Journal of Innovative Agriculture: 8(1) : 26-31, 2021
DOI: 10.37446/jinagri/ra/8.1.2021.26-31
Review paper

Phytoremediation - a green technology adapted to eradication of harmful heavy toxic metals from contaminated soil

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Received: 08 October 2020
Accepted: 15 March 2021
Published: 31 March 2021

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Phytoremediation is an alternative technology which makes use of biological processes for detoxifying the harmful pollutants in the environment. Rapid increase of industrialization and various other factors such as agricultural activities, the excessive use of fertilizers, untreated waste, and untreated laboratory effluents lead to degradation of soil as well as environment. The heavy toxic metals plays major role because these are basically crucial for development of plants. These are generally take part in various reduction and oxidation reactions, elementary role in metabolisms of nucleic acids, electrons transferring as a direct participant and being a fundamental part of some essential enzymes. The presence at a minimum threshold amount of these heavy toxic metals in a normal growing medium is vital, but excessive high amount lead to numerous lethal effects. Hence, it becomes our foremost duty towards sustainable development goals to eradicate the toxic harmful metal ions. Certain physical and chemical technologies are used to eradicate such toxins, but due to certain limitations, natural method is preferred which is plants-based technology for eradication of noxious heavy metals from contaminated soil. This technology used from last two decades to solve the problem of eradication of harmful metal ions through plants metabolic pathway in sustainable, environment friendly way. The plants which are used as phytoremediator are generally hyperaccumulators, that can accumulate metal ions in concentration of more than 1000ppm and they must have certain properties such as branched root system, less biomass, easy harvestable. This review article focuses on the sources, harmful effects and various technologies to eradicate heavy metals by using hyperaccumulating plants.

Key words: phytoremediation, heavy metals, toxic effects, uptake mechanisms, hyperaccumulators

INTRODUCTION

Phytoremediation consists of *Phyto* which means plant and *remedium* which means to remove (Erakhrumen et al., 2007). Phytoremediation is a highly evolved technique used to get rid of harmful pollutants from our environment and render it harmless through various biological ways using plant as remediant (Salt et al., 1998). In this process, the plants or various plant parts are used to remove many organic and as well as inorganic contaminants from soil, sludge, ground water, surface water, or any other contamination source. Increasing urbanization and industrialization greatly affect the land and degrade it, making it unfit for cultivation, and even unfit for organisms such as plants and animals. Untreated industrial effluent released in nearby water resource as well as home waste which is dumped as landfills are causing the slow extinction of flora and fauna in natural environment through toxic pollution. Untreated pollutants from industries contaminate air and result in increase in pollution in atmosphere, this contaminated air falls back on earth surface through acid rain and result in degradation of land and affect vegetation in other non-contaminated areas also. Currently, earth's surface, ground water as well as other water bodies are getting polluted by toxic heavy metal that are discarded in the environment from laboratories, research fields, agriculture activities, radioactive processes, mining sites, lead-acid batteries, fertilizers, pesticides motor vehicle emissions, industrial wastes, municipal wastes, and all sewage-derived materials. Soil is being contaminated day by day with industrial advancement and due to long term and excessive use of harmful chemical fertilizers, synthetic pesticides, synthetic fungicides & sewage sludge (Yadav, 2010). Harmful effects of these contaminants pose a potential threat to various terrestrial and aquatic organisms and severe problems and disease to human. Among different heavy metals, lead and mercury are known to be most hazardous in comparison to other toxic metals like copper, zinc, arsenic, cadmium, chromium. Nickel is also considered as harmful metal because of its high presence and its serious effect on primary plants metabolism and plant biology grown in soils. Heavy metals are toxic because they replace essential nutrients such as enzymes, pigments from soil (Henry, 2000). Phytotoxicity results in poor plant growth and low yield. Therefore, there is need to develop effective and feasible technology for removing contaminants from the soil and thus prepare soils for cultivation. There are number of traditional technologies for cleaning up the environment but all such methods are very costly and time-consuming. The use of chemicals and heating of waste with electric equipment's also result in degradation of minerals which are anyway important in soil. Thus, such methods are avoided as there is a risk to lose valuable soil minerals along with waste. Chemical techniques produce large amount of sludge and are costly methods (Rakhshaee et al., 2009). Phytoremediation is cheap, affordable and eco-friendly method. It is natural way for the remediation of contaminants using various plant physiological processes. Physiological processes used by plants are general processes like transpiration, water and nutrient uptake, gas exchange, photosynthetic metabolism translocation, accumulation, and also exudate release.

Phytoremediation and various other methods are mostly implemented in developed countries and less in developing countries due to inadequate information and less availability of resource. There are many advantages of Phytoremediation technique over other traditional remediation techniques (Sharma and Reddy, 2004). These are:

1) It saves transportation cost.
2) It is eco-friendly technique.
3) It is classical way and is preferred by public widely
4) Widespread nature of this technique.
5) This method is applicable for removal of contamination present at single site.
6) It has low maintenance and establishment cost

The untreated wastes from industries, laboratories, household etc. are continuously degrading soil thus leading to soil contamination for heavy metal in soil. Waste from mining sites, uncontrolled disposal of waste from industries, oil spills, smelting metal ores and the sewage sludge is used as manures to field are some of the factors which are accountable for movement of lethal contaminants into the various fresh non-contaminated locations. The various types of organic and inorganic contaminants like heavy metal contaminants, hazardous waste, oil or petroleum contamination etc. pose threat to mankind. Out of these contaminants, heavy metal contamination is much dangerous than organic contaminants (Logan, 1987; Alloway, 1990). Heavy metals pose much greater threat because heavy metals have ability to replace enzymes and essential metals from soil (Henry, 2000). Heavy metals are considered as hazardous because they get accumulating in plants and animals. Due to toxic effects of heavy metals it is also reported, "the loss of livestock". Heavy metals also affect whole food chain through biomagnification and thus affecting various organs in human body (Bonada and Ma, 2003). Thus, to remove such contaminants various techniques are used out of which phytoremediation is the best eco-friendly technique which is also known as green method of remediation (Hartman, 1975). (Baumann, 1885) reported that *Viola calaminaria* and *Thalspi caerulescens* are the very first plants which act as phytoremediation plant by accumulation and storage of high amount of toxic metals in plant parts such as leaves and shoot biomass. Later on, (Byers, 1935), reported that genus *Astragalus* accumulate 0.6% selenium concentration in dry shoot biomass. In 1989, Chaney reintroduce the technique of remediation through plants (Chaney, 1989). The first field trial was conducted in 1991 on phytoextraction of zinc and cadmium. Now before decade of two this technique of phytoremediation is greatly studied and is highly acceptable technique in terms of ecological and economical point of view.

SOURCES OF HEAVY METALS

Various natural and manmade activities lead to contamination of environment. Natural activities like soil erosion, breakdown of minerals, volcanic eruption, earthquakes etc. and man-made sources are mining, pesticides and fertilizers use, electroplating, smelting, industrial effluents, sludge dumping etc. many basic types of sources are discussed in Table 1. (Modaihsh et al., 2004; Fulekar et al., 2009; Wuana and Okieimen, 2011).

Table 1. Heavy toxic metals from various sources

Name of Heavy metals	Types of Sources
Arsenic (As)	Wood preservatives and various pesticides.
Cadmium (Cd)	Plastic stabilizers, paints and pigments, electroplating, plastics, phosphate fertilizers.
Chromium (Cr)	Tanneries, steel industries, fly ash.
Copper (Cu)	Pesticides and fertilizers.
Mercury (Hg)	Medical waste and release from Au–Ag mining and coal combustion.
Nickel (Ni)	Industrial effluents, kitchen appliances, surgical instruments, automobile batteries.
Lead (Pb)	Combustion of leaded petrol by aerial emissions, battery manufacture, herbicides and insecticides.
Zinc (Zn)	Mining activities/smelting, Coal mining and steel processing industries
Iron (Fe)	Ores/mineral dissolution
Manganese (Mn)	Ores/mineral dissolution, industrialization and urbanization

(Table Source: H. Ali et al., 2013)

DESTRUCTIVE IMPACT OF HEAVY METALS ON HUMAN HEALTH

Heavy metals have many harmful effects on primary human health. Heavy metals are very toxic and thus cause adverse and severe problems and diseases even when present at very much low concentration (Kara, 2005; Ampiah-Bonney et al., 2007; Memon and Schroder, 2009). Oxidative stress which is referred as formation of ROS, reactive oxygen species which suppresses antioxidant defenses and results into cell death, this oxidative stress is also caused by heavy metals (Mudipalli, 2008; Das et al., 2008; Krystofova et al., 2009; Sancher-Chardi et al., 2009). The list of harmful metals with their harmful effects on human health is discussed below in Table 2.

Table 2. Harmful impact of heavy metals on human health

Name of Heavy metal	Harmful effects of toxic heavy metals on human health
Zinc (Zn)	Dizziness and fatigue when used over dosage.
Chromium (Cr)	Hair loss.
Nickel (Ni)	Allergic dermatitis known as nickel itch; cancer of the lungs, nose, and sinuses;

	hematotoxic, genotoxic, neurotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, hepatotoxic; and causes hair loss.
Mercury (Hg)	Anxiety, autoimmune diseases, drowsiness, fatigue, hair loss, insomnia, irritability, memory loss, restlessness, vision disturbances, tremors, tempers, and damage to brain, kidney and lungs.
Cadmium (Cd)	Carcinogenic, mutagenic, and teratogenic; endocrine disruptor; interferes with calcium regulation in biological systems; causes renal failure and chronic anemia.
Lead (Pb)	Its poisoning causes problems in children such as impaired development, reduced intelligence, loss of short term memory, learning disabilities and coordination problems; causes renal failure; increased risk for development of cardiovascular disease.
Arsenic (As)	Arsenate is an analogue of phosphate and thus interferes with oxidative phosphorylation and ATP synthesis.
Copper (Cu)	Liver cirrhosis, brain and kidney damage, and chronic anemia, stomach and intestinal irritation are caused are high elevated level of dosage.

(Table Source: H. Ali et al., 2013)

TECHNIQUES EMPLOYED IN PHYTOREMEDIATION

These methods also help in removing of contaminations through different mechanisms. The different phytoremediation techniques for removal of toxic metal contaminants from polluted water, soil, and as well as sediment are phytoextraction, phytovolatilization, phytostabilization phytodegradation, rhizodegradation, each of which have specific properties in Table 3 and Fig. 1. (Sarma, 2011; Prasad, 2004).

Table 3. Different phytoremediation techniques

Phytoremediation techniques	General meanings of these techniques
Phyto-extraction	Accumulation of toxic pollutants in harvestable biomass of plants parts i.e., shoots
Phyto-filtration	Sequestration of toxic pollutants from contaminated waters by plants.
Phyto-stabilization	Limiting the mobility and also bioavailability of pollutants in soil by plant roots
Phyto-volatilization	Conversion of toxic pollutants to volatile form and their subsequent release to the atmosphere.
Phyto-desalination	Removal of excess salts from saline soils by halophytes
Rhizo-degradation	Degradation of organic xenobiotics in the rhizosphere by microorganism's living in the rhizosphere regions.
Phyto-degradation	Degradation of organic xenobiotics by various plant enzymes within plant tissues.

(Table Source: H. Ali et al., 2013)

MECHANISM OF UPTAKE OF TOXIC HEAVY METAL BY PLANTS

Phytoremediation is the method by which plants and microbes present in rhizosphere help in removing contaminants thus reducing contamination from environment (Greipsson, 2011). Plants are very efficient and are highly specific to take micronutrients even when they are present at low parts per million level in the environment. Plants act as both as accumulator and as excluders (Sinha et al., 2004). Accumulators accumulate toxic substance in its aerial parts and still can survive whereas excluded does not take contaminants into its biomass. Plants absorb nutrients through roots with the help of various chelating agent which may be produced by plants or can be externally applied, through plants induced pH change and various reduction oxidation reactions which help in solubilization and absorption of micronutrients even when their present at very low concentration in soil. There is also a specific mechanism for translocation and storage of micronutrients. Various employed mechanisms for transportation are as follows:

Proton pumps: In this case electrochemical gradient is generated for exchange of ions, ATPase consume energy and help in generation of such gradient which help in the transport of ions including metal ions.

Co-transporters and anti-transporters: It involves the transportation through plasma membrane through proteins that use electrochemical gradient for exchange of ions which is generated by ATPase.

Channels: These are proteins that help in transportation of ions across cell membrane.

These mechanisms are helpful in taking large number of ions across plasma membrane. The difference arises when the heavy metal contaminants interact with the micronutrients and such ionic species. Then, they are absorbed by roots and then they are translocated into shoots. The uptake mechanism by plants is regulated but beyond the metabolic needs plant do not accumulate trace elements and the metabolic requirement ranges from 10 to 15 parts per million for most micronutrients. But the hyperaccumulators can take up high concentration of heavy metal even in thousands of parts per million. Certain studies reveal the storage of heavy metals in vacuoles. Transpiration, is one method for the absorption of nutrients and other metals that is iron from soil which result in transpiration through leaves thus helping in translocation of contaminants from plants roots to shoots. Certain plants which can accumulate heavy metals to concentration of 1000 ppm and more are termed as hyperaccumulators. In this plant shoot to root metal concentration ratio is more than one whereas the plants which are known hyperaccumulators have shoot to root ratio of metal concentration ideally less than one. Ideal hyperaccumulator includes those plants which can survive easily in toxic environment with less maintenance cost and can produce high biomass. But these requirements are fulfilled by very a smaller number of plants (Salido *et al.*, 2003). Hyperaccumulator plant species can accumulate heavy metal like Lead, cadmium, Mercury nickel, manganese, zinc up to concentration of hundred thousand time more than and that of non-accumulator that is, excluder plants. In several cases microorganisms such as bacteria example *Pseudomonas spp.,* Fungi etc. which are present in rhizosphere also help in the process of phytoremediation, this type of method is called as rhizoremediation. These microbes play a substantial role in case of toxic organic contaminants rather than inorganic contaminants (Figure 2).

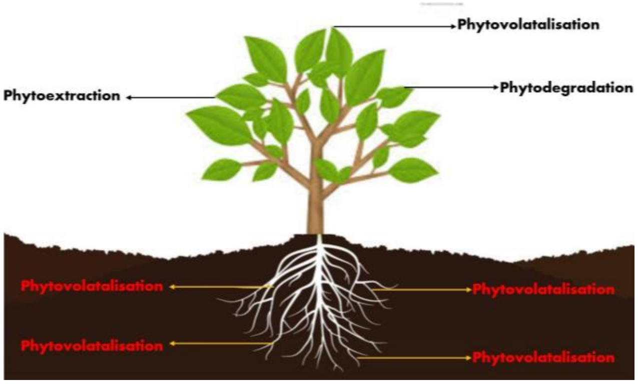

Figure 1. Different phytoremediation techniques

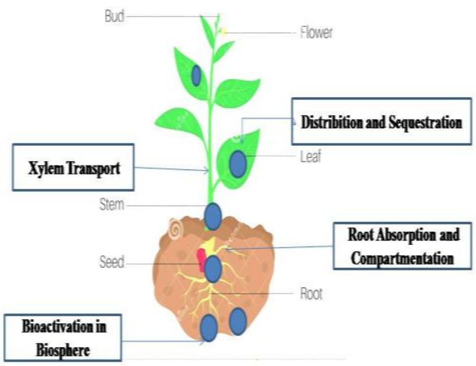

Figure 2. Mechanisms employed in heavy toxic metal hyper-accumulation by plants

UPTAKE OF METAL, TOLERANCE AND TRANSLOCATION

The process of phytoextraction include the moment for mobility of heavy toxic metal in contaminant soil, absorption and uptake of heavy metal by plants roots from rhizosphere, transport of heavy metal from plants roots to above ground parts such as shoots, removal of many known heavy toxic metal ions and finally tolerance of such metal. *i.e.,* how much a plant can accumulate metal (Clemens, 2001; Tong et al., 2004). Metal tolerance is necessary process for metal accession and thus helping in phytoremediation method (Clemens, 2001; Tong et al., 2004). Heavy metal tolerance in phytoremediant plant is organized to mechanism like cell wall binding mechanism, metal ion complex formation, Deposition of metals to vacuoles by active ion transfer which help in sequestration of heavy metal in vacuoles (Memon and Schroder, 2009). The whole process of metal uptake starts from the uptake of heavy metals from soil by plants roots, this uptake is generally governed by microbes present in rhizosphere. After absorption from roots the heavy metal and its ions are translocated to above ground part tissues through xylem vessels or can also remain stored in the roots (Prasad, 2004; Jabeen et al., 2009). Heavy metals are generally stored in plant vacuoles. Heavy metal ions are degraded in vacuoles of plant and thus decreasing the interaction of heavy metal ions with other metabolic processes of plants (Assuncao et al., 2003; Sheoran et al., 2011). The process of uptake of metallic ions is governed by number of molecules. Some of these molecules help in transport of ions across the membrane and others help in degradation of these ions, starting from absorption of heavy metal ions from soil is governed by the transporter protein also called as tunnel protein or through carrier proteins which is coupled with the transfer of H+ ion (Greipsson, 2011). For example: zinc iron permease (ZIP) these are the protein present in plasma membrane which help in the transfer of zinc ions and ferric ions (Clemens, 2001). Non-essential or heavy metal contamination ion also transfer through the same pathway as followed by essential metal ions if they have same oxidation number or ionic radii of that of the metal (Thangavel and Subbhuraam, 2004; Alford et al., 2010). Also, some known chelating agents such as amino acids and organic acid such as citric acid acts as ligands because of presence of various donor atoms like S, N and O thus enhancing the rate of absorption (Shah and Nongkynrih, 2007; Sheoran et al., 2011).

METALLOTHIONEINS (MT) AND PHYTOCHELATINS (PC) FUNCTIONS

PC and MT are the most important peptides which help in aggregation and endurance of heavy metals. These proteins help in segregation of heavy metal ions by binding them to make them to make stable complexes because they are rich in cysteine sulfhydryl groups which help in binding with the metal ions and in the formation of stable complexes. Phytochelatins are glutathionine derived peptides synthesized by enzymes which help them to attach to the metals and help in metal detoxification. Phytochelatins work as metal detoxification system of plants (Clemens, 2001; Fulekar et al., 2009). Phytochelatins synthase enzymes are activated by different heavy metals results in production of Phytochelatins (Sarma, 2011). Activities of PC synthase and production of PC is more in root than in shoots (Ghosh, 2010). Metallothioneins are metal binding proteins helping plant against the toxic effect of heavy metal ions. These are gene encoded proteins with low molecular weight (Cobbet and Goldsbrough, 2002, Jabeen et al., 2009). More production of chelators such as PC and MT and organic acids not only facilitate the easy entry of metallic ions in two plants but also help in easy translocation to above ground parts with the help of xylem vessels (Wu et al., 2010).

QUANTIFICATION OF HYPERACCUMULATOR BY BCF AND TF

Effectiveness of hyperaccumulator can be calculated in terms of bioconcentration factor (BCF) and translocation factor (TF).

Bioconcentration Factor (BCF): This factor tells us about how efficient is the hyperaccumulator plant in accumulation of heavy metal into the above ground biomass (Zhuang *et al.,* 2007; Zhuang *et al.,* 2005).

$$BCF = \frac{C\ harvest\ tissue}{C\ soil}$$

$C_{harvest\ tissue}$ is concentration of heavy metal in the above ground plant biomass or plant harvest part. C_{soil} is the concentration of same metal in the ground or soil in which the plant has grown. BCF or Accumulating factor can also be calculated in terms of percentage. According to the equation (Wilson and Pyalt, 2007).

Accumulating factor (A) or $BCF = \frac{C\ harvest\ tissue}{C\ soil} x100$

Translocation factor (Padmavathiamma and Li, 2007).

$$TF = \frac{C\ shoot}{C\ root}$$

Here C_{shoot} is the concentration of heavy metal which is accumulated in shoots and C_{root} is that concentration of heavy metal which is accumulated in root.

Translocation factor can also be represented in terms of percentage according to the following equation (Zacchin et al., 2009; Zhuang et al., 2005).

$$\textbf{TF} = \frac{C\ shoot}{C\ soil} x100$$

BCF and TF are very important in helping in the selection of plants which can be used for phytoremediation thus calculating the values of BCF and TF hyperaccumulator species can be selected (Wu et al., 2011). If value of translocation factor TF is greater than one which indicates the movement of metal from roots to shoots (Jamil et al., 2009). It is also reported that the value of the BCF and TF must be greater than 1 then only the plant can be used for two extraction purpose (Yoon et al., 2006). BCF can also help in quantification of bioavailability of heavy metals to phytoremediator plant species (Nassem et al., 2009; Zhuang et al., 2005).

CONCLUSION

Meanwhile soil contamination by harmful toxic heavy metals is a solemn environmental problem. So the effective heavy metals removal methods are necessary. Phytoremediation is ecologically responsible technology and environment friendly which have high public acceptance. In this field current research is in rigor improvement to screen and select various natural plants for phytoremediation techniques of heavy toxic metals and also to assess the impact of diverse parameters on effectiveness of phytoremediation. Likewise, high research is effectively shown across the globe to genetically modify some appropriate plants for improved and competent phytoremediation of toxic heavy metals as well as other xenobiotics.

ACKNOWLEDGMENTS

I am thankful to School of Life Sciences, Central University of Himachal Pradesh for supporting and contributing during my work.

AUTHOR CONTRIBUTIONS

All the three authors contributed equally in drafting the manuscript.

COMPETING INTERESTS

The authors declare no conflict of interest in preparing this article.

ETHICS APPROVAL

Not applicable.

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