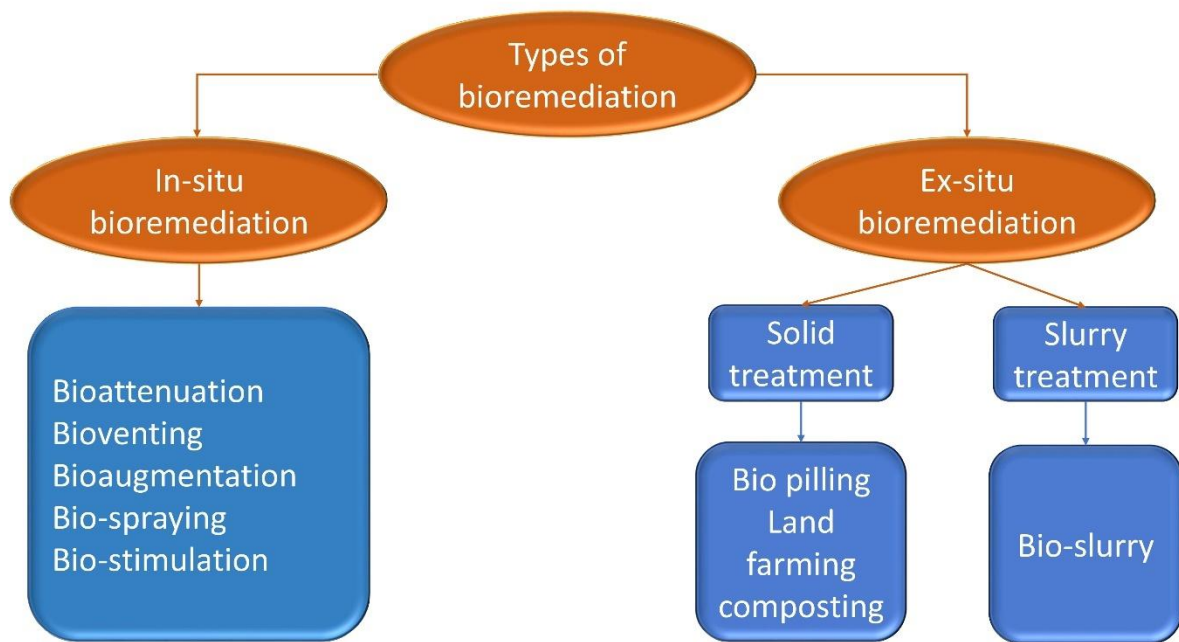


Figure 2. Types of bioremediation

A. In situ bioremediation

i) Bioattenuation

Also referred as monitored natural attenuation (MNA), bioattenuation is the process by which naturally occurring microorganisms are maintained in polluted places to lower their population to a desired level, with few or no involvement of human (Dung et al., 2022). It is recommended for usage in comparison to other cleanup procedures due to its low cost.

Natural hydrocarbon-degrading microbes can be used to clean up contaminated soils and lower levels of risk to the environment and human health (Eriakha et al.,2023).

ii) Bioventing

One of the first large-scale technologies to be used in the 1990s, bioventing is now widely used in commercial applications and in common bioventing technique keeping the subsurface oxygen level around 5% (sharma et al.,2023). It supplies oxygen to unsaturated zones or injects it directly into contaminated areas via vertical and horizontal wells. Organic contaminants are broken down and absorbed into the soil by indigenous microbes (Fragkou et al.,2021).

iii) Bioaugmentation

The practice of introducing bacteria to soil to speed up the breakdown of contaminants is known as bioaugmentation. Bioaugmentation is frequently used for petroleum hydrocarbons, chlorinated pollutants, etc. (Sayed et al.,2021). Organic and inorganic chemicals, including phenolic compounds, acrylic acid, nitrite, acetone, and other hazardous substances, are present in a lot of industrial waste. bioaugmentation products are proffer, which makes them easy to handle and degrade (Bhatia et al.,2021).

iv) Bio-spraying

By supplying gas to the saturated zone, a technique known as "bio spraying" improves the bioavailability of pollutants by promoting aerobic biodegradation and interaction between the air, water, and aquifer, advised in locations where there is fuel, diesel or petrol residue (Sravya et al.,2022 and jabbar et al.,2022). Its target chemicals include BTEXN (benzene, toluene, ethylbenzene, xylene, and naphthalene), which is biodegradable in an aerobic environment (Chipu et al.,2022).

v) Bio-stimulation

Microbes cannot sustain their population by decomposing toxins; instead, they require additional energy for development and growth, here bio stimulation plays a significant role (Maqsood et al.,2023). Bio-stimulation, a procedure in which additional nutrients are added to the soil to encourage microbe growth and the biodegradation of pollutants (Aloja et al.,2022).

A.Ex situ bioremediation

There are mainly two types of ex situ methods i.e. solid treatment and slurry treatment which are discussed elaborately.

1. Solid treatment

In solid treatment, amendments are applied to the piles to treat the impurities. It is typically used to remove contaminants from household, industrial, and organic waste (Angmo et al., 2020). It involves the following techniques:

i) Bio piling

The disposal of soil contaminated with petroleum compounds or other organic chemicals is a part of bio piling bioremediation. The contaminated soil is disposed of in pile form in this technique of bioremediation (Muttaleb et al., 2022). A multitude of fertilizers and supplements are employed in order to augment the population of microorganisms. The piles are two to three meters high, and a vacuum pressure system is built beneath the soil. This system is coated with polythene to either boost heating or decrease evaporation loss. It is an economical method that aids in cleaning up extremely contaminated environments (Philp et al., 2014).

ii) Land farming

In land farming, contaminated soil is disposed of in line beds, and aeration is kept up to date by turning. Soil is treated to change its chemical and physical characteristics in order to break down pollutants (Ajibade et al., 2021). It may occur both in situ and ex situ. It's an easy procedure that uses the least amount of energy to treat contaminated soil. In aerobic circumstances, it aids in the remediation of soil (Alori et al., 2022).

iii) Composting

Another name for it is windrows. During this procedure, organic materials, such as organic amendments, are combined with the polluted soil to improve the microbial characteristics by providing additional nutrients and carbon (Khan et al., 2023). Utilizing these organic components for their metabolic qualities, it aids in the breakdown of organic waste products with the help of microbes (Deena et al., 2022).

2. Slurry treatments

Using this method, pollutants are removed from the polluted soil by treating it with water. Thus, this approach has an adequate supply of oxygen and nutrients, which is beneficial for

the growth of microorganisms. Compared to previous approaches, this bioremediation technique is quicker (Alori et al.,2022). This technique consists of:

i)Bio-slurry

In bioreactor techniques, water is used to remediate the polluted soil to create slurry. The amount of pollutants in the soil determines how much water is needed (Srivastava et al.,2022). Polluted soil might be moved into the vessel where the degrading process takes place in order to break down contaminants.The contaminants are fed in a slurry or dry form. It offers an atmosphere that is conducive to the growth of microbes(sharma et al.,2022).

Use of microorganisms in soil remediation

i) Bacteria

Bacteria are beneficial heavy metal absorbent. Heavy metal transformation is carried out by the bacterial population. It is one of the better approaches for detoxifying heavy metals(Thakare et al,2021). Organic pollutant is treated with the help plant growth promoting rhizobacteria (PGPR). Bacteria like Bacillus, pseudomonas, rhizobium, and Klebsiella are among the species found in PGPR. These species typically encourage plant development and aid in the removal of hazardous contaminants. For the purpose of eliminating chromium contamination, Pseudomonasaeruginosa is a more efficient species of bacteria (Xiang et al. 2022). The bacteria produces exopolysaccharides (ESP), which aid in the binding or chelation of heavy metals. These ESP trap the metal and detoxify the metal ion, preventing the bacterial cells from drying up (Guo et al. 2021). Hydrocarbon pollution can also be broken down by the bacterial population. Hydrocarbons are the energy or carbon source that these bacteria use for growth and metabolic processes(Kebede et al.,2021). Aromatic and aliphatic groups are present in the hydrocarbon. Pseudomonas, bacteria, and Xanthomonas species completely destroy the aliphatic group of hydrocarbons, while the aromatic group's degradation is contingent upon the complexity of its structure and the existence of benzene rings (Stepanova et al.,2022).There are two types of bacteria: gram positive and gramnegative. Pseudomonas, bacillus group is one of the gram-negative species that is best in eliminating pollutants (Hussain et al.,2022).

ii) Fungi

Fungi are heterotrophic creatures that, because of their shape and metabolic capabilities, contribute to the processes of degradation in soil and aquatic systems. Mycoremediation is a type of bioremediation that incorporates fungus species (Li et al., 2020). When compared to traditional approaches, the use of fungus species is a more efficient and economical means of remediating soil (Khatoun et al., 2021). *Aspergillus* species of fungus efficiently breakdown pesticides in soil and reduce their persistency; nevertheless, *Microsporum*, *Penicillium*, and *Trichoderma* species of fungus are the most frequently used in bioremediation processes (Mohapatra et al., 2021). White rot fungus (*Phanerochaete chrysosporium*) is beneficial in breaking down organic contaminants in soil and reducing their persistence (Bumpus et al., 2021). With the aid of extracellular lignin-modifying enzymes, these fungi are mostly utilized for the breakdown of polycyclic aromatic hydrocarbons (Medaura et al., 2021). The Arbuscularmycorrhizal fungal species is capable of controlling the pollution caused by heavy metals. For their growth and metabolic processes, they utilize the organic components from these pollutants (Tomar et al., 2021). Certain indigenous fungal species, such *Aspergillus hiratsukae* and *Aspergillus terreus*, have extracellular polymeric compounds that are utilized to break down copper (Cu) in soil contaminated with heavy metals (Palanivel et al., 2023). Table 1 contains the use of different types of fungal species for the removal of contaminants.

Table 1. Different fungal species used in removal of contaminants.

Sr no.	Fungal species	Host plant	Contaminants Removed	Reference
1	<i>Fusarium</i> spp	Wheat (Triticum aestivum)	Lead(Pb), Cadmium(Cd)	Saad et al., (2022)
2	<i>Aspergillus</i> spp	Sunflower (Helianthus annuus)	Chromium (Cr)	Quadir et al., (2022)
3	<i>Penicillium</i>	Bell bean (Vicia faba)	Cadmium (Cd), Lead(Pb)	Mehdy et al., (2021)
4	<i>Colletotrichum</i> spp	Rice (Oryza sativa)	Cadmium(Cd)	Mukharjee et al., (2022)
5	<i>Trichoderma</i>	NA	Cadmium (Cd), Lead (Pb)	Khilji et al., (2021)

iii) Algae

Algae are important in the bioremediation process because they help in changing harmful substances into less harmful or neutral forms. There are two different kinds of algae employed in the bioremediation process: macro algae and micro algae (Singhal et al., 2021). Cyanobacteria and Bacillariophyta are examples of microalgae, whereas Phaeophyta, Rhodophyta, and Chlorophyta are examples of macroalgae (Ansar et al., 2023). Microalgae are primarily employed in the bioremediation process, which lowers the need for both biological and chemical oxygen demand (Viegas et al., 2021). The microalgae *Chlorella fusca* are used to extract iron (Fe^{2+}) from water and soil. In addition to their high metal absorption ability, algae species are also reasonably priced, environmentally benign, and non-toxic in nature (Zada et al., 2021). In order to remediate salt-affected soil, such as saline soil, indigenous species of microalgae called *Coelestrella* are employed because of their ability to withstand salinity (Chen et al., 2024). Table 2 contains removal of contaminants by using different algal species.

Table 2. Contaminants bioremediation by different algal species.

Sl.no	Algal species	Contaminants removed	References
1	<i>Chlorella vulgaris</i>	Ethidium Bromide	Almeida et al., 2020
2	<i>Cyanophyta</i> (hyper accumulator)	Arsenic (As)	Ankit et al., 2022
3	<i>Tetradesmus</i>	Phenolic compounds	Lorenzo et al., 2023
4	<i>Scenedesmus</i>	Captan	Hamed et al., 2022
5	<i>S. obliquus</i>	Lead (Pb)	Danouche et al., 2020
6	<i>Nostoc</i>	Cadmium (Cd)	Hameed et al., 2021

iv) Yeast

Yeast effectively aids in the bioremediation process by removing heavy metals from soil and contaminated sites, aiding in the mineralization of organic materials, and having the ability to synthesize intracellular materials like mannoproteins (Kreling et al., 2019). Yeast feeds the

microorganisms involved in bioremediation with nutrients and occasionally with enzymes. Yeast species that are tolerant can be employed to get rid of infectious agents. For instance, the yeast species *Rhodotorulamucilaginosa* is utilized in the bioremediation of dumpsites (Masih et al., 2020). It can produce substances like indole acetic acid (IAA) that stimulate plant development, or it can be resistant to heavy metals and aid in the biosorption of lead (Pb) (De Paz et al., 2023). Additionally, yeast contains extracellular enzymes that aid in the biodegradation of textile dyes, such as xylanases and proteinases. These enzymes are found in certain yeast species, such as *Mrakia*, *Cystobasidium*, and *Vishniacozyma*, which can biodiscolorize the textile dyes (Bezus et al., 2021). *Saccharomyces cerevisiae*, a wild species of yeast that can be transformed by including the *populus* gene is used for cadmium (Cd) metal removal (Oliveira et al., 2020). Nowadays, it's common practice to use live or dead yeast cells to remove stubborn pollutants because they're so affordable and it is used in the bioremediation of synthetic dye (Danouche et al., 2021). Table 3 contains removal of contaminants by using different yeast species.

Table 3. Different yeast species for contaminant removal.

Sl. no	Yeast species	Contaminants removed	References
1	<i>Saccharomyces</i>	Lead (Pb)	Espinoza et al., 2022
2	<i>Pichia</i>	Synthetic dye	Saravanan et al., 2021
3	<i>Wickerhamomyces</i>	Copper (Cu)	Kouchou et al., 2020
4	<i>Lipomyces</i>	Oil effluents	Karim et al., 2021
5	<i>Candida</i>	Petroleum hydrocarbon	Hegazy et al., 2024

Phytoremediation

Plants and bacteria in association clean up the environment is known as phytoremediation. Using naturally occurring mechanisms, this method breaks down and sequesters organic and inorganic contaminants in the rhizosphere of plants (Smits et al., 2005). This technique helpful in clearing polluted soil of heavy metals including lead, cadmium, and arsenic. After the pollutants are absorbed by the plants, they may be harvested and appropriately disposed of, eliminating the toxins from the surrounding environment (Saravanan et al., 2021) Figure 3

different types of phytoremediation. Table 4 contains different types of phytoremediation used in bioremediation process.

Table 4. Types of phytoremediation used in bioremediation.

Sl. No.	Type	Contaminants adsorbed	Use	Plant used	Reference
1	Phytoextraction	Ag, Cr, cd	Contaminant chelation	<i>Alyssum murale</i> , <i>Berkheyacoddii</i> , <i>Pteris vittate</i> ,	Remigio et al.,2020
2	Rhizofiltration	Cu, Ag, Cd, Hg, Pb, Mn, Cr	Contaminant absorbed by roots	<i>Brassica juncea</i> , <i>Helianthus</i>	Kristanti et al.,2021
3	Phytodegradation	Malachite Green, DDT	Contaminant decayed by plants	<i>Elodea canadensis</i> , <i>Datura innoxia</i>	Kafle et al.,2022
4	Phytovolatilization	Organochlorines, Se, As	Contaminant convert to volatile form	<i>Polypogonmonspeliensis</i> , <i>Phragmites australis</i>	Guarino et al.,2020
5	Phytodesalination	Na, cl	Desalinization of soil by halophyte plants	<i>Typha latifolia</i> , <i>Lonicera japonica</i> <i>Thunb</i>	Kafle et al.,2022
6	Phytostabilization	Zn, Cd, Hs, Pb	Contaminant immobilization	<i>Haumaniastrumsp.</i> , <i>Commelina sp.</i>	Lacalle et al.,2023
7	Phytostimulation	Polycyclic Aromatic Hydrocarbons (PAHs), Ar, Cd	Contaminant decayed by plant root exudates	<i>Jatropha curcas</i> , <i>Populus spp.</i>	Islam et al.,2022

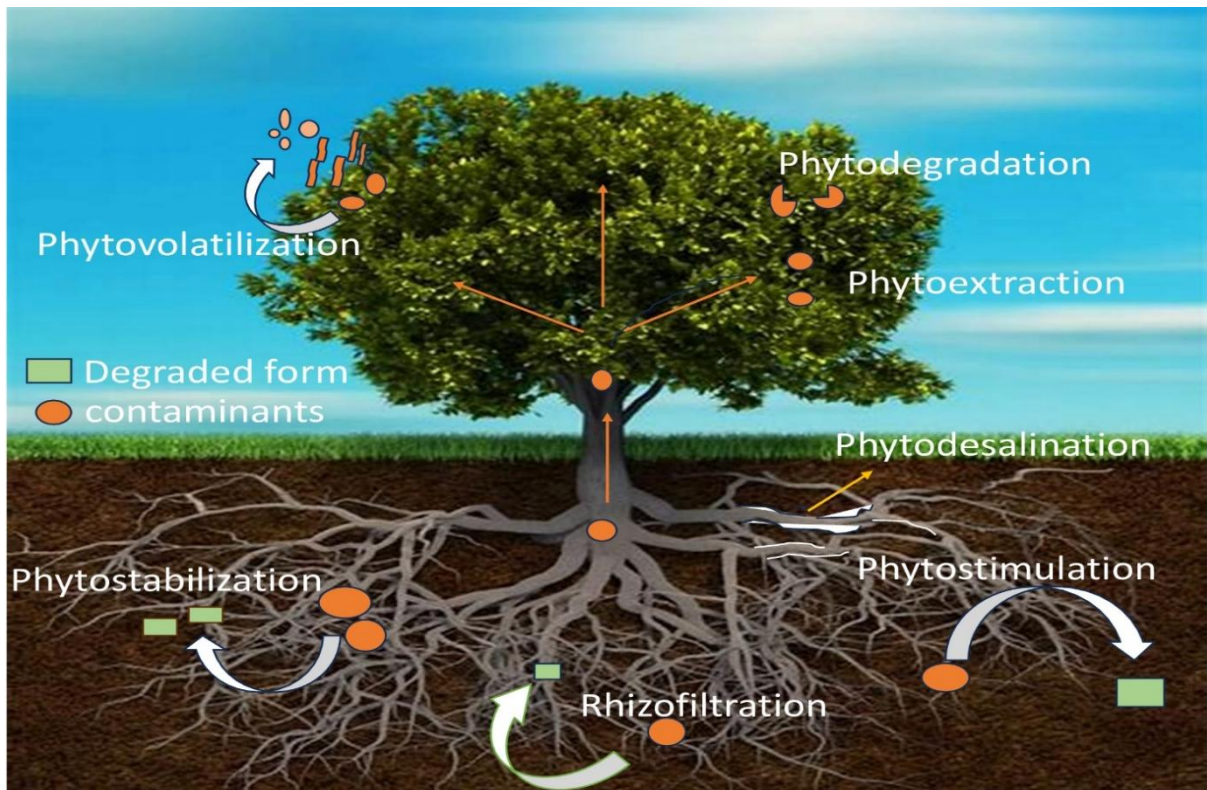


Figure 3. Types of phytoremediation

Genetic engineering of bioremediation

Pollutant degradation is greatly aided by organisms. Their capabilities are improved by genetic tools. Microorganisms are being actively modified for bioremediation through the use of gene editing techniques such as CRISPR-Cas9, ZFNs, and TALEN. With the use of these techniques, bacteria target contaminants more efficiently (Bora.,2023). These genetically modified organisms (GMOs) have the ability to degrade a variety of pollutants, such as radioactive waste, heavy metals, polychlorinated biphenyls (PCBs), and petroleum hydrocarbons. The term "genetic bioremediation" is frequently used for GMOs (Kumar et al.,2020). In order to enable an organism to break down contaminants, fresh genetic material is injected into it during the genetic bioremediation process. Numerous techniques, like as plasmid conjugation, transduction, and transformation, can be used to accomplish this (Cai et al.,2021). For example, the mutant NiCoT efflux gene (*rcnA*) is eliminated by the presence of the NiCoT gene of *E. coli*, which is used to remove cobalt and nickel. *Rhodospseudomonas palustris*, CGA009 (RP), and *Novosphingobium aromaticivorans* were the sources of the NiCoT gen (Sharma et al.,2020). *Bacillus cereus* NWUAB01, which produces biosurfactants has metal removal efficacy of Pb 69%, Cd 54%, and Cr 43% respectively

(Malik.,2022).Figure 4 different methods of genetic engineering used in bioremediation.

Table 5 contains different techniques of gene editing used in bioremediation.

Table 5. Different techniques of gene editing.

S. No.	Genetic engineering	Specific use	Reference
1	CRISPR-Cas9	CRISPR sequences identify and eliminate DNA from related bacteriophages. Cas9 functions as molecular scissors, cutting the target DNA at certain spots after it has identified it.	Massafra et al.,2021
2	ZFNs	ZFN produce site-specific DSB which is made up of a chain of zinc finger proteins. Since zinc finger proteins individually recognise a DNA sequence, they allow for site-specific targeting.	Janik et al.,2020
3	TALEs	The DNA-binding domain of the TAL effector is injected into plant cells, they find their way to the nucleus, attach to the desired promoters, and start the production of genes.	Beckeret al.,2021
4	Cre-loxP recombination system	Cre-loxP target gene function which provide both spatial and temporal control over gene expression and preventing the total deletion of certain genes that may result in embryonic death.	Son er al.,2021
5	RNAi technology	Ribonucleic acid molecules target genes and can regulate microbe activity involved in the breakdown of pollutants.	Bharagava et al.,2022
6	Modular Cloning System (MoClo)	MoClo assembles many DNA fragments in a predetermined linear sequence by using the activity of Type IIS restriction enzymes, such as BsaI and BpiI/BbsI.	Ardi et al.,2023

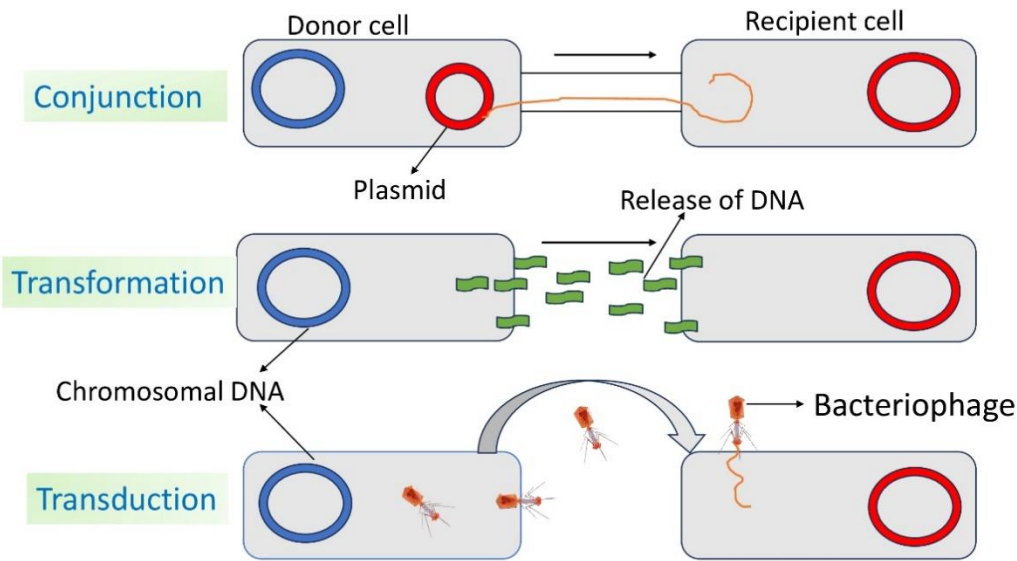


Figure 4. Different methods of genetic engineering used for bioremediation

Modern techniques for improving bioremediation

i) Nanotechnology

The process of producing nanoparticles, which is not expensive, involves the utilization of microorganisms. Environmentally friendly materials are used in nanotechnology (Mandeep et al., 2020). The heavy metals lead (Pb), arsenic (As), and cadmium (Cd), which are harmful to both individuals and the environment, are absorbed by these well-absorbent nanoparticles (Mallikarjunaiah et al., 2020). By employing biosynthetic nanoparticles, nanotechnology offers an affordable method of eliminating pollutants from the air, water, and soil. It is an environmentally friendly method of removing pollutants from contaminated materials (Naik et al., 2023). To clean up the contaminated materials, these nanoparticles which include carbon nanotubes, nano enzymes, and nano iron particles are utilized (Tulasingh et al., 2023). These days, polluted substances are treated with a combination of metal nanoparticles, microbial biomolecules, and biogenic nanoparticles (Shahi et al., 2021). Seventy percent of industrial pollutants end up in water bodies, harming aquatic life or posing health risks to humans due to their entry into the food chain (Kaul et al., 2022). The primary source of pollution for aquatic bodies is the ever-increasing need for plastic. The size of this microplastic, which ranges from 5 to 1000 μm , is found in large amounts in the stomachs of marine creatures (Priyadarsini et al., 2021).

ii) Electro bioremediation

A relatively new technique called electro bioremediation uses electroactive microbes to control the oxidation and reduction process in conjunction with electrodes. These microorganisms are able to transfer or accept electrons (Escalera et al., 2021). This technology uses electrokinetic processes such as electromigration, electroosmosis, electrophoresis, and electrochemical oxidation to remediate soil. These processes aid in the destruction of microorganisms since they alter their physical makeup (Wang et al., 2023). By exposing the microbial community to direct current, it aids in the breakdown of polycyclic aromatic hydrocarbons. The voltage of current is 2V per centimetres (Li et al., 2020). This approach is multi-step, involving constant electric field, biostimulation, and bioaugmentation. Increased activity of soil-dwelling microorganisms is referred to as biostimulation. The addition of microorganisms to the soil is known as bioaugmentation, and a constant electric field of 1 V per cent is applied (Barba et al., 2021). This process causes the saline soil that is contaminated with petroleum hydrocarbons to become more ion-rich and capable of retaining more water. As time passes, the amount of soil ions surrounding the electrode rises (Meng et al., 2020).

limitations of bioremediation

While enabling toxins to biodegrade through bioremediation is a promising, cost-effective, and ecologically friendly approach, there are a number of challenges associated with it. Bioremediation may take a long time for complex contaminants with several links (Wang et al., 2021). The environment's pH, temperature, moisture content, and nutrient availability all affect efficiency of phytoremediation (Hajabbasi et al., 2016). Certain contaminants are resistant to biodegradation because they are complex or have persistent chemical bonds. Developing adaptable ways to deal with a variety of pollutants is crucial (Intisar et al., 2023). Different contaminants need different microbial species, or bacteria that have undergone genetic modification, to break down. Finding the ideal combination might be challenging, especially in a new setting (Sharma et al., 2020). Products that break down might still be hazardous after bioremediation. Ensuring the safety of these byproducts is crucial (Duodu et al., 2022).

Conclusion

Removal of hazardous contaminants from soil through modern sophisticated techniques requires more time and money while emerging eco-friendly techniques like genetic engineering, nanotechnology, and electro bioremediation are making the process of

bioremediation less time or capital consuming and more successful. Environmental pollution is getting worse day by day as a result of rapid urbanization and industry growth. In order to eliminate these harmful effluents from soil or water bodies, bioremediation is an environment friendly process that boosts soil fertility and microbial activity with enhancing economic production. Bioremediation is becoming more popular than traditional remediation techniques because of environmental awareness and higher efficiency. Modern bioremediation techniques require huge capital and skilled workers, scientists are trying to developing cheaper tools and methods that can eliminate contaminants from soil to improve soil health in a sustainable way.

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