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

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**APSTRACT**

Soil pollution from heavy metals (HMs) poses a critical issue in developing nations owing to their resistance to natural decomposition and substantial capacity to harm ecosystems and related benefits. Swift industrial growth and practices like mining, production, and building construction are producing vast amounts of hazardous waste, leading to environmental risks. Microorganisms and flora utilize distinct mechanisms for the bioremediation of contaminated soils. Opting for plant-based solutions in the treatment of polluted soils is a more prevalent approach when addressing heavy metal pollution. Integrating both microorganisms and plants represents a strategy for bioremediation that ensures a more effective purification of heavy metal-contaminated soils. Nevertheless, the triumph of this strategy is heavily reliant on the specific organisms engaged in the process. This comprehensive analysis explores novel facets and perspectives of remediating heavy metal-contaminated soils. The analysis underscores an improved comprehension of the challenges linked to the poisonous nature of heavy metals within compromised ecosystems, as well as their viable, sustainable, and environmentally friendly bioremediation methodologies. This particularly encompasses the mechanisms behind phytoremediation of heavy metals, accompanied by illustrative case studies from both India and overseas. Furthermore, the analysis delves into factors that impact the efficiency of bioremediation efforts.

**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 is 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, 10,11].

In-situ and ex-situ is the process that the branches of bioremediation-situ is the bioremediation process that performed in the place of origin based on the procedure. Ex-situ implies that the procedure done by the transportation of the other site [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 method we can develop the healthy environment and ensure the sustainable environment.

**Bioremediation:**

Bioremediation involves harnessing the capabilities of microorganisms and/or plants to purify contaminated soil. This method is widely recognized as a natural process and is embraced for its affordability in revitalizing soil quality. In contrast to conventional techniques like excavation and landfill disposal, a study by Blaylock et al. [13] highlighted cost savings ranging from 50% to 65% when opting for bioremediation to address 1 acre of soil contaminated with lead (Pb). While bioremediation stands as a minimally invasive method for soil restoration, it commonly demands a substantial time investment. Its application for addressing heavy metal-contaminated soils might encounter hindrances stemming from the climatic and geological attributes of the targeted remediation site [14]. Bioremediation doesn't achieve total elimination of heavy metals; instead, it aids in transforming them from one organic complex or oxidation state to a different form. This transformation of heavy metals can yield reduced toxicity, enhanced volatility, heightened water solubility (enabling easier environmental extraction), or diminished bioavailability through alterations in their oxidation states. [15].

According to a study, addressing metal-contaminated sediments and soils using techniques like landfilling and chemical treatment results in costs spanning around 100 to 500 USD per ton for remediation. The verification process for these methods amounts to about 90 to 870 USD per ton. In contrast, bioremediation presents a cost range of around 15 to 200 USD per ton, and phytoremediation stands at approximately 5 to 40 USD per ton [16]. Rough estimates indicate that employing bioremediation could potentially reduce expenses by 50-65% when rehabilitating an acre of soil contaminated with lead (Pb), in comparison to the conventional practices of excavation and disposal [17, 18].

Moreover, bioremediation stands as a non-disruptive technology capable of potentially permanent toxin removal, all while preserving the integrity of the ecosystem. It can also be employed in conjunction with chemical and physical treatments [19]. Bioremediation processes exclusively harness the innate biological capabilities of nature. The effectiveness of the majority of bioremediation approaches depends on a range of factors. These encompass soil composition, pH values at polluted sites, moisture levels, pollutant characteristics, availability of extra nutrients, variety of microorganisms, temperature at the treatment area, and oxygen presence [19, 20, 21, 22]. The strategies utilized in bioremediation are divided into two categories: 'in-situ' and 'ex-situ' methods [23].

**On-site bioremediation:**

Furthermore, bioremediation represents a non-intrusive approach that has the potential to eliminate harmful substances in a lasting manner, all the while preserving the integrity of the ecosystem. It can also be combined with chemical and physical treatments for enhanced results [19]. The processes of bioremediation exclusively harness the inherent biological capabilities present in the natural environment. Most bioremediation methods are impacted by various elements, including the arrangement of the soil, pH levels at polluted locations, level of moisture, the nature of the pollutants, supplementary nutrient availability, diversity of microorganisms, temperature at the treatment site, and the presence of oxygen, as discussed in references [19, 20, 21, 22]. Bioremediation is categorized as either 'in-situ' or 'ex-situ' techniques [23]. The key benefits linked with in situ bioremediation include its cost-efficiency, elimination of the need for digging, minimal disturbance to the location, reduced creation of dust, and the eventual possibility of addressing both soil and groundwater simultaneously over the long term. Nevertheless, notable drawbacks include the time investment needed, fluctuations in microbial activity due to changing seasons, and the intricate task of applying treatment chemicals within the natural surroundings [27].

**Ex-situ bioremediation:**

On the other hand, ex-situ bioremediation techniques involve the removal of contaminated soil and water from their original site to undergo treatment elsewhere. This process can be categorized into two main groups: solid-phase systems and slurry phase systems. In solid-phase bioremediation, polluted waste materials such as industrial byproducts, domestic refuse, urban solid waste, and sewage sludge are mixed with organic waste elements like dung, plant matter, and leftover agricultural materials. The treatment procedures include composting, soil biopiles, hydroponics, and land farming. All of these methods create suitable environments that encourage the natural anaerobic and aerobic microorganisms to assist in the process of reclamation [26, 27]. A biopile, functioning as a temporary bioremediation unit, involves mixing excavated soils with soil additives, shaping them into compost heaps or elevated enclosures, and enclosing them for treatment with an aeration system [28]. Conversely, slurry phase bioremediation, a swifter technique, entails blending polluted soil with water and additional substances within a sizable vessel known as a bioreactor. This mixture is agitated to ensure sustained interaction between the microorganisms and the contaminants within the soil. This optimized setting fosters an environment conducive to the degradation of contaminants by microorganisms. Achieving successful ex situ bioremediation relies on ensuring proper sampling procedures and upholding regulated conditions through the utilization of obtained core samples. Land farming represents a straightforward approach where polluted soil is dug up and distributed across a prepared surface. It is then regularly plowed to encourage the degradation of pollutants by activating the native biodegrading microorganisms. This method is confined to treating the uppermost 10-35 cm of soil. The existence of these organic constituents fosters the development of a varied microbial population [29]. This approach is employed in combination with additional remedial tactics to attain effective hydroponic-based bioremediation. A wastewater treatment plant that merges conventional biological treatment with hydroponics and microalgae was established within a greenhouse close to Stockholm, Sweden [30].

As a general observation, the frequency and extent of biodegradation tend to be more pronounced within a bioreactor system as compared to in situ scenarios. This disparity arises due to the confined nature of the environment, which facilitates enhanced management, regulation, and predictability. Nonetheless, a notable drawback linked to this approach is the potential scenario where pollutants could be extracted from the soil through methods like soil washing or physical removal before being introduced into the bioreactor. Additional bioremediation strategies are explored in greater detail below;

**Bioventing:**

Among the various in situ treatments, one particularly prevalent method involves introducing air and nutrients into polluted soil via wells. This strategy aims to bolster the activity of native aerobic bacteria and serves as an illustration of subsurface bioremediation. This method employs regulated airflow rates and provides precisely the required quantity of oxygen needed for biodegradation. This strategy is devised to reduce both the volatilization of substances and the release of pollutants into the surrounding environment. Normally, contaminants experience biodegradation within conditions rich in oxygen, aided by the naturally occurring heterotrophic bacteria indigenous to the soil or underlying subsurface [31]. In the domain of subsurface bioremediation, superficial aquifers are purified through geological actions, which encompass the control of redox potential and the intricacies of heavy metal adsorption. Ultimately, this procedure detoxifies soils from heavy metal pollutants and guarantees the availability of uncontaminated groundwater suitable for both drinking and agricultural use [32].

**Biosparging:**

This process entails injecting compressed air beneath the water table to elevate oxygen levels in groundwater, thereby accelerating the biological decomposition of contaminants through the actions of indigenous microorganisms [33]. This method improves the blending within the saturated region, consequently increasing the interplay between the soil and groundwater.
Biosparging is utilized to reduce the presence of petroleum compounds in groundwater, subsurface soil, and even in the capillary fringe. Its efficacy is especially remarkable in lowering petroleum product concentrations in locations where underground storage tanks are present [31]. Interestingly, very similar principles to those guiding the remediation of soils tainted with heavy metals, akin to the scenario of bioventing, are also at play in this approach.

During 1997-2001[34], In a specific instance, a biosparging mechanism was conceived and activated at oilfield service locations in Odessa, Texas, USA. This system targeted an arsenic-hydrocarbon co-contaminated aquifer. The undertaking to address widespread petroleum contamination impacting both soil and groundwater provided valuable insights into the efficiency of biosparging, particularly in settings characterized by sandstone sedimentary bedrock [35]. Temperature's impact is crucial, as bacterial growth speed is linked to temperature variations. The ideal pH for bacterial proliferation is around 7, and biosparging functions effectively within a pH spectrum of 6 to 8. In the context of biosparging, evaluations of bacterial density are commonly measured as colony-forming units (CFUs) per gram of soil.

Biosparging was adopted as a proactive remedial approach at the previous Soviet Army air station situated in the Czech Republic for a period of ten years (1997-2008). Within the cleanup zones, a discernible elevation in the average groundwater temperature was recorded, likely attributable to biological processes taking place during the remediation endeavor. Notably, a substantial enhancement in biodegradation rates was observed following the intensification of air sparging activities. Equally significant was the robust linear correlation established between rates of air injection and levels of biodegradation activity. This correlation underscored the paramount role played by the air injection rate in determining the efficiency of biodegradation, especially within heavily contaminated regions [35].

**Bioaugmentation:**

Bioaugmentation involves introducing pre-cultivated microbial cultures, whether they originate from the local environment or are introduced from outside sources, into contaminated sites. This step aims to enhance the decomposition of undesirable compounds [36]. Infrequently, introduced microbial cultures can establish robust competitive presence that is sustainable enough to foster viable population levels. Typically, however, soils that have been exposed to biodegradable waste for extended periods boast indigenous microbial populations that are well-equipped for efficient degradation. In such cases, the successful degradation of pollutants is primarily contingent upon the effective management of the land treatment unit [37].

Similar to various other bioremediation approaches, bioaugmentation might not be sufficient as a standalone method. However, when combined, bioaugmentation and biostimulation, which involve the addition of nutrient boosters (such NH4NO3 and K2HPO4), degrading bacteria, biosurfactants, natural carbon obtained from kitchen waste or compost, and organic carbon from these sources, can produce improved results. Optimal results were achieved when these strategies were implemented while maintaining a moisture content of approximately 15-25% and a temperature of 30°C [38].

**Mechanism of bioremediation:**

Bioremediation operates through various mechanisms such as reduction, detoxification, decomposition, mineralization, or transformation of more harmful metals into less harmful forms. Cleanup techniques are employed to eliminate noxious waste from polluted environments. Through the comprehensive actions of bacteria, bioremediation is widely acknowledged for its role in breaking down, eliminating, and immobilizing diverse chemical and physically hazardous substances from the surrounding ecosystem. The idea of biotransformation/biodegradation serves as the foundation for both directly and outside remediation techniques. The primary assumption behind this theory is that plants and microorganisms (bacteria, fungus, and yeast) can help remove, mobilize, immobilize, or decontaminate a variety of toxins from the environment. This is achieved through the actions of these organisms, especially during biotransformation, where microbes utilize chemical pollutants as an energy source, subjecting the target contaminants to redox reactions that result in their transformation into usable energy.

When contrasted with primary pollutants, the by-products or metabolites generated are generally less harmful to the environment. For instance, aerobic respiration allows bacteria to degrade petroleum compounds in the presence of oxygen. During this process, the hydrocarbon undergoes oxidation, resulting in the loss of electrons, while oxygen undergoes reduction, leading to the gain of electrons. This redox reaction culminates in the production of water and carbon dioxide [40]. Due to the various mechanisms that microorganisms have evolved to counteract the adverse impacts of heavy metals (HMs), they assume a vital function in the remediation of HMs from contaminated soil. Microorganisms are capable of sequestering, precipitating, biosorbing, and altering the oxidation states of metals [41,42].

The process of metal sequestration takes place through various mechanisms, including the involvement of cell wall constituents and the binding of metals by peptides and proteins present both within and outside the cells. Notable examples include metallothionein, phytochelatins, and bacterial siderophores [43]. On the other hand, the biosorption technique hinges on two pivotal factors. The first is associated with cell metabolism, while the second revolves around the specific location within the cell where the removal of the heavy metal occurs. In the context of bioremediation, three key elements come into play: the presence of the pollutant itself, the electron acceptor, and the availability of microorganisms capable of breaking down the specific contaminant.

Generally, the process of biodegradation proves to be straightforward when dealing with contaminants that either naturally occur or bear chemical resemblances to naturally existing compounds. This simplicity arises from the innate capacity of microorganisms to break down pollutants. For instance, petroleum hydrocarbons are compounds that are naturally produced, making microbes well-acquainted with these toxins and enabling them to readily metabolize them. Harmful chemicals are removed from polluted land using a variety of approaches used in the microbial remediation process, such as bioattenuation, biostimulation, and bioaugmentation.

**Bioattenuation**:

In the process of bioattenuation, pollutants undergo conversions into forms that are less hazardous or immobilized. These immobilization and transformation actions can primarily be ascribed to microbial biodegradation and biological transformation [44]. In addition, interactions with naturally occurring substances and sorption onto geological mediums play a minor role in these processes. For the remediation of fuel-related pollutants, methods based on natural attenuation processes unique to particular contaminants are regarded as successful approaches, as in the instance of biosparging used on toluene, benzene ethylbenzene, and xylene (BTEX) elements. Other forms of contaminants, like sulfides and ferrous iron, are less amenable to this strategy [45].

**Biostimulation:**

By using techniques like fertilizers with slow release, biosurfactants, and biopolymers to manage the flow of nutrients, this approach involves altering the environment's physical and biological propertiesHeavy metals, hydrocarbons, and oils can be removed with the help of these procedures [46,47]. Additionally, this method promotes higher absorption, translocation, and rates of biodegradation of heavy metals, hydrocarbons, pesticides, and herbicides by the naturally occurring bacteria present at the site [48]. It also improves the availability of some elements, such as copper, lead, zinc. To promote bacterial activity, a range of fertilizers are available, including soluble in water options like NaNO3, KNO3, and NH3NO3 and slow-release options including custom formulations like max-bac and IBDU as well as oleophilic substances like Inipol EAP22, MM80 and S200.

**Bioaugmentation:**

By adding pre-cultivated microorganisms, bioaugmentation increases the efficiency of heavy metal removal. This technique involves the deliberate introduction of natural, exotic, or engineered microorganisms into soils contaminated with heavy metals [49]. Microorganisms are gathered from the location that necessitates remediation, cultivated individually, and occasionally modified genetically before being reintroduced to the site. This process stimulates the growth and abundance of microorganisms, consequently amplifying the ability to address heavy metal contamination by improving solubility, movement, accumulation, and overall remediation effectiveness [50]. Furthermore, this method reduces the threat posed by these pollutants, either through chemical transformations in their composition or by reducing their potential to be taken up by living organisms [51,52]. Recent applications of this method have involved a variety of bacterial and fungal strains in the remediation of various soils contaminated with heavy metals, including Oscillatoria sp., Leptolyngbya sp., Portulaca oleracea, Perenniporia subtephropora, Aspergillus niger MH541017, Daldinia starbaeckii, Tremates versicolor, and Tremates versicolor [53,54].

**Heavy metal-contaminated soils can be cleaned up using microorganisms:**

It has been successfully used to transform poisonous Cr(VI) into the less dangerous Cr(III) form by a variety of microbes, primarily bacteria like Bacillus subtilis, Pseudomonas putida, and Enterobacter cloacae [54,55,56,57]. Additionally, it has been proven that B. subtilis reduces non-metallic components. According to research by Garbisu et al. [58], B. subtilis can change selenite into the less harmful elemental Se. Additionally, it has been demonstrated that B. cereus and B. thuringiensis can improve the extraction of Cd and Zn from soils that are high in Cd and soils that have been contaminated by metal-industry runoff [59]. This increase in metal extraction is related to the siderophores that these bacteria produce, which form compounds with iron. This is significant since studies have demonstrated that heavy metals might affect siderophores' bioavailability [60]. For instance, Azotobacter vinelandii produced more siderophores when Zn(II) was present [61].

As a result, heavy metals impact the actions of bacteria that generate siderophores, thus intensifying the movement and retrieval of these metals within the soil. An alternative avenue of bioremediation is through the process of bioprecipitation, wherein sulfate-reducing bacteria (such as Desulfovibrio desulfuricans) facilitate the reduction of sulfate to hydrogen sulfide. Subsequently, this hydrogen sulfide reacts with heavy metals like Cd and Zn to produce forms of metal sulfide that are intractable [62]. A particularly noteworthy in situ utilization of microbe-assisted remediation involves the microbial reduction of soluble mercuric ions Hg(II) to volatile metallic mercury and Hg(0). While the majority of the aforementioned ex situ (outside of the original environment) microbial-assisted remediation occurs, this is not the case for all of it. Mercury-resistant bacteria carry out this change [63]. The resulting Hg(0) quickly evaporates into the air and diffuses into the environment[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] |

Improving the soil's conditions to support soil microorganisms is a tactic employed in the remediation of polluted soils. This technique encompasses a biostimulation process wherein nutrients, often in the form of manure or other organic additives, are introduced as a carbon source for soil microorganisms. These additional nutrients stimulate the expansion and functionality of microorganisms engaged in the remediation process, thus boosting the efficacy of bioremediation. Although biostimulation is commonly utilized for the breakdown of organic pollutants [65], it can also be useful for treating soils tainted with heavy metals. Given that heavy metals are not subject to biodegradation, biostimulation can indirectly enhance heavy metal remediation by influencing the pH of the soil. This pH alteration is facilitated through the introduction of organic materials into the soil. Organic material incorporation lowers the pH of the soil [66], which increases the solubility and, as a result, the bioavailability of heavy metals. The easy removal of heavy metals from the soil is made possible by this increased bioavailability [67].

Biochar, an organic material, is currently under investigation for its potential application in addressing soils impacted by heavy metal pollution. It was noticed in a study by Namgay et al. [68] that adding biochar to contaminated soil reduced the accessibility of heavy metals. This decreased uptake of certain metals by plants as a result of this reduction. Notably, biochar possesses the ability to elevate soil pH, a characteristic that distinguishes it from many other organic amendments [69]. This pH adjustment may have facilitated improved metal sorption, limiting the bioavailability of certain metals for plant absorption. It is important to stress that depending on the production process and the source material used, the properties of biochar exhibit considerable variety. Due to this, the effects of various biochar treatments on the availability of heavy metals in soil would likewise vary. Furthermore, more research is required to fully understand the impact of biochar on soil microorganisms due to the paucity of such studies in the body of existing literature. This involves looking at how biochar and soil microbial interaction affects the process of cleaning up heavy metal-polluted soils.

**Phytoremediation:**

Phytoremediation is a facet of bioremediation that harnesses plants for remediating contaminated soils. The development of various remediation techniques has been sparked by recognition of the possible ecological and human health risks associated with pollution. But because certain technologies are so expensive, attention has shifted to developing complementary or alternative approaches, such incorporating plants and bacteria as bioremediators [70,71,72]. Phytoremediation is the "use of plants and their associated microbes for environmental cleanup," according to Pilon-Smits (2005) [73]. It is described as a "cost-effective, silent alternative technology to engineering-based restoration methods." Among these methods, phytoremediation stands out because it makes use of microbes and metabolically active green plants to reduce hazards and/or remove pollutants from contaminated soil, water, sediments, and air in situ. This approach involves the deployment of meticulously selected or genetically engineered plants [74]. Pollutant elimination, degradation, or a combination of these activities can reduce risk. Phytoremediation emerges as an energy-efficient and visually appealing approach for remediating areas with low to moderate pollution levels. It can also serve as a final step in conjunction with more conventional remedial methods. In terms of metal contamination, Helianthus annuus L. is particularly sensitive to Cr and Zn, according to a recent study (Mani et al., 2012b) [75] looking at the phytoremediation capacities of the plant in Indo-Gangetic alluvial soils irrigated with sewage. In particular, the treatment with humic acid at a rate of 500 mL/acre markedly increased the phytoremediation capability of H. annuus L., leading to the accumulation of 3.21 mg/kg of Cr in the roots and 3.16 mg/kg of Cr in the shoots.

Kumar et al. (2008a) [76] reported a significant increase in the deposition of heavy metals in Nelumbo nucifera in the Pariyej community reserve (a wetland) in Gujarat State, India. Among the six heavy metals examined, the order was arranged in decreasing concentration and toxicity within the vegetation of the lake as follows: Zn > Cu > Pb > Ni > Co > Cd. Ipomoea aquatica and Typha angustata were also taken into consideration as possible bioremediation agents. According to Wu et al. (2010) [77], the present investigation into non-crop hyperaccumulators will possess limited relevance in future applications. They propose that the pragmatic advancement should focus on cropped hyperaccumulators (newly referred to as 'cropaccumulators'), which can be developed through transgenic or symbiotic techniques. Wu et al. outlined a series of universally applicable approaches that are innovative, provisional, and flexible, designed to assess the feasibility of hyperaccumulators prior to large-scale commercial implementation.

Additionally, Wu et al. (2012) [78] found that applying EDDS significantly increased the levels of dissolved organic carbon (DOC) and pH in soil solution, as well as improving the availability of metals for plants in Sedum plumbizincicola. However, this led to increased levels of soluble metals in the soil and an increased risk of groundwater pollution. The effectiveness of metal removal decreases with time when the concentrations of bioavailable metal components fall as a result of recurrent phytoremediation of metal-contaminated soils. Notably, conventional organic materials like rice straw and clover demonstrate much greater efficacy and environmental friendliness than EDDS supplements in improving the phytoremediation efficiency of Cd-contaminated soil. **Table 2** showcases a selection of recent biotechnological breakthroughs within the realm of phytoremediation. The authors advise using this plant for the phytoremediation of Cr in sewage-irrigated Gangetic alluvial soils. **Figure 1** presents a comprehensive overview of biotechnological approaches applied in phytoremediation. The above figure, taken from Dhankher et al. (2011) [79], demonstrates how excreted chemicals aid in mobilization, root cell membrane penetration, and shoot translocation. Increased levels of root and xylem chelators (such acids and GSH) help plants tolerate stress better and mobilize their xylem. Elevated enzyme levels modify, conjugate, or break down contaminants, enabling them to be tolerated, degraded, sequestered, or volatilized. Additionally, heightened phloem chelator levels facilitate remobilization to reproductive tissues.



  **Fig. 1 A synopsis of biotechnological strategies for phytoremediation (79)**

Recent research in phytotechnology has advanced our comprehension of plant and soil sciences. However, there remains a requirement for more efficient and economically feasible methodologies to enhance the commercial attractiveness of phytotechnology. Conesa et al. (2012) [80] put forward the suggestion of harnessing novel economic opportunities, including the cultivation of bioenergy, biochar, and biofortified crops. Additionally, they advocate for the incorporation of economic research, financial evaluations, and a fresh approach to phytotechnology implementation.

**Using Plants to Clean Up Heavy Metal-Polluted Soils:**

A component of bioremediation called phytoremediation uses plants to treat soils that have been polluted with heavy metals. Heavy metal - polluted soil phytoremediation involves a variety of methods. This strategy consists of a number of different strategies, including (1) phytoextraction, (2) rhizofiltration, (3) phytostabilization, (4) phytodegradation (or phytotransformation), (5) rhizodegradation, and (6) phytovolatilization.

1. **Phytoextracxtion:**

The process by which plant roots draw metals from the earth and then move them to the aerial portions of the plant is known as phytoextraction. Given that different plants exhibit varying capacities to absorb and endure substantial levels of pollutants, a diverse range of plants can be employed. This adaptability is particularly crucial for sites afflicted by the contamination of multiple metals. The discovery of plants that accumulate metal has greatly increased interest in phytoremediation. Hyperaccumulators are species that may accumulate metals at levels that are 100 times higher than those typically observed in non-accumulator plants. A hyperaccumulator would therefore store more than 10 ppm Hg, 100 ppm Cd, 1,000 ppm Co, Cr, Cu, or Pb, and 10,000 ppm Ni or Zn. These plants are picked and properly disposed of once they have grown and assimilated the metals [81,82]. Approximately 400 metal hyperaccumulators are currently known [83], and this number continues to expand. The sluggish development and limited capacity for biomass generation of many of these plants, however, restrict their potential for remediation. Ideal plant species for phytoremediation should have a notable capacity to collect metals in their shoot tissues as well as a sizeable biomass [84,85,86]. This process is reiterated multiple times until the contamination level reaches an acceptable threshold. Metals can occasionally be recycled through a process called phytomining, albeit this is often only done with valuable metals. Successful phytoextracted metal compounds include Zn, Cu, and Ni, and promising research into plants that can absorb Pb and Cr is continuing [87,88,89].

1. **Rhizofiltration**

Rhizofiltration operates on a similar principle as phytoextraction but is targeted at treating contaminated groundwater instead of impaired soils. Either the roots of the plant absorb contaminants or they are adsorbed onto the root surface. However, rhizofiltration involves the acclimatization of plants to the pollutant before being introduced to the actual contaminated site. Plants are initially cultivated using a hydroponic system with pure water instead of soil until a strong and resilient root structure is established. Once the plant has established significant roots, the water source is switched to a contaminated supply to acclimate the plant. Subsequently, the acclimatized plants are transplanted to the polluted site, where their roots take in both the polluted water and its contaminants. Once the roots are fully saturated, they are carefully collected and disposed of with great attention. Implementing this procedure multiple times at the location can gradually reduce the contamination to levels that meet the acceptable standards. A prime example is the utilization of sunflowers (Helianthus annuus) for the remediation of radioactively polluted pools in Chernobyl, showcasing its effectiveness [90,91].

1. **Phytostabilization**

Phytostabilization involves the absorption and accumulation of substances like arsenic (As) in the rhizosphere. This cost-effective technique is particularly vital for curbing overall bioavailability and the magnification of contaminants within the food chain [92]. While this method stabilizes contaminants within a specific environment, it does not lead to the complete elimination of these substances, potentially allowing for their resurgence in the future. Its effectiveness can be further enhanced by incorporating supplements such as compost, phosphates, bone meal, furnace slag, fly ash, and similar materials [93]. This intervention curtails or even hampers the movement of pollutants into groundwater or the atmosphere, as well as their availability to living organisms, thus preventing the spread of metals through the food chain. Furthermore, this method has the potential to support the recovery of a plant ecosystem in regions where extensive metal contamination has led to depletion. Once a group of resilient plant species forms a community, the chances of wind-induced erosion (and subsequently the spread of pollutants) are diminished, along with the risk of contaminants leaching into the soil [94,95].

1. **Phytodegradation**

Phytodegradation is the term used to describe the breakdown or disintegration of organic pollutants via both internal and external metabolic mechanisms set in motion by the plant itself. Within this context, organic compounds experience hydrolysis due to metabolic activities occurring outside the plant, resulting in the creation of smaller components that the plant can absorb [96]. Some pollutants are taken up by the plant and subsequently broken down by specialized plant enzymes. These reduced pollutant molecules can potentially serve as building blocks for the developing plant, ultimately becoming part of its tissue structure [97]. This procedure is occasionally referred to as phytotransformation. It encompasses the disintegration of pollutants taken up by plants through internal metabolic procedures within the plant itself, as well as the decomposition of pollutants external to the plant influenced by substances generated by the plant, like enzymes. The core mechanism revolves around the plant's absorption and metabolic processes, ultimately resulting in the breakdown of pollutants. Additionally, the degradation might occur beyond the plant due to the emission of chemicals that provoke transformation [98].

1. **Rhizodegradation**

The existence of the root zone encourages the degradation of organic pollutants within soil through microbial activity. Terms such as rhizodegradation, plant-assisted degradation, plant-assisted bioremediation, and increased rhizosphere biodegradation are used interchangeably. In these processes, plants and their associated microbes are utilized to remediate polluted environments by extracting, transforming, degrading, and/or stabilizing both organic and inorganic contaminants. Rhizodegradation is specifically defined as 'degradation by microorganisms in the plant rhizosphere' [98,99].

**Table 2: Certain plants possess the capacity for phytoremediation and exhibit effectiveness in remediating specific heavy metals**

|  |  |  |  |
| --- | --- | --- | --- |
| **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**

Phytodegradation involves the sequence of a plant taking in a pollutant, then expelling either the pollutant itself or a altered version of it into the air. This process occurs through the amalgamation of the pollutant, the plant's metabolic activities, and the release of moisture into the atmosphere through transpiration. This type of phytoremediation can occur alongside phytovolatilization. For example, tobacco plants have been observed to volatilize certain inorganic components like mercury. Within this mechanism, the leaves of plants absorb extremely hazardous methyl mercury, modify its chemical structure, and then emit a less injurious elemental mercury into the air. This process is known as 'leaf-mediated volatilization' [98,99,100]. The key advantage of this method is the potential transformation of a pollutant, such as mercuric ions, into a less harmful form, like elemental mercury. However, the released mercury into the atmosphere can be subject to recycling through precipitation and subsequent deposition in lakes and oceans, creating challenges. Examples of well-documented cases of mercury volatilization comprise genetically modified tobacco (N. tabacum) and Arabidopsis thaliana [101]. Additionally, there are instances of selenium volatilization observed in Indian mustard and canola plants (Brassica napus) [102].

**Utilizing the Synergy of Plants and Microbes for Soil Remediation of Heavy Metals:**

The collaborative utilization of both microorganisms and plants in soil remediation expedites and enhances the efficiency of cleaning up areas tainted by pollution [103]. Mycorrhizal fungi have been harnessed in numerous investigations targeting the restoration of soils contaminated with heavy metals, revealing diverse strategies employed by mycorrhizae in rehabilitating such polluted environments. For example, certain studies have reported escalated phytoextraction due to the accumulation of heavy metals in plants [104,105,106], while others have noted augmented phytostabilization arising from the immobilization of metals and decreased metal levels in plants [107,108]. In general, the benefits offered by mycorrhizal partnerships, including enhanced absorption of nutrients and water, the creation of a consistent soil milieu conducive to plant growth, and reinforced resistance against plant diseases [109,110,111], are thought to bolster the viability of plants in contaminated soils. This, in turn, aids in the development of vegetation on treated soils [112]. Heavy metals have also been demonstrated to affect the activities of mycorrhizal fungi [113,114]. Moreover, Weissenhorn and Leyval [115] noted that certain types of mycorrhizal fungi (specifically arbuscular mycorrhizal fungi) are more susceptible to the negative impacts of pollution compared to plants.

Other than mycorrhizal fungi, additional microorganisms have been utilized in conjunction with plants to remediate soils affected by heavy metals. Among these microorganisms, a prominent group is composed of plant growth-promoting rhizobacteria (PGPR), frequently found in the rhizosphere. These PGPR contribute to plant development via various means, including the synthesis of phytohormones and the supply of nutrients [116], the production of siderophores and other agents that bind to metals [117], specific enzyme activities and nitrogen fixation [118], and the modulation of ethylene production, which in turn stimulates root growth [119].

Nonetheless, Madhaiyan et al. [120] showcased an improvement in the growth of tomato plants attributed to reduced accumulation of Cd and Ni in both shoot and root tissues. This was observed when the plants were introduced to Methylobacterium oryzae and Burkholderia spp. This finding underscores the possibility that the mechanisms PGPR employ for phytoremediation in heavy metal-contaminated soils might vary depending on the specific PGPR strains and plant species engaged in the process. Although research combining mycorrhizal fungi and PGPR is relatively limited, Vivas et al. [121] highlighted that certain PGPR (specifically Brevibacillus sp.) amplified mycorrhizal efficacy, subsequently diminishing metal accumulation and promoting the growth of white clover in an environment contaminated with heavy metals (Zn).

**FACTORS AFFECTING BIOREMEDIATION EFFICIENCY:**

The success of bioremediation is significantly influenced by site-specific conditions. Moreover, environmental aspects including water levels, temperature, pH, nutrient supply, moisture levels, and the accessibility of pollutants can potentially impede the effectiveness of bioremediation [122,123]. Furthermore, the process of bioremediation is intricate, shaped by a multitude of factors. The interplay between pollutants, microorganisms, nutrient accessibility, and environmental circumstances impacts the availability of contaminants and their subsequent breakdown through biological processes.

**Site Characteristics:**

The primary and foremost elements influencing the bioremediation process are the attributes and position of the site. The composition and quantity of pollutants present in the surroundings directly dictate the effectiveness of the remediation efforts [39]. These concerns can be addressed and controlled by conducting a comprehensive site assessment and characterization before commencing the remediation process.

**Temperature:**

Temperature assumes a vital role in deciding the viability and expansion of microorganisms, as well as the structure of hydrocarbons [124]. It carries weight in microbe-facilitated remediation by impacting the chemical and physical conditions of pollutants within polluted areas. Furthermore, temperature affects microbial metabolism, the pace of growth, soil characteristics, and the solubility of gases [125]. Elevated temperatures can disrupt bacterial cell metabolism and impede the bioaccumulation process [126,127]. Moreover, temperature strongly influences microbial physiological traits, potentially accelerating or decelerating the remediation process. Temperature impacts the structure and stability of fungal membranes through ionization of chemical groups [128].

**pH:**

The metabolic activity of bacteria is influenced by pH, which can either enhance or hinder the remediation process. Bioremediation is applicable across an extensive spectrum of pH values. Nevertheless, for the remediation of both land and water environments, a pH range from 6.5 to 8.5 is typically regarded as the most favorable [39]. The pH level exerts influence over the movement and solubility of heavy metals due to its effect on the separation of functional groups on fungal membranes during the process of biosorption [129]. The ability of Exiguo bacteria sp. to biosorb Cd was found to increase with rising pH up to 7.0 and then plateaued as the pH exceeded 7.0 [130]. pH and ionic strength also play a role in microbial adsorption [131].

**Nutrient Availability:**

Similarly, nutrient content, availability, and type are crucial factors in the bioremediation process for supporting microbial growth and activity. Necessary components such as carbon, nitrogen, and phosphorus aid bacteria in producing the requisite enzymes for the degradation of pollutants. In colder weather conditions, an abundant provision of nutrients boosts the metabolic functioning of microorganisms, culminating in a hastened pace of remediation [132,133]. An excessive presence of nitrogen in polluted environments has been observed to inhibit microbial growth [134]. Furthermore, increased levels of nitrogen, phosphorus, and potassium have been demonstrated to impede the natural breakdown of hydrocarbon pollutants through biodegradation.

**Moisture Content:**

The moisture content in the soil can have ramifications for microorganisms. Moisture influences the way pollutants are metabolized by influencing the composition and quantity of soluble compounds, along with the pH and osmotic pressure of both land and water environments [39].

**Water Content:**

Microorganisms require water activity levels ranging from 0.9 to 1.0 to facilitate their metabolism and growth. A majority of bacteria exhibit their optimal performance at the extreme points of the water activity range [135]. Consequently, the level of moisture present in contaminated soil emerges as a critical determinant affecting the pace of bioremediation. Notably, [124] underlined in recent times that factors like water scarcity, sodicity, and salinity all hold significance in determining the efficacy of bioremediation.

**Pollutant Bioavailability:**

The limited presence of heavy metals (HMs) in soil contaminated with them significantly affects the effectiveness of bioremediation. The accessibility of contaminants is subject to diverse physicochemical processes, encompassing sorption, diffusion, desorption, and dissolution. This challenge can be tackled by incorporating various surfactants and chelating agents, which augment the accessibility of HMs for both microbial breakdown and uptake by plants. More recent approaches involve substances such as ethylenediamine tetraacetic acid (EDTA), [S,S]-ethylenediaminedisuccinic acid (EDDS), ethylenediamine-di-o-hydroxyphenylacetic acid (EDDHA), diethylenetriaminepentaacetic acid (DTPA), n-hydroxyethylenediaminetriacetic acid (HEDTA), citric acid, acetic acid, and malic acid. The application of these chelating agents has effectively demonstrated their capacity to create complexes with HMs and enhance their availability [136].

**Conclusion:**

The current investigation furnishes essential scientific insights for leveraging natural mechanisms and devising strategies that can accelerate these processes to remediate soil environments tainted by pollution. Despite its limitations, bioremediation, especially phytoremediation, holds significant potential as a method for addressing metal-contaminated soils. Additionally, rapid advancements in scientific understanding have facilitated the improved comprehension and implementation of this technique in areas affected by heavy metal pollution.
Human actions have led to the discharge of significant amounts of detrimental metals into the surroundings, affecting the vital functions of all living organisms through both direct and indirect routes. It has been observed that multiple types of heavy metals often coexist in polluted soil, and conventional approaches for detoxifying pollutants are generally not considerably more effective than the bioremediation process. To this end, collaborative efforts involving experts from diverse fields such as plant biology, soil chemistry, microbiology, and environmental engineering are essential for enhancing the efficiency of bioremediation and phytoremediation. This interdisciplinary approach holds the promise of providing a viable solution for addressing contaminated soil challenges.

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