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The Use of Plant-Based Technology for Degradation and Bioremediation of Pollutants and Heavy Metals from Contaminated Soils

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Abstract

Over the past centuries, increasing levels of environmental pollution can be owing to increased human activities. The risky agricultural practices and the enormous growth of industrialization have led to raised levels of anthropogenic chemicals into the environment. Amongst the various pollutants, heavy metals and metalloids have significantly contributed to widespread soil contamination. Different physical, chemical and biological processes have been used for the efficient remediation of polluted soil. The use of traditional physicochemical treatments of soil has led to the development of new technologies for remediation. Phytoremediation involves the use of plants to bioremediate pollutants from the contaminated soils. Plants that are hyperaccumulators can take up heavy metals from soil and offer a possibility to clean up the polluted sites. This plant-mediated technology is emerging as an efficient and environmental friendly treatment technique that could be used as an in situ, non-invasive and aesthetic solution. It is economical, easy to manipulate and can be engaged in high-risk contamination regions by planting particular species for preventive measures.

Keywords: Phytoremediation; Heavy metals; Hyperaccumulation; Environment; Pollutants

Abbreviations: AM: Arbuscular Mycorrhizal; TNT: 2,4,6-trinitrotoluene; DNT: 2,4-dinitrotoluene; TNT: 2,4,6-Trinitrotoluene; DANTs: Diaminonitrotoluenes; ADNTs: Andaminodinitrotoluenes; GST: Glutathione S-Transferase; Px: Peroxidase.

Introduction

The term “heavy metals” denotes a class of “toxic elements” which are both naturally and industrially imperative. The pervasive contamination of soil with heavy metals exemplified an extreme environmental issue with disturbing ecological quality and human wellbeing. The known sources for the release of heavy metals in the environment include both natural as well as anthropogenic. Interestingly, these metals when introduced tend to persevere for a longer time depending upon variable characteristic soils. In this context, the remedial procedure encompasses an extensive range of techniques physical, chemical and natural actions. The procedures are usually applied in synergism to ensure economical and proficient remediation of polluted soil. Rapid industrialization has substantially increased the disposal of heavy metals and radionuclides from certain industries into the environment. They are released into the environment through effluents from industries such as electroplating, paint pigment, electrical accumulators and batteries. Additionally, the application of agrochemicals and sewage from agricultural fields also adds a considerable amount of metals pollution in the soils [1]. Heavy metals and metalloids in excess is a serious worldwide environmental problem due to its hazardous properties and being able to get accumulated in living organisms including plants, animals, microorganisms and human [2] and are accountable for many metabolic and functional disorders [3]. Heavy metals and metalloids accumulate in soil and water and pose a risk to human health and the health of other living organisms.

Essential heavy metals (Fe, Co, Cu, etc.) already exist in the ecosystem and are required by organisms for normal physiological processes. But some heavy metals, often termed as non-essential heavy metals (As, Cd, Cr,etc.) have been reported to be toxic or lethal to organisms even in low concentrations [4]. Further, essential heavy metals above permissible limits are also known to cause damage to the physiological system of organisms [5]. Keeping in view the reported toxic effects of heavy metals, their removal, as well as management from soil, becomes compulsory. Soil washing, chemical reduction, vitrification, pneumatic fracturing are some of the techniques available for heavy metal removal from the soil [6]. Despite the effectiveness of the mentioned techniques, it poses high cost, disturbs existing natural microflora and has been known to generate pollutants as byproducts. Thus, in the present scenario, phytoremediation has emerged as a cost-effective, eco-friendly sustainable tool for the decontamination of polluted soils.

Phytoremediation is based on the unearthing of hyperaccumulators and the improved understanding of the mechanisms involved in hyperaccumulation. The use of hyperaccumulators to clean the environment is termed as Phytoextraction which is an emerging remediation technology. It is environmentally friendly and cost-effective. Several plant species are already known to able to accumulate and detoxify very high levels of heavy metals. About 450 hyperaccumulator plant species from 45 families have been reported in the literature including metal-accumulating woody species [7,8]. In this context, forest plant species have been commonly used for environmental assessment and restoration projects because of their potential for biomonitoring and bioremediation. Hyperaccumulators are not only the efficient cleaners of the environment but are able to produce high production of biomass, which can be used in producing energy [9]. Fast-growing, high biomass-producing woody plants that tend to accumulate metals in aerial tissues could be a source for the development of feasible phytoextraction technology.

Physical Remediation

Soil Replacement

The process of soil replacement is based upon replacing contaminated soil with non-contaminated soil. This method tends to dilute the existing content of heavy metals in the site, therefore expanding site usefulness [10]. The replacement of contaminated soil is followed byits treatment to eliminate pollutants or dumping it in any other site. The replacement procedure is achieved via "soil spading" and "new soil importing". The soil spading involves digging of contaminated sites deeply in order to spread heavy metals into deep sites for achieving metal diluting. However, in the process of new soil importing clean soil is added to polluted soil which can be introduced to the surface or mixed to decrease metal concentration [11]. Although soil replacement method effectively isolates the contaminated soil and ecosystem, it is costly due to the high labor involved and is usually suitable for heavily contaminated soils having a smallarea.

Soil Isolation

The method of soil isolation focuses on the separation of contaminated soil from the uncontaminated soil by using auxiliary engineering measures [12]. Such isolation technologies focus on the restriction of heavy metals and other contaminants movement within a specific area. This technique is also used to prevent further groundwater contamination by heavy metals since other remediation methods are not feasible physically or economically [13]. Temporary isolation is also preferred for further avoiding transport during site assessment as well as remediation. Barriers are installed beneath the landfill to avoid restrict the contamination of groundwater or other water bodies [14]. The lateral movement of groundwater is usually restricted by vertical subsurface barriers which can be installed downstream, upstream, or completely surrounding the contaminated site. However, such barriers have limitations to be installed at deep soil (up to 30 ft). Therefore for efficient isolation of the contaminated part, the barriers should comprise of the low-permeability layer which is usually comprised of clay. Other materials used for sub-surface barriers are grout curtains, sheet piles, and slurry walls.

Vitrification

The method of vitrification reduces the mobility of heavy metals from the contaminated site/soil via application of high temperature leading to the formation of vitreous material [15]. During vitrification, metals such as mercury (Hg) get volatilized that can further be collected and disposed of. This technique can also be effectively applied to the majority of soils contaminated with organic as well as inorganic contaminants. Interestingly, temperature plays a crucial part in the immobilization of heavy metals in soil. Keeping this in view, vitrification can be adopted for both *in situ* and *ex-situ*. The process of *in situ* vitrification is carried out by passing an electric current through the soil by vertical insertion of electrodes into the contaminated site/ area [16]. But the *insitu* remediation is preferred via wet soil with low levels of alkali content as high alkali content leads to poor current conduction. In contrast, the energy is provided burning of fossil fuel or directly heating electrode in the case of *ex-situ* [17]. The material which is vitrified is further introduced to additives such as native soil, sand or clay in order to obtain a product with characteristics, which can enhance the effectiveness of this technology. Therefore, it can be applied for small scale remediation of polluted sites. In conclusion, the phenomenon of vitrifying heavy metals is one of the efficient technology inspite of its complications and high energy requirement.

Chemical Remediation

Chemical Immobilization

The process of "immobilization" refers to a reduction in mobility, bioavailability, and bio-accessibility of heavy metals in the presence of immobilizing agents. This can be attained by precipitation and adsorption reactions in the soil respectively. This technique causes the distribution of heavy metals from soil solution to the soil particles resulting in their limited transport as well as bioavailability [18,19]. Immobilization of heavy metals is attained by using organic and inorganic amendments of soil including cement, clay, phosphates, zeolites, and microbes [20]. The organic amendments can immobilize contaminants by adsorptions or the formation of stable complexes. The important organic amendments used for the immobilization of heavy metals include animal manures and biosolids [21]. In addition to this, manure by-products can also be employed for the remediation of metals from soil [22,23] have confirmed the usage and efficiency to remediate Pb, Mn, Fe, Cr and Ni using farmyard manure.

The main constituents of organic matter are cellulose and lignin. Other components includes hemicellulose, proteins, simple sugars, hydrocarbons and functional groups (carbonyl, phenolic, esters, etc) and structural polysaccharides. It is a well known fact that metals are capable to form complexes with organic components in soils [24]. The connotation of metals with organic matter varies the type and nature of the latter. The soils with a higher content of phenolics, hydroxyl and carboxylic groups are more suitable and show greater affinity toward metals [25]. The increased soil pH also favors the extraction and immobilization of pollutants via organic manures due to the prevention of sulfide oxidation or hydrolysis [26]. They also result in a reduction of metal bioavailability by escalating surface charge respectively. Recently, biomaterials have been used extensively to immobilize heavy metals in soils due to their low cost and easy availability. Among various biomaterials use of biochar obtained from organic residues such as municipal or animal wastes, wood, etc has been significantly explored to immobilize the contaminants in soil. Its use also efficiently enhances the sorption of heavy metals and significantly decreases their phytoavailability and mobility. Interestingly, the use of biochar results in the alteration of the chemical, physical as well as biological properties of soil. The change of properties such as an increase in pH can further lead to their precipitation thus heavy metals get immobilized in soil [27,28].

Chemical Encapsulation

"Encapsulation" refers to the amalgamation of the contaminated soils with other products *viz.,* concrete, asphalt or lime [29]. As a result contaminated area/soil surrounding material becomes immobilized preventing the contamination of the surrounding area. To attain the encapsulation various binding materials are used however cement is ideal component, owing to its easy availability, and cost-effectiveness [30]. Interestingly, the process of leaching can also be prevented efficiently by encapsulation via various mobilization agents such as polyvinylalcohol, alginate, polyacrylamide and polyurethanes [31]. However, such methods are also associated with certain limitations due to the formation of a final product where encapsulation is carried out by asphalt for hydrocarbons contaminated soils. This drawback has helped in the development of an alternate method based on the silica encapsulation. Algin has also been observed to be an excellent encapsulating agent due to its efficient sorbent properties which results from the presence of the carboxylic group. Such reactive groups show tendency to interact with metal cations via chelations, thus leading to metal remediation. In recent years, new technology based upon metallic nanoparticles (NPs) of Fe has attained focus due to their exclusive optical, magnetic and electrochemical and chemical catalytic properties.

Soil Washing

One of the important approaches for remediation of contaminated area/soil is "soil washing". This process is based on the removal of heavy metals from soil implying various reagents and extracts that are able to leach contaminants [32,33]. For remediation, the method involves the mixing of contaminated soil with specific extractant to extract the specific metal. The process is followed by precipitation, ion exchange, adsorption or chelation leads to the transfer of heavy metals from soil into liquid phase consequently avoiding it to enter ground levels via leaching [34]. Consequently, the processed soil which fulfills the regulatory criteria can be refilled back to the original site. This method is a frequently used the technique for remediation because of its cost-effectiveness, rapidness, and effectiveness in completely removing heavy metals.

Several reagents have also been used to mobilize metals from soil such as chelating agents, organic acids, surfactants, etc. The effectiveness of soil washing depends upon the competence of extractant to dissolve the metals in soil. Among various extractants, synthetic chelates *viz.,* EDTA is most efficient for soil washing due to their ability to form stable complexes with a wide array of heavy metal contaminants [35]. Other chemicals used for soil washing are a salt-chloride solution such as calcium chloride and iron (III) chloride. Similarly, the use of chelators in combination has improved heavy metal toxicity in soil respectively. Several studies have reported the use of several washing chelators for the successive washing of heavy metals. It has been observed that phosphoric-oxalicacid-Na_2EDTA dependent washing led to the enhanced removal of heavy metals by 41.9% for as and 89.6% for Cd [36].

Biological Remediation

In recent years, biological remediation has gathered enough attention as a potent remedy for establishing the natural condition of the soil and maintains its balance. This process involves the use of microorganisms/plants for removal/detoxification of heavy metals from the soil. It is considered as one of the most viable, cost-effective and permanent solutions for soil remediation. Although heavy metals are not completely degraded during bioremediation they tend to get transformed from one organic state to others. As a result, heavy metals become less toxic, easily volatized, water soluble (removal through leaching or precipitation), and less bioavailable [37] in the environment. Biological remediation is broadly categorized into phytoremediation using plants, bioremediation using microorganisms and the combination of both the techniques.

Phytoremediation

The term "phytoremediation", known as vegetative remediation or botanoremediation constitutes technologies using plants as the site of detoxification. Althoughthe method of using plants as a source of heavy metal accumulation was first presented in 1983; it has been explored from the past 300 years. It is considered as energy-efficient, environmentally friendly, non-invasive and cost-effective technology. Phytoremediation can be explored successfully in combination with several other traditional methods as a finishing measure for the removal of heavy metals. The efficacy of phytoremediation mainly depends on the properties of plants and soil *viz.,* physicochemical properties, the bioavailability of metals; microbial exudates along with the abilityto live organisms to uptake, accumulate, request it as well as translocate metals after detoxification.

Overall phytoremediation constitutes several techniques and methods which differ in their application/process/mechanism using which plants can remove or immobilize heavy metals. The four important pillars of phytoremediation are normally categorized into (i) phytostabilization, (ii) phytoevaporation, (iii) phytoextraction which is based upon different uptake mechanism. During phytostabilization plants are used to reduce the bioavailability and agility of heavy metals in soils (Sylvain et al., 2016). But the process does not remove the contaminants but prevents their offsite movement. It basically targets the restriction of heavy metals in the plants via accumulation in roots in the vadose zone or precipitation (at rhizosphere). During stabilization, plants can inhibit the movement of metals various methods *viz.,* reduction of leaching, regulation of flow generation by plant transpiration, reduction in soil erosion, etc. This process further does not generate contaminated secondary waste that requires further management. Moreover, stabilization helps in the restoration of the ecosystem as it results in increase in soil fertility. However, this process demands regular monitoring for maintaining optimal stabilizing conditions. Phytovolatilization tends to convert heavy metals from the soil into less toxic vapourized form, followed by their release as biomolecules in the atmosphere through transpiration in plants. Plant species such as *Arabidopsis thaliana* and *Chara canescens* have been known to regulate uptake of heavy metals followed by converting them into gaseous forms to release in the environment [38]. The process of conversion of toxic metals to gaseous form requires specific mechanisms that are regulated by enzymes and genes inside the plant. Unfortunately, there is very few numbers of naturally occurring plant species that are proficient in converting metal to volatized form. Therefore this technique is generally used by genetically modifing plants to attain maximum volatizition ability. Plants such as *Arabidopsis thaliana* and *N. tabacum*have had shown the ability to volatize Hg [39]. In comparison to this, the process of phytoextraction is the uptake of contaminants from the soil via plant during transpiration. Plant roots uptake the heavy metals, translocate and concentrate by storing it in cellular compartments of aerial parts that are harvestable. Phytoextraction leads to the transfer of metals to plant biomass from the soil which is comparatively easy to recycle or dispose of or oxidize. However, it removes heavy metals from the low or moderately affected area due to fewer plant species that can survive at highly polluted sites [21].

In recent times, the exclusion of pollutants such as heavy metals through the process of phytoremediation has been widely accepted due to its ecofriendly approach and potential [40]. The standard values used for Cu, Co, Cr, and Pbare 1000 g kg^{-1} of dry mass and for Cd, it is 100 mg·kg^{-1} of dry mass. As compared to other plants, hyperaccumulators such as *Solanum nigrum* and *Arabidopsis halleri* in the case of Cd absorption have been observed to display higher heavy metal accumulation and tolerance (Table 1). One of the major limitations of the use of hyperaccumulators is difficulty in finding plants that can act as metal hyperaccumulators due to their lower biomass yield and slow growth.

S.No.	Plant Species	Heavy Metal	Reference
1	*Populus* spp. (*Populus deltoides, Populus nigra, Populus trichocarpa*)	Cd, Cu, Pb, Zn	Ruttens, et al. [41]
2	*Ricinus communis*	Cd	Huang, et al. [42]
3	Zea mays	Cd, Pb, Zn	Meers, et al. [43]
4	*Salix* spp. (*Salix viminalis, Salix fragilis*)	Cd, Cu, Pb, Zn	Volka et al., [44]
5	*Brassica juncea, Astragalus bisulcatus*	Se	Bither, et al. [45]
6	*Populus deltoides*	Hg	Che et al., 2003

Table 1: Important plants reported for phytoremediation of heavy metals.

Additionally, it is time-consuming and thus is not viable for rapidly contaminated sites or sewage remediation [46]. On the other hand, different rhizosphere microbes playing a critical role in plant growth or metal tolerance via different mechanisms. These mechanisms can help in designing multifunctional microbial synergism in phytoremediation. Rhizosphere has a crucial role in the phytoremediation process and helpful for the synergistic action of plants and microbes to enhance the efficiency of removing heavy metals from the soil [47]. Overall, plants and microbes release a variety of active agents and metal chelating molecules (siderophores) that can change the mobility of heavy metals.

Bioremediation

Microorganisms have been evolved and have developed various strategies to endure in heavy metal-polluted environments [48]. The microorganisms employ various mechanisms including biosorption, bioaccumulation, biotransformation, and biomineralization to survive. These detoxifying mechanism can be efficiently used to remediate the soil *ex-situ* or *in situ* [49-51]. The bioremediation of heavy metals aims at utilizing microorganisms which can affect the migration as well as the transformation of heavy metals. The remediation process is coupled with precipitation, extracellular complexation and redox reactions along with intracellular accumulation. The phenomenon of microbial leaching is an easy and efficient technique for the extraction of valuable metals from low-grade mineral concentrates. Therefore this property of microbes has the potential for remediation of mining areas and sites polluted with heavy metals [52]. They can also enzymatically carry out the reduction of metals in metabolic processes along with directing oxidation of simple organic acids and alcohols, hydrogen, or aromatic compounds.

There is an array of important mechanisms by which microbes carry out remediation and detoxification. Bioremediation by adsorption involves biosorption of heavy metals through the action of microbes and binding them on the binding sites positioned in cells without energy expenditure. Extracellular polymeric substances present on microbial cell walls play a critical role in acid-base properties as well as in metal adsorption which further include proton exchange and micro-precipitation of metals. The process of biosorption generally includes a higher affinity of a biosorbent to metal ions till equilibrium is established. *Saccharomyces cerevisiae* has shown the potential to act as bio sorbent via ion exchange mechanism for removing Zn (II) and Cd (II) [53].

Similarly, fungi such as *Klebsiella oxytoca*, *Pleurotus pulmonarius*, *Botryosphaeria rhodina* has emerged potent bioremediation agents [54]. In order to achieve detoxification aerobic or anaerobic microbial activities play an important role. Aerobic degradation introduces oxygen atoms into the reactions catalyzed by enzymes ligninases, peroxidases, mono-oxygenases, oxidative dehalogenases or hydroxylases. In contrast to this anaerobic degradation of heavy metals is carried out by initial activation reactions along with oxidative catabolism through anoxic electron acceptors. In addition to this, microorganisms remove contaminants from affected sites by two main mechanisms *viz.,* detoxification and active efflux i.e., pumping of the toxic metal from cells.

Interestingly, a basic redox reaction is carried out in between microorganisms and toxic metals in the soil [55] that leads to their removal. Oxygen is known to act as an electron acceptor in aerobic conditions while in anaerobic environment microorganisms oxidize organic contaminants by reduced electron acceptors. Studies have shown metals being used as terminal electron acceptors and this process is called “dissimilatory metal reduction” [56]. Some species of microbes such as *Geobactor* spp. reduce the metals state and alter their solubility resulting in a reduction of Uranium soluble state (U^{6+}) to insoluble state (U^{4+}) [57]. In addition to this, heavy metal accumulation *via*. Microbes involve the expression of various metal-binding proteins or peptides. Transcription factors in protein metal interactions are known to control both hormone and redox signaling pathways in perspective to toxic metals *viz.,* Cd, Zn, Hg, Cu, Au, Ag, Co and Ni [58]. *Synechococcus spp*. has also been known to express some gene and production of metal-binding protein [59]. *Escherichia coli* also showed ability to regulates the range of accumulation of Cd [60] thus, indicating fascinating role of microorganisms in the process of bioremediation.

Phytoextraction

Phytoextraction process uses crop species known to accumulate metals, metalloids or radionuclides from metalliferous soil into the aboveground parts of plants and harvested for recycling or less expensive disposal. The plant materials can be burned for energy/electricity production and the ash processed to recover metals [61]. Phytoextraction is chiefly used to treat the contaminated soils. The metal hyperaccumulator species of plants can efficiently remove metals from contaminated soils and there are approximately 400 known plant species, out of these 45 families are reported to hyperaccumulate metals. Most of these plants belong to the family of *Fabaceae*, *Brassicaceae, Euphorbiaceae*, *Lamiaceae*, *Asteraceae* and *Scrophulariaceae* [62,63]. However, most plants can only hyperaccumulate one specific metal. Specific plant species can absorb and hyperaccumulate metal contaminants and/ or excess nutrients in harvestable root and shoot tissue, from the growth substrate through the phytoextraction process.

A lot of research has explained the role of endophytes in the process of phytoextraction. Endophytes are the microbes that reside inside plant tissues without causing any damage to the host. This mechanism of phytoextraction has been slow because of a lack of valuable strains having heavy metal resistance and detoxification capacities [64]. Moreover, arbuscular mycorrhizal (AM) fungi, soil-borne obligate biotrophs, was also found to be very useful in extracting the heavy metals from soil [65].

Phytovolatilization

Phytovolatilization is the release of pollutants from the aerial parts of the plant to the atmosphere in the form of a gas. Plants have the ability to accumulate heavy metal in close association with microorganisms and convert them into non-toxic volatile forms. Members of the *Brassica* genus and some microorganisms are particularly good volatilizers of Se [66]. Phytovolatilization may be a useful, inexpensive method of removing heavy metals from contaminated sites. Similarly, many authors revealed the efficient volatilization by some transgenic plants like *Arabidopsis thaliana, N. tabacum* [39,67]*, Liriodendron tulipifera*, [68] that have converted organic and inorganic mercury salts to the volatile, elemental form.

The advantage of this method is that this method is eco-friendly and inexpensive to transform toxic pollutants to less toxic form (i.e., elemental mercury Hg). But the elemental mercury is released via its recycling through rainfall leading to its re-deposition in the atmosphere into water bodies and repeating the process of production of methyl mercury by anaerobic bacteria.

Rhizofilteration

Rhizofilteration process refers to the adsorption or absorption of low contaminant concentrations of groundwater, surface water and wastewater surrounding the root zone. Lead, Cadmium, Zinc, Nickel, Chromium are predominantly reserved inside the roots. *Helianthus* spp., *Brassicajuncea* (L.) Czern., *Nicotianatabacum* L., *Secalecereale*, *Spinaciaoleracea,* and *Zeamays* are some of the plants reported with the ability to absorb lead from polluted sites, *Helianthus* spp. was found to be the most efficient to remove contaminants from the sites [69]. Plants are first acclimatized to grow in the polluted sites followed by the process of uptake of contaminants from the soil or water. After a certain period of time ensuring the roots are completely saturated, the plants are harvested followed by disposal in safe sites. Repetitive treatments are helpful to reduce the contagion to suitable levels. A well-known example to exemplify the process was Chernobyl where sunflower (*Helianthus annuus*) was grown in radioactively contaminated pools [70].

Phytostabilization

The process of phytostabilization is applicable for the remediation of soil, sediment, and sludge according to USEPA, 2000. The process involves certain plant species to immobilize contaminants in the soil and groundwater through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants (rhizosphere). This process reduces the mobility of the contaminant and prevents migration to the groundwater and it reduces the bio-availability of metal into the food chain. This technique can also be used to re-establish vegetation cover at sites where natural vegetation fails to survive due to high metals concentrations in surface soils or physical disturbances to surface materials. Metal-tolerant species is used to restore vegetation at contaminated sites, thereby decreasing the potential migration of pollutants through wind erosion and transport of exposed surface soils and leaching of soil contamination to groundwater. Phytostabilization can be achieved via sorption of heavy metals followed by its precipitation, or its complexation via metal valence reduction. It is usually applied in the treatment or removal of lead, cadmium, copper, zinc, chromium, and arsenic.

Factors Affecting Uptake Mechanism

Soil Factors

Many soil factors like water holding capacity, heavy metal content, soil pH, organic content affect the accumulation of metals in plants. Generally, only a fraction of soil metal is readily available (bioavailable) for plant uptake since the bulk of soil metals are commonly found as insoluble compounds unavailable for transport into roots [71]. The nature of soil determines the metal solubility and availability and is strongly affected by soil pH, which is considered as the major factor influencing the availability of elements in the soil for plant uptake. A lower soil pH increases the concentration of heavy metals in the solution by decreasing their adsorption. In soil, the solution concentrations of metal contaminants tend to increase with decreasing pH because of their displacement from exchangeable sites on solid surfaces by increasing the activity of hydrogen ions as there is a decrease in pH. Many metal cations like Cd, Cu, Hg, Pb, and Zn are reported to be more soluble and available in the soil solution at low pH (below 5.5) [65]. The organic content of the soil also has a strong bearing on the extent of the phytoextraction of heavy metals. Narwal, et al. [72] reported that the addition of organic material (peat and manure) in the soil leads to increase accumulation of heavy metals like Cu, Zn, and Ni in the wheatplants.

Microorganisms

Many microorganisms have the ability to secrete some chelating compounds known as siderophores [73] which may solubilize Cd [74] at moderate pH range [75]. A bacterium can produce many types of siderophores, including hydroxamates and carboxylic acids [76]. Microorganisms may also decrease Cd solubility by the formation of insoluble metal sulfides and also lowers the sequestration of the toxic metal via the cell walls or by proteins and extracellular polymers, etc [77,78].

Genetic Factor

Plant genotype is considered as the most important factor affecting heavy metal uptake by plants. Some genotypes respond positively to increased heavy metal concentration in soil, while others may be inert or show negative growth.

Addition of Chelating Agent

The increase of the uptake of heavy metals by the energy crops can be influenced by increasing the bioavailability of heavy metals through the addition of biodegradable physicochemical factors such as chelating agents, and micronutrients, and also by stimulating the heavy-metal-uptake capacity of the microbial community in and around the plant. This faster uptake of heavy metals will result in shorter and, therefore, less expensive remediation periods. The use of chelating agents in heavy-metal-contaminated soils could promote leaching ofinto the soil.

Environmental Condition

Temperature is also one of the important factor influencing phytoremediation as it controls many physiological processes like absorption of water from root hair, the ascent of sap, transpiration, growth, uptake, and elimination of pollutants [79]. It was found that with an increasein temperature, the rate of elimination of metal also increases [80]. Baghour, et al. [81] mentioned that high temperature promotes the Cr uptake by potato plants as compared to plants grown at low temperatures.

Effectiveness of Heavy Metal Uptake by Plants

A number of studies have confirmed the usage and efficiency of plants as heavy metal accumulators from the contaminated source whether soil or water. Literature studies have shown the practice of using phytoremediation as an alternative approach to remediate and clean the target sites. Crop plant (*H. annuus*) was chosen in this study based on its high biomass, fast growth rate and its ability to remove heavy metals from contaminated soils. Phytoremediation can be an alternative solution as a green technology to treat heavy metal contaminated areas. As per literature studies, a number of plants are there with a substantial efficiency for the removal of heavy metals from the soil via the process of bioaccumulation. On the other hand, there are certain limitations to the phytoremediation system. Among them are being timed consuming method, the amount of produced biomass, the root depth, soil chemistry and the level of contamination, the age of the plant, the contaminant concentration, the impacts of contaminated vegetation, and climatic condition. Phytoremediation can be a time-consuming process, and it may take at least several growing seasons to clean up a site.

Heavy Metal Uptake by Plants

Numerous studies have demonstrated the potential of plants as bioaccumulators of heavy metals from contaminated sites. Literature studies have shown the practice of using phytoremediation as an alternative approach to remediate and clean the target sites. Various plants belonging to different families have been reported as an efficient bioaccumulators. The heavy metals taken up *via* roots are either translocated upward to shoots or stored in roots it. This process is governed by several factors that include vacuolar compartmentalization, phytochelatin production, metal exclusion, etc. [82]. Bakers, et al. in 1977 [83] gave the concept and criteria for plants as hyperaccumulators of heavy metals while growing in their natural habitat. The secondcriterion used to identify hyperaccumulators was based on Bio-concentration Factor and Translocation Factor values (>1) [84]. Hyperaccumulators are the plants with unique efficiency of tolerating and accumulating high content of heavy metals (both essential and non-essential) to levels exceeding those present in the soil or nearby growing plants. Throughout the world, about 400-500 plant species have been identified as hyperaccumulators belonging to families *Brassicaceae, Compositae, Caryophyllaceae, Leguminosae, Cyperaceae,*etc. [85,86]. Plants like *Pterisvittata* L., *Piricumsativum* L., *Pterisbiaurita*, *Pteriscretica*, *Pterisquadriaurita,* and *Pterisryukyuensis* have been reported as hyperaccumulators for As; *Azollapinnata*, *Eleocharisacicularis*, *Lemnaminor*L., *Oryzasativa* L., *Rorippaglobosa*, *Solanumphoteinocarpum*, *Thlaspicaerulescens*, and *Vettiveriazizanioides* L., has been reported as potential hyperaccumulators for **Cd**.; *Alyssumwulfenienum* Bernh.,*Arrhenatherumelatius* (L.) Beauv., *Chenopodiumalbum* L., *Cepaefolium* (Wulfen) Rouy & Fouc, *Euphorbiacheiradenia*, *Festucaovina* L., *Hemidesmusindicus* L., *Thlaspirotundifolium* (L.) Gaudinand*Vetiveriazizanioides* L. as hyperaccumulators of **Pb**; *Alyssumbertolonii*, *Alyssumcaricum*, *Alyssumcorsicum*, *Alyssumheldreichii*, *Alyssummarkgrafii*, *Alyssummurale*, *Alyssumpterocarpum*, *Alyssumserpyllifolium*, *Alyssumlesbiacum* (Candargy) Rech. f., *Agropyronelongatum* (Host.)P. Beauv., *Berkheyacoddii*, *Isatispinnatiloba*and*Lemnaminor* L. as hyperacumulators of **Ni**; *Brassicajuncea* (L.) Czern.,*Eleocharisacicularis*, *Elsholtziasplendens*Nakai ex Maekawa, *Festucarubra* L., *Lemnaminor* L., and *Vallisneria Americana* Michx. ashyeraccumulators of **Cu**; *Brassicajuncea* L., *Pterisvittata* L. and *Vallisneriaamericana* for **Cr;** *Berkheyacoddii* Roessler and *Haumaniastrumrobertii* (Robyns) P .A. Duvign. & Plancke for **Co**; *Agrostiscastellana* Boiss. & Reuter, *Phytolaccaamericana* L., and *Schima* superb as hyperaccumulator for **Mn**; *Marrubiumvulgare* L. and *Pistiastratiotes* L. as hyperaccumulator for **Hg** and *Brassicajuncea* L., *Cynodondactylon* (L.) Pers., *Cardaminopsis* spp., *Eleocharisacicularis* for **Zn** [4,87].

Herbaceous plants are the great candidate for the accumulation of higher concentration of heavy metals but it leads to a significant reduction in its biomass. Also, these are unable to accumulate an insoluble fraction of metals in soil via natural mechanisms. Therefore, to overcome these drawbacks, woody plants can become a very useful ecological solution for cleaning contaminated areas due to their good accumulation potential, fast and high biomass production [88,89]. Woody plants can prevent down leaching of heavy metals in the soil, soil erosion by water, dispersion by wind combined with providing economic benefits [90]. Phytoextraction through woody plants enables the recovery of contaminant sites and can significantly reduce the cost factors. The fast-growing trees such as *Salix* sp. (willow) and *Populus* sp. belonging to Salicaceae have been identified as a promising candidate for treating heavy metal contaminated sites [7-9,91]. However, comparatively long duration of the process, enhanced absorption of pollutants in shallow layers within the rhizosphere, and the possibility of propagation of contaminated plant organs (leaves) combined with the risk of spread of contaminants to other sites are the drawbacks of this approach.

Application of Tissue Culture in Phytoremediation

In contrast to traditional methods used for removal of contamination from specific sites, phytoremediation provides a cleaner, cost-effective and eco-friendly approach. It has been proven to remove inorganic as well as organic pollutants from the environment. On the basis of the type of mechanism involved by plants to remove pollutants can be classified further as phytoextraction, phytostabilization, phytovolatilization, rhizofilteration, etc [92]. Uptake of pollutants by plants results in various physiological processes to occur involving various metabolic pathways, enzyme actions, and other unknown mechanisms. Due to this complexity, hairy root cultures provided a great option to unravel the unknown mechanisms involved or responsible for toxicity tolerances [93]. In the last few years, hairy root cultures have immensely enhanced our knowledge about the complex molecular as well as biochemical mechanisms involved in phytoremediation. It allows us to study the detoxification potential of the plant without the interference of soil matrix and microbes combined with short sub cultivation period and good biomass production. The subculture period varies from 2-3 weeks without any dependence on seasonal effects including photoperiods, frost, heat, etc. It also provides a microbial free environment and allows to differentiate responses of rhizospheric microbes with the other in the vicinity of the rhizosphere. Further, hairy root cultures providean opportunity to incorporate contaminants in high concentrations followed by efficient recovery of the metabolites produced for analysis [94]. Uptake of pollutants through sorption is the first step towards phytoremediation and it varies for inorganic and organic pollutants [95]. There are no specific transporters for organic pollutants in plant membranes, so its uptake occurs through diffusion. Followed by uptake, the plants convert them into their less toxic form through the process termed as phytodegradation/phytotransformation. Polychlorinated biphenyls are chlorinated aromatic compounds that have a large scale application as flame retardants, heat transfer fluids, hydraulic lubricants, etc. But its improper disposal has led to its leak in the environment posing threat to the ecosystem. It has even reported being accumulated in living organisms [96]. Using phytoremediation, Morita, et al. showed the enhanced potential of hairy root cultures of *Atropabelladonna* to metabolize in comparison to its natural root system [97]. Later, extensive work supported the enhanced potential of hairy root cultures with callus culture as well as normal roots in other plant systems [98,99]. In another study, phytoremediation has contributed to the removal of 2,4,6-trinitrotoluene (TNT), a persistent explosive compound using *Catharanthusroses*(periwinkle) converting it into its non-toxic derivative dinitroamino-derivatives using hairy root cultures [100]. It also revealed that there was no role of microbial or symbiotic relationships involved in the phytodegradation of TNT indicating the role of different enzymatic systems, with variable substrate specificity depending upon different plant species. Thus, the hairy root culture approach can be an excellent candidate for studying the transformation of toxic compounds to non-toxic forms. If a compound is metabolized by *invitro* hairy root cultures, it clearly indicates the efficiency of a plant has the genetic capacity to biotransform the toxic compound [94]. Similarly, *Armoraciarusticana* (horse radish) hairy root cultures have been demonstrated to metabolize 2,4-dinitrotoluene (DNT), 2,4,6-trinitrotoluene (TNT), diaminonitrotoluenes (DANTs) and aminodinitrotoluenes (ADNTs). The study clearly revealed the role of enzymes in pollutant metabolism including glutathione *S*-transferase (GST) and peroxidase (Px). Such studies can help in elucidation of various transformation pathways that can help in the efficient removal of these explosives from the environment. Another area is pharmaceutical industries, their metabolites are often detected in the environment due to wrong disposal practices such as *N*-acetyl-4-aminophenol (paracetamol) and some antibiotics were found to be harmful to the aquatic system. A study conducted in 2009 on the metabolism of paracetamol (antipyretic agent) using hairy root cultures of *Armoracia rusticana* L. It was revealed that the *invitro* hairy root cultures were able to take up and detoxify paracetamol converting it into paracetamol–glucoside [101]. Further, *Helianthusannus* (sunflower) hairy root cultures were found to be able to remove tetracycline and oxytetracycline from aqueous media [102]. Removal of textile dyes through phytoremediation has been one of the most neglected areas of research. The knowledge regarding basic mechanisms and pathways involved in this aspect is limited [102]. Hairy root cultures from Marigold (*Tagetespatula* L.) (Reactive Red 198), *Brassicajuncea* (textile dyes, phenol), *Solanum aviculare* (phenol, chlorophenol), *Brassica napus*, *Solanum Lycopersicon*, and *Nicotianatabacum* (phenol and 2,4-DCP) were found to be efficient in removing the contaminants from the aqueous medium [95,103,104]. Thus from the above discussion, we can conclude that hairy root cultures are greatly helpful in understanding the various physiological and biochemical processes suggesting underlying mechanisms for phytotransformation.

Conclusion

It is a well-known fact that heavy metals in polluted soil are able to affect the physiological and biochemical characteristics of plant metabolism causing deteriorated growth. Althoughto some extent, these heavy metals may be crucial for the plant when no beneficial role is involved, it leads to serious consequences. The natural and ecofriendly approach of bioremediation is a great alternative for the curing of the contaminated site as well as its restoration [105-109]. It can be of great importance in case of lands involved in crop production. Involving plants rather of microorganisms is a more common approach to remove heavy metals when compared with the use of microorganisms. Therefore, coalescing plants and microorganisms in bioremediation can intensify the efficiency remediation process. In context to this, mycorrhizal fungi have also been efficaciously amalgamated in many phytoremediation programs.

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