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Strategies for the management of toxic heavy metal contaminants through green clean technology for a sustainable ecosystem

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Abstract
Rapid urbanization, industrialization and modernization activities lead to extensive environmental problems and one of the most challenging problems is heavy metal contamination. Heavy metals are important as they cannot be broken down to non-toxic forms and are released into the environment by both natural and anthropogenic sources, especially, mining and industrial activities, and automobile exhausts (for lead). Heavy metals from the soil pass on to the ground water system which in turn causes unhealthiness for human health and environment thereby posing a threat for “Man and Biosphere” by contamination of food chain. In order to reduce the harmful effects, protocols are currently being used for decreasing heavy metal load into the food chain. Since, many of the conventional methods used for rectification are either inconvenient or involves heavy economics, a comparatively sustainable, promising, relatively new eco-friendly green technology has been developed for removal of heavy metals from contaminated sites by utilizing the plants, named phytoremediation. It includes phytoextraction, rhizofiltration, phytostabilization, phytovolatization, and phytodegradation or phytotransformation. Many of the common crops involving more than 400 species of sunflower, maize, mustard, barley, beet, bitter gourd, cauliflower, brinjal, chili, fenugreek, garlic, coriander, ivy gourd, lufa, lady’s finger, mint, radish, spinach, tomato and white gourd are very much in use for remediation of heavy metals. Keeping the importance of the topic in view, the present article deals with different approaches for containment of contaminants from the soil.

Keywords: heavy metals, phytoextraction, rhizofiltration, phytostabilization, phytovolatilization, phytodegradation, phytotransformation, phytorestoration

Introduction
The contamination of the environment by toxic metals as a result of modernization, urbanization and industrialization has become a global problem which affects the crop productivity, soil biomass and fertility contributing to bioaccumulation in the food chain. Due to high occurrence, as a contaminant the metallic compound can influence the quality of the atmosphere and surface water and also threaten the health of animals and humans upon entering into the food chain system. Generally these are heavy metals which are toxicants and cannot be broken down to non toxic forms and persist for a longer time (Jabeen *et al.*, 2009) [32]. Over the period of time the concentration of such metals has increased drastically due to industrialization and mining activities (Ana *et al.*, 2009) [5]. Such metals or metalloids of densities greater than 5g cm^{-3} are heavy metals which usually cause pollution and toxicity. But, this definition of heavy metals also varies depending on circumstances (Muhammad *et al.*, 2017) [53] Some of these heavy metals are essential elements required by the organisms at low concentrations. In addition, several metals have been classified as carcinogenic and mutagenic (Beyersmann and Hartwig 2008) [12]. The common heavy metals include: Chromium (Cr), Copper (Cu), Lead(Pb), Zinc(Zn), Cadmium(Cd), Mercury(Hg), Nickel(Ni),etc. Translocation of such toxic metals to foliage and even to the edible fruits is a matter of serious concern (Singh *et al.*, 2010a) [72].
Remediation of polluted soil has been a matter of concern that is why many remediation technologies such as soil flushing, pneumatic fracturing, solidification, vitrification, electrophoresis, chemical reduction, soil washing, excavation, etc. have been practised. Because of some limitations in their respective applications (expensive and environmentally destructive) these traditional methods were found unsuitable (Abourlroos *et al.*, 2006) [1].

And for the same reason scientists globally have been in search of an innovative, eco-friendly and low cost technology. Phytoremediation is one such technology which involves the green plants to clean the soil and cure the environment because the plants are very well known for their property to absorb, accumulate and detoxify the toxic components present in the soil, water and air through various physicochemical and biological processes (Hooda, 2007) [31].

Heavy Metals

The global accumulation of heavy metals in soils has emerged as a serious problem as a result of growing industrial activity all over. Mining wastes, paper mill wastes, smelting processes, utilization of agricultural chemicals, small scale industries like battery production, cable coating industries, brick kilns, coal combustion and toxic elements from atmospheric emissions have all contributed to the continuous deposition, resulting in accumulation of toxic metals in the environment. Due to the growth of various industries, approximately 1000 new chemicals are being synthesized every year and added to the market (Shukla *et al.*, 2010) [70]. Currently, around 60,000 to 90,000 different types of chemicals are in commercial use which result in soil pollution, as a result of which the concentration of toxic metals present in the polluted soil is almost hundred fold more than the requirement of the higher plants. Such toxic metals can have a prolonged existence in the soil and thus, can affect the biosphere for longer periods and can be leached through the soil surfaces and reach the underground water table to contaminate. In turn, plants use this contaminated soil and the use of these plants contaminated by heavy metals for food purposes cause a serious health hazard for the humans and animals.

Effect of Toxic Metals on Plants

Heavy metal when enters in the plant system can bring about different changes in the physiology and yield of plants. Different metals leave varied impacts on the plants such as: Cd containing soil results in chlorosis, inhibition of growth, root tip browning and ultimately death (Mohanpuria *et al.,* 2007) [51]. Similarly, as a result of heavy metal toxicity, it leads to stunted growth of plants, chlorosis of leaves and changes in the activity of several key enzymes of important metabolic systems. The metal stress also causes reduction in plant growth by causing low water potential, reduced nutrient uptake and secondary stress such as oxidative stress (Prasad *et al.* 2005) [61]. It can also disturb microtubule organization in meristematic cells and can also inhibit the growth of the roots (Bakos *et al.,* 2008) [10]. Ahmad *et al.* (2008) [3] reported regarding the undesirable growth effects of plants as a result of high translocation of such metals from the toxic soils. Reduction of photosynthetic activity of plants due to heavy metals has been reported due to the damaged ultrastructure of chloroplast (Prasad *et al*., 2005) [61]. Reduction of rate of photosynthesis was also reported due to the action of Cd which makes an impact on the stomatal conductance, chloroplast organization, chlorophyll biosynthesis, electron transport mechanism as well as alteration of enzyme action of carbon fixation mechanisms (Yordanova *et al.*, 2009) [86]. Action of Cu showed an increase in the inhibition of roots and leaves, dry biomass with the increase of time and concentration of availability of the metal (Upadhyay and Panda, 2009) [78].

These metals also cause major health problems in humans which include the onset of cognitive impairment, cardiovascular issues, anemia, development of malignancy, renal disfunction, neurological problems affecting brain and nerves (Muhammad *et al*., 2017) [53]. Teeth, bones and skin also reacts to these heavy metals causing damage and deformities (Ullah *et al.*, 2015a) [77].

The increased concentration of Cr results in the decrease in growth rate of *Hordeum vulgare* (Aery and Rana, 2003) [2]. The presence of Ni and Pb has an adverse impact on the plants, microbes and aquatics (Khan and Moheman, 2006) [38].

Path of movement and Transporters

Heavy metal contaminants are generally available in the soil in an insoluble state and are mostly not bioavailable. In order to make those bioavailable, plants release some substances thus helping the change of soil pH and solubility of the contaminants (Dalvi and Bhalerao, 2013) [18]. Now the metals which become bioavailable get absorbed at the root surface and tend to move across the cellular membranes of the root cells by either taking apoplastic way or through the symplast. The movement through symplast is an energy dependent process being regulated by some carriers or complex agents (Peer *et al*., 2005) [58]. When the heavy metals enter into root cells they bind with some chelators like organic acids and form many complexes such as phosphate, sulphate, carbonate, etc. These complexes get deposited at either the walls of apoplast or the compartments of symplast (Ali *et al*., 2013) [4].

Benefit of Phytoremediation research

Phytoremediation is a method of introduction of plants into the toxic environment and thereby allowing plants to assimilate the metals into their sub- and aerial systems. This way cleaning of heavy metals, pesticides and xenobiotics (Suresh and Ravishankar, 2004) [73], organic compounds (Newman and Reynolds, 2004) [56], toxic aromatic pollutants (Singh and Jain, 2003) [71] and acid mine drainage (Archer and Caldwell, 2004) [6] have been reported.

Today's phytoremediation is not a new development, rather this has been documented 300 years ago but the scientific analysis and specific plant study in relation to particular metals were not conducted till 1980s (Lasat, 2000) [41].

Why this technology is in lime light today is because it is an environment friendly technology which is quite safe and a cheap method of removal of contaminants. This technology is doing the job of a group of engineers very economically i.e., at one tenth of the cost of the engineers. But the major bottleneck of this technology is, this becomes less effective if the toxic metals run too deep into the soil or the concentration of contaminants is very high.

Methods of detoxification of contaminated soil

There are some conventional methodologies practised in the early days which seem to be uneconomical, non-technical and labor intensive processes. Some of those are discussed below:

a. **Washing of the soil:** This is a methodology where acids (HCI and HNO_3), chelators (EDTA, nitrilotriacetic acid, DTPA,etc.) and other anionic surfactant (bio surfactant) are used to solubilize the polluting metals (Neilson *et al.,* 2003) [55]. This process may either involve soil flushing with pumps (Neilson *et al.,* 2003) [55] or washing an excavated portion of the contaminated site with these agents followed by the return of clean soil residue to the site which is expensive and can have lots of adverse effects (Lone *et al.,* 2008).
b. **Excavation:** This method involves the removal of polluted soil with special landfills (Jing *et al.,* 2007) [33]

which is the most common way of reclamation but does not actually remediate the soil (Lombi *et al.*, 2001) [44].

c. Capping method: Since the contaminated soil is present in the upper strata of the soil surface this method helps in replacing the uncontaminated soil from the offsite and placing them in the soil as a cap which is also not a permanent solution as the toxic metals can still pass into the underground water column.

d. Chelating agents: There are chemicals, called chelating agents which make the availability of solid heavy metal contaminants water soluble complexes which are easily getting amenable to the plants from the soil either by phytoremediation or soil washing. EDTA (Ethylene diamine tetra acetic acid) is one such chelating agent which significantly increases the metal absorbing capacity of plants more specifically Pb (Luo *et al*., 2005; Tandy *et al*., 2004) [46, 74]. Studies reveal that EDTA is more potent compared to EDDS (Ethylenediamine-N,N'-disuccinic acid) for the Pb and Cd accumulation where as for Cu and Zn, EDDS is more effective (Luo *et al*., 2005) [46]. Further, the combination of both EDDS and EDTA surpass the individual efficiency of these chelating agents and could accumulate more of Cu, Zn, Cd and Pb in plants.

Besides these, there are some other methods available which are not found to be very successful and cost effective in the long run.

In contrast, some new eco-friendly sustainable technologies have developed which can easily combat the heavy metal availability and have been proved beneficial too and is called Phytoremediation.

Phytoremediation

This eco-friendly means help in removal, transfer and stabilization of different metals from contaminated soil areas (Garbisu *et al.*, 2010) [25]. There are some 500 plants identified as hyper accumulators of toxic metals which amount to only 0.2% of the total angiosperms available (Sarma, 2011) [67]. Mostly the members belonging to the families Fabaceae, Scrophulariaceae, Lamiaceae and Asteraceae are said to be good Cobalt hyperaccumulators (Baker *et al.*, 2000) [9], Brassicaceae for Nickel (Kramer, 2010) [40], Arsenic (Karimi *et al*., 2009) [35], Crassulaceae for Cadmium (Deng *et al.*, 2007) [20]. Apart from this, the other plants which are good phytoaccumulators belong to Cyperaceae, Cunoiuniaceae, Poaceae, Violaceae, Euphorbiaceae, etc.

The phytoaccumulator plants can accumulate toxic metals in their aerial part and also have the tolerance to such metals (Baker *et al*., 2000) [9]. Simultaneously, some plant hyperaccumulators are slowly growing with the production of less biomass. There are some macrophytes which can accumulate multiple toxic metals at a time i.e., multiple hyperaccumulators.

The aim of the phytoremediation is to utilize specific plants against particular heavy metals to detoxify the soil or water present in the rhizosphere. The phytoremediation technology is further subdivided into categories based on the processes and applicability: phytoextraction, rhizofiltration, phytostabilization, phytovolatization, phytodegradation or phytotransformation and phytorestoration.

i. Phytoextraction: This is also known as phytomining where specific planting is made which is known to accumulate toxic metals in the shoots and leaves of the plants from the sub soil area and those plants are harvested, thus removing the contaminant from the site. Due to such accumulations at the aerial shoots, this is also called phytoaccumulation. Generally the shoot biomass are subject to harvesting and they get disposed off at demarcated places or can be burnt for extraction of absorbed metals. This method of extraction is very economical because it would cost less than ten times per hectare cost of conventional remediation processes (Salt *et al.*, 1995) [66]. The contaminant level gets depleted with successive plantation of the phytoextracting plants (Vandenhove *et al*., 2001) [79]. Even ferns are of immense help as the contamination level of Ar gets reduced by the deposition in their tissues (Ma *et al*., 2001) [47]. Some plants such as Sesamum, Brassica, Sedum etc. have been found most suitable against some heavy metals. Mojiri (2011) [52] showed that for the heavy metal Pb, the corn plant can be a better hyperaccumulator and the accumulation of Pb is more in the roots of maize than the shoot.

However, there are some drawbacks which limit the phytoextraction efficacy such as: (a) phytovolatility of metals in soil, (b) ease of translocation of metals to the aerial parts of the plant.

ii. Rhizofiltration: It is a groundwater curing protocol where the toxic metals are absorbed by the plants which are getting concentrated and precipitated in the plant tissues, mostly roots. Both terrestrial and aquatic plants are resourceful for this cause to clear the low concentration contaminants. This is very similar to phytoextraction but the only difference is, in rhizofiltration the ground water is remediated but not the soil (Ensley, 2000) [22]. As rhizofiltration utilizes the root system of a plant, terrestrial plants with either fibrous or long, substantial roots are treated as efficient systems for this purpose (Raskin and Ensley, 2000) [63]. By this method the heavy metals which get absorbed mostly are Cr, V, Ni, Cd, Cu, Pb, As and many radionuclides like U, St, Cs, etc. Some of the known plant species utilized for this remediation include Indian mustard, spinach, rye, sunflower, maize and also pistia, water hyacinth, duckweed, etc.

Phytoremediation ability of *Ailanthus altissima*, *Salix viminalis* and *Phragmites australiswere* experimentally observedin irrigated waters with 10mg/l of hexavalent Cr (Ranieri and Gikas (2014) [62]. The *Phragmites* and *Salix* plants could absorb 56% and 70% Cr from the irrigated water, respectively and help significantly in the reduction of Cr content of the soil after 360 days. Cr were seen to be accumulated in the roots of the above plants but the accumulation was higher in *Phragmites* and *Salix* (1800 and 2029 mg kg^{-1}, respectively) but lower i.e. 380mg kg^{-1} in *Ailanthus* (Muhammad *et al*., 2017) [53].

iii. Phytostabilisation: This process aims at the reduction of the mobility of heavy metals in soil. The heavy metals present in the contaminated soil get absorbed and precipitated by the plants thereby reducing their bioavailability in the soil. (Jing *et al.*, 2007) [33]. This is mostly called in-place inactivation and is practised for the curing of soil, sediment and sludge. This absorption or adsorption in roots restricts the heavy metals to move into the underground water or air. Through this method, heavy metals like As, Pb, Cr, Cd, Cu, Zn etc. can be remediated.

iv. Phytovolatilization: This process utilizes plants to absorb heavy metals from the contaminated soil and convert it to the volatile form before releasing them into the atmosphere through transpiration (USPA, 2000). Before transpiration, heavy metals get modified as the contaminants enter the water column of the plant xylem before getting evaporated or volatilized into the surrounding air. Ghosh and Singh (2005) [29] reported that the primary use of phytovolatilization is to remove mercury, as during this process mercuric ion get converted to less toxic elemental Hg. This method has been successfully applied in the removal of tritium (3H), which is a radio isotope of Hydrogen. A number of plants like *Chara, Arabidopsis, Brassica*, etc. have been found to be useful in absorbing toxic metals and to volatilize them before release. It was observed that *Pteris vittata*, under a greenhouse Arsenic (As) contaminated soil, could remove 90% of the total heavy metal (Sakakibara *et al*., 2007) [65]. But, once the volatilization occurs and the toxic metals get released into the atmosphere nobody has control over the same and they can migrate to any area. Similar reports to these findings have been experimentally observed by Tangahu *et al*. (2011) [75].

v. Phytodegradation or phytotransformation

It is a process of enzymatic breakdown of the accumulated contaminants inside the plant tissues. Some enzymes such as halogenase, oxygenase, etc. are produced inside the plants that help in catalyzing the phytodegradation process (Wani *et al*., 2012). In other words, this can be explained as a process where degradation or breakdown of organic contaminants occur involving internal and external means being regulated by the plants (Prasad and Freitas., 2003) [59, 60]. The contaminants present outside the plants get converted to smaller units which get absorbed by the plants, which in turn, are broken down enzymatically inside the plant tissues for the use of the plants during their growth. Those plant enzymes responsible for such breakdown of organic contaminants in the soil get released from the plants to the rhizosphere and eventually these enzymes play vital roles in transformation of contaminants. Some of such enzymes detected from plant rhizosphere include dehalogenase, nitrilase, peroxidase, nitroreductase, laccase, etc. The major organic contaminants getting degraded by this means are ammunition wastes, trichloroethylene, some organic herbicides, insecticides and many inorganic nutrients (Schnoor *et al*., 1995) [69].

A "Green Liver Model" has been demonstrated to explain the behavior of plants as compared to a human liver while reacting with xenobiotics (i.e., foreign substance). As the human liver tends to increase polarity when it gets a signal of xenobiotic drugs, similar reactions occur in plants too, where the enzymes present increase their polarity towards the contaminants by addition of hydroxyl (OH) functional groups. This kind of reaction is called Phase-I metabolism. During Phase-I reaction in the human liver, the initial reactions are facilitated by the enzyme Cytochrome 450 (Yoon *et al*., 2008) [85] whilst some plant enzymes like nitroreductase reportedly performs similar functions (Wani *et al*., 2012) [81].

vi. Phytorestoration: This means bringing back or curing of contaminated soil to a fully functioning soil (Bradshaw 1997) [14]. More particularly in this section of Phytoremediation, the plants native to a particular area is utilized to make the site a natural land. Basically phytoremediation should aim at "to what extent has the decontamination been achieved?" It is because there is much difference between the removal of enough contaminants from the soil and making the soil contamination free so that it can be used once again i.e. bringing the soil from a contaminated state to an environmentally uncontaminated one. The aim of an ecologist is always to concentrate on how to bring the soil back to a legally acceptable level of contaminants. For this, it is recommended to use combinations of phytoremediation methods so that at a time different toxic wastes can be removed for getting optimum results in soil restoration. An ideal remediation system for this should be use of plants that can be good hyperaccumulators and plants which can boost the microbial activity for degrading organic contaminants (Wendy *et al*., 2005) [82].

vii. Metallophytes: A group of plants having a specialized mechanism for the tolerance of high concentrations of metals are referred to as metallophytes (Kavamura and Esposito, 2010) [36]. Such metallophytes population have been originated with the genetic ability based on the variations related to the quality of survival against the natural selection from the heavy metals. These plants can be variously named such as accumulators, excluders, indicators, etc. depending on the quantity of heavy metal deposition in them (Baker, 1981) [8]. The accumulation of heavy metals no doubt inhibits the growth potential of the plants. All heavy metals are neither evenly absorbed nor distributed amongst the plant systems equally indicates that the plants are metal specific and devoid of universal metal tolerance (Kavamura and Esposito, 2010) [36].

Expanding the horizon of bioavailability

In addition to selection of better performing plants, the phytoremediation also requires the maximum bioavailability of toxic heavy metals. It is because the presence of heavy metals in the soil does not confirm its availability to the plants for accumulation in plant tissues. Only those metals which remain in a soluble form in the soil get readily absorbed (Blaylock and Huang, 2000) [13]. As per the bioavailability of heavy metals, a classification can be made. The cheaply available ones include Zn, Ni, As, Cd, Se, Cu; medium available are Fe, Co, Mn whereas unobtainable ones are Cr, Pb (Prasad, 2003) [59, 60]. The bioavailability of heavy metals is completely dependent on some of the factors like inherent solubility, richness of soil, soil binding capacity of heavy metals, occurrence of chealting agents, pH of soil and activity of microbes in the soil (Wang *et al*., 2006) [80]. The less the bioavailability of these metals the lesser will be the phytoextraction.

The plants also devise its own methods to increase the bioavailability of metals in the soil such as lowering of pH by acidification of rhizosphere (Thangavel and Subbhuraam, 2004) [76] secretion of compounds which help in metal availability like phytosiderophores, organic acids, carboxylates, etc. (Padmavathiamma and and Li, 2012; Gerhardt *et al.*, 2009; Robinson *et al*., 2009) [57, 7, 64]

Interaction of microorganisms and bioavailability of metals

Plants and microbes present in the soil have a combined role in increasing the phytoremediating efficiency of the plants. Their presence in the rhizosphere not only acts as a stimulation for the healthy root development but also boosts the growth of plants and heavy metal tolerance (Fasani *et al*., 2018) [23]. This ability of the microbes to increase the plant growth and in turn, enhance of the capacity of phytoremediation has been studied by different workers and it has been found that this all-round increase of metal tolerance, plant growth, defence against pathogens, translocation, etc.

occur due to the interaction of plant growth promoting bacteria (PGPB). This gives an impression that PGPB does help in the promotion of some enzymes, antibiotics, organic acids, siderophores and phytohormones, etc. (Ma *et al*., 2011). These PGPB help in the lowering of the formation of ethylene, a known growth retarder. This is achieved by the formation of 1- aminocyclopropane-1-carboxylate (ACC) deaminase enzyme which is responsible for degrading the precursor of ethylene i.e., ACC. Thus, plant growth gets promoted by PGPB (Glick, 2014) [30] by way of root and shoot growth, increased metal uptake (Arshard *et al*., 2007) [7], lateral root as well as root hair formation (Dal Corso *et al*., 2019; Glick, 2010) [17] which facilitates better phytoremediation. Some fungi are also known to have roles in enhancing the phytoremediation efficiency of plants through better absorption process, bioavailability and nutrient uptake (Gohre and Paszkowski, 2006) [28]. A bacteria (*Burkholderia cepacia*) have been found effective in increasing the growth and metal uptake specially Zn, Cd and P in some hyperaccumulators such as *Sedum alfredii* (Li *et al*., 2007) [42]. Microorganism mediated mechanisms like CBA efflux pumps regulated by some proteins, cation diffusion facilitators and chromate proteins, NreB and CnrT resistance factors operate in the plants which help in the conversion of metals into their soluble and insoluble forms (Gadd, 2000) [24]. Similarly, there are some bacteria known which can lower the solubility of metals and their portability thereby reducing the phytotoxicity (Lasat, 2000) [41] which is compared to the biosurfactant production. The biosurfactants produced from *Pseudomonas aeruginosa* has been tested to remediate the soils contaminated with Cd and Pb (Juwarkar *et al*., 2007) [34]. *Enterobacter cloacae* and *Bacillus* strain QC 1-2 are successful in reducing the toxicity of Cr(VI) to less toxic Cr(III) (Campos *et al*., 1995) [16].

Drawbacks with the plants used in phytoremediation and probable remedies

The slow growth of the plants used in phytoremediation is a major bottleneck in their large scale application (Sarwar *et al*., 2017) [68] and adaptability to nonfertile soil (Gerhard *et al.*, 2017) [6]. Therefore, it becomes a challenge for plant scientists to modify the plants having characters such as abundant biomass production, sharp growth rate, well developed root system and increased capability of hyperaccumulation, so that they can meet the phytoremediation related challenges. These required modifications can be brought by employing either conventional breeding methods or genetic engineering (DalCorso *et al*., 2019) [17].

a. Application of Somatic hybridization and Mutation

Attempts have also been made in this regard to utilize the method of parasexual hybridization through protoplast fusion between a Zn hyperaccumulator *Thalpsi caerulescens* and high growth and biomass capable *Brassica napus* and resulted in the formation of somatic hybrids having the higher biomass and hyperaccumulation capability with respect to Zn and Cd metals (Brewer *et al*., 1999) [15]. This success amply indicates that such transfer of useful characters of phytoremediation is very much possible through somatic fusion. Likewise, a useful giant mutant was obtained by Nehnevajova *et al.* (2007) [54] in Sunflower by using ethyl methanesulfonate (EMS) which possessed much higher accumulation capability for the toxic metals like Cd, Zn and Pb.

b. Application of Genetic Engineering

Currently, genetic engineering is found to be an effective tool to incorporate a foreign gene in a target plant so that the desired transgenic can be obtained within a very short span. It has been proved that through such genetic modifications, the phytoremediation ability of plants can be enhanced to meet the growing challenges of metal pollution. Genetic engineering is a promising tool because it crosses easily the boundaries of sexual incompatibility and has been successful in transferring target genes to a genotype, which is otherwise impossible through conventional plant breeding methods (Marques *et al*., 2009) [50]. As far as the phytoremediation is concerned, the important characters of a plant that need to be genetically modified include: ability to grow speedily, production of abundant biomass to enhance the degree of accumulation, profuse roots, enhanced ability to tolerate toxic metals, etc.

It is known that the heavy metals can increase the production of ROS (Reactive Oxygen Species) which result in oxidative stress in plants. So it is required to devise mechanisms to improve the antioxidative stress management in plants and this can be achieved by the over expression of genes responsible for the antioxidative activity (Kozminska *et al*., 2018) [39]. The efficiency of hyperaccumulation of heavy metals can be strategically enhanced by the introduction of over-expressive genes responsible for absorption, translocation and sequestration (Das *et al*., 2016; Mani and Kumar, 2014) [19, 49]. Thus, the genes responsible for heavy metal transportation can be considered for transformation in the target plants and their overexpression will lead to the hyperaccumulation capacity build up of plants (Yan *et al*., 2020) [84]. Similarly, different chelators work as the metal binding ligands which are responsible for the heavy metal uptake and upward translocation in plants. So, over expressions of chelators though transgenes is an effective alternative which will certainly boost the heavy metal accumulation in the plants (Wu *et al*., 2010) [83].

Dominguez-Solis *et al.* (2004) [21] created one transgenic line of *Arabidopsis thaliana* where overexpression of cystein synthetase was marked which showed tolerance to the concentration of Cd and was a good hyperaccumulator particularly in the trichome regions instead of leaves. In contrast, in transgenic tobacco plant overexpression of cystein synthetase in cytosol or chloroplast was detected which was more tolerant to Se, Cd and Ni. The F_1 of these transgenic plants showed hyperaccumulation of Cd in the shoots by overexpression in cytosol as well as chloroplast (Kawashima *et al*., 2004) [37]. The γ-glutamylcystein synthetase (γ-ECS) transgenic plants were shown to have 2.5 to 3 times more hyperaccumulation capacity than their wild counterparts for Cu,Cr and Pb (Bennett *et.al*., 2003) [11]. In *A. thaliana* when transgenic plants were obtained by manipulation of overexpressing phytochelatin synthetase(PCS), the transgenics were found to be tolerant to As and can have more accumulation(20-30times) in contrast to the wild plant (Li *et al*., 2004) [43]. Therefore, genetic engineering sets ample opportunities in this sector which can increase the efficiency of the plants in phytoremediation.

Conclusion

Phytoremediation is an eco friendly technique to clean the contaminated soil by using either micro-organisms or the plant itself. This process aims at isolation, destruction, transportation or removal of organic and inorganic contaminants from the medium. This is gaining importance because the rapid accumulation of heavy metals in the soil environment is causing a threat to the agriculture and human health. It has been experimented that phytoremediation is capable of revegetating the heavy metal contaminated soil easily and at the same time have much more advantages over contemporary physicochemical approaches. This process is successful now, after the identification of hundreds of hyperaccumulator plants which serve to clean the soil contaminants. Since it is a natural process, it is time consuming and takes a longer period of time for soil reclamation especially in heavily contaminated areas. The potential of the hyperaccumulators to clean the soil is completely dependent on the rate of growth and the production of biomass. Hence, designing the best performing hyperaccumulators to meet this requirement is an important challenge before the present plant scientists. Thus, the significance of genetic engineering can not be overlooked here as this can bring immediate solutions by creating ideal plants through transformation procedures. The genes responsible for luxuriant growth, biomass production metal uptake, translocation, sequestration, etc. should be identified from the available germplasm to use in the better genetic manipulation for generating the best efficient hyperaccumulators. Similarly, mutagenesis and activity of microbes and chelating agents also play a significant role in this by increasing the bioavailability of heavy metals to plants and in enhancing soil health.

It is needless to mention that the heavy metals are natural constituents of the environment and due to the indiscriminate use by the modern society they have changed their geochemical cycles and biochemical balance. This balance is going to deteriorate further due to rapid modernization and industrialization and the pace of human life. So, a single lined approach is certainly not a panacea which can curb and control such contaminations, rather a unified approach involving multidisciplinary biotechnology, microbiology, genetic engineering only can be successful in generating some ideal best performing hyperaccumulators.

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