**Recent Developments in Bioremediation Strategy via Crops and Microbes for Decontaminating Hazardous Metal-Polluted Soils**

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**APSTRACT**

Soil contamination with heavy metals (HMs) is a serious concern for the developing world due to its non-biodegradability and significant potential to damage the ecosystem and associated services. Rapid industrialization and activities such as mining, manufacturing, and construction are generating a huge quantity of toxic waste which causes environmental hazards. Microorganisms and plants employ different mechanisms for the bioremediation of polluted soils. Using plants for the treatment of polluted soils is a more common approach in the bioremediation of heavy metal polluted soils. Combining both microorganisms and plants is an approach to bioremediation that ensures a more efficient clean-up of heavy metal polluted soils. However, success of this approach largely depends on the species of organisms involved in the process. This review covers some new aspects and dimensions of bioremediation of heavy metal polluted soils. This review highlights better understanding of the problems associated with the toxicity of heavy metals to the contaminated ecosystems and their viable, sustainable and eco-friendly bioremediation technologies, especially the mechanisms of phytoremediation of heavy metals along with some case studies in India and abroad. Further, factors affecting the bioremediation efficiency are discussed.

**Keywords**;

Heavy metals, Environmental hazards, Bioremediation, Eco-friendly, Phytoremediation.

**INTRODUCTION**

To promote the soil growth in the environment, such various techniques used to remove the contamination or the pollutant in the soil. incase bioremediation is one of the techniques thus by using microbes, plants the toxic be removed and reduce the contamination. The forest and more vegetation get destroyed and that get rebuild by the farmers with crops and the plantation of edible plants thus various pesticide be used to grow the plant, land turn into more toxic, in such cases soil bioremediation is used to keep the soil in usable or healthier condition [1]. Likewise, the impact of heavy metals is high in the soil, the soil has the natural sources of heavy metals, the anthropogenic activities in it get increase the activation of heavy metals leads to soil contamination. The industrial waste or the heavy metal that release from the industry can cause damage to the land, in this cause the process of soil bioremediation plays the major role to keep the eco-friendly environment by removing the contamination by using microbes [2,3,4]. The advanced biotechnological process has result in the arrangement of different element of science and role in the society [5]. Thus the cell be manipulated to develop the alternative by the technological process by the chemistry of microorganism to maintain the natural surroundings the production of traditional product is the effective and innovative method. There are more researches that facilitates the beneficial term of food, growth among the beings. Thus human activities exploit the natural resources in various ways by the chemical fertilizers and the waste release from the industries [6].

Thus the ecosystem get polluted make a more impact on the plant, living organism as well. The contaminated resources get treated in various biological system, in this the more effective technology of phytoremediation be used to decreased the pollutants by plants. As we know the bioremediation is the process that employs the removal of the toxin substances in the soil by the use of plant, fungi, microorganism to provide a healthy environment thus bring the original state from the contamination [7,8].

The recent advancement in the microbe pesticide interaction the new strain be discovered that help in the degradation of the wide range of pesticide in the toxic substances get breakdown into less harmful by the process to reduce the toxicity of soil and to prevent the toxic accumulation in the soil. The key advancement in the field is the low risk of contaminated soil, land, water and the development of the pesticide from the natural resources like microorganism, plant etc. Pesticide and bio pesticide are just opposite thus the bio pesticide are less harmful the combination of microbe-pesticide and the bio pesticide interaction is the most effective pest controlling method and it is very useful to promote the growth of the soil. Microorganism that used in this enhanced the growth and the nutrient uptake of the resources, this has the efficiency of the more production of the crops, whereas there is no need of the toxic chemical to be dumped in the environment [9-11].

In-situ and ex-situ is the process that the branches of bioremediation. In-situ is the bioremediation process that performed in the place of origin based on the procedure, the contaminated soil is used to clean with the procedure of in-situ to avoid the maximal damage to the soil structure. As this technology is an non-invasive it permanently eliminate the toxins. This process involve in the technologies of physical, chemical methods. Ex-situ implies that the procedure done by the transportation of the other site. Solid and slurry phase system are the two systems involve in the ex-situ bioremediation. Solid phase system involving in the treatment of land and the soil piles. Slurry phase system acts on the treatment of solid-liquid suspension in the bioreactors. Aging, nutrients, mixing, pH, moisture content, mmicrobia population, temperature, reactor operation are the most important factors affecting biodegradation in the slurry phase [12].

By this bioremediation process the interaction between the microbes, we can solve the most modern agricultural problems such as soil degradation, pesticide resistance etc. By enhancing this methods we can develop the healthy environment and ensure the sustainable environment.

**Bioremediation:**

Bioremediation is the use of organisms (microorganisms and/or plants) to clean contaminated soil. It is a commonly accepted approach of soil rehabilitation since it is thought to occur naturally. It is also a low-cost soil treatment strategy. When compared to a traditional procedure (excavation and landfill), Blaylock et al. [13] noted a 50% to 65% savings when using bioremediation for the treatment of 1 acre of Pb poisoned soil. Although bioremediation is a non-disruptive approach of soil remediation, it is generally time consuming, and its usage for the treatment of heavy metal polluted soils can be affected by the climatic and geological nature of the site to be remediated [14]. Heavy metals cannot be eliminated during bioremediation, but can only be converted from one organic complex or oxidation state to another. Heavy metals can be transformed to become less toxic, easily volatilized, more water soluble (and so easily removed from the environment) or less bioavailable due to modifying their oxidation state [15].

According to one research, cleaning metal-polluted sediments and soils by landfilling and chemical treatment costs around 100-500 USD/ton, and vitrification costs about 90-870 USD/ton, while bioremediation costs about 15-200 USD/ton and phytoremediation costs about 5-40 USD/ton [16]. It is estimated that bioremediation can save 50-65% of the cost of clearing an acre of Pb-contaminated soil when compared to standard excavation and disposal [17,18].

Furthermore, bioremediation is a non-invasive technology that may eliminate toxins permanently while leaving the ecosystem intact and can be used with chemical and physical treatments [19]. The bioremediation procedures rely exclusively on natural biological potency. The majority of bioremediation procedures are dependent on numerous criteria such as soil structure, pH of contaminated sites, moisture content, type of pollutants, nutrient supplement, microbial diversity, treatment site temperature, and oxygen availability [19-22]. Bioremediation techniques are classified as 'in-situ' and 'ex-situ' [23].

**On-site bioremediation**

Additionally, bioremediation is a non-invasive technology that may permanently eliminate toxins while leaving the ecosystem unharmed and can be used with chemical and physical therapies [19]. The bioremediation procedures rely exclusively on biological potency found in nature. The majority of bioremediation procedures are influenced by factors such as soil structure, contaminated site pH, moisture content, pollutant type, nutritional supplement, microbial diversity, treatment site temperature, and oxygen availability [19-22]. Bioremediation is classified as 'in-situ' or 'ex-situ' [23]. The main benefits of in situ bioremediation are its low cost, lack of excavation, minimum site disruption, low dust production, and the future possibility of treating soil and groundwater simultaneously. However, the main disadvantages are the time required, seasonal variations in microbial activity, and the challenging application of treatment chemicals in the natural environment. [27].

**Ex-situ bioremediation**

Ex-situ bioremediation procedures, on the other hand, necessitate the extraction of contaminated soil and water from its original place for treatment. This is divided into two categories: solid-phase systems and slurry phase systems. Contaminated waste, such as industrial trash, home garbage, municipal solid waste, and sewage sludge, is combined with organic waste, such as manure, leaves, and agricultural waste, in solid-phase bioremediation. Composting, soil biopiles, hydroponics, and land farming are part of the treatment process, which creates favorable circumstances for indigenous anaerobic and aerobic microorganisms to aid in the reclamation process [26,27]. A biopile is a short-term bioremediation device in which excavated soils are blended with soil additives, shaped into compost heaps or aboveground cells, and enclosed for treatment with an aeration system [28]. Slurry phase bioremediation, a faster process, combines contaminated soil with water and other additives in a large tank known as a bioreactor and is mixed to keep the microorganisms in contact with the contaminants in the soil, creating the optimal environment for the microorganisms to degrade the contaminants. Effective ex situ bioremediation can be done by assuring adequate sampling practice and maintaining controlled conditions with gained core samples. Land farming is a simple technique in which contaminated soil is excavated and spread over a prepared bed, then tilled on a regular basis until pollutants are degraded by stimulating indigenous biodegradative microorganisms; the practice is limited to the treatment of the highest 10-35 cm of soil. The presence of these organic components encourages the growth of a diverse microbial community [29]. This technology is used in conjunction with other remedial strategies to achieve successful bioremediation using hydroponics. A wastewater treatment facility employing traditional biological treatment mixed with hydroponics and microalgae was built in a greenhouse near Stockholm, Sweden [30].

In general, the frequency and quantity of biodegradation are greater in a bioreactor system than in situ because the enclosed environment is more manageable and hence more regulated and predictable. The main disadvantage of this approach is that the pollutant may be removed from the soil via soil washing or physical extraction before being put in a bioreactor. Other bioremediation strategies are further discussed below;

**Bioventing**

It is the most popular in situ treatment and includes delivering air and nutrients through wells to a polluted soil in order to promote the indigenous aerobic bacteria. It is an example of subsurface bioremediation. It uses low air flow rates and delivers only the quantity of oxygen required for biodegradation while minimizing volatilization and the release of pollutants into the atmosphere. Pollutants are often biodegraded under aerobic events by indigenous heterotrophic bacteria found naturally in the soil or subsurface soil [31]. Subsurface bioremediation cleans shallow aquifers through geochemical processes (including the redox potential and dynamics of heavy metal adsorption), which eventually cleans soils of heavy metals and delivers safe groundwater for drinking and irrigation [32].

**Biosparging**

It means putting compressed air underground the water table in order to raise groundwater oxygen concentrations and speed up the biological breakdown of pollutants by naturally present microorganisms [33]. It improves mixing in the saturated zone, increasing soil-groundwater interaction. Biosparging can be used to lower the concentration of petroleum elements in groundwater, soil below the water table, and within the capillary fringe. It is extremely efficient in reducing petroleum products at underground storage tank sites [31]. How ever, almost similar phenomena in this method are involved in the remediation of soils from heavy metals as in the case of bioventing.

During 1997-2001[34], a biosparging system was built and implemented to a co-contaminated arsenic-hydrocarbon aquifer at oilfield services sites in Odessa, Texas, USA. The cleanup of large-scale petroleum pollution of soil and groundwater has produced useful information concerning biosparging effectiveness in sandstone sedimentary bedrock [35]. Temperature is also an essential aspect since bacterial growth rate is temperature dependent. The optimum pH for bacterial growth is approximately 7, and the acceptable range for biosparging is between 6 and 8. For biosparging, plate count reports are usually given in terms of colony-forming units (CFUs) per gramme of soil.

Biosparging was used as an innovative cleanup solution at the old Soviet Army air station in the Czech Republic for ten years (1997-2008). An increase in average groundwater temperature was detected in the cleaning areas, most likely as a result of biological activity during the cleanup process. The significant increase in biodegradation rates observed after air sparging intensification, as well as the strong linear correlation between air injection rates and biodegradation activities, have demonstrated that the air injection rate is the most important factor in biodegradation efficiency in heavily contaminated areas [35].

**Bioaugmentation**

Bioaugmentation is the addition of pre-grown microbial cultures, either indigenous or foreign, to polluted locations in order to improve the breakdown of undesired compounds [36]. Exogenous cultures seldom compete well enough with indigenous populations to generate and sustain viable population levels, and most soils with long-term exposure to biodegradable garbage have indigenous microbes that are efficiently degraded provided the land treatment unit is adequately managed [37].

Bioaugmentation, like other bioremediation techniques, may not be able to stand on its own. The combined bioaugmentation and biostimulation with degrading bacteria, biosurfactants, organic carbon source with kitchen waste or compost, and nutrient enhancement (like NH4NO3, K2HPO4) at maintaining around 15-25% moisture content and 30 2 C temperature produced better results [38].

**Mechanism of bioremediation**

Bioremediation works by lowering, detoxifying, decomposing, mineralizing, or changing more hazardous metals into less hazardous metals. Cleaning methods are used to eliminate toxic waste from a contaminated environment. Through the all-encompassing activity of bacteria, bioremediation is widely assumed in the degradation, eradication, and immobilisation of several chemical wastes and physically hazardous chemicals from the surrounding environment. Both in-situ and ex-situ remediation strategies rely on the biotransformation/biodegradation concept, which involves the removal, mobilisation, immobilisation, or decontamination of different contaminants from the environment by the activity of microorganisms (bacteria, fungus, and yeast) and plants [39]. During biotransformation, microbes utilise chemical pollutants as an energy source and metabolise the target contaminant into usable energy through redox reactions.

When compared to main pollutants, by-products or metabolites are typically less hazardous to the environment. Microorganisms, for example, may destroy petroleum hydrocarbons in the presence of oxygen via aerobic respiration. The hydrocarbon loses electrons as it oxidises, whereas oxygen gains electrons as it decreases. As a result of this redox reaction, water and carbon dioxide are produced [40].

Because microorganisms have developed numerous methods to resist the harmful effects of HMs, they play an essential role in HM cleanup from polluted soil. Microorganisms can sequester, precipitate, biosorb, and modify metal oxidation states [41,42].

Metal sequestration occurs via cell wall components and intercellular metal binding peptides and proteins such as metallothionein, phytochelatins, and bacterial siderophores [43]. However, the biosorption method is based on two factors: the first is cell metabolism, and the second is the location of the cell where the HM is eliminated. The presence of a pollutant, the acceptor of electrons, and the presence of microorganisms that may digest a specific contamination are three crucial bioremediation elements.

In general, the biodegradation process is simple for naturally existing contaminants or those that have chemical similarities with naturally occurring chemicals. It is due to a ability of microbes to degrade pollutants. Petroleum hydrocarbons, for example, are naturally generated chemical compounds; hence, microbes are used to these toxins and may quickly digest them. Various methodologies used in the microbial remediation process, such as bioattenuation, biostimulation, and bioaugmentation for eliminating hazardous contaminants from contaminated land, are mentioned here.

**Bioattenuation**

During bioattenuation, pollutants are transformed to less dangerous or immobilised forms. Such immobilisation and transformation processes are generally attributed to microbial biodegradation and biological transformation [44], as well as, to a lesser extent, to interactions with naturally occurring chemicals and geological medium sorption. Contaminant-specific natural attenuation processes are regarded techniques for the remediation of fuel components [e.g., biosparging of benzene, toluene, ethylbenzene, and xylene (BTEX)], but not for other types of pollutants (e.g., sulphide and ferrous iron) [45].

**Biostimulation**

This involves modifying environmental characteristics such as restricting nutritional supplements such as slow-release fertilisers, biosurfactants, and biopolymers, which aid in the removal of heavy metal, hydrocarbon, and oil contaminants [46,47]. It also improves the bioavailability of Cu, Cd, Pb, and Zn, as well as heavy metal absorption, translocation, and biodegradation rate of hydrocarbons, pesticides, and herbicides by naturally occurring microbes on the site [48]. There are several fertilisers available to encourage bacteria, such as water-soluble NaNO3, KNO3, NH3NO3, slow-release customised, max-bac, IBDU, and oleophilic Inipol EAP22, MM80, F1, S200.

**Bioaugmentation**

Bioaugmentation boosts heavy metal removal effectiveness by adding pre-grown microorganisms. Natural/exotic/engineered microorganisms are artificially integrated into heavy metal-contaminated soil in this process [49]. Microbes are gathered from the remediation site, cultivated separately, genetically grown, and reintroduced to the site. This process promotes the proliferation and population of microorganisms, which improves HM solubility, motility, accumulation, and remediation efficacy [50]. It does, however, lessen the danger of these contaminants by either chemically modifying their chemical structure or decreasing their bioavailability [51,52]. This method has recently been applied to various HM contaminated soils using various bacteria and fungal strains such as Oscillatoria sp., Leptolyngbya sp., Portulaca oleracea, Perenniporia subtephropora, Aspergillus niger MH541017, Daldinia starbaeckii, Tremates versicolor, and Tremates versicolor [53,54].

**Micro bioremediation of Heavy Metal Polluted Soils**

Several microorganisms, particularly bacteria (Bacillus subtilis, Pseudomonas putida, and Enterobacter cloacae), have been utilised successfully to reduce Cr (VI) to the less hazardous Cr (III) [54,55,56,57]. Nonmetallic elements have also been observed to be reduced by B. subtilis. Garbisu et al. [58] discovered that B. subtilis converted selenite to the less poisonous elemental Se. Furthermore, B.cereus and B. thuringiensis have been demonstrated to boost Cd and Zn extraction from Cd-rich soil and soil contaminated with metal-industry effluent[59]. It is assumed that the production of siderophore (Fe complexing molecules) by bacteria facilitated the extraction of these metals from the soil; this is because heavy metals have been reported to simulate the production of siderophore, which affects their bioavailability [60]. For example, in the presence of Zn (II), Azotobacter vinelandii produced more siderophores [61].

As a result, heavy metals influence the activities of siderophore producing bacteria, enhancing the mobility and extraction of these metals in soil. Indirect bioremediation can also occur by bioprecipitation by sulphate reducing bacteria (Desulfovibrio desulfuricans), which reduces sulphate to hydrogen sulphate, which then combines with heavy metals such as Cd and Zn to generate insoluble forms of these metal sulphides [62]. The majority of the aforementioned microbial aided cleanup is done ex situ. However, one extremely significant in situ microbe aided remediation is the microbial reduction of soluble mercuric ions Hg (II) to volatile metallic mercury and Hg (0) carried out by mercury resistant bacteria [63]. The reduced Hg (0) can readily volatilize out of the surroundings and get diffused in the atmosphere [64].

 **TABLE :1 MICROBE MEDIATED REMIDIATION OF HEAVY METALS**

|  |  |  |  |
| --- | --- | --- | --- |
| **MICROBIAL GROUP** | **CONTAMINATION HMs** | **MICROORGANISMS** | **REFERENCES** |
|  Bacteria | LeadCadmiumNickel MercuryCopper,Cadmium and ZincCadmium and Zinc | Bacillus subtilisPseudomonas aeruginossBacillus sp. KL 1Bacillus firmusDesulfovibriodesulfuriaSynechococcuss sp | [137][138][139][140][141][142] |
|  Algae | Ar(V)Cadmium,zinc,lead and nickelLead,nickel,cadmium and zincLead, nickel and cadmium | LessonianigrescensAsparagopsisarmataCodiumvermilaraCystoseirabarbata | [143][144][144][145] |
|  Fungi | LeadCopper,lead and Cr(V)SilverCopper | Batrytis cinerealAspergillus nigerPieurotus platypusRhizopusoryzae | [146][147][148][149] |

Making the soil more conducive to soil microorganisms is one method used in contaminated soil bioremediation. This biostimulation procedure comprises the provision of nutrients in the form of manure or other organic amendments that act as a C source for soil microorganisms. The additional nutrients promote the development and activity of microorganisms engaged in the remediation process, increasing the effectiveness of bioremediation. Although biostimulation is often utilised for organic pollutant biodegradation [65], it may also be used for heavy metal contaminated soil remediation. Because heavy metals cannot be biodegraded, biostimulation can indirectly improve heavy metal remediation by altering soil pH. The addition of organic materials lowers the pH of the soil [66], increasing the solubility and hence bioavailability of heavy metals, which may then be easily removed from the soil [67].

Biochar is one organic substance that is now being researched for its potential in the treatment of heavy metal damaged soils. Namgay et al. [68] found that adding biochar to contaminated soil reduced the availability of heavy metals, which in turn lowered plant uptake of the metals. The capacity of biochar to elevate soil pH, in contrast to most other organic additions [69], may have boosted sorption of certain metals, limiting their bioavailability for plant uptake. It is crucial to note that because the features of biochar vary greatly depending on the method of production and the feedstock used in its production, the effect of various biochar additions on the availability of heavy metals in soil will likewise vary. Furthermore, because such studies are rare in the literature, additional study is required to understand the effect of biochar on soil microorganisms and how the interaction between biochar and soil microbes effects remediation of heavy metal polluted soils.

**Phytoremediation**

Phytoremediation is an aspect of bioremediation that uses plants for the treatment of polluted soils. Recognising the ecological and human health risks posed by contaminants has resulted in the development of many remediation solutions. However, due to the high expense of some of these technologies, focus has shifted to creating alternative/complementary solutions such as the employment of plants and microbes as bioremediators [70-72]. Pilon-Smits (2005) [73] described phytoremediation as "the use of plants and their associated microbes for environmental cleanup," describing it as a "cost-effective, noninvasive alternative technology to engineering-based remediation methods." Phytoremediation is one of these strategies that includes the use of metabolically viable green plants and their associated microbes for in-situ risk reduction and/or removal of pollutants from polluted soil, water, sediments, and air. The procedure employs specially chosen or developed plants [74]. Risk reduction can be accomplished by removal, degradation of a pollutant, or a combination of these factors. Phytoremediation is an energy-efficient and aseptically appealing way of remediating areas with low to moderate levels of pollution, and it may be employed as a finishing step in conjunction with other more traditional remedial procedures. A recent study regarding the phytoremediation potential of Helianthus annuus L. in sewage-irrigated Indo-Gangetic alluvial soils in India (Mani et al. 2012b) [75] indicates that H. annuus L. is highly sensitive to Cr and Zn in terms of metal pollution, and for Cr phytoremediation, humic acid treatment at 500 mL/acre increased the phytoremediation potential of H. annuus L. and induced 3.21 and 3.16 mg/kg of Cr accumulation in the roots and shoots, respectively.

Kumar et al. (2008a) [76] observed a higher deposition of heavy metals in Nelumbo nucifera in the Pariyej community reserve (wetland) of Gujarat State, India. The six heavy metals were ordered in descending order based on concentration and toxicity status in the lake's vegetation: Zn>Cu>Pb>Ni>Co>Cd. Typha angustata and Ipomoea aquatica were also considered as bioremediants. According to Wu et al. (2010) [77], the current inquiry of non-crop-hyperaccumulators will be of limited relevance in the future application and pragmatic development should be cropped hyperaccumulators (newly dubbed as 'cropaccumulators') through transgenic or symbiotic technique. They proposed a set of universal approaches that are unique, tentative, and adaptable in order to assess the viability of hyperaccumulators before large-scale commercialization.

Furthermore, Wu et al. (2012) [78] found that EDDS significantly increased soil solution dissolved organic carbon (DOC) and pH, as well as soil plant-available metals, in Sedum plumbizincicola, resulting in high soil concentrations of soluble metals and a high risk of groundwater contamination. The efficiency of metal removal decreases as the concentrations of bioavailable metal fractions decrease after repeated phytoremediation of metal-contaminated soils; however, traditional organic materials (rice straw and clover) are far more effective and environmentally friendly amendments than EDDS in enhancing phytoremediation efficiency of Cd contaminated soil. **Table 2** shows some recent biotechnology breakthroughs in the field of phytoremediation. The authors suggest that this plant be used for Cr phytoremediation in sewage-irrigated Gangetic alluvial soils. **Fig. 1** depicts an overview of biotechnological techniques for phytoremediation. The modified figure from **Dhankher et al. (2011)** [79] demonstrates how excreted chemicals aid in mobilisation, improve transit in the root cell membrane, and aid in translocation to the shoot. Increased levels of root and xylem chelators (acids, GSH) improve plant tolerance and xylem mobilisation. Increased enzyme levels alter, conjugate, or degrade contaminants, allowing them to be tolerated, degraded, sequestered, or volatilized. Increased phloem chelator levels aid in remobilization to reproductive tissues.



 **Fig. 1 An overview of biotechnological approaches for phytoremediation (79)**

Recent phytotechnology research has increased our understanding of plant and soil sciences; yet, more effective and financially viable procedures are still needed to make phytotechnology more commercially appealing. Conesa et al. (2012) [80] proposed taking use of new economic prospects such as the production of bioenergy, biochar, and biofortified crops, as well as the use of economic research and economic assessments, as well as a new phytotechnology implementation procedure.

**Using Plants for Remediation of Heavy Metal Polluted Soils**.

Phytoremediation is an aspect of bioremediation that uses plants for the treatment of polluted soils. Phytoremediation of heavy metal polluted soils can be achieved via different mechanisms. Phytoremediation includes (1) phytoextraction (phytoaccumulation), (2) rhizofiltration, (3) phytostabilization, (4) phytodegradation (phytotransformation), (5) rhizodegradation, and (6) phytovolatilization

1. **Phytoextracxtion:**

Phytoextraction is the process by which plant roots remove metals from the soil and transport them to above-ground tissues. Because various plants have varying ability to absorb and survive high amounts of pollutants, a variety of plants may be employed. This is especially important for sites that have been contaminated with more than one metal. Following the discovery of metal hyperaccumulator plants, there has been a surge of interest in phytoremediation. Hyperaccumulators are commonly described as species capable of collecting metals at levels 100-fold higher than those typically recorded in non-accumulator plants. Thus, a hyperaccumulator will concentrate more than 10 ppm Hg; 100 ppm Cd; 1,000 ppm Co, Cr, Cu, or Pb; and 10,000 ppm Ni or Zn. The plants are picked and carefully disposed of once they have grown and absorbed the metals [81,82]. There are about 400 known metal hyperaccumulators [83], and their number is growing. However, due of their sluggish development and low biomass generating capabilities, many of these plants' remediation potential is restricted. While the optimum plant species for phytoremediation should have a high biomass as well as high metal accumulation in the shoot tissues [84,85,86]. This procedure is performed multiple times until the contamination level is acceptable. Metals may be recycled in some situations by a process called as phytomining, albeit this is normally reserved for precious metals. Metal compounds that have been effectively phytoextracted include Zn, Cu, and Ni, although there is promising research being conducted on Pb and Cr absorbing plants [87,88,89].

1. **Rhizofiltration**

Rhizofiltration is conceptually similar to phytoextraction, however it is focused with the treatment of contaminated groundwater rather than damaged soils. The pollutants are absorbed by the plant roots or adsorbed onto the root surface. Plants used for rhizofiltration are not planted immediately in the ground, but are first acclimated to the pollutant. Hydroponically grown plants are cultivated in clean water rather than soil until a substantial root system develops. To acclimatise the plant, the water source is changed with a dirty water supply once a significant root system has been established. After the plants have been acclimatised, they are planted in a contaminated location, where the roots absorb the polluted water as well as the toxins. When the roots become soaked, they are collected and carefully disposed of. Repeated treatments of the site can reduce contamination to acceptable levels, as demonstrated at Chernobyl by the cultivation of sunflower (Helianthus annuus) in radioactively polluted pools [90,91].

1. **Phytostabilization**

Phytostabilization, on the other hand, entails halting As absorption and accumulation in the rhizosphere. This low-cost procedure is especially significant for restricting overall bioavailability and biomagnification in the food chain [92]. This method stabilises it in a certain environment but does not result in total elimination of contaminants, which may cause revival in the future. Its potential can be further boosted by adding supplements such as compost, phosphates, bone mill, furnace slag, fy ash, and so on [93]. This decreases or even prohibits pollutant mobility into groundwater or air, as well as contaminant bioavailability, preventing metals from spreading through the food chain. This strategy can also be used to rebuild a plant community on areas that have been depleted owing to high levels of metal pollution. Once a community of tolerant species has been created, the possibility for wind erosion (and consequently the spread of the pollutant) is diminished, as is the potential for soil contaminant leaching [94,95].

1. **Phytodegradation**

The decomposition or breakdown of organic pollutants by internal and external metabolic processes triggered by the plant is known as phytodegradation. Organic substances are hydrolyzed by ex-planta metabolic activities into smaller units that may be taken by the plant [96]. Some pollutants are absorbed by the plant and subsequently degraded by plant enzymes. These smaller pollutant molecules may subsequently be utilised as metabolites by the plant as it grows, resulting in their incorporation into plant tissues [97]. It is sometimes referred to as phytotransformation. It is the breakdown of pollutants taken in by plants via metabolic processes within the plant or the breakdown of contaminants external to the plant via the impact of substances generated by the plants (such as enzymes). The primary process is plant absorption and metabolism, which results in plant destruction. Degradation may also occur outside the plant as a result of the release of chemicals that trigger the transformation [98].

1. **Rhizodegradation**

The presence of the root zone promotes the degradation of an organic pollutant in soil via microbial activity. Plant-assisted degradation, plant-assisted bioremediation, plantaided in situ biodegradation, and increased rhizosphere biodegradation are all terms for rhizodegradation. Plants and their associated microbes are used to remediate polluted matrices by extracting, transforming, degrading, and/or stabilising organic and inorganic contaminants. Rhizodegradation is defined as 'degradation by plant rhizospheric microorganisms'[98,99].

**Table 2 Some plants having phytoremediation potential and heavy metals they can remediate**

|  |  |  |  |
| --- | --- | --- | --- |
| **S.No** | **SPECIES** | **METALS** | **REFERENCES** |
| 1 | *Pteris vittata* | Cu,Ni,Zn,As | [150] |
| 2 | *Brassica juncea* | Se,Cd | [151] |
| 3 | *Populus sp.* | Hg | [152] |
| 4 | *Brassica napus* | Cd | [153] |
| 5 | *Typha latifolia* | Pb | [154] |
| 6 | *Nelumbo nucifera* | Zn,Cu,Pb,Ni,Co,Cd | [155] |
| 7 | *Amaranthus viridis* | Cr | [156] |
| 8 | *Helianthus annuus* | Cu,Zn,Pb,Hg,As,Cd,Ni | [157] |
| 9 | *Trifolium pretense* | Cs | [158] |
| 10 | *Spinacea oleracea* | Pb,Zn | [159] |
| 11 | *Brassica juncea* | Pb | [160] |
| 12 | *Lupinus luteus* | Cu,Cd,Pb | [161] |
| 13 | *Populus tremula* | Zn,Cd,Cu | [162] |
| 14 | *Beta maritime* | Pb | [163] |
| 15 | *Pistia stratiotes* | Cd,Pb,Zn | [164] |
| 16 | *Spinacea oleracea* | Cd,Pb | [165,166] |
| 17 | *Helianthus annuus* | Cr,Zn | [167] |
| 18 | *Arabidopsis thaliana* | Cd,As | [168] |
| 19 | *Alyssum lesbiacum* | Ni | [169] |
| 20 | *Gmelina arborea* | Al | [170] |

**Phytovolatilization**

It is the absorption and transpiration of a pollutant by a plant, followed by the release of the contaminant or a modified version of the contamination into the atmosphere via contaminant uptake, plant metabolism, and plant transpiration. Phytodegradation is a kind of phytoremediation that can occur in conjunction with phytovolatilization. Tobacco plants may volatilize some inorganic components such as mercury. Plant leaves absorb extremely dangerous methyl mercury, change the chemical speciation, and phytovolatilize less harmful elemental mercury into the atmosphere. 'Volatilization by leaves' is the mechanism of phytovolatilization [98-100]. The primary benefit of this approach is that the pollutant (for example, mercuric ion) may be converted into a less hazardous compound (elemental Hg). However, mercury discharged into the atmosphere may be recycled by precipitation and redeposited in lakes and seas, causing difficulties. There has been documented mercury volatilization by genetically engineered tobacco (N. tabacum) and Arabidopsis thaliana [101], as well as selenium volatilization by Indian mustard and canola (Brassica napus) [102].

**Combining Plants and Microbes for Heavy Metal Remediation in Soils.**

The combination of both microorganisms and plants for contaminated soil remediation leads in a faster and more efficient clean-up of the polluted site [103]. Mycorrhizal fungi have been utilised in various heavy metal remediation studies, and the results suggest that mycorrhizae utilise multiple methods for the repair of heavy metal polluted soils. For example, whereas some research found increased phytoextraction due to heavy metal buildup in plants [104-106], others observed increased phytostabilization due to metal immobilisation and decreased metal concentration in plants [107,108]. In general, the benefits of mycorrhizal associations, which range from increased nutrient and water acquisition to the provision of a stable soil for plant growth and an increase in plant resistance to diseases [109-111], are thought to aid plant survival in polluted soils and thus aid in the vegetation/revegetation of remediated soils [112].

Heavy metals have also been demonstrated to affect the activities of mycorrhizal fungi [113,114]. Furthermore, Weissenhorn and Leyval [115] observed that some mycorrhizal fungi (arbuscular mycorrhizal fungi) are more vulnerable to pollution than plants.

Other microorganisms, other than mycorrhizal fungi, have been employed in combination with plants to remediate heavy metal damaged soils. The majority of these microorganisms are plant growth-promoting rhizobacteria (PGPR), which are often found in the rhizosphere. These PGPR promote plant growth through a variety of mechanisms, including the production of phytohormones and the supply of nutrients [116], the production of siderophores and other chelating agents [117], specific enzyme activity and N fixation [118], and a reduction in ethylene production, which promotes root growth [119].

Madhaiyan et al. [120], on the other hand, showed improved plant growth due to a decrease in Cd and Ni accumulation in tomato shoot and root tissues after inoculation with Methylobacterium oryzae and Burkholderia spp. As a result, the processes used by PGPR in the phytoremediation of heavy metal polluted soils may differ depending on the type of PGRP and plant engaged in the process. Although studies involving both mycorrhizal fungi and PGPR are uncommon, Vivas et al. [121] reported that PGPR (Brevibacillus sp.) increased mycorrhizal efficiency, which in turn decreased metal accumulation and increased the growth of white clover growing on a heavy metal (Zn) polluted soil.

**FACTORS AFFECTING BIOREMEDIATION EFFICIENCY**

Site parameters are the most critical element influencing bioremediation effectiveness. Second, environmental parameters including water content, temperature, pH, nutrient availability, moisture content, and pollutant bioavailability can all reduce bioremediation efficiency [122,123]. Aside from that, the bioremediation process is a complicated system that is optimised and regulated by a variety of variables. The interplay between pollutants, microorganisms, nutritional availability, and environmental conditions influence contaminant bioavailability and biodegradation.

**Site Characteristics**

The first and most critical parameters influencing the bioremediation process are the location and features of the site. The extent and kind of pollutants present in the area impact the efficacy of cleanup [39]. These issues may be mitigated and managed by doing thorough site assessment and characterization prior to beginning the cleanup procedure.

**Temperature:**

Temperature is an important consideration in determining microorganism survival and development, as well as the composition of hydrocarbon [124]. It is essential in the microbe-assisted remediation process because it affects both the physical and chemical states of pollutants present in contaminated areas and disrupts microbial metabolisms, growth rate, soil matrix, and gas solubilities [125]. High temperatures have been shown to disrupt bacterial cell metabolic function and interfere with the bioaccumulation process [126,127]. Furthermore, temperature has a strong impact on microbial physiological features, which can speed up or slow down the repair process. The interaction between fungal membrane binding sites and heavy metal ions is temperature dependent. Temperature influences the shape and stability of fungal membranes by ionising chemical moieties [128].

**pH**

The metabolic activity of bacteria is affected by pH, which can boost or reduce the elimination process. Bioremediation can be used across a wide variety of pH levels. However, a pH of 6.5 to 8.5 is thought to have the greatest potential for remediation of most terrestrial and aquatic systems [39]. The pH value impacts heavy metal mobility and solubility via dissociating functional groups on the fungal membrane during the biosorption process [129]. The Cd biosorption capability of Exiguo bacteria sp. improved with increasing pH up to 7.0 and remained neutral when the pH exceeded 7.0 [130]. pH and ionic strength also influence microbial adsorption [131].

**Nutrient Availability**

Similarly, nutrient content, availability, and type are critical in the bioremediation process for microbial growth and activity. The essential elements (such as carbon, nitrogen, and phosphorus) aid bacteria in producing the enzymes required to break down contaminants. In a cooler climate, an adequate supply of nutrients increases the metabolic activity of microorganisms, resulting in an increase in remediation rate [132,133]. It has been observed that microbial inhabitation was caused by an excess of nitrogen in the polluted media [134]. Furthermore, greater nitrogen, phosphorus, and potassium concentrations impede the biodegradation of hydrocarbon pollutants.

**Moisture Content**

The moisture content of the soil might be harmful to microorganisms. Moisture influences pollutant metabolism by affecting the quantity and type of soluble materials, as well as the pH and osmotic pressure of terrestrial and aquatic locations [39].

**Water Content**

Microorganisms require water activity levels ranging from 0.9 to 1.0 for metabolism and growth. The majority of bacteria thrive best at the extremes of water activity [135]. As a result, the amount of water in contaminated soil is an important component that may influence the pace of bioremediation. [124] recently stated that water scarcity, sodicity, and salinity are all critical variables influencing bioremediation efficiency.

**Pollutant Bioavailability**

The limited bioavailability of HMs in the polluted soil had a significant impact on bioremediation effectiveness. Contaminant bioavailability is influenced by a variety of physicochemical processes such as sorption, diffusion, desorption, and dissolution. This issue may be addressed by adding different surfactants and chelating agents, which increase the bioavailability of HMs for microbial breakdown and plant absorption. Recent applications include ethylenediamine tetraacetic acid (EDTA), [S,S]-ethylenediaminedisuccinic acid (EDDS), ethylenediamine-di-ohydroxyphenylacetic acid (EDDHA), diethylenetriaminepentaacetic acid (DTPA), nhydroxyethylenediaminetriacetic acid (HEDTA), citric acid, acetic acid, and malic acid. The use of these chelating agents has successfully demonstrated that they efficiently form a complex with HMs and enhance bioavailability [136].

**Conclusion**

The current study presents the scientific understanding needed to harness natural processes and create and design techniques to speed up these processes for bioremediation of polluted soil settings. Despite its limitations, bioremediation, particularly phytoremediation, is seen as a promising approach for decontaminating metal-polluted soils. Furthermore, eager advances in science allowed us to better fully understand and implement this technique on heavy metal-contaminated areas. Man-made activities have released a large amount of harmful metals into the environment, influencing the life processes of all living species in both direct and indirect ways. It has been found that more than one type of heavy metal is present on polluted soil at the same time, and existing conventional procedures are not much more efficient at detoxifying pollutants than the bioremediation process. It has been demonstrated that bioremediation procedures are significantly less expensive than other physicochemical remediation strategies. To accomplish this, a multidisciplinary strategy comprising plant biologists, soil chemists, microbiologists, and environmental engineers is necessary for increased effectiveness of bioremediation/ phytoremediation, which might serve as a feasible soil cleanup solution.

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