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A Review

Scope and future perspectives of phytoremediation

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SUMMARY

Phytoremediation is the plant-based technology that has emerged as a novel cost effective and ecofriendly technology in which green plants are used for extraction, sequestration and/or detoxification of the pollutants. Plants possess the natural ability to degrade heavy metals and this property of plants to detoxify contaminants can be used by genetic engineering approach. Currently, the quality of soil and water has degraded considerably due heavy metal accumulation through discharge of industrial, agricultural and domestic waste. Heavy metal pollution is a global concern and a major health threat worldwide. They are toxic, and can damage living organisms even at low concentrations and tend to accumulate in the food chain. The most common heavy metal contaminants are: As, Cd, Cr, Cu, Hg, Pb and Zn. High levels of metals in soil can be phytotoxic, leading to poor plant growth and soil cover due to metal toxicity and can lead to metal mobilization in runoff water and thus have a negative impact on the whole ecosystem. Phytoremediation is a green strategy that uses hyperaccumulator plants and their rhizospheric micro-organisms to stabilize, transfer or degrade pollutants in soil, water and environment. Mechanisms used to remediate contaminated soil includes phytoextraction, phytostabilization, phytotransformation, phytostimulation, phytovolatilization and rhizofiltration. Traditional phytoremediation method presents some limitations regarding their applications at large scale, so the application of genetic engineering approaches such as transgenic transformation, nanoparticles addition and phytoremediation assisted with phytohormones, plant growth-promoting bacteria and Arbuscular mycorrhizal fungi (AMF) inoculation has been applied to ameliorate the efficacy of plants for heavy metals decontamination. In this review, some recent innovative technologies for improving phytoremediation and heavy metals toxicity and their depollution procedures are highlighted.

Key Words : Decontamination, Hyper-accumulator plants, Heavy metals, Phytoremediation

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The quality of soil is degraded day by day due to the complications associated with heavy metal pollution. Heavy metal pollution is of global concern and a major health threat worldwide. Approximately 300 years ago the technology Phytoremediation was used for the treatment of waste water (Hartman, 1975). Baumann in the year 1885, firstly documented two plant species, *viz., Thlaspi caerulescens* and *Viola calaminaria*, which accumulate high concentrations of heavy metals in leaves. Byers, 1935 analysed the dry shoot biomass of the genus *Astragalus* and findings revealed that it has the capacity to accumulate selenium (Se) concentration up to 0.6%. Then in 1948, more plants were identified as accommodating Nickel (Ni) concentration up to the level of 1% (Minguzzi and Vergnano, 1948). Rascio, 1977 reported *Thlaspi caerulescens* possesses a high resistance to Zn and accumulates high Zn concentration in its shoots. Phytoremediation is a green strategy that uses hyperaccumulator plants and their rhizospheric microorganisms to stabilize, transfer or degrade pollutants in soil, water and environment (Liu S. et al.,2020) This technology is considered as well-efficient, cheap and adaptable with the environment (Nedjimi, 2020). According to the soil conditions, pollutant and the species of plants used, five types of phytoremediation have been applied: phytodegradation, Phytofiltration, phytoextraction, phytostabilization and phytovolatilization. Plants were classified to be tolerant and/or hyperaccumulator to Heavy Metals (HMs) when they show rapid growth, high biomass and are capable to extract and accumulate high amounts of HMs in their shoots, without signs of toxicity when grown in contaminated soils (Table 1). Hence this green technology can be very useful for remediation of HMs contaminated soils/agro-ecosystems. Plant hyper-accumulators have received greater attention in recent decades, due to its potential to HMs contamination. However, there are some limitations for these plants to become efficient at large scale. These limitations need to be overcome by transgenic approach applications to improve HMs tolerance/accumulation of these plants (Rai *et al.*, 2020). This critical review describes the effective mechanisms of phytoremediation, the promising potential of hyperaccumulator plants and the biotechnological approach for HMs decontamination.

Table 1 : Some potential species for phytoremediation

Sr. No.	Species	Heavy metals	Reference
1.	*Acacia nilotica*	Cd	Shabir *et al.* (2018)
2.	*Atriplex lentiformis*	Cd	Eissa and Abeed (2019)
3.	*Boehmeria nivea*	Cd	Pan *et al.* (2019)
4.	*Canna indica*	Cd	Solanki *et al.* (2018)
5.	*Lagerstroemia indica*	Cd	Wang *et al.* (2016)
6.	*Atriplex halimus*	Zn	Lutts *et al.* (2004)
7.	*Brassica Juncea*	As	Ko *et al.* (2008)
8.	*Pteris vittata*	As	Wang *et al.* (2002)
9.	*Berkheya coddii*	Ni	Robinson *et al.* (1997)
10.	*Salix viminalis*	Ni	Korzeniowska and Stanislawska-Glubiak (2019)
11.	*Genipa americana*	Cr	Santana *et al.* (2012)
12.	*Pistia* sp.	Cr	Mondal and Nayek (2020)
13.	*Dalbergia sissoo*	Cu/Ni	Kalam *et al.* (2019)
14.	*Helianthus annuus*	Cu/Pb	Forte and Mutiti (2017)
15.	*Hydrocotyle ranunculoides*	Cu/Zn	Demarco *et al.* (2018)
16.	*Lathyrus sativus*	Cd/Pb	Abdelkrim *et al.* (2019)
17.	*Ludwigia peploides*	Pb/Zn	Fernandez San Juan *et al.* (2018)
18.	*Noccaea caerulescens*	Zn/Cd/Ni	Kozhevnikova *et al.* (2020)
19.	*Spartina sp.*	Hg	Tian *et al.* (2004)
20.	*Suaeda fruticose*	Cd/Cu	Bankaji *et al.* (2015)
21.	*Thlaspi caerulescens*	Zn/Pb/Cd	Banasova *et al.* (2008)
22.	*Usnea amblyoclada*	Pb	Carreras *et al.* (2005)
23.	*Haumaniastrum robertii*	Co	Kabeya *et al.* (2018)
24.	*Vetiveria zizanioides*	Cd/Cu	Kumar *et al.* (2018)
25.	*Solanum nigrum*	Cd	Wei *et al.* (2013)

Last, some recent innovative technologies for improving phytoremediation and future prospects like over expression of foreign genes in non-tolerant plants, nanoparticles (NPs) addition and phytoremediation assisted with phytohormones, microbial and AMF inoculation are presented. Finally, it should be kept in mind that phytoremediation is an interdisciplinary area of research where plant biology, microbiology, soil science, genetic engineering and environmental modelling converge. In addition, there is a novel phenomenon known as phytomining, one of the aspects of green technology that utilizes metal-hyperaccumulating plant species to extract metal ores generally termed as bio-ore from soil.

Phytoremediation techniques:

For removal of different hazardous compounds from contaminated soil and water, plant potentials have been exploited that resulted in several technological subsets (Fig. 1). Schwitzguebel (2000) has defined the following techniques: Phytoextraction is the use of pollutant accumulating plants to remove pollutants like metal organics from soil by concentrating them in harvestable plant parts. Phytostabilization is the use of plants to reduce the mobility and bioavailability of pollutants in the environment, thus preventing their migration to groundwater or their entry into the food chain. Phytotransformation is the degradation of complex organic to simple molecules or the incorporation of these molecules into plants tissues (Fig.3). Phytostimulation is plant-assisted bioremediation or the stimulation of microbial and fungal degradation by release of exudates/ enzymes into the root zone (rhizosphere). Phytovolatilization is the use of plants to volatilize pollutants or metabolites. Phytodegradation is enzymatic breakdown of organic pollutants such as trichloroethylene (TCE) and herbicides, both internally and externally and through secreted plant enzymes. Rhizofiltration is the use of plant roots to ab/adsorb pollutants, mainly metals, but also organic pollutants, from water and aqueous waste streams. Dendroremediation is the use of trees to evaporate water and thus to extract pollutants from the soil.

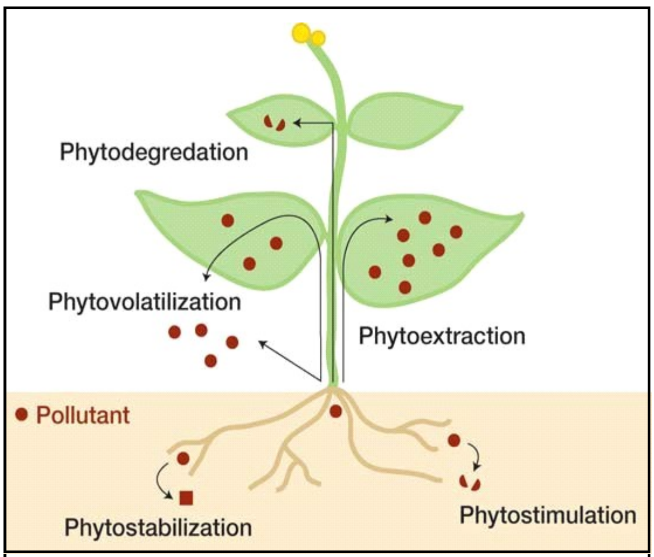

Fig.1: Possible fates of pollutants during phytoremediation: the pollutant (represented by red circles) can be stabilized or degraded in the rhizosphere, sequestered or degraded inside the plant tissue, or volatilized (Pilon Smith, 2005)

Limitations of phytoremediation:

Phytoremediation offers a low-cost, low maintenance, environment-friendly and renewable resource for the remediation of contaminated environments. In some situations, for example pesticide-laden soil or ground water, phytoremediation may be the only practical and economical in situ technique that can be used to remove pollutant chemicals. However, phytoremediation has its own limitations. The uptake and translocation of organic pollutants from soil through the plant root may be limited to compounds that are not highly hydrophobic, and through foliage to compounds that are not hydrophilic. Also, the technology would probably have little impact in situations where low levels of a pesticide were widely distributed in the environment. Even when large amounts of pesticides were present in a given environmental compartment, such as soil or water, phytoremediation could at best be useful as a long-term strategy. This is because plant growth is dependent on a number of environmental and climatic factors, availability of water and nutrients, soil type and pH, etc. Even under the most favourable conditions, plant growth and removal/ degradation of pesticides may not exceed a certain rate. These limitations require integration of phytoremediation with other more immediate clean up options, as well as more economical utilisation of the biomass produced for non-food purposes, for example energy generation. However, the role of vegetation and plant biomass as an absorbent and a long-term sink for diffused pesticides must not be underestimated. There is also a need for finding ways to enhance the absorption and degradation of pesticides in plants. As microbial activity in the rhizosphere is known to aid the release of bound pesticide residues in soil, which can enhance uptake and transformation by plants, a combination of microbial bioremediation and phytoremediation is likely to be more successful in the field. Whilst the advancement in biotechnological sciences has revolutionised a number of areas of conventional science, this has had a little impact on phytoremediation until now. The amount of knowledge generated in plant biotechnology in recent years is enormous, and there are many more yet unexplored avenues that can be pursued to engineer or enhance a particular pathway for the phytodegradation of persistent pesticides in the environment.

Technologies for improving phytoremediation:

The selected plant species with phytoremediation potential have few limitations, such as slow growing, which limit rapid and large-scale applications of these plants (Sarwar *et al*., 2017) and adaptation to a variety of environmental conditions like nutrient-poor soils (Gerhardt *et al*., 2017). Hence, to minimize these limitations, a strategy is developed through modifying and improving certain traits of these plants to ensure their ability for effective phytoremediation. Traditional breeding (plant hybridization) or genetic engineering (creation of transgenic plants) are employed to either improve growth rate and biomass of hyperaccumulator or introduce hyperaccumulation traits to fast growth, high biomass plants (DalCorso *et al*., 2019). Brewer *et al*. (1999) used electrofusion to fuse protoplasts isolated from the Zn hyperaccumulator *T. caerulescens* and *Brassica napus*. The selected somatic hybrids which have enhanced hyperaccumulation capability and tolerance derived from *T. caerulescens* and higher biomass production derived from *B. napus* (Brewer *et al*., 1999), showed the ability to accumulate high levels of Zn and Cd. This study indicated that transfer of the metal hyperaccumulation trait to high biomass plants is feasible through somatic hybridization. Similarly, Nehnevajova *et al*. (2007) used chemical mutagen ethyl methanesulfonate (EMS) to treat sunflowers and obtained sunflower "giant mutant," which exhibited a significantly enhanced heavy metal extraction ability with 7.5 times accumulation for Cd, 9.2 times for Zn, and 8.2 times for Pb compared to control plants (Nehnevajova *et al*., 2007).

Genetic engineering has been proved as a promising technique for improving phytoremediation abilities of plants toward heavy metal pollution. As compared to the traditional breeding, genetic engineering has the advantages to modify plants with desirable traits for phytoremediation in a much shorter time. Moreover, genetic engineering can even transfer desirable genes from hyperaccumulator to sexually incompatible plant species, which is impossible to achieve through traditional breeding methods such as crossing (Berken *et al*., 2002; Marques *et al*., 2009). Therefore, using genetic engineering to develop transgenic plants with the desired traits has shown attractive prospects in the field of phytoremediation (Fig.2). Technically, modifying fast-growing, high-biomass species to obtain high tolerance and high heavy metal accumulation ability is more applicable than engineering hyperaccumulators to get high-biomass production. Hence, in most applications, fast-growing, high-biomass plants are engineered either to enhance tolerance against heavy metals or to increase heavy metal-accumulation ability, which are the key properties of hyperaccumulators. Therefore, the selection of genes for genetic engineering should base on the knowledge of heavy metal tolerance and accumulation mechanisms in plants. Heavy metals may cause excessive production of ROS and result in oxidative stress, so heavy metal tolerance is usually manifested by the strength of oxidative stress defense system. Therefore, the most common strategy to increase heavy metal tolerance is to enhance antioxidant activity (Koz´min´ska *et al*., 2018), which can be achieved by overexpression of genes involved in antioxidant machinery. To increase heavy metal accumulation through genetic engineering, the common strategy is to introduce and overexpress genes that are involved in the uptake, translocation and sequestration of heavy metals (Mani and Kumar, 2014; Das *et al*., 2016). Hence, genes encoding heavy metal/metalloid transporters can be transferred and overexpressed in target plants to improve heavy metal accumulation. These genes encode metal ion transporters including ZIP, MTP, MATE and HMA. As metal chelators act as metal- binding ligands to improve heavy metal bioavailability, promote heavy metal uptake and root-to-shoot translocation as well as mediate intracellular sequestration of heavy metal ions in organelles, it is a promising strategy to increasing heavy metal accumulation by promoting the production of metal chelators via genetic engineering. By overexpression of genes encoding natural chelators, heavy metal uptake and translocation can be improved (Wu *et al*., 2010).

Although genetic engineering approach has shown attractive prospects on improving plant performance in phytoremediation of heavy metals, there are also a few setbacks that remain.As the mechanisms of detoxification and accumulation of heavy metals are very complicated and involve a number of genes, genetic manipulation of multiple genes to improve desired traits is time and effort consuming and usually not successful. Another issue is that genetically modified plants are difficult to gain approval for field testing in some areas of the world due to the risk raised on food and ecosystem safety. Therefore, alternative approaches are required to improve plant performance in phytoextraction once genetic engineering is impracticable. Another best alternative is using micro-organisms to improve plant performance.

Use of plant-associated microorganisms (rhizosperic microorganisms) is another approach to improve plant performance for phytoremediation. The microbial community of the rhizosphere may directly stimulate root proliferation and, thus, promote plant growth, increase heavy metal tolerance and plant fitness (Gupta *et al.*, 2013a; Fasani *et al.*, 2018). It has been shown that plant growth-promoting rhizobacteria (PGPR) have large potential to improve phytoremediation efficiency. PGPR can promote plant growth and fitness, protect plants against pathogens, increase plant tolerance to heavy metals, improve plant nutrient uptake as well as heavy metal uptake, and translocation (Ma *et al.*, 2011). This is achieved by producing various compounds, such as organic acids, siderophores, antibiotics, enzymes, and phytohormones (Ma *et al.,* 2011). PGPR can synthesize the 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which degrades the ethylene precursor ACC. Through producing ACC deaminase, PGPR is able to lower ethylene production, thus, promote plant growth (Arshad *et al.*, 2007; Glick, 2014). Plants inoculated with PGPR containing ACC deaminase showed enhanced biomass production as manifested by extensive root and shoot densities, resulting in enhanced uptake of heavy metals and increased phytoremediation efficiency (Huang *et al.*, 2004; Arshad *et al.*, 2007). PGPR can also produce bacterial auxin (IAA) to stimulate lateral root initiation and root hair development, thus promoting plant growth and facilitating phytoremediation (Glick, 2010; Dal Corso *et al.*,2019). Ji *et al.* (77) revealed that application of 10, 100 and 1000 mgL^{-1} gibberellic acid 3 (GA_3) can significantly increase biomass and phytoremediation efciency of Solanum nigrum. Recently Song *et al.* (180) demonstrated that the growth enhancement efect of supplemental ABA on Zn-stressed Vitis vinifera was due to the expression of ZIP and detoxifcation-related genes. Similarly, Leng *et al.* (102) found that supplementation of 5, 10 and 15 µm ABA alleviates adverse efects of Cd on the growth of mung bean (*Vigna radiate*) plants. Arbuscular mycorrhizal fungi (AMF) are another important microbial community that can assist plants for phytoremediation. The presence of AMF in rhizospheres increases the absorptive surface area of plant roots through the extensive hyphal network, thus, enhancing water and nutrient uptake as well as heavy metal bioavailability (Göhre and Paszkowski, 2006). AMF can also produce phytohormones to promote plant growth and aid phytoremediation (Vamerali *et al.*, 2010).

Earthworms known as 'ecosystem engineers' are the main group of soil macroinvertebrates. They play a vital role in organic matter decomposition, nutrient cycling and ameliorant of soil conditions. Wang *et al.* (2002) demonstrated that integration of earthworms in culture medium enhances the phytoremediation capacity of Cd in Solanum nigrum. Bongoua-Devisme *et al.* revealed that Pontoscolex corethrurus can alleviate Cr and Ni

Table 2 : Some plant growth promoting bacteria (PGRB) - assisted phytoremediation

Sr. No.	Name of bacteria	Host species	Heavy metals	References
1.	*Arthrobacter* sp.	*Glycine max*	Cd	Rojjanateeranaj *et al.* (2017)
2.	*Bacillus cereus*	*Vetiveria zizanioides*	Cr/Fe	Nayak *et al.* (2018)
3.	*Chaetomium cupreum*	*Miscanthus sinensis*	Al	Haruma *et al.* (2019)
4.	*Cupriavidus basilensis*	*Pteris vittata*	As	Yang *et al.* (2020)
5.	*Enterobacter sp. strain EG16*	*Hibiscus cannabinus*	Cd/Fe	Chen *et al.* (2017)
6.	*Mesorhizobium loti HZ76*	*Robinia pseudoacacia*	Pb/Zn	Fan *et al.* (2018)
7.	*Microbacterium arborescens*	*Leptochloa fusca*	U/Pb	Ahsan *et al.* (2017)
8.	*Pseudomonas libanensis*	*Helianthus annuus*	Ni	Ma *et al.* (2019)
9.	*Pseudomonas fuorescens*	*Trifolium repens*	Sb	Daryabeigi *et al.* (2020)
10.	*Streptomyces* sp.	*Salix dasyclados*	Zn/Cd/Pb	Zloch *et al.* (2017)

tolerance of Acacia mangium. Incorporation of Brassica juncea plants with Eisenia fetida earthworm enhances significantly the detoxification efficiency of Cd. Addition of vermicompost using Ensenia Andrei to HM-contaminated soil increases the ability of black oat (Avena strigosa Schreb) plants to remove Cd, Cr and Pb. Various plant growth promoting bacteria (PGRB) -assisted phytoremediation also enlisted in Table 2.

The strategies used to improve heavy metal phytoremediation, including genetic engineering, microbe-assisted, and chelate- assisted phytoremediation, are illustrated in Fig. 2.

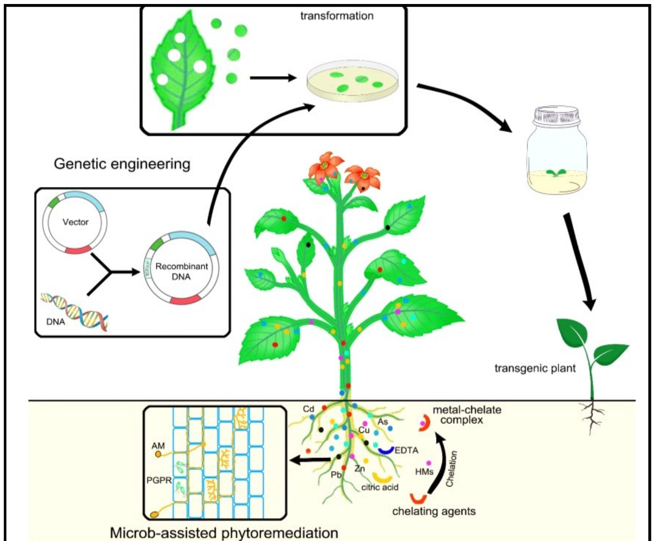

Fig.2: Schematic diagram illustrates strategies used to im prove phytoremediation

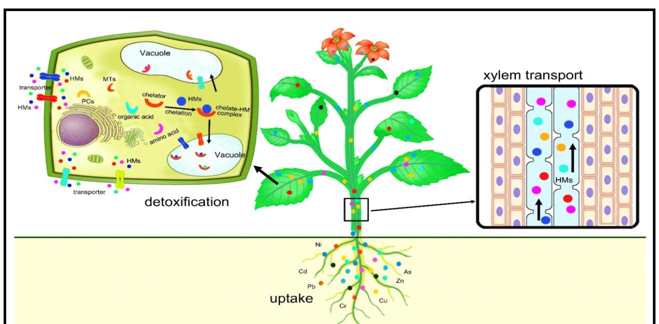

Fig. 3: Schematic diagram shows the uptake, translocation, and sequestration of heavy metals in plants

Future prospects and research needs:

Phytoremediation is one of the most promising techniques for the eco-rehabilitation of polluted sites, but further investigations and research are also necessary to enhance our knowledge inefficient phytoremediation of HMs. To avoid any failure during field cultures, it has become an essential requirement to tend toward future ways clarify mechanisms, metabolites and genes using latest techniques. These methods can aid in defining new metabolites and trails implicated in stabilization or extraction of HMs by hyper-accumulator plants. Utilization of hyper-accumulator shrubs and plants to remove HMs from soil requires novel strategies for its progress. This can be accomplished either by finding and valorising a vast diversity of new hyper-accumulator species or by genetic engineering. Multiplication and intensification of hyper-accumulator plants with higher translocation rates; high green biomass; or depth root system provide research about phenotypic differences between tolerant plants. Overexpression of foreign genes in non-tolerant plants for HMs depollution of soil is practicable. Although several plants and shrubs were established to clean up HMs through genetic engineering, no perfect model can be established for HMs detoxification until the obtainability of whole genome data is certified. Urgent works about the role of plant hormone such as IAA, GA cytokine were also needed to understand their role to increase potential of HMs detoxification plants. Plant–microorganism interaction (bacteria, fungi) was also an effective approach to uptake and translocation of HMs in plants. These approaches can aid in discovering novel metabolites and trails contributed in degradation of contaminants through plant–microorganism interactions Mishra I, Arora NK (2019). An additional advance technique is the study of hologenome of the microorganism of plants which could be applied in manipulation of microbial niches to increase resistance against HMs contamination Mueller UG, Sachs JL (2015). Nano remediation is a novel technology assisted by microbial cells integration to enhance remediation process more effectiveness to removing HMs from high contaminated soils Song *et al.* (2019) and Zhu *et al.* (2019). However, the relation between the molecular approaches of phytoremediation and nanoparticles needs to be clearly elucidated to expand the prospect of polluted soil remediation.

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