



IJRASET

International Journal For Research in
Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 11 Issue: VIII Month of publication: Aug 2023

DOI: <https://doi.org/10.22214/ijraset.2023.54636>

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Bioremediation: A Sustainable Green Biotechnology- A Review

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Abstract: *The industry, healthcare, agricultural, and municipal sectors all contribute to environmental pollution today by dumping harmful chemicals and wastes into the water, soil, and air. The risk of heavy metal accumulation in living systems primarily affects people, animals, plants, and microorganisms. The quality of the air, water, and soil are all being negatively impacted by these wastes. "Bioremediation" uses microorganisms to quickly and economically decompose harmful contaminants, making it an environmentally friendly and cost-effective technique of cleanup. Toxic compounds are converted into less dangerous versions. Due to its cost-effectiveness and environmentally friendly methods, bioremediation has grown in popularity. Bioremediation is one mitigation technique to combat this pollution. The word "bioremediation" refers to a broad range of biologically mediated processes used to alter, degrade, sequester, and/or completely remove undesirable compounds from an environment. Bioremediation may be mediated by organisms from all spheres of life, but fungi stand out as particularly interesting options. Fungi are nature's most effective degraders of resistant organic matter because they have the metabolic capacity to break down complicated compounds. The current Literature emphasises bioremediation as a powerful method for removing contaminants by growing mushrooms to aid in biosorption. The focus of this review is also on the many bioremediation techniques, such as phytoremediation, mycoremediation, and biosorption, for cleaning up environmental pollutants.*

Keywords: *Pollutants; Bioremediation; Environment*

I. INTRODUCTION

An approach to waste management called bioremediation uses living things to remove or neutralise contaminants from contaminated sites. Using naturally occurring organisms to transform toxic compounds into less toxic or non-toxic ones is a process known as bioremediation, according to the EPA. In general, technologies can be divided into ex situ and in situ categories. Ex situ bioremediation entails removing the contaminated material from the site to be treated elsewhere, whereas in situ bioremediation treats the polluted material on the spot. Recent developments that increase the capacity of the resident microbe population to degrade contaminants by introducing matched microbe strains to the medium have also been successful.

Bio-remediators are microorganisms that are utilised in the process of bioremediation. However, not all contaminants can be effectively removed using microorganisms in bioremediation. Heavy metals like cadmium and lead, for instance, are not easily absorbed or trapped by microorganisms. Mercury incorporation into the food chain could become worse matter. In these situations, phytoremediation is beneficial because natural plants or transgenic. These poisons can bioaccumulate in plants' above-ground components, which are then collected for removal. The heavy metals in the gathered biomass could be recycled for industrial use or even further concentrated by incineration.

Natural attenuation, also known as intrinsic bioremediation, can take place on its own in the environment, but it can also be sped up by adding fertilisers to the mix to increase the bioavailability of the medium (a process known as "bio-stimulation"). Examples of bioremediation techniques include bioventing, bioleaching, bioreactor, bioaugmentation, composting, bio stimulation, land farming, phytoremediation, and rhizo filtration. Bioremediation technology can be categorised as in-situ and ex-situ depending on how wastes are removed and moved. Ex-situ bioremediation is completely removing contaminated material from one site and moving it to another site where it has been treated using biological agents.

In-situ bioremediation involves treating contaminated material at the same site. It was discovered through the comparison of the two approaches that the in-situ and ex-situ approaches have different rates of biodegradation and consistency of process output. Ex situ bioremediation is more expensive than in-situ because it requires the excavation of contaminated samples for treatment. Although both in-situ and ex-situ bioremediation techniques rely heavily on microbial metabolism, ex-situ techniques are currently preferred for ecological restoration of contaminated soil, water, and environment.

A. Significance of this Study

The significance of current study is to study different types of Bioremediation processes and to study potential of these bioremediation processes in remediating different type of contamination in waste water and soil. This study will also focus on the different type of Bio- microorganisms that are used in for remediating environment.

B. Novelty of this Study

Bioremediation is an environment friendly process and helps to create an environment for sustainable development. The mycoremediation technology based on mushroom cultivation for the remediation of waste water and we can commercialize the mushroom as a product for its different use. Different plant species and micro-organisms have been listed out which are used for removing different type of contaminants from water and soil.

II. LITERATURE REVIEW

Synthetic organic compound pollution of the environment has grown to be a serious issue on a global scale. Given that they are substances that do not normally present in the biosphere and that many of them are difficult for local flora and fauna to digest, many of these novel compounds that have been introduced to nature are referred to as xenobiotics. (Sullia, 2004).

Therefore, in order to encourage the sustainable growth of our society with minimal negative effects on the environment, the removal of numerous pollutants and wastes from the environment is a must. Given the severity of the issue and the lack of a workable solution, an expedient, affordable, and environmentally responsible method of cleanup is urgently required. (Hamman, 2004).

According to Atlas and Bartha (1992), To break down hydro-carbon and organic toxins, bioremediation is the on-site stimulation of living soil organisms like fungi, bacteria, and green plants. It entails adding organisms and nutrients to contaminated soil in order to speed up biodegradation. The microorganisms that are employed in bioremediation must have undergone testing and demonstrated their viability in laboratory tests. To combat certain soil contaminants, such as the bacterial breakdown of chlorinated hydrocarbons, bioremediation may be used. The use of nitrate and phosphate fertilisers to oil spill cleanup efforts is merely a general strategy to encourage native or exogenous microbes to break down crude oil.

Enhancing natural biodegradation by living organisms is the general strategy for bioremediation (intrinsic bioaugmentation). Bioremediation, in contrast to conventional approaches, can be done both in- and ex-situ. Ex-situ bioremediation entails removing the contaminated material to be treated elsewhere, whereas in-situ bioremediation treats polluted materials at the site. (<http://en.wikipedia.org/wiki/bioremediation>).

Alexander (1994) claimed that there is interest in using fungi in bioremediation due to their capacity to convert a wide range of dangerous compounds. With their potent enzyme secretions, fungi are some of nature's most effective decomposers. Due to their rapid growth, high biomass output, and extended hyphae reach in the environment, fungi have a considerable potential for bioremediation. (Ashoka et al., 2002).

Oyster mushrooms, which belong to the *Pleurotus* genus, are the primary fundamental decomposers of plant and wood waste. They thrive primarily on decaying wood, although they can parasitize living trees and spread a white mould infection. (Warszawa 2006).

White rot, or oyster mushrooms, has the ability to bioremediate by removing heavy metals, polycyclic aromatic hydrocarbons, or oil-derived contaminants from contaminated soil. (Pointing SB).

In addition to tropical and subtropical forests, oyster mushrooms can be found naturally in practically all latitudes, with the exception of Antarctica. Mycologists have identified numerous species and types as a result of various growing conditions. (Gapiński M, 2001).

This kind of mushroom is beneficial to grow and eat. Their fruiting bodies, which are characterised by low fat concentration, are sources of easily absorbed proteins, carbohydrates, amino acids, B vitamins (thiamine, riboflavin, and niacin), vitamin D, and mineral salts (calcium, phosphorus, iron). (Bonatti M et.al, 2004)

III. TYPES OF BIOREMEDIATION

A. Phyto Remediation

It has been suggested that phytoremediation, the use of plants to remove or degrade contaminants from soils and surface waters, is a less expensive, sustainable, efficient, and eco-friendly option than traditional remediation technologies. To absorb chemicals from the soil and deposit them in the above-ground portion of their bodies or to change them into a less poisonous form, plants require sun energy (via photosynthesis). The pollutants can subsequently be removed by harvesting and treating these plants. High levels of contamination tolerance, the capacity to concentrate or degrade contaminants at high levels in biomass, broad root systems, the ability to absorb significant amounts of water from the soil, rapid growth rates, and high levels of biomass are all characteristics of an ideal phytoremediator.

Even though some species can thrive in contaminated areas, they usually grow very slowly, produce very little biomass, and are adapted to very particular environmental conditions. Furthermore, trees do not accumulate pollutants despite having deep roots, a high biomass, and low agricultural input requirements. Therefore, conventional plants fall short of the standards for effective phytoremediators.

An good illustration of the method of plant-facilitated bioremediation and its function in reducing environmental stress is phytoremediation of heavy metals from the environment. A species that produces a lot of biomass, develops quickly, has a deep root system, and can be easily farmed and harvested is the best kind of phytoremediator (Clemens et al., 2002). The only issue with this standard is that natural phytoremediators frequently fall short of it. *Thalasspirotundifolium* and *Brassica juncea* are two plant species that hyperaccumulate lead, one of the non-essential chemicals

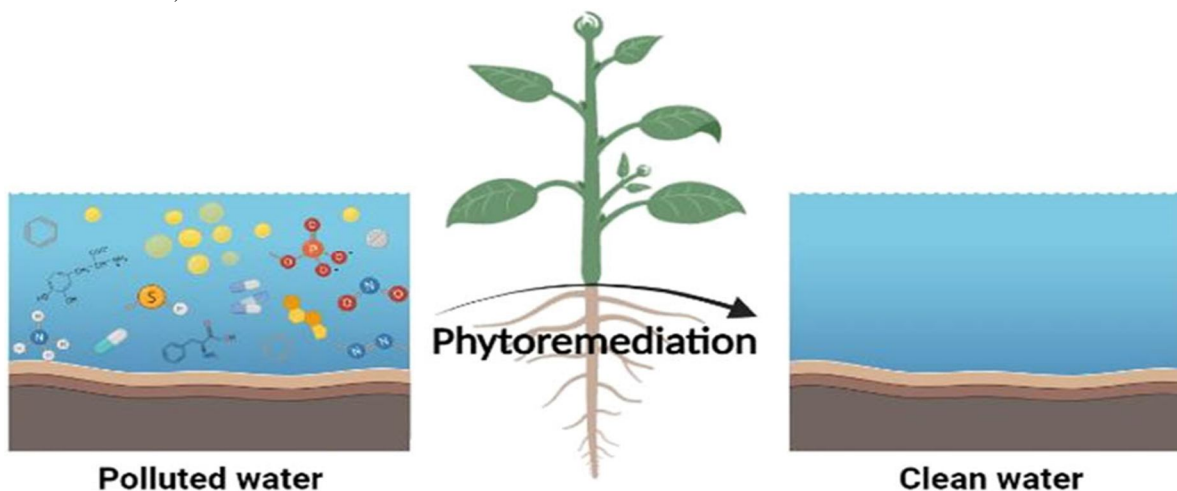


Figure 1: Pollutant phytoremediation is depicted.

Both apoplastic and symplastic pathways are used in the complex process of heavy metal entrance into the plant system. (i) Active metal transport across root membranes (ii) Metal entry into symplast during translocation from root to shoot (iii) and chelation and sequestration of metals into particular compartments in the leaves (Maestri et al. 2010) are the three main steps in inorganic ion transport in the symplastic pathways.

Metal chelation inside the plant enables xylem loading and transport in addition to sequestration. These mechanisms involve numerous metal-specific chelators, also known as ligands and organic acids, many of which are still under investigation or have not yet been fully understood. Chelators' function in hyper-accumulation is to combine with heavy ions to form complexes. This can either help with transport or act as the ion's termination point, causing sequestration in the plant's shoot. Two kinds of chelators implicated in metal buildup include metallothioneins and phyto chelatins.

It has been discovered that various organic acids and ligands are connected with various metals in different regions of various plants. For instance, in *Thalasspi caerulescens*, the majority of Zn was coupled with histidine in the roots and with organic acids in the shoots (Verbruggen et al. 2009). Additionally, Cd was discovered to be bound to sulphur ligands in the leaves of *Thalasspi caerulescens* (Verbruggen et al. 2009). Zn was mostly stored in the mesophyll vacuoles of *Arabidopsis halleri*, whereas *Thalassia caerulescens* stored it in the vacuoles of the epidermal cells (Verbruggen et al. 2009). The main detoxification mechanism in both cases was believed to be vacuolar sequestration of Zn in the leaves, and this is a characteristic that sets hyperaccumulators apart from non-hyperaccumulators.

Phytoextraction has a number of benefits. When compared to traditional procedures, phytoextraction is quite inexpensive. The contamination is completely eliminated from the soil, which is another advantage. In some situations, the contaminant can be recycled from the biomass of the contaminated plant, and the amount of waste that must be disposed of is also significantly reduced (up to 95%). Because of their slow growth, shallow root systems, and low biomass production, hyperaccumulator species are not widely used. The plant biomass must also be gathered and appropriately disposed of. The amount of metal phytoextraction is constrained by a number of reasons, including:

Rhizosphere bioavailability of metals

Roots' rate of metal absorption percentage of metal that is "fixed" inside the roots

The speed at which xylem loads and moves to shoots cellular resistance to harmful metals


The plant's tolerance to the pollutant has an impact on the success of remediation, and pollutants gathered in senescing tissues may be more difficult to remove. These limitations are some of those related with phytoremediation. released back into the environment during specific seasons; the time required to clean up a site takes far longer than it would with other methods; and the solubility of the contaminants may increase, causing more environmental harm and the likelihood of leaching.

B. Mycoremediation

Mycoremediation is another name for remediation utilising fungus. Due to their potential to breakdown a wide range of environmentally persistent contaminants and turn industrial and agro-industrial wastes into products, mushrooms and their enzymes are used in mycoremediation.



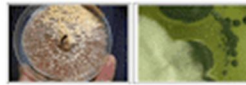





Scientists from the U.S. Geological Survey applied this hypothesis in 1992. Pleurotus ostreatus and Ganoderma lucidum were utilised in mycoremediation to clean up the San Francisco Bay oil disaster in 2007.

People have consumed mushrooms for millennia because of their robust flavour and high protein content, but mushrooms are so much more than simply a vegetable. According to Ali and Di (2017), fungi have a special affinity for absorbing or binding heavy metals, including radiation, as well as chemical contaminants such as oil and pesticides. Even water can be filtered by fungi, enabling an endless number of ecosystem-regenerating life cycles.



MYCOREMEDIATION

BIOREMEDIATING ENVIRONMENTAL TOXINS USING FUNGI

BIOLOGICAL	CHEMICAL	INDUSTRIAL
<p>Many fungi prey and feed on bacteria that are pathogenic to humans.</p>  <p>E.Coli and Salmonella bacteria are destroyed by mushrooms using natural biofilters placed near livestock farms and shoreline plantings where runoff occurs.</p>  <p>There are as much as 8 miles of mycelium (mushroom filaments) in 1 cubic inch of soil! These make a perfect filter for trapping contaminants from site runoff. Strains of endangered polypore mushrooms are fighting pox and avian flu viruses.</p> 	<p>Natural microbial communities participate with the fungi to break down contaminants, eventually into carbon dioxide and water.</p>  <p>Wood-degrading fungi are particularly effective in breaking down aromatic pollutants (toxic components of petroleum) herbicides, pesticides.</p>  <p>Mushrooms can be "trained" to break down TNT, PCBs, dioxins, and other dangerous toxins.</p> 	<p>Living filters can capture hazardous site runoff and chemicals from Papermills, Dye manufacturers, and Power Plants.</p>  <p>Contaminated "Brown Fields" and Quarantined mill sites can be bioremediated to break down complex, carcinogenic compounds before they leach into the groundwater.</p> 

Mycelium, or the vegetative portion of a fungus, is used in the mycoremediation technique to treat contaminated soil locations. A mushroom's enzymes are effective at degrading a wide variety of pollutants. This strategy is fundamentally about using the natural decomposition skills of fungi to rejuvenate and restore land.

A critical and ongoing problem is the buildup of heavy metals and hazardous substances in our environment. Heavy metals, PCBs, and dioxins are just a few examples of the toxins that enter our food chain and bioaccumulate, which is defined as the "gradual accumulation of a certain chemical into the living tissue of an organism from its environment [and] may result from direct absorption from the environment or from ingestion of food particles" (Institute, 2020). Before the toxins in the soil may reach our food supply and ultimately us, fungus mycelia can eliminate them.

The enzymatic machinery for the degradation of waste/pollutants is present in mushrooms and other fungi, and it can be used for a wide range of pollutants (Purnomo et al. 2013; Kulshreshtha et al. 2013). However, mushrooms, a basidiomycetous fungus, are growing in popularity as a bioremediation method because they also produce mycelium or fruiting bodies, which are a source of protein. While most pollutants are broken down by fungi into non-toxic byproducts, heavy metals are simply accumulated dynamically by fungi. Oyster Additionally, mushrooms are effective cadmium and mercury absorbers.

Their mycelium transports mercury from the earth to the mushroom. Enzymes from the fungal mycelia can separate specific atoms from bigger compounds, such as chloride (Cl⁻), and subsequently dissolve the hydrogen-carbon link. These substances can be further broken down by bacteria into end products such carbon dioxide (CO₂), water, and possibly methane (CH₄). As shown in Table 1, fungi have also shown promise in the removal of heavy metals including lead (Pb) and cadmium (Cd). Fungi may take these metals from soil or water and store them in their tissues since they are already in their most basic form and cannot be further broken down. The numerous flies and other insects that were drawn to the mushroom fruit bodies transformed the once-polluted soil into a habitat that supported their own existence

Table 1 lists the types of plants and fungi utilised in the bioremediation of heavy metals.

Heavy metal	Plant species	Fungus
Cd	Trifolium repens Hordeum vulgare	Glomus mosseae
Ni	BERkheya coddii	Gigaspora sp. Glomus tenue
Zn	Viola calaminaria	Glomus sp.
Pb	Zea mays	Glomus intraradices
Cd, Cu	Zea mays	Glomus caledonium
Zn, Pb	Lygeum spartum	Glomus mosseae, Glomus macrocarpum
Cd, Zn	Glycine max	Glomus mosseae
Zn, Cd, Cu, Ni, Pb	Sorghum bicolor	Glomus caledonium

According to recent reports, certain types of mushrooms can break down polymers, including plastics (da Luz et al. 2013; Table 2). The biodegradation process is extremely intricate. The cause is the interaction of ligninolytic enzymes with the cytochrome P450 monooxygenase system, hydroxyl radicals, and the amount of H₂O₂ produced by the mushroom, as well as the influence of other biochemical systems.

Table 2: How mushrooms help pollutants degrade

S.no.	Mushroom spp.	Waste/Pollutants	Remarks	Refrences
1.	Pleurotus ostreatus	Oxo-Biodegradable	Mushrooms degraded the plastic and grew on it.	da Luz et al. (2013)
2.	Lentinula edodes	2,4-dichlorophenol	Mushrooms degraded 2,4dichlorophenol (DCP) by using vanillin as an activator.	Tsujiyama et al (2013)
3.	Pleurotus pulmonarius	Radioactive cellulosic-based Waste	Waste containing mushroom mycellium was solidified with Portland cement and then this solidified waste act as barrier against the release of radiocontaminants	Eskander et al. (2012)
4.	Jelly sp,Schizophyllum commune and polyporous sp.	Malachite green	99.75% (jelly sp.), 97.5% (Schizophyllum commune),68.5% (Polyporous sp.2) dye was degraded in 10 days	Rajput et al. (2011)
5.	Pleurotus Pulmonarius	crude oil	Crude oil was degraded	Olusola and Anslem (2010)
6.	Coriolus versicolor MKACC 52492	PAH	Mushroom possesses ability to degrade Poly-R 478 which decides its suitability to degrade PAH.	Jang et al. (2009)

C. Biosorption

Biosorption is the process by which microorganisms remove metals and other contaminants from the environment. Industrial wastewater cleanup and the recovery of metals from effluent are both alternatives that are being explored. Live or dried biomass that frequently displays a marked sorption of metallic ions, contaminants, and xenobiotics from wastewater is used in the biosorption process. tolerance for negative effects and metals. A definition of biosorption is the capacity of metabolically mediated biological materials to remove heavy metals from wastewater or physical-chemical uptake mechanisms. Since Arden and Lockett found that certain living bacteria cultures could remove nitrogen and phosphorus from raw sewage when it was combined in an aeration tank in the early 1900s, the idea of employing biomass as a tool for environmental remediation has been around. This discovery led to the development of the activated sludge process, a wastewater treatment method still in use today that is based on the idea of bioaccumulation. The discovery of the sequestering property of dead biomass in the late 1970s led to a change in focus in study from bioaccumulation to biosorption.

Table 3 lists the organisms that are employed to biosorb heavy metals.

Microorganism	References	Elements	References
Bacillus spp.		Cu, Zn	Philip et al., 2000; Gunasekaran et al., 2003
Pseudomonas aeruginosa		U, Cu, Ni	Sar et al., 1999; Sar and D'Souza, 2001
Zooglea spp.		Co, Ni, Cd	Gunasekaran et al., 2003; Yan and Viraraghavan, 2001;
Citrobacter spp.		Cd, U, Pb	Gunasekaran et al., 2003; Pearson, 1969;
Chlorella vulgaris		Au, Cu, Ni, U, Pb, Hg, Zn	Gunasekaran et al.,2003
Aspergillus niger		Cd, Zn Zn, Ag, Th, U	Guibal et al., 1995;
Pleurotus ostreatus		Cd, Cu, Zn, Ag, Hg, P	Gunasekaran et al., 2003

Kulshreshtha et al., (2010)

Important Elements in Microbial Bioremediation

Factor	Desired conditions
microbial population	suitable kinds of organisms that can biodegrade all of the contaminants
oxygen	enough to support aerobic biodegradation (about 2% oxygen in the gas phase or 0.4 mg/liter in the soil water)
water	soil moisture should be from 50–70% of the water holding capacity of the soil
nutrients	nitrogen, phosphorus, sulfur, and other nutrients to support good microbial growth
temperature	appropriate temperatures for microbial growth (0–40 °c)
ph	best range is from 6.5 to 7.5

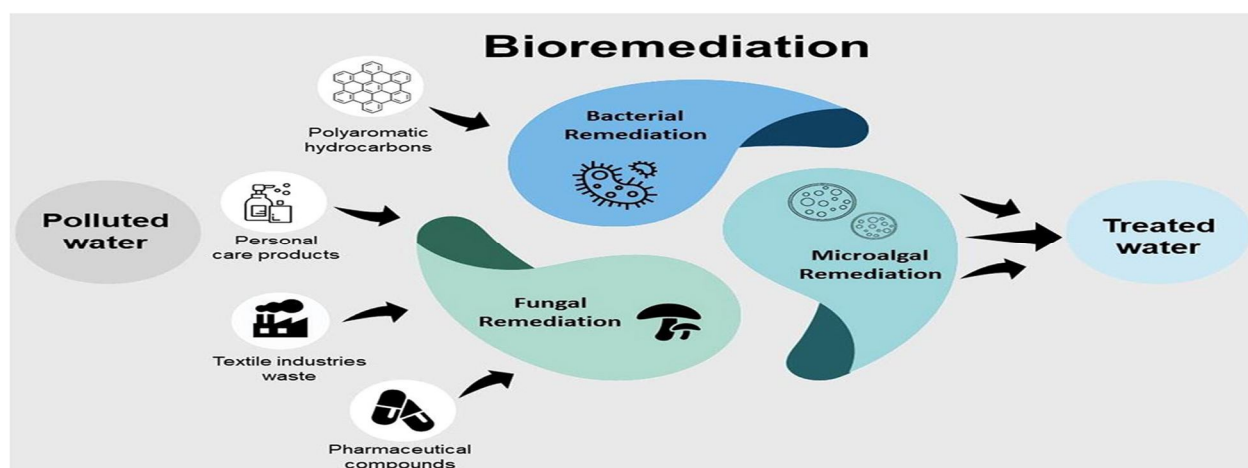


Fig. 2 illustrates the bioremediation process.

Contaminants are broken down by microbes since doing so gives them energy to develop and reproduce. By rupturing chemical bonds and transferring electrons from the contaminants to an electron acceptor, such as oxygen, microbes obtain energy from the pollutants. To create additional cells, they "invest" the energy along with some electrons and contaminant-derived carbon. Metals can precipitate as a result of reduced or oxidised species produced by microorganisms. For instance, when Fe^{2+} is oxidised to Fe^{3+} , ferric hydroxide ($Fe(OH)_3(s)$) precipitates reduction of SO_4^{2-} to sulfide (S^{2-}), which precipitates with Fe^{2+} as pyrite ($FeS(s)$) or with mercury (Hg^{2+}) as mercuric sulfide ($HgS(s)$); reduction of hexavalent chromium (Cr^{6+}) to trivalent chromium (Cr^{3+}), which can precipitate as chromium oxides, sulfides, or phosphates; and, as mentioned previously, reduction of soluble uranium to insoluble U^{4+} , which precipitates as uraninite (UO_2). Microorganisms can biodegrade organic compounds that bind with metals and keep the metals in solution. Unbound metals often precipitate and are immobilized.

IV. POSITIVE ASPECTS OF BIOREMEDIATION

Since bioremediation is a natural process, it is seen as a suitable method of waste treatment for contaminated materials like soil. When the contamination is present, the number of microbes that can breakdown it rises; when the contaminant is eliminated, the population of biodegradative microbes decreases. The treatment's residues, which comprise carbon dioxide, water, and cell biomass, are often safe byproducts.

- 1) Using bioremediation, a variety of contaminants that are deemed hazardous and can be converted into harmless products can be completely destroyed. By doing this, any potential future liability for the handling and disposal of tainted material is eliminated.
- 2) It is feasible to completely destroy target pollutants rather than moving them from one environmental medium to another, such as from land to water or air.
- 3) On-site bioremediation can frequently be done without significantly interfering with daily operations. Additionally, this avoids the need to move large amounts of garbage off-site and any associated risks to public health and the environment.
- 4) Compared to other approaches, bioremediation can show to be more affordable for the cleanup of hazardous waste.

V. CONCLUSION

When it comes to remediating, cleaning, maintaining, and recovering methods for resolving a polluted environment through microbial activity, biodegradation is a very profitable and alluring alternative. The rivalry between biological agents like fungi, bacteria, and algae as well as unfavourable external abiotic factors (aeration, moisture, pH, and temperature) and limited bioavailability dictate how quickly undesired waste materials degrade. The effectiveness of bioremediation depends on a number of variables, including but not limited to cost, site features, and the kind and quantity of pollutants. Site description is the first step in a successful bioremediation since it aids in the creation of the most effective and promising bioremediation technique (ex-situ or in-situ). Due to the excavation and removal from the archaeological site, ex-situ bioremediation methods are typically more expensive. They can, however, be utilised to cure a variety of contaminants. Contrarily, in-situ techniques do not incur additional costs for excavation; yet, some inefficient in-situ bioremediation approaches can be reduced by the on-site installation cost of equipment, attached with successfully, and controlling the subsurface of a polluted site. When choosing the most effective bioremediation method to successfully treat polluted sites, geological properties of the polluted site, including soil, pollutant kind and depth, human habitation site, and performance of each bioremediation approach, should be taken into consideration.

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