Heavy metal phytoremediation: Potential and advancement

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Abstract. Industrial activities lead to the release of different types of toxic metals into the environment. Phytoremediation has been established as one of the environmental-friendly and economical processes that have the potential for the remediation of industrial waste. Phytoremediation is used to extract metals from industrial effluents using *ex-situ* and *in-situ* treatments. Also, phytoremediation may be used to reclaim the polluted land resource for agricultural purposes. Moreover, this also prevents the bioaccumulation and biomagnification of xenobiotics from farming activities if carried out from polluted land. Phyto-mining can be done to recover and reuse the heavy metals from plant tissues after phytoremediation by plants. This study aimed to give a comprehensive review of recent research work in heavy metal phytoremediation.

Keywords: Bioaccumulation, biomagnification, contaminants, heavy metals, phytoremediation

INTRODUCTION

Pollution by heavy metals has a serious impact on the environment (Ali *et al.*, 2013). The high concentration of heavy metals has very harmful effects on human health, plants, and animals (Djingova *et al.*, 2000). Major sources of heavy metal in the soil are fertilisers, wastewater, sewage sludge, pesticides, urban soil waste, smelting of ores and parent materials (Mahar *et al.*, 2015). Apart from these sources, there are still many major sources that release heavy metals in huge amounts (Figure 1). Heavy metal pollution in land and water resources has an extensive deleterious effect on the ecosystem and food web (Gaur *et al.*, 2004). Some of the heavy metals are rapidly absorbed. Therefore, they have a huge impact on animal health. The toxicity of heavy metals is dose-dependent. Therefore as the *in vivo* concentration of heavy metal increases, the

toxicity to vital organs increases. Heavy metal toxicity has been implicated as a causative factor for serious disease conditions such as Alzheimer's disease, other mutagenic diseases, and cancer (Dorne *et al.*, 2011). Some harmful effects of heavy metals on human health are enlisted in Table 1.

Unlike organic substances, heavy metals cannot be degraded and accumulate in the environment for a long time. Conventional methods for metal removal are costly. It is difficult to control due to pollutant migration (Tangahu *et al.*, 2011). Many techniques such as microbial-remediation, physio-chemical sorption and nano-particle remediation help to accumulate heavy metals efficiently. However, phytoremediation is an economical and effective alternative to these techniques. These techniques

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are widely used in the sorption of not only heavy metals but also many other pollutants such as dyes, pesticides, and herbicides. The accumulation of heavy metals can be carried out by agro-waste materials, micro-organisms and plants, also known as phytoremediation (Panchal, 2020; Dixit *et al.*, 2015). Phytoremediation by algae is another evolving strategy to accumulate heavy metals from contaminated water bodies. It is found that some algae contain phytochelatin and metallothionein that made complex with heavy metals and then translocate them to vacuoles. Other techniques involve so many processes to remove heavy metals such as sedimentation, flocculation, complexation, cation and anion exchange, precipitation and microbial activity but the accumulation of heavy metals can be done directly by algae. So, the degradation of heavy metals via algae is another way to treat contaminated soil and water bodies (Chekrounand Baghour, 2015).

Plants accumulate heavy metals from the contaminated land. Nowadays, phytoremediation has thus gained importance for remediation of soil and water pollution due to its sustainability and cost-effectiveness (Sarwar *et al.*, 2017; Paz-Ferreiro *et al.*, 2014; Pandey *et al.*, 2015). Phytoremediation occurs through many different mechanisms such as phytoextraction, phytotransformation, phytostabilisation, phytodegradation, rhizofiltration, phytovolatilisation, and hydraulic control (Ghosh and Manchanda, 2019). The present review presents the latest developments in the field of phytoremediation of heavy metals.

Mechanisms of phytoremediation

Phytoremediation is an *in-situ* solar energy-driven remediation technology. Plants take up metals leading to their immobilisation and hence preventing their transport. It has been reported that the capability of phytoaccumulation of contaminants is higher in tropical regions because of their high prevailing climate factors that favour plant growth and induces microbial activity (Etim *et al.*, 2012). The properties of soil such as soil texture, soil pH, soil organic matter and redox potential play a key role in the accumulation of heavy metals from contaminated land (Laghlimi *et al.*, 2015; Yobouet *et al.*, 2010). The bioavailability of contaminants is another factor in phytoremediation. At low soil pH, the accumulation and mobility of metals cations increases. However, in alkaline soil pH condition, these metals are present in the form of metal oxide. As a result, the mobility of metals gets decreased (Semple *et al.*, 2003).

Plant enzymes and rhizosphere microbiota help to modify the soil properties. Clayey soil benefits the process of phytoremediation of heavy metals since it reduces the phytotoxicity of the heavy metals by named metal-organic complexation (Elekes *et al.*, 2014; Brown *et al.*, 2003; Sherene *et al.*, 2010; Sing *et al.*, 2013). The phytoremediation is accomplished through different mechanisms such as phytoextraction, phytotransformation, phytostabilisation, phytodegradation, rhizofiltration, phytovolatilisation, and hydraulic control (Kong *et al.*, 2017) (Figure 2).

Table 1. Permissible limits and toxic effects of heavy metals.

Heavy Metals	Permissible Limits (ppm)	Toxic Effect	References
Ag	0.10	Throat and lung infection, stomach pain	Hall <i>et al.</i> , 1992
As	0.01	Affect ATP synthesis and phosphorylation	Tripathi <i>et al.</i> , 2007
Hg	2.0	Brain damage, hair loss, autoimmune diseases	Neustadt <i>et al.</i> , 2007; Gulati <i>et al.</i> , 2010
Zn	0.5	Fatigue	Salzman <i>et al.</i> , 2002
Pb	15	Reduction in intelligence, risk of cardiovascular diseases	Salem <i>et al.</i> , 2000; Padmavathiamma <i>et al.</i> , 2007
Cu	1.3	Intestine irritation	Wuana <i>et al.</i> , 2011
Ba	2.0	Respiratory failure, gastrointestinal dysfunction	Jacobs <i>et al.</i> , 2002
Ni	0.2	Lung cancer, infertility and genotoxicity	Das <i>et al.</i> , 2008

Figure 1. Representation of sources of heavy metals.

Phytoextraction

Phytoextraction also has known as phytoaccumulation where heavy metals are taken up directly by the plants (heavy metals Pb, As, Zn and Cr). Heavy metals are stored in harvestable parts (root, shoot, stem, and leaf) of the plant (Kumar *et al.*, 1995). However, all plants are known to take up contaminants to some extent some of the plant species have been now recognised as hyperaccumulators. These plants accumulate heavy metals in their parts and yet unaffected by those toxic effects of the accumulated heavy metals. The plants get adapted to survive in the highly contaminated matrix (soil and water) (Suman *et al.*, 2018). Most of the hyperaccumulator plants belong to *Tiliaceae*, *Proteaceae*, *Rubiaceae* and *Brassicaceae* families. These hyperaccumulator plants varieties have been reported to accumulate different metals like Zn, Cd and Ni (Milner *et al.*, 2008). Some the important factors in phytoextraction are (i) plants variety, (ii) plant growth rate -higher the rate of growth higher is accumulation, (iii) to be applied *in situ* as a remediation tool the plant needs to be distributed widely, (iv) the site for hyperaccumulator plants should be chosen in such a way that they are separated from the fodder plants for herbivores so as not to disturb the food chain (Sarwar *et al.*, 2017).

Phytotransformation

Phytodegradation is another common name of phytotransformation. Plants take up the heavy metals through the cortical tissue of root due to their similarity with some essential micro-nutrient and use the apoplastic and symbiotic pathway to reach xylem. These heavy metals also increase water stress in plants by decreasing the transpiration rate and stomatal conductance (Sarwar *et al.*, 2017). So, in phytotransformation plants can degrade toxic molecules and then convert into the non-toxic molecule, and this made possible by different plant enzymes and enzyme co-factors (Sharma and Sachdeva, 2013). Different plant enzymes such as nitroreductase and nitrilase actively participate in the breakdown of toxic compounds. It also enhances microbial degradation in the rhizosphere (Leguizamo *et al.*, 2016). There are some species of plants like *Eurasian watermilfoil* and *hydrilla* that helps to treat polluted water bodies (McCutcheon and Schnoor, 2004). Dec and Bollag (1994) observed that plant species could degrade aromatic rings even in the absence of micro-organism. However, this mechanism is not effective for heavy metal remediation.

Phytostabilisation

It is also known as phytoimmobilization because, in this technique, plants can reduce the mobility of heavy metals. So, it could not reach the groundwater. This process takes place by the root zone of the plants. As the phytostabilisation technique restricts the heavy metals to move from soil to groundwater, it should be monitored regularly to get an efficient result. But this technique is not enough to restrict the mobilisation of heavy metals into the water. Other techniques should also be concerned (Erakhrumen and Agbontalor, 2007).

Rhizofiltration

The free-living rhizobacteria enter into roots and play different roles (i) helps to produce several hormones that stimulate plant growth (ii) release antibiotics so that plants could not be affected by diseases (Gupta *et al.*, 2017). Sobariu *et al.* (2017) reported that *Azobacter* microbial population increase the growth of *Lepidium sativa* plant in the presence of toxic heavy metals. The plant root

length was increased by 22%, stem length increased by 34%, and dry biomass was observed 53%. The plants are first grown in greenhouse nursery. After a satisfactory growth of plant roots, the plants are harvested and planted on the contaminated sites. Then these plants start to accumulate heavy metals from contaminated wastewater and reached saturation point, and after that again, these plants are harvested from the sites. Many plants such as tobacco, rye, spinach and Indian mustard have excellent capabilities to accumulate lead from contaminated water (Lebrun, 2001). The pH of water is an important factor in this process and is needed to be monitored regularly. Diazotrophic bacteria also increase the efficiency of phytoremediation. These bacteria convert metal to more bioavailable form by process of a redox reaction, leaching, and methylation. Diazotrophs also promotes the growth of plants by fixing nitrogen phytochrome synthesis and phosphorous solubilisation (Ullah *et al.*, 2015).

Figure 2. Mechanism of phytoremediation.

Phytovolatilization

Phytovolatilization is a subprocess of phytoremediation. In this process, plants after accumulating the heavy metals from the contaminated matrix and release them in the form of volatile compounds into the atmosphere through transpiration. Usually, this process takes place when plants are in their growing stage and take water and organic compounds for the same. During this process, plants convert high toxic metals into less toxic metallic compounds then release them into the environment. Some heavy metals like mercury, selenium and volatile hydrocarbons are occurring through phytovolatilisation (Kamusoko *et al.*, 2017). Dushenkov (2003) reported in their studies that plant species such as *N. tabacum*, *Tritium* and *liriodendron tulipifera* effectively absorb and volatilised Hg from highly contaminated soil.

Hydraulic control

In this process, the roots of the plants function as a pump drawing contaminated groundwater and prevent it from mixing with clean water sources (Muthusaravanan *et al.*, 2018). There are lots of plant species that show effectual results in the removal of heavy metals such as *Populus species* (Hirsh *et al.*, 2003), *Salix species* (Negri *et al.*, 2004) and *Eucalyptus species* (Ferro *et al.*, 2003).

Advanced techniques for phytoremediation

Metal hyper-accumulator plant species

Several varieties of plants have been reported to be accumulators of heavy metals from contaminated soil (Hao *et al.*, 2014). Many species of aquatic plants help in up taking of heavy metals from contaminated water (Rahman and Hasegawa, 2011). Some of the excellent examples are water hyacinth, duckweed, water fern, watercresses, and water cabbage. Young trees are known to accumulate heavy metals more rapidly as compared to the adult trees. Alvarado *et al.* (2008) studied that phytoremediation of Arsenic (As) using *E. Crassipes* species of plant is capable of accumulating up to 600 mg/ha/d from contaminated soil. *Solanum photeinocarpum* shows effective results in uptake of Cadmium metal (71%) (Zhang *et al.*, 2011). Barbosa *et al.* (2015) found that *Arundodonax L.* species of plant can

accumulate Zn metal up to 60% from contaminated soil. Altinozlu *et al.* (2012) reported that *Isatis pinnatilota* could remove inactive Ni from the soil very effectively up to 1441 mg/kg. Sukumaran *et al.* (2013) studied phytoremediation of lead (Pb), copper (Cu), arsenic (As) and cadmium (Cd). These metals were accumulated by four different types of plant species *Typhalatifolia*, *Eichhornia crassipes*, *Salviniamolesta*, and *Pistiastratiotes*.

All types of heavy metal accumulation were observed in both the root and leaf of a plant. Lead and Arsenic highly accumulated by *Eichhornia crassipes* species. But copper and cadmium were removed prominently by *Typhalatifolia* species. Ahmadpour *et al.* (2014) took four plants that are belonging to different species *Jatropha curcas*, *Acacia*, *Dyera costulata* and *Hopea odorata* in his study to remediate copper metal. *Hopea odorata* had the greatest potential for remediation of copper contamination metal. Garcia-Salgado *et al.* (2012) find out that *Corrigiola telephifolia* was able to accumulate arsenic metal from contaminated soil (up to 2110 mg/kg. *Hydrilla verticillata* plant was used to uptake Cadmium metal (He *et al.*, 2016). Pandharipande *et al.* (2016) study two aquatic plant species *E. crassipes* and *Azolla* to accumulate copper and chromium. *Azolla* was able to uptake both the metals (29% and 14% respectively). The accumulation was found to increase with time of exposure. *E. crassipes* uptake both metals up to 13% and 71% respectively. Niazi *et al.* (2017) observed that the addition of phosphate in the root of *Brassica napus* and *Brassica juncea* lead to an increase in the rate of accumulation of metals and made the plant more metal tolerant. In this study, both of these species were used for remediation of Arsenic metal from the contaminated soil. *Brassica could* accumulate as metal up to 75% (Table 2).

Ali *et al.* (2012) study the uptake of Cd, Pb, Cu, and Zn by *T. alexandrinum* plant species. The maximum removal was observed in plant roots and also in leaves, and stem in decreasing order. Zinc was the highest accumulated heavy metal in the study. Mojiri (2012) treated heavy metal contamination from municipal wastewater by using *Typhadomingensis* plant species. In 2005, Yanqun studied the phytoremediation capacity of 129 species of plants belonging to 50 families for

his experiment. All the plant species were grown on the lead-zinc mining area in Yunnan (China), from which 16 species of plants were chosen as a best-performing specimens. Out of those 16 species, 10 species were found to accumulate Pb, 4 species Zn and 5 species for Cd. *Corydalis pterygopetala* was found to accumulate all of the metals under the study. Turgut *et al.* (2001) worked on

synthetic chelators to investigate their ability in phytoremediation of Cd, Ni, and Cr contaminated soil. Ethylene diamine tetraacetic acid (EDTA) and Hydroxy ethylene diamine tetraacetic acid (HEDTA) were applied to the soil at a different dosage. After all the experiments it was concluded that EDTA increases 59 µg/plant and 42 µg/plant accumulation.

Table 2. Plant species reported for heavy metal sequestration.

Plants	Heavy metal sequestration	Reference
<i>Asteraceae</i>	Ni	Slatter <i>et al.</i> , 1998
<i>Brassicaceae</i>	Zn, Cd, As	Zhang <i>et al.</i> , 2017; Niaz <i>et al.</i> , 2017
<i>Violaceae</i>	Pb, Zn, Cd	Zhuang <i>et al.</i> , 2005
<i>Crassulaceae</i>	Pb	Chen <i>et al.</i> , 2013
<i>Poaceae</i>	Cr	Nalla <i>et al.</i> , 2012
<i>Fabaceae</i>	Se	Alford <i>et al.</i> , 2012
<i>Acanthus ilicifolius</i> L.	Cd	Shackira and Puthur, 2017
<i>E. crassipes</i>	As, Cu	Alvarado <i>et al.</i> , 2008; Ebel <i>et al.</i> , 2007; Pandharipande <i>et al.</i> , 2016
<i>Solanum photeinocarpum</i>	Cd	Zhang <i>et al.</i> , 2011
<i>Arundodonax</i> L.	Zn	Barbosa <i>et al.</i> , 2015
<i>Isatis pinnatifida</i>	Ni	Altinozlu <i>et al.</i> , 2012
<i>Eichhornia</i> sp.	Pb	Sukumaran <i>et al.</i> , 2013
<i>Typha</i> sp.	As, Cu, Cd	Sukumaran <i>et al.</i> , 2013
<i>Hopea odorata</i> (Merwansiput jantum)	Cu	Ahmadpour <i>et al.</i> , 2014
<i>Corrigiolatelephifolia</i>	As	Garcia-Salgado <i>et al.</i> , 2012
<i>Hydrilla verticillata</i>	As	He <i>et al.</i> , 2016
<i>Azolla</i>	Cr	Pandharipande <i>et al.</i> , 2016; Rai and Tripathi, 2009
<i>L. gibba</i>	As	Mkandawire <i>et al.</i> , 2005
<i>N. microphyllum</i>	As	Robinson <i>et al.</i> , 2006
<i>Pteris vittata</i>	As	Sakakibara <i>et al.</i> , 2010
<i>Liriodendron tulipifer</i>	Methyl-Hg	Greipsson <i>et al.</i> , 2011
<i>Trifolium alexandrinum</i>	Cd, Pb, Cu, Zn	Ali <i>et al.</i> , 2012
<i>Typhadomingensis</i>	Fe, Mn, Zn, Ni, Cd	Mojiri <i>et al.</i> , 2012
<i>Corydalis pterygopetala</i>	Zn, Cd	Yanqun <i>et al.</i> , 2005
<i>Sonchus asper</i>	Pb, Zn	Yanqun <i>et al.</i> , 2005
<i>Helianthus annuus</i>	Cd, Cr, Ni	Turgut <i>et al.</i> , 2001
<i>Thlaspi caerulescens</i>	Zn	Lasat <i>et al.</i> , 2000
<i>Salix schwerinii</i>	Cr, Zn, Cu, Ni, Petroleum hydrocarbons	Salam <i>et al.</i> , 2016
<i>Jatropha curcas</i>	Cd, Cr, Co, Ni	Luhach and Chaudhry, 2012
<i>Tagetes patula</i>	Cr, Pb, Co, Zn	Choudhary <i>et al.</i> , 2016
<i>Brassica juncea</i>		
<i>Arbuscular mycorrhiza</i>	Zn, Cu, Fe, Mn	Khan <i>et al.</i> , 2014
<i>Pteris vittata</i>	As, Pb	Sun <i>et al.</i> , 2014
<i>Pteris critical</i>		
<i>Lavandula vera</i> L.	Pb, Zn, Cd	Angelova <i>et al.</i> , 2015
<i>Glyceria maxima</i> Phragmites australis typhalatifolia	Zn, Fe, Mn, Ni, Cu	Parzych <i>et al.</i> , 2015
<i>Phalaris arundinacea</i>		

Mixture of <i>Typhaangustifolia</i> sp. and <i>Limnocharisflava</i> sp.	Cd, Cr, Mn, Cu, Ni, Pb, Zn, Fe	Syukor <i>et al.</i> , 2016
<i>Trapanatans</i>	Cd, Cu	Bauddh <i>et al.</i> , 2015
<i>Elodea canadensis</i>	Fe, Co, Cd, Cu, Ni, Mn, Cr, Mo, Zn, Se	Miranda <i>et al.</i> , 2014
<i>Callitrichestagnalis</i> <i>Potamogeton</i> <i>Pectinatus</i> L.	U	Pratas <i>et al.</i> , 2014
<i>Phragmitescommunis</i> <i>Typha angustifolia</i> <i>Cyperus esculentus</i>	Cd, Cr, Cu, Mn, Fe, Ni, Pb, Zn	Chandra <i>et al.</i> , 2011

In 2000, Lasat used *Typha caerulea* plant species to accumulate Zn from contaminated sites. *Typha caerulea* accumulate 3% dry zinc in their shoot zone, but radiotracer flux techniques were used to track accumulation of zinc in the shoot and root of the plant. The accumulation of zinc was very high in the shoot area without any associated toxicity symptom. Salam *et al.* (2016) selected *Salix schwerinii* plant species to accumulate Cr, Zn, Cu, Ni and petroleum hydrocarbons. All the experiments were performed in pots, to estimate accumulation and plant growth performance under the high level of heavy metals. The soil used in the pot experiment was collected from the landfill area in Finland. The whole experiment was conducted in 141 days at 4 and 6 pH. The maximum accumulation of heavy metals was observed at pH 6. The total amount of Cr, Zn, Cu and Ni was 250.45 mg/kg, 1616.59 mg/kg, 223.74 and 75.90 mg/kg. The whole study investigates that *Salix schwerinii* has great potential to accumulate heavy metals from contaminated soil.

Luhach and Chaudhry (2012) accumulate heavy metals from oily sludge of petroleum refinery by *Jatropha curcas* plant species. All the experiments were planned to perform in growth media with sludge at six levels. The growth of *Jatropha* seeds was controlled in the first three experiments. The maximum plant biomass was observed at S1 and S2 treatment, whereas maximum multiple heavy metal removal was observed at S2 and S3 treatment. From the oily sludge maximum removal was observed of cadmium, followed by Cr, Cu and Ni. Choudhury *et al.* (2015) used Mustard and Marigold plants to accumulate heavy metals from Buriganga Riverbed sediment. Buriganga riverbed was contaminated with Cr, Pb, Cu and Zn. The maximum removal of all the heavy metals by the

mustard plant was observed 102.6, 28.9, 53 and 1861.5 mg/kg respectively whereas Marigold plant accumulates all the heavy metals up to 90%. Khan *et al.* (2014) used AM fungi in the accumulation of heavy metals of Zn, Cu, Fe and Mn and its effect on the yield of wheat crop. All the experiments were conducted in pots by CR design with four replications during 2012-13. It was observed from this study that the potential of phytoremediation of contaminated soil could be improved by the inoculation of crops with AM fungi. Angelova *et al.* (2013) conduct the field study to evaluate the efficiency of *Lavandula vera* L. in the accumulation of heavy metals from contaminated soil. The maximum accumulation was observed at the upper part of the plant that is stem, leaves and petals without harming the quantity and quality of the essential oil. Parzych *et al.* (2015) used four aquatic plant species that were *Glyceria maxima*, *Phragmites australis*, *Typhalatifolia* and *Phalaris arundinacea* to accumulate different heavy metals (Zn, Fe, Mn, Ni and Cu). *T. latifolia* maximum removed Zn and Ni whereas *G. maxima* highly accumulate Mn and Fe that observed in rhizomes of the plant. *P. australis* and *T. latifolia* plant uptakes Mn in their leaves at high concentration.

Syukor *et al.* (2016) focused on investigating the improvement in the conventional oxidation process integrated by aquatic plant species. The experiment was continued to 12-13 days. The maximum removal of Cu 79.07%, Mg 68%, Cd 61.07%, Cr 69.17%, Ni 74.87%, Fe 81.17%, Pb 62.07% and Zn 63% was observed. Miranda *et al.* (2014) used duckweed, elodea and water clover plant species to accumulate selenium from wastewater and also focused on producing biofuel from that waste. After the accumulation of selenium, some amount of bio-gas, bio-solid and

pyrolysis of these plants produce a large straight chain of c14-c20 alkanes that can directly use as diesel fuel. Chandra *et al.* (2011) used three plant species *P. communis*, *T. angustifolia*, and *C. esculentus* to accumulate Cd, Cr, Cu, Mn, Fe, Ni, Pb and Zn. *P. communis* were shoot accumulator plants whereas *T. angustifolia* was root accumulator plants. *C. esculentus* accumulate all the heavy metals by their root zone except Mn and Fe that was uptake by shoot and found in leaves. From this whole study, it was analysed that *P. communis* and *T. angustifolia* has great potential to accumulate heavy metals as compared to *C. esculentus*. Rodríguez *et al.* in 2018 compared the heavy metal accumulation by two plant species *B. juncea* and *S. lycopersicum*. It was observed that *B. juncea* was absorbed heavy metals in their root and shoot from that contaminated soil.

Plant-microbe interactions for enhancing phytoremediation

In recent years, scientists have focussed on discovering and establishing the diverse mechanisms of phytoremediation as detailed in the previous sections. Exciting research is being carried out to establish new plant-microbe interactions for enhancing phytoremediation of heavy metal contaminated sites (Saxena *et al.*, 2019). The plant growth-promoting rhizobacteria have been reported to improve host plant growth at metal-contaminated sites through the production of different growth hormones (auxins, cytokinins, gibberellins, ethylene, etc.) Arbuscular mycorrhizal fungi have been identified to protect the host plant from heavy metal toxicity and help in the phytoremediation process (Meier *et al.*, 2012; Khan *et al.*, 2014). Endophytes that reside in the inner tissue of the host plant and help in plant growth. It is highly tolerant of high heavy metal concentration and thereby helps in phytoremediation (Luo *et al.*, 2011; Ma *et al.*, 2015).

Enhancing the bioavailability of metals for uptake by plants

Several technologies have been developed that assist the plants in increasing phytoremediation or by protecting the plants from the toxic effects of the heavy metals. Vermicomposting is one such technique used to increase the phytoremediation process because earthworms are used from the

ancient period in the decomposition of heavy metals from soil. By using these two processes (phytoremediation and vermicomposting) efficient results could be obtained. Naturally occurring species such as aromatic plants and energy crops can grow in such contaminated sites that accumulate heavy metals very well. In order to enhance the metal uptake by plants, strategies such as the addition of chelating agents such as ethylenediaminetetraacetic acid (EDTA), ethylenediamine-N, N'-disuccinic acid (EDDS), humic substances have been successful (Luo *et al.*, 2018).

Genetically engineered hyper-accumulator plant

Genetic engineering of plant species with an objective to create hyperaccumulators for phytoremediation is an attractive strategy. Several researchers have successfully applied genetic engineering for overexpression of genes responsible for phytoremediation. However, these experiments are performed only on a small scale and tested to get desirable results (Koźmińska *et al.*, 2018). Table 3 summarises the genetically engineered plant species used for phytoremediation.

Pan *et al.* (1994) cloned the MT-1 gene from *Mus musculus* into *Nicotiana tabacum* plant to improve cadmium resistance. This genetically engineered plant could effectively remove shows very good results that were up to 10-200 µm. Similarly, modification of *Brassica napus* L. and *Nicotiana tabacum* plant by MT-2 gene obtained from *Saccharomyces cerevisiae* led to the improvement in their tolerance (KaËrenlampi *et al.*, 2000).

Genetic manipulation also showed a promising technique to enhance phytoremediation. The aim is to introduce metal tolerant genes into the plant tissues for a hyperaccumulation of heavy metals from highly contaminated sites (Sarwar *et al.*, 2017; Chandra *et al.*, 2015). Also, genetically modified bacteria in symbiotic plant-associated microbes relationship help in phytoremediation (Ullah *et al.*, 2015; Huang *et al.*, 2016). However, a major impediment in the studies involving genetically modified organisms is restrictions on large-scale field trials. This needs to be addressed using systematic technological innovations that ensure the safety of

the intervention in the field and also by changing the outlook of the policy-makers. However, comprehensive risk assessment studies need to be

undertaken before this technique can be used effectively.

Table 3. Hyperaccumulator transgene types and host plant species.

Transferred genes	Function of the transgene	Target plant	Heavy metal	Reference
NRAMP1	Spanning protein: Fe transporter in bacteria, fungi, animals and plants	<i>A. Thaliana</i>	As, Cd, Fe	Tiwari <i>et al.</i> , 2014
γ-ECS	1) Key enzyme GSH pathway. Overexpression of γ-ECS in <i>E.coli</i> increase Cd tolerance and accumulation	<i>P. tremula</i> <i>P. alba</i>	Cd	He <i>et al.</i> , 2015
NcZNT1	1) ZIP family transporter of Zn	<i>A. thaliana</i>	Zn	Milner <i>et al.</i> , 2012
AtABCC1 AtABCC2	1) ZIP family transporter 2) Accumulation of Cd 3) Double knockout of AtABCC1 and AtABCC2 enhance tolerance and accumulation of Hg	<i>A. thaliana</i>	Cd, Hg	Park <i>et al.</i> , 2012
SPDS1	1) Used to encode a functional protein by complementation of the spe3 yeast mutant that lacks SPDS 2) Increased abiotic stress of plants and also produced transgenic fruits	<i>Pyruscommunis</i>	Cu	Wen <i>et al.</i> , 2008
EhMT1	1) EhMT1 overexpression led to Cu accumulation in roots	<i>N. tabacum</i>	Cu	Xia <i>et al.</i> , 2012
SPMT1	1) SPMT1 similar to MT proteins hyperaccumulation of Cd in leaves of <i>Sedum plumbizincicola</i>	<i>Sedum plumbizincicola</i>	Cd	Peng <i>et al.</i> , 2017
MT-1	1) Induction by metal ion binding	<i>Nicotiana tabacum</i> L.	Cd	Pan <i>et al.</i> , 1994
MT-2	1) Human metallothionein – 2 gene 2) Increased accumulation of Cd	<i>Brassica Napus</i> L. <i>Nicotiana tabacum</i>	Cd	Misra and Gedamu, 1989

CONCLUSION

Phytoremediation is a commercially viable and promising technology that helps to accumulate heavy metals from contaminated land and water bodies. This solar-driven technique does not lead to the production of sludge; it doesn't lead to environmental disturbance and is cost-effective, making it an attractive technology to use. Although phytoremediation has lots of advantages and it helps to reduce soil pollution

caused by metalloids. However, the *in-situ* effectiveness of phytoremediation is heavily dependent on several factors like (i) climate of the region (ii) soil type (iii) plant capability to utilise metals. Therefore field trials are required to improve the accuracy of predictive models. Naturally occurring plants have very limited maintenance requirements and a low-cost process. In conclusion, it needs to be stressed that non-edible plants should be used to avoid bioaccumulation and biomagnification. Phytomining process can be implemented for the

recovery of the heavy metals by incinerating the metal accumulator crops. Large scale application of various phytoremediation techniques such as phytotransformation, phytoextraction, phytovolatilisation, and phytovolatilisation, etc. will be an excellent sustainable method for recycling and reusing of heavy metals to keep the environment safe, green, and resourceful for future generations.

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