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Recent Advances of Phytoremediation in Environmental Detoxification

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ABSTRACT

Phytoremediation is a process of environmental clean-up that uses plants and associated microbes to clean up pollutants from the air, water, and soil by producing substances beneficial for plant growth and through controlling plant pathogens. There is an adverse effect of heavy metals on both aquatic life forms as well as terrestrial living beings including humans. Being recalcitrant, heavy metals accumulate in the environment and are eventually biomagnified via the food chain. There are advanced molecular tools for a better understanding of the mechanism of metal absorption, translocation, sequestration, and tolerance in plants and microbes.

This review article describes the accumulation of heavy metals in the environment, its effect on the environment, and the current role of phytoremediation in the extinction of heavy metals.

Keywords- Environment, clean up, Heavy metal, Pollution, Phytoremediation, Phytoextraction, Phytofiltration, Phytostimulation.

I. INTRODUCTION

Phytoremediation is a process of environmental clean-up that uses plants and associated microbes to clean up pollutants from the air, water, and soil [1]. Transgenic plants can be used to take up heavy metal. Metal polluted soil can be treated with phytoremediation assisted by bacterial endophytes since metal toxicity in plants can be alleviated through the metal resistant system of endophytic bacteria and facilitate plant growth under metal stress conditions. There are 2 different ways by which endophytic bacteria improve plant growth in metal stress conditions.

a) By producing substances beneficial for plant growth including solubilization/transformation of mineral nutrients, phytohormones production;
b) Through controlling plant pathogens or by inducing a systemic resistance of plants against pathogens.[2]

The aquatic system is polluted by metals through volcanic eruption, weathering of soil and rocks, and from several anthropogenic activities such as mining, processing, and industrial effluents having the substances that contain metal pollutants. The release of heavy metals through volcanic eruption, fossil fuel combustion, etc can pollute the air. Mining, industrial wastes, sewage sludge, manure, fertilizers, herbicides, pesticides applied on the field can introduce heavy metal in the soil as well as water.

Heavy metals cannot be degraded, and it is harmful. Hence, it needs to be controlled. The physicochemical remediation method is very expensive and can destroy the soil ecosystem. Phytoremediation uses plants and assisted microbes to remove, reduce or stabilize heavy metals or make them less toxic in situ in an efficient, cost-effective, and environment-friendly manner. Rapid industrialization is responsible for the accumulation of heavy metals in the environment. It may lead to assert toxicity to plants, impairing their metabolism, biomass production, and yield. Heavy metals come in contact with humans through numerous anthropogenic activities and it gets accumulated. It can lead to damage of multiple organs such as the liver, bones, lungs, kidneys, brains, etc via the formation of nonspecific complexes at high concentrations. Due to rapid urbanization, industrialization and intensive agriculture, heavy metal pollution occurs. 23 metals out of 35 naturally existing metals possess high density above $5g/cm^3$ with atomic weight greater than 40.04 and are generally considered as heavy metals.

Fe (II) causes iron-mediated toxicity by reacting with oxygen-producing free radicals and leads to damage to macromolecules causing cell death. Cu (II) (cupric) and Ni^{2+} are toxic as they can damage DNA by binding with it [3][4].

Interaction among plant-microorganisms-heavy metals in rhizosphere soil has been studied by many researchers to enhance the efficiency of phytoremediation. Phytoremediation of metal-polluted soil has attained prominence by inoculating plants with selected and acclimatized microbes (Bioaugmentation). Some studies have found that metal-induced phytotoxicity can be alleviated by plant growth-promoting rhizobacteria and the biomass production of plants will be enhanced when grown in metal contaminated soil. Phytoremediation process includes Phytoextraction, Phytostabilization, Rhizofiltration, and Phytovolatilization. Phytoextraction is a process of up-taking metals with translocation. Phytostabilization refers to the maintenance of toxic ions in the polluted substrate being immobilized in the roots. Hence, in the rhizosphere heavy metals are stabilized. Besides, water erosion of the ground is prevented by the vegetation cover. Rhizofiltration is a process of filtering

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contaminated groundwater, wastewater, and surface water by removing toxic substances or excess nutrients through a mass of roots. Harvesting of the plant biomass with accumulated toxic metals is required for Phytoextraction and Rhizofiltration. The preparation of eco-catalysts for the chemical industry is an innovative trend in using the harvested biomass from metal hyper-accumulating plants. Inorganic pollutants can also be removed by plants through volatilization. In this process, gaseous forms are produced via the biological conversion of metals and released into the atmosphere [5][6].

Therefore, the objective of this review is to summarize and discuss the sources and biochemistry of heavy metals along with their effect and the strategies to prevent heavy metal contamination in the environment.

II. CLASSIFICATION AND BIOCHEMISTRY OF HEAVY METALS

The toxicity of heavy metals depends on chemical nature, concentration, oxidative state, and bioavailability. Heavy metals are classified into two categories: [7]

1. Essential heavy metals: Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), and zinc (Zn).

2. Nonessential heavy metals: Lead (Pb), Cadmium (Cd), and Mercury (Hg)

Depending on the level of toxicity heavy metals are of 3 types:

a) Extremely poisonous,
b) Moderately poisonous,
c) Relatively less poisonous.

Among these metals bioavailability of Cd is more than the other metals and it is highly capable of being bioaccumulated because of its high solubility. Cd does not have any biological function. It is present as a divalent ion in the compound form. Cd is mostly found to be present with Zn in heavy metal contaminated soil [8][9]. A high concentration of Zn causes toxicity in plants while phytotoxicity is rarely caused by Cd [10]. Vetiver grass can uptake and accumulate large concentrations of Cd [11]. Hence, it can be utilized for the reclamation of Cd contaminated soil [12]. In the rhizosphere, sequestration of Cd is caused by microorganisms and thus Cd uptake by plants is influenced [13].

A high concentration of glutathione and glutathione-s-transferase is present in the roots of plants that are used for remediation of contaminated soil [14]. It is associated with the detoxification process [15]. Among edible crops researchers have found the highest level of Cd and Zn concentration in maize and a high level of Pb concentration in wheat [16].

Antioxidative enzymes mainly glutathione reductase can be suppressed by Cd. Removal of Pb from contaminated soil is very difficult. The higher the pH cation exchange capacity, organic matter content capacity of the soil, the higher the capacity to bind with Pb. Hg is found in different forms. Oxidized forms of Hg are mercuric and mercurous, while elemental Hg has been reduced from organic and inorganic Hg under reducing conditions and it can be converted to alkylated form [17] [18] [19]. Among all these, the most toxic form is alkylated mercury because it has a strong affinity for sulfur-bound compounds including enzymes and proteins.

Chromium (Cr) mainly comes from the mining industry. It is found in the environment as trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) forms [20] [21]. Cr^{3+} acts as a cofactor for many enzymes [22]. Under the aerobic condition, chromium is present as Cr^{6+}, and under anaerobic conditions, Cr^{6+} is converted to Cr^{3+} by organic matter. Cr^{3+} forms a complex with Cl^-, CN^-, SO_4^{2-}, NH3, OH^- at low pH [23]. Among heavy metals, the least toxic metal is cobalt (Co). Cobalt works as a cofactor for different enzymes in a biological system. But high concentrations of cobalt and nickel are toxic for humans. Copper (Cu) is the most useful metal. Plants and animals use Cu as a micronutrient. Cu is an essential component for hemoglobin production. Cu helps in water regulation and seed production in plants. Cu can form complexes with organic matter. Due to its radical property, it can form a superoxide radical which can interact with thiol compounds in cell membranes [24]. Hence, Cu is a toxic metal.

The most abundant metalloid is arsenic. It exists in two forms: arsenate (As^{5+}); arsenite (As^{3+}). Arsenate and arsenite are dominant under aerobic and anaerobic conditions respectively [25] [26]. Volatile methylated compounds are formed by arsenic. Arsenate mimics phosphate and enters the microbial cells. Thus, phosphate-dependent energy production is halted and hence, oxidative phosphorylation is prevented [27].

III. EFFECT OF HEAVY METALS

Non-biodegradable heavy metal is bioaccumulated through food webs and affects the biota by getting transferred from soils and water to all the trophic levels. Heavy metal toxicity can occur by chronic exposure (long-time exposure) or acute exposure (short-time exposure) to heavy metals. Particulates formed by heavy metals sink to the bottom sediment and affect the aquatic organisms. Heavy metals react as redox catalysts in microorganisms in the production of ROS (Reactive Oxygen Species) and lead to malfunctioning of biomolecules, disruption of enzyme activity and ion regulations; and produce harmful or non-functional DNA and proteins. Heavy metal contaminated water drinking, and consuming crops grown in heavy metal contaminated environmental conditions are the main source for heavy metal exposure to humans [28].

Toxic effects of heavy metal include cardiovascular disorder, respiratory problems, hepatic, and renal problems including proteinuria, neuronal damage, skin corrosion and irritation, blood vessel damage, digestive problems, anemia, and risk of diabetes and cancer. After being accumulated in soft tissues if heavy metals remain in a non-metabolized state; it results in the generation of ROS and oxidative damage of the tissues.

In chronic toxicity, symptoms arrive after a long period of exposure to heavy metals. Heavy metal gets easily absorbed by the digestive tract, abdominal cavity, and muscles due to its stability and solubility. Heavy metals such as Pb, Hg, and Al can easily cross the blood-brain barrier. Cd can form Cd-complexes by binding to the ligands. Through passive diffusion or marine food chain. Cd is bioaccumulated in fishes and enters the human body through diet. Cd causes "Itai-Itai" disease in humans and may lead to death [29]. Cd toxicity causes chlorosis, leaf rolls, and stunting in plants. Cd disrupts the uptake, transport, and use of several elements such as Ca, Mg, P, K, and water in plants [30].

Immediate toxicity is caused by soluble inorganic arsenic. Ingestion of a large amount causes severe vomiting, hampers blood circulation and nervous system, and leads to death. Plant roots can easily uptake arsenate and arsenite. It induces ROS production in plants. Hence, it leads to the production of antioxidant metabolites and various enzymes involved in antioxidant defense. But gaseous forms of arsenic cannot cause injury to plants. Through photometry or bacterial methylation, inorganic Hg can be converted to organic forms. The most toxic chemical form of Hg is methyl-mercury which has a longer half-life [31][32]. It can be readily absorbed into the food chain and pass through the cell membrane and results in the formation of a stable organometallic compound with a sulfhydryl group of amino acids with a high affinity toward it [33]. Hg hampers the plant growth, modulation, and nitrogen fixation of legumes and the development of plants. Mercury stress can disrupt the cellular structure. The inhalation of mercury vapor can cause damage to the nervous system, digestive and immune system, lungs, kidneys, and it may be fatal [34].

Heavy metals have adverse effects on macrophytes that aid phytoremediation by inhibition of chlorophyll synthesis and hampers the sugar production and proteins by increasing consumption of sugar at the period of metal stress and forming peptide-metal complexes (Figure 1).

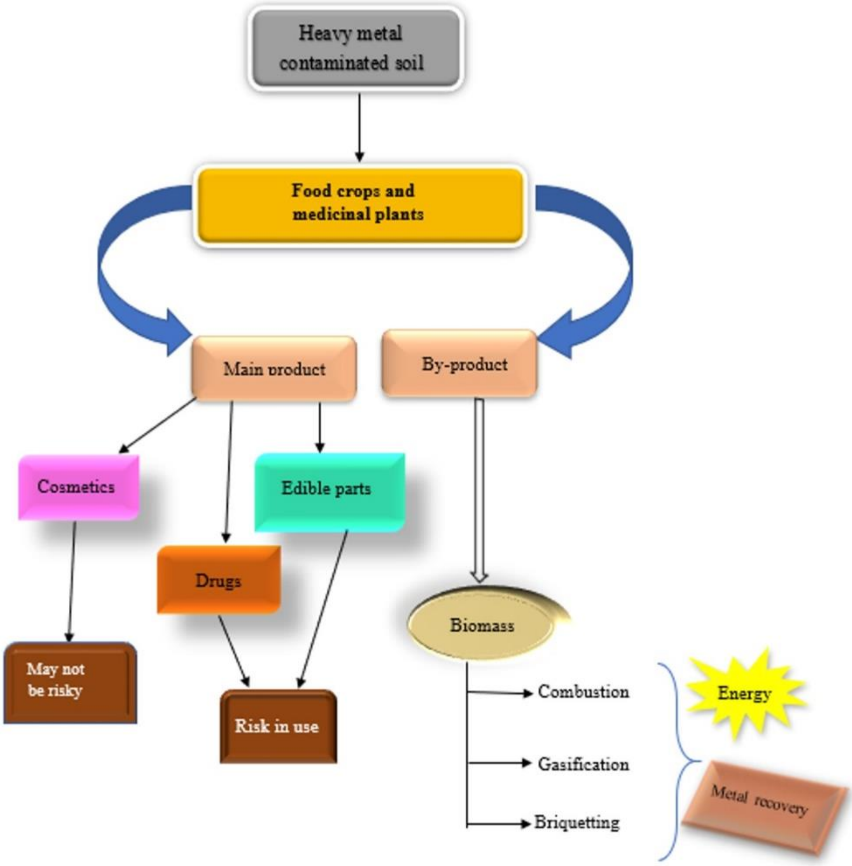

Figure 1. End products of phytoremediation in heavy metal contaminated soil

IV. PHYTOREMEDIATION STRATEGIES

Phytoremediation process is also known as "botano-remediation", "agro-remediation", and "green-remediation". Phytoremediation is a suitable process for environmental clean-up.

The phytoremediation technique is still at its testing stage since 1983.

There are 5 subclasses in the phytoremediation technique.

1. Phytostabilization
2. Phytostimulation
3. Phytotransformation
4. Phytofiltration
5. Phytoextraction

The objective of phytostabilization is to reduce mobility and bioavailability of heavy metals and it leads to the prevention of heavy metal entry into groundwater or food chain, respectively. In this process, a secondary role is played by the plants as compared to soil amendments. This process is carried out by physical and chemical immobilization of contaminants via root absorption and fixation with various soil amendments. The most efficient soil amendments for heavy metal immobilization are organic matter, clay minerals, biosolids, and phosphate fertilizer. Lowering water penetration, limitation of contact with contaminants, decrease migration of contaminants are the main objectives of plants. It is not a long-lasting way rather it is a management strategy. Since, eventually, the metal contaminants get persisted in the soil.

Phytostimulation is also called “photodegradation”. Organic pollutants are disintegrated in the rhizosphere with improved microbial activity. The rhizosphere is a soil volume about 1mm nearby the root and is influenced by root activity.

There are different ways through which microbial activity is enhanced in the rhizosphere.

- Root exudes having amino acid and indigenous microorganisms that are carbohydrate rich.
- For aerobic transformation oxygen supply in the rhizosphere is ensured by roots.
- The availability of organic carbon is increased by root biomass.
- Compounds that can be broken down by bacteria are degraded by mycorrhizae fungi.
- Habitat for an enhanced microbial population is provided by plants.

In the phytoremediation process, organic compounds are broken down by either metabolic processes of plants or enzymes produced by plants and it is not dependent on the microbial community. Hence, plants are called "green-livers of the biosphere".

Phytovolatilization is a process of releasing volatile compounds to the environment through the transpiration of plants. In this process, contaminants are taken up by plants and are converted to volatile compounds and eventually, volatile compounds are discharged into the atmosphere. This technique is useful for organic compounds, Hg and Se heavy metals.

Phytofiltration is the process where plant roots are used for the reclamation of surface water, wastewater, and groundwater with the minimum level of contaminants. Firstly, for acclimation of the plants, they are supplied with contaminated water, and then those are shifted to the site of contamination for remediation. Saturated roots are harvested and can be used for remediation. Phytofiltration is also called "rhizofiltration" when roots are used, "blastofiltration" when seedlings are used and "caulofiltration" when plant shoots are used. In this process, contaminants are precipitated or absorbed and their seeping into water is reduced.

Rhizosphere pH can be changed as root exudates and Metals can be precipitated on plant roots. In rhizofiltration, Cd, Cr, Cu, Ni, Zn can be extracted by the implementation of terrestrial and aquatic fast-growing plants because of their fibrous and longer root system.

In phytoextraction, the removal of heavy metal from the environment is mediated by the implementation of fast-growing plants.

There are two approaches:

1. Continuous or natural.
2. Chemically induced.

In natural or continuous processes, heavy metals are removed by a network of roots and then transferred to upper plant tissues, unlike chemically induced processes where a chemical is used to increase the heavy metal uptake and degradation rate. The plants must have a rapid growth rate, extended root network, and high biomass [35].

Biogas can be produced using harvested biomass and can be combusted. Metal recovery can be achieved by using combusted plant biomass. This process is also called "phytomining" or "biomining". This process does not affect the soil properties. The metals can be restored from the harvested plant parts. Levels of contaminants in soil can be reduced by continuous cropping and harvesting systems. Plants having rapid growth, high biomass, and extended root network, able to store and tolerate high levels of heavy metals are used for phytoextraction processes.

Natural hyperaccumulators are used in continuous phytoextraction. At metalliferous sites, hyper-accumulators can be used.

There are various processes involved in phytoextraction:

- Root surface absorbs some metal fraction.

- Entry of bioavailable metals in roots is mediated through the cell membrane.
- Roots take up a small fraction of metals and it becomes immobilized in the vacuole.
- Entry of Mobile metals in roots and transported to the xylem.
- Translocation of metals occurs from roots to stem tissues and leaves.

Plants that can tolerate and live-in heavy metal contaminated environments are metallophytes that belong to the Brassicaceae family of the plant [36].

Metallophytes have 3 categories:

i. Hyperaccumulators
ii. Indicators
iii. excluders.

Metal excluders can take up heavy metal and can accumulate it into roots. But it reduces the transport of heavy metals.

Heavy metals are taken up and accumulated into the upper parts of the plant system by metal indicators.

Heavy metal hyperaccumulators react against plant infective agents. More than 400 hyperaccumulators have been reported to have sluggish growth and low biomass production capacity [37].

There are 3 ways to design plants for phytoremediation-

- Increasing production of ligands for metalloids.
- Metal/metalloid transporter genes manipulation.
- Transferring metal and metalloid to produce less toxic and volatile compounds.

To increase the efficiency of phytoremediation using transgenesis, the genes which are involved in the uptake, translation, and sequestration of metals need to be over-expressed and a plant antioxidant activity strategy can be used for studying heavy metal stress conditions. Metal mobilization, formation of complexes with ligands and chelators, detoxification by deposition in vacuoles, transport to shoots via apoplast or symplast are some potential strategies to improve the accumulation of metals in plants.

The inherent ability of endophytic bacteria may help host plants to adapt to unfavorable soil conditions and enhance the efficiency of phytoremediation by promoting plant growth, alleviating heavy metal stress, reducing metal phytotoxicity, altering metal bioavailability in soil and metal translocation in the plant.

V. ADVANTAGES AND LIMITATIONS OF PHYTOREMEDIATION

Nowadays, phytoremediation is a widely used technology. The use of this technology cut down the costs in comparison with traditional remediation technologies. Phytoremediation is an eco-friendly as well as an efficient process of environmental clean-up. Being typical in situ technique this process can be applied to a broad range of sites. Bioenergy can be produced by using harvested plant biomass after phytoremediation. Along with advantages, there are also some limitations of phytoremediation. The main limitation is that it requires a long duration of time for cleaning up mechanisms. It depends on the ability of the plant to uptake and accumulates heavy metals and its ability to survive and grow in a heavy metal contaminated environmental condition. Slow growth and low biomass accretion are also important reasons for limited plant remediation potential. In places where the pollution level is low and roots can reach the contaminants, phytoremediation is effective. Plants used for phytoremediation are often ecotypes. Those plants inhabit a specific ecosystem, and it becomes difficult to cultivate them in other environmental conditions and heavy metals may get accumulated in fruits and other edible parts.

There is also a risk of food chain contamination with heavy metal may increase because of improper biomass disposal. The use of modified or transgenic plants for phytoremediation may overcome many limitations and enhance its efficiency.

VI. FUTURE PERSPECTIVE AND CONCLUSION

Heavy metals are persistent and toxic pollutants which of the alarming environmental concern. Genetic engineering is a useful technique to develop desirable plant lines for phytoremediation. By applying plant material in isolated industrial districts instead of agricultural areas can reduce the risk of gene escape. To improve crop quality and enhance biotic stress tolerance in plant cisgenesis and intragenesis can be used. In these methods, plants are introduced with modified copies of natural genes. In cisgenesis, complete genetic elements from a sexually compatible gene donor must be present in the gene construct. In intragenesis, there must be genetic elements from different genes within a sexually compatible gene pool. Inbreeding programs, to facilitate biomass production and natural hyperaccumulators growth cisgenesis and intragenesis approaches may be valuable.

Plants belonging to the Brassicaceae family such as *Arabidopsis* or *Thlaspi* are good for genetic modification due to scientific Knowledge of their close phylogenetic relationships. Production of cisgenic plants with increased/decreased ability to accumulate cadmium in leaf tissues may be facilitated by the recent findings in genetic mapping in maize [38]. This may be used in the phytoremediation of contaminated soil. Although, there is no report to date on the use of cisgenesis/intragenesis in modulating plant response to heavy metal stress.

Different factors affect the phytoremediation process such as distribution of pollutants, pH, soil, nutrients, pathogens, moisture, and temperature. Identification of plant species capable of producing high biomass and heavy metal accumulation and efficiency of phytoremediation technique.

This review described that heavy metals are a critical threat to the environment as well as human health. There are various sources of heavy metals. The use of conventional techniques for environmental clean-up is expensive and harmful for the environment. On the other hand, phytoremediation is the cheapest and eco-friendly process for environmental clean-up. This is an effective process that uses plants and associated microorganisms to clean up the environment without causing any harm to it. The Plants uptake and accumulate heavy metals and reduce the concentration of contaminants. Transgenic plants can be efficiently used for phytoremediation by over-expressing the genes that are responsible for heavy metal uptake. Information provided in this review may be useful for further research towards biotechnological development of phytoremediation efficiency.

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