HEAVY METAL DETOXIFICATION FROM THE ENVIRONMENT

UNCOVERING MOLECULAR APPROACHES

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PREFACE

Heavy metal remediation has gained much more attention in the past few years with the exploration of varied methods and detoxification approaches. They have acquired immense importance due to their hazardous effects on the environment. Heavy metals occur naturally in the environment, but their concentrations have been exponentially increased due to anthropogenic activities. They are continuously persisting in the environment in enormous quantities, thereby, entering the food chain through crops and consequent accumulation in the living cells via biomagnification. Novel remediation strategies have attained a specific position in the present era and also attracted interests of researchers through the implementation of bioremediation processes as well as various assistants to remediate the polluted soils. This in turn has become the talk of the town owing to its sustainable properties. The molecular insights provide excellent and new perspectives to unravel the heavy metal accumulation and detoxification. The metagenomic studies as well as screening strategies are used to extract the genomic information for detoxification mechanisms. Moreover, the development of next-generation sequencing studies has also provided us the unparalleled perspectives through key remediation mechanisms. This book mainly focuses on the impact of heavy metals in the environment, and their detoxification potential with special emphasis on the sustainable approaches, molecular patterns, omics approaches and metagenomics analysis. This book discusses the novel and new generation strategies for heavy metal detoxification and remediation by the use of eco-friendly methods such as microbes, plant hormones, earthworms, mycorrhizae, microalgae, probiotics etc. It further focuses on the function and characteristics of the remediating agents used for metal detoxification at molecular or genetic levels.

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INTRODUCTION

For decades, the environment has been found disparaging due to heavy metal toxicity and sustainability has become a crucial aspect in the present scenario. In other words, environmental engineering ensures societal development and the use of environmental resources in a crucial manner. This requires the collaborative role of biologists, researchers, chemical engineers, industrialists, biotechnologists, microbiologists, botanists, molecular biologists, biochemists, and genetic engineers. All these environmental engineers are directly linked with problems associated with air, water, and soil contamination and they provide the technical solutions in order to resolve or attenuate such situations in a sustainable way that complies with legislative, social, economic, and political concerns. Moreover, the researchers should find a reasonable way to check the heavy metal contamination of groundwater, surface water, and soil along with the remediation of the contaminated sites, bioremediation, and waste management. Heavy metal toxicity is the most crucial concern that damages the entire environment and affects the plants followed by the complete food chain. It is therefore the need of the hour to find a suitable solution for the detoxification of heavy metals from the environment. Over the decades, biologists and researchers have contributed a lot in this realm and generated a vast information. The life sciences have provided descriptive approaches and this phenomenon is now better understood for the benefit of society. We have now focussed on the approaches that provide spectacular reductions in cost with minimal risks and more effectiveness. Our aim has been now more oriented towards realistic solutions with advanced biotechnological and molecular approaches in order to find the solutions.

This book is written by multiple authors concerned with heavy metal detoxification after the advent of molecular techniques. It intends to serve the professionals as well as encourage researchers to conduct various research in this field. The book is organised into various areas that are important for the environmentalists, biologists, and researchers that are working in this field for sustainability and removal of heavy metals and other toxic compounds from the environment. This book has covered all the main aspects and methods for heavy metal toxicity as this field is changing and moving very rapidly. The authors have included all the fundamental topics and aspects of heavy metal toxicity, and detoxification strategies through novel approaches. In addition, the chapters on bottlenecks in sustainable technology of heavy metal remediation along with future prospects are also included. Further, the topics associated with sustainable amelioration of heavy metal contaminated areas through the use of probiotics, microbes, genetic engineering, nanotechnology, biotechnology, phytoremediation, etc. along with the hurdles and futuristic strategies are also included. The book deals with some hitherto neglected areas for sustainable treatment technologies for heavy metal detoxification. It is most likely expected that these contributors will target many other researchers to contribute their research and facilitate in disseminating of knowledge in this discipline.

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CHAPTER 1

Hazardous Effects of Heavy Metals towards Plants: Recent Trends and Challenges

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Abstract: The prevalence of heavy metal soil pollution has increased as a result of increased geology and human activities. Metal pollution issues are becoming more prevalent in India and other nations. Numerous reports of metal toxicity in coal-burning power plants, foundries, smelters, mining, and agriculture have been made. As a result of industrialization and urbanisation processes, pesticides, petroleum products, acids, and heavy metals have been integrated into natural resources. Due to this, the quality of the environment has declined, affecting both biotic and abiotic components and consequently has an impact on the ecosystem. Some metals are required in trace levels for plant metabolism. However, they can be dangerous to plants when present in larger amounts. Lead, nickel, cadmium, copper, cobalt, chromium, and mercury are heavy metals that are significant environmental contaminants and have hazardous effects on plants. Plant growth, performance, and output are all reduced in heavy metal-contaminated soil-grown plants. Plants that are exposed to heavy metals experience oxidative stress, which damages their cellular structure. Metal ions build up in plants and upset the balance of cells. Plants have developed detoxifying systems to decrease the harmful effects of exposure to the accumulation of heavy metals (HMs). To treat heavy metals-contaminated soils, several in-situ and ex-situ remediation methods have been used, but they also have a number of drawbacks, such as high capital costs, toxicity, and environmental health hazards. The risks that heavy metals pose to plants are the main topics of the current chapter.

Keywords: Environmental contamination, Heavy metals, Plant metabolism, Toxicity.

INTRODUCTION

Different concentrations of heavy metals in soil, water, and air cause pollution after a certain threshold is achieved. Different factors contribute to this type of pollution. The natural weathering of rocks, volcanic eruptions, industrial proces-

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ses including mining, the burning of fossil fuels, and the discharge of sewage release metals into the atmosphere on a constant basis [1 - 3]. Copper, lead, zinc, and cadmium are particularly prevalent in agricultural soils [4]. Because of changes in biochemical processes, such as the suppression of enzyme activity, protein penetration, and decreased nutrition, *etc*., these metals are frequently readily taken up by plants and prove hazardous to them, which can be seen as growth failure [5]. The persistence of hazardous compounds in the environment, as well as their bioaccumulation and biomagnification in the trophic chain—where plants are the primary producers at the base of terrestrial ecosystems—pose a serious threat to human health [5]. Patients who consumed food contaminated with Cd, Pb, As, or Hg manifested neurological problems, stomach discomfort, and various ailments related to the heart, kidney, liver, digestive system, and several forms of cancer [6 - 9].

In plants, the toxic symptoms may include DNA deterioration and cell death, as well as the suppression of root development, photosynthesis, and mitochondrial respiration impairment. Under different conditions (Cd, Hg, As, or Cu), different plant species display different stress signals [10 - 13].

To cope with the stress, the primary mechanism for maintaining cellular redox homeostasis in plants is the SOD-mediated conversion of superoxide radicals into hydrogen peroxide, which in turn metabolized into non-toxic products by catalase and ascorbate peroxidize [14]. Glutathione reductase (GR) is another important antioxidant enzyme that converts GSSG into Glutathione (GSH) by employing NADPH as an electron source and preserving the GSH cellular pool [15].

TOXIC EFFECTS OF CADMIUM

A very toxic metal pollutant of soils, Cadmium (Cd) impairs homeostasis and nutrient absorption, impedes crop production and root and shoot growth, enters the food chain, and poses a major risk to human and animal health [16 - 18]. The development of seedlings and seed germination are both influenced by cadmium.

Effects of Cadmium on Germination and Plant Growth

Plant tissues that have accumulated Cd exhibit various toxicological signs as well as decreased development. Cadmium stress reduced the development of *Nicotiana tobacum*; adverse results included plant dwarfism, loss of green leaves, leaf detachment, and even plant mortality [19]. Rao *et al*. [20] investigated the effects of various cadmium concentrations (10 mg/L, to 250 mg/L) on *Brassica napus* and discovered a decline in different growth parameters. Similar results were observed in plants like *Cucumis sativus*, *Hordeum distichum*, *Lactuca sativa, etc*. [21 - 23]. When *Peganum harmala* seedlings were exposed to 100, 200, or 300 μM concentrations of Cd for 15 days, Nedjimi [24] observed a decrease in germination rate, length of hypocotyl, and index of tolerance. When *Pisum sativum* was grown and treated with Cd concentrations of 500, 750, 1000, and 1250 mg kg-1 in order to study the rate of germination, length of hypocotyls and root along with dry biomass, and tolerance index. After 15 days of treatment with various Cd doses, the researchers observed that all metrics decreased as Cd concentrations increased [25]. After 6 days of treatment, Zayneb *et al*. [26] found that *Trigonella foenum graecum's* germination rate, root length, and hypocotyl elongation decreased as Cd concentrations increased from 0.1, 0.5, 1 to 10 μM concentrations. El Rasafi *et al*. [27] discovered that after 7 days of Cd treatment, (10, 50, 100, 250, 500, 750, 1000 mg/l) *Triticum aestivum* showed a decrease in morphological parameters. Goel *et al*. [28] investigated the harmful effects of cadmium on the development and metabolic responses of rice and maize. Heidari and Sarani [29] evaluated that in mustard plant, seed germination and root development steadily decreased as Cd concentration increased.

Effects of Cadmium on Lipid Peroxidation

Dey *et al*. [30] identified a link between metal toxicity and oxidative stress. Malondialdehyde (MDA) and hydrogen peroxide concentration are quantified to determine the level of oxidative stress using the Cd application. Wu *et al*. [31] found that treating barley with 1 or 5M Cd increased the MDA content. Zayneb *et al*. [26] reported an increase in the content of MDA in plants (fenugreek) treated with Cd for 30 days. In a hydroponic experiment, Meng *et al*. [32], investigated the impact of cadmium and discovered a rise in the amount of MDA in *Lactuca sativa* and *Chrysanthemum coronarium*. According to the investigations, Cd-induced lipid peroxidation damages membranes, as shown by an increase in electrolyte leakage. Numerous authors have discussed the effects of cadmium at various concentrations on a variety of plant families, including Poaceae [33], Brassicaceae [34 - 37], Fabaceae [~~45~~, 38, 39], Asteraceae [32, 40] and Lamiaceae [41].

Effects of Cadmium on Photosynthesis

It has been observed that heavy metals can prevent the production of chlorophyll, especially by preventing the activity of the enzymes protochlorophyllide reductase and δ-aminolevulinic acid dehydrogenase [42]. Ekmekci *et al.* [43] studied the effect of Cd in two different varieties of maize *i.e*. 3223 and 32D99 and observed that eight days of treated seedlings of both cultivars showed signs of membrane damage and chlorophyll and carotenoid loss with an increase in Cd concentration. Chlorophyll fluorescence measurements showed that 3223 was significantly more impacted by Cd levels than 32D99 in terms of photochemical efficiency.

CHAPTER 2

Bioaccumulation and Detoxification of Heavy Metals in Plants

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Abstract: Rapid industrialization and urbanization have exacerbated the accumulation of HMs (HMs) in the soil. Their greater mobility enables them to readily accumulate in the body of living organisms, which is referred to as bioaccumulation, thereby leading to their magnification in the biological systems. As a result, they have become an inevitable part of our food chain affecting the lives of organisms at all trophic levels; however, plants are at greater risk due to their sessile habitat. The bioaccumulation of HMs is affected by several factors like the type of plant species, its development stage and plant parts as well the environmental factors like temperature, pH, and composition of soil as well as the form of HM present in water and soil. Furthermore, the entry of noxious HMs into the plant system triggers several detrimental effects on the plants ROS burst, inhibition of enzymatic activities, reduced mineral nutrition, and many more resulting in reduced growth and development of plants. Therefore, in order to tolerate HMs in their system, plants incorporate several detoxification mechanisms like the efflux of HMs from the plant body, sequestration and compartmentalization through GSH (GSH), phytochelatins (PCs), and metallothionein (MTs), chelation of HMs with some organic compounds and activating antioxidant defense system to

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mitigate the deleterious effects produced by ROS generated due to HM stress. The role of miRNAs in helping the plants battle against the HMs and their toxicity has also been acknowledged. The bioaccumulation potential and detoxification mechanisms advocated by the plants enable them to become potential candidates for phytoremediation. This chapter will unveil the bioaccumulation potential of plants along with presenting detailed mechanisms underlying the detoxification of HMs that protects plants from their toxic effects.

Keywords: Bioremediation, HMs, Hyperaccumulation, ROS, Phytochelatins.

INTRODUCTION

The inclination of the human race towards rapid industrialization and urbanization has led to the contamination of natural resources thereby making it a global concern threatening the survival of biological creatures [1]. Also, the abrupt rise in the global population has exerted significant pressure on agriculture to enhance food production which further deteriorates the condition of soil and water contamination [2]. Among pollutants, Heavy Metals (HMs) have become an inevitable part of the biological food chain thereby posing detrimental health effects [3]. Plants rooted in a single spot are most prone to HM contamination in different natural sources. The uptake of HMs by plants is affected by several factors like soil composition, pH, microbiotics, type of ions present in the soil and water, and several others [4, 5]. Such factors impact the amount of uptake of HMs in plants and influence the type of plant parts accumulating HMs. Based on the bioaccumulation capacity of plants, they can be classified into excluders, metal indicators, and metal accumulators or hyperaccumulators [6]. This becomes the basis for the exploitation of plants for the process of bioremediation of HM-contaminated soils.

Upon the entry of HMs into the plant body, they pose several hazardous impacts including oxidative stress, enzyme activity inhibition, reduced photosynthetic pigments and photosynthetic efficiency, and affecting overall metabolic machinery [7 - 9]. Therefore, to curb the deleterious effects of HMs upon their accumulation into the plant body, plants regulate a wide array of responses against HMs. Firstly, the plants tend to incorporate efflux mechanisms that tend to use metal transporters for the same. Furthermore, upon entry into the plant body, HMs disturb cellular homeostasis. The biochemical responses of plants to maintain cellular integrity are through the process of osmoregulation, which involves the synthesis of osmoprotectants like proline, glycine betaine, and sugars [10, 11]. Further, plants tend to immobilize the HMs and render them inactive through the process of sequestration and chelation. They also synthesize several organic phytochelatins that sequester HMs and restrict their deleterious impacts by rendering them inactive [12]. Metallothioneins (MTs) play a major role in chelation of HMs along with GSH (GSH) [13, 14]. Furthermore, the HMs disturb metabolic machinery and lead to oxidative stress in plants. In order to scavenge oxidative species, plants enhance their antioxidant machinery which comprises both enzymatic and non-enzymatic antioxidants that neutralize the reactive oxygen species (ROS) [1, 7, 15]. Lastly, the role of miRNAs (microRNAs) has greatly been acknowledged in altering the gene expression of plants facing HM stress. Therefore, the chapter will summarize the HM bioaccumulation tendency of plants and the various factors impacting the uptake of HMs along with discussing the mechanisms initiated as a response to HM accumulation in plants in order to shield their metabolic machinery.

Factors Affecting the uptake of HMs in Plants

With the advent of industrialisation and population explosion, a large number of anthropogenic activities are being carried out on a large scale, which is responsible for HMs contamination of the basic components of the environment may it be air, water, or soil. These HMs make their way into plant systems through roots or foliar adsorption thereby posing a serious threat to the normal metabolic machinery of plants. The entry of HMs into the plant body by either means is controlled by various factors grouped as physical, chemical, biological, and meteorological factors (Fig. **1**). Each of these factors impacts the amount and type of HM that gets accumulated within the root system and aerial parts of plants.

The soil-root transfer of HMs is regulated by a variety of factors including the ionic state of metals, soil particle size, soil pH, soil microbiota composition, organic matter content, and cation exchange capacity of plant roots [16]. Mostly, HMs are uptaken by plants at low pH levels of soil (4.5-5). Also, the diverse microbial composition of soil lessens the movement of HMs to plant systems as microbes adsorb their large amounts within themselves [17]. The foliar uptake of HMs depends on a variety of aspects *i.e*., leaf size, surface area, cuticular and epidermal composition, physical form, trichome length and number, speciation of metal ion, plant habitat, duration of HM exposure, stomatal density and rate of gaseous exchange [18]. Plants with smaller leaves of rough surfaces are found to be more potent accumulators of atmospheric HMs than those with larger and smooth-surfaced leaves [19]. Mostly small-sized and lipophilic HMs enter through cuticular penetration and large-sized and hydrophilic ones make their entry *via* stomatal pores or other aqueous pores in the cuticle [20].

The permeability of the cuticle and the ionic state of HMs are held accountable for the rate of foliar adsorption of HMs by the plants. Usually, metals forming ionic compounds are easily absorbed as compared to those forming covalent

CHAPTER 3

Molecular Interactions During Heavy Metal Detoxification in Plants

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Abstract: Heavy metal stress is one of the abiotic stresses that are damaging plants. Heavy metals cause toxicity in plant cells by targeting key molecules and essential processes. The toxicity of these heavy metals is caused by their discharge from both natural and anthropogenic sources. When these heavy metals are absorbed by plants, they activate multiple components, including reactive oxygen species (ROS), phytohormones, and nitric oxide (NO), thereby triggering the Mitogen-Activated Protein Kinases (MAPK) cascade. Signals detected by the MAPK cascade through the cell membrane receptor cells are then transmitted to various transcriptional factors and proteins that aid in stress management. This chapter discusses the activation of the MAPK cascade by heavy metals. In addition, the involvement of transcriptional genes and factors regulated by the MAPK cascade as well as their interconnections have been examined in depth.

Keywords: Heavy metals, MAPKs, ROS, Sources, Toxicity.

INTRODUCTION

In addition to other soil pollutants, heavy metal (HM) pollution is a global environmental concern. Since these HMs cannot be decomposed, they are always present in the soil, and their concentration continues to increase dramatically [1]. Several organisms, including plants, are adversely affected by these HMs emitted by natural and anthropogenic sources. When plants are subjected to persistent HM

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stress, their biological systems are irreparably damaged. Low yield and productivity are the results [2]. To overcome this HM stress, plants endure several biochemical and physiological changes, including a complex series of signalling pathways [3]. Through phosphorylation and dephosphorylation, surface receptors are amplified, and these signals are transmitted to cells when the cell membrane detects the signals [4]. When signals reach the cell, specific proteins such as enzymes, kinases, and several transcriptional factors are activated in the nucleus and cytoplasm to regulate gene expression.

Due to HM stress, various transcriptional factors are activated in plants. This causes the expression of several antioxidant enzymes. Protein phosphorylation is a crucial method of signal transduction in plants. These reactions are catalysed by mitogen-activated protein kinases (MAPKs). Some authors have reported that these MAPKs act downstream to coordinate numerous cellular responses required for proper plant growth and development [5]. Multiple MAPK pathways are present in plant cells. Each signal transmission pathway is independent and intertwined for normal transmission. In order to form a molecular network, these pathways are interconnected with other transduction pathways in the cell [6]. During times of pressure, MAPKs regulate the transcriptional factors bZIP, MYC, MYB, and WRKY [7]. These MAPKs are predominantly found in the cytoplasm and/or nucleus, although in some instances, they may be transferred from the cytoplasm to the nucleus for signalling purposes. This chapter discusses the sources and effects of HMs on plants. In addition, background information on plants under HM stress and new molecular approaches to transcriptional factors for accumulation and tolerance of HMs have been presented.

SOURCE SOF HEAVY METALS

Soils are often susceptible to environmental changes and HM contamination has become a global issue due to the related ecological dangers [8]. Due to the bioavailability, bioaccumulation, toxicity, and incapacity to break down HMs, soil pollution with HMs is a major concern [9, 10]. It is important to note that the physical, chemical, and biological characteristics of the soil affect the bioavailability of HMs there [11, 12]. Studies have demonstrated that prolonged contact with contaminated soils can have hazardous effects on living things like plants and animals, especially in areas where environmental protection laws are not strictly enforced [13 - 15]. In rural or urbanized areas, most soils could accumulate HMs over the permitted average levels, significantly enough to pose health risks to people, flora and fauna, ecosystems, or other media. The distribution of HMs in soils is associated with both natural and anthropogenic sources [16, 17] (Fig. **1**).

Fig. (1). Different sources of heavy metals in soil.

Natural Sources

The main natural sources of HM pollution in soil have been identified as soil parent materials, pedogenic processes, mineral degradation, volcanic eruptions, forest fires, sea spray, and evaporation from soil and water surfaces [18, 19]. The parent substance from which soils are formed is the soil's main source of HMs. The release of HMs into the soil is also caused by biological causes such as destructive forest fires, earthy weathering of metal-bearing rocks and other geological substances, volcanic occurrences, and natural processes including erosion and surface winds blowing dust particles [20 - 22]. Another source for the movement of HMs in nature is the gaseous exchange and bubbles that create bursts in the water [23]. However, anthropogenic sources are more substantial than natural ones.

Anthropogenic Sources

Metals are natural elements but increased levels of HMs have been found in soils as a result of anthropogenic inputs such as extensive use of agrochemicals (fertilizers and pesticides), wastewater irrigation/sewage sludge supplementation, use of organic wastes and manures, higher atmospheric depositions by industrial units, combustion of fossil fuels and other aerial sources [24, 25]. Mining and other industries also add up to HMs pollution in soil [26, 27]. The leading sub-sections provide explanations for some of these sources.

CHAPTER 4

Bioremediation Strategies for Heavy Metal Detoxification in Plants

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Abstract: Contamination of vital environmental components especially water and soil by heavy metals (HMs) due to effluents from various industries and the use of inorganic chemicals for farming is an issue of major concern these days. These heavy metals are non-biodegradable and cannot be vanished by any kind of natural biological or chemical decomposition. Therefore, eco-sustainable methods of heavy metal treatment need to be adopted to eliminate these harmful pollutants. Utilization of microorganisms and plants, living organisms for the eradication of HMs from soil and water has proven to be a highly efficient as well as cost-effective strategy. All types of microbes have the tendency to accumulate the optimum amount of heavy metals within them, however most common are bacteria and algae. Similarly, phytoremediation is a common detoxification mechanism, in which the plants absorb heavy metals from their surroundings and accumulate them in harvestable parts. By studying this chapter, students will become aware of the need for detoxification of heavy metals, various bioremediation strategies (phytoremediation, cyanoremediation, and mycoremediation), and biotechnological advances in these mechanisms for the removal of heavy metals from the ecosystem.

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Keywords: Bioremediation, Phytoremediation, Cyanoremediation, Mycoremediation.

INTRODUCTION

HMs are the concerning contaminants that are being added to the ecosystem on a large scale due to different reasons, both natural as well as anthropogenic. Uncontrolled accumulation of these HMs in soil and water leads to their entry into plant systems and further to the animal and human bodies through the food chain. Most of the HMs have no or little physiological function in living organisms and that too at very low concentrations. Therefore, they have been reported to pose serious threats to normal biological processes in living beings ultimately resulting in plant stress. In order to cope with the deleterious effects of these HMs, some sustainable, long-lasting, and cost-effective strategies must be chalked out. A vast number of research studies done in the past and recently have evidenced the implication of living organisms themselves to protect themselves from the noxious effects of HMs. This method of exploitation of living beings to get rid of HMs is termed Bio-remediation. The living organisms which are being utilised for this purpose include microbes and plants. Among microorganisms, algae, bacteria, and fungi have been reported to show potent efficiency in the elimination of HMs from agricultural soils. Various plants also possess the potential to collect the HMs within their roots and not transport them to the aerial parts, making them suitable remediation agents. This chapter mainly covers the details of processes of different bioremediation strategies along with the most common examples of living organisms being utilised for this purpose. In addition to this, the recent advances in molecular biology pertaining to the genetic engineering of plants for more effective utilisation as bio-remedial agents have also been discussed.

NEED FOR HEAVY METAL DETOXIFICATION

HMs are chemicals with a very high density as a result of which they are potentially toxic to all living organisms. A variety of natural as well as anthropogenic activities may result in HM accumulation in the environment (Fig. **1**). One of the various natural processes that lead to the accumulation of Zn, Co, Ni, Cr, Fe, and Mn HMs in the biosphere is weathering of rocks. Waste from gasoline, paint industries, mining, coal combustion, pesticide usage, and leather tanning are the most common human-related causes of HM contamination [1, 2]. Some of the most common sources of various heavy metals have been enlisted briefly in Table **1**.

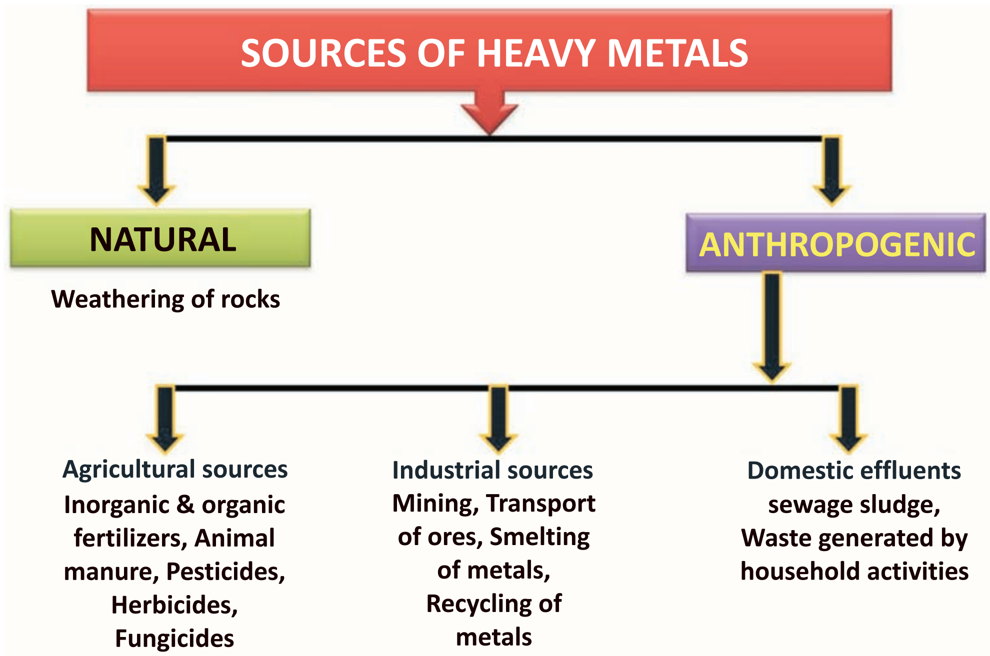

Fig. (1). Natural and anthropogenic sources of heavy metals.

Table 1. Various sources of heavy metals.

Sr. No.	Heavy Metals	Source of Heavy Metals	References
1.	Ar	Arsenical pesticides, Weathering and erosion of As-containing rocks.	[3 - 5]
2.	Hg	Industrial effluents, mining, agricultural waste, wastewater treatment and incineration; ore smelting, biogeochemical cycles.	[6 - 8]
3.	Pb	Fertilizers, Pesticides ore smelting, battery and paints industry effluents.	[9 - 11]
4.	Cr	Electroplating, metal processing, agricultural by-products, dye manufacturing, leather tanning, and paper manufacturing.	[12 - 14]
5.	Al	Acid rain, mining, natural deposition, and soil acidity from ore leaching.	[15, 16]
6.	Cd	Ore outcrops, smelting metal, sewage sludge, and home garbage, agricultural waste and pesticides.	[17 - 19]
7.	Au	Food manufacturing and packaging, jewelry production, pharmaceutical manufacturing, as well as ore leaching and smelting processes.	[20 - 23]

Enormous quantities of HMs pose serious threats to human health. Drainage of water from agricultural fields, mining, and industrial waste is mainly responsible for increasing levels of heavy metals in water bodies [24]. Therefore, the upsurging levels of heavy metals and their derivatives in landfills cause ecological

CHAPTER 5

Probiotics as a New Means for Heavy Metal Detoxification

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Abstract: Heavy metals can harm the bodies of all living organisms in terrible ways. This frightening situation will grow into a severe crisis when polluted living beings containing harmful heavy metals enter the human food chain. Probiotics have a great potential to minimize such kind of heavy metal poisoning in living beings. Probiotics have tremendous potential to effectively bioremediate various toxic heavy metals or metalloids (Cadmium (Cd), Mercury (Hg), Lead (Pb), Arsenic (As), *etc*.) from a variety of polluted environments. Endogenous presence of probiotics or exogenous application of probiotics can be proven to lessen the toxicity of these heavy metals inside living beings' bodies. Due to this reason, probiotics are also popularly known as biological detoxification tools. This book chapter provides comprehensive details related to probiotic strains, their mechanism of action, and their role in various heavy metal detoxification processes. This chapter also sheds light on recent progress in generating genetically engineered probiotics for treating HM toxicity.

Keywords: Detoxification, Heavy metals, Probiotics, Toxicity.

INTRODUCTION

Modern industrialization has caused major contamination inside soil, water and air ecosystems. Among the different types of contamination, heavy metals (HMs) are one of the most common and inescapable encounters a variety of organisms face either through direct or indirect sources, for example, HMs entering the food chain. Conventional ways to detox heavy metals from organism bodies, especially the organisms like fish, goats, *etc*., that are an essential part of the human food chain, are an extremely expensive, risky, and tedious process. In contrast to this,

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probiotic methods for heavy metal detoxification are very cheap, easy, specific, safe, effective, and green alternatives with lots of health-beneficial impacts on the organisms. Moreover, after examining the previous literature on probiotics' reactions to a variety of toxins, including heavy metals, we found that some strains and combinations improve health and detoxification processes *in vitro* and *in vivo*. There are diverse varieties of traditional and next generation probiotics strains like *Lactobacillus, Lactococcus, Bifidobacterium*, *Escherichia coli*, some species of *Bacillus, Streptococcus, Enterococcus, Pediococcus, Akkermansia, Bacteroides, Faecalibacterium, Eubacterium* and *Saccharomyces* yeast used for effective removal of toxic heavy metals from the organism bodies [1]. These probiotics remove heavy metals from the organisms' bodies mainly by the processes known as biosorption and bioaccumulation. The primary mechanism adopted by most probiotics especially *Lactobacillus* for heavy metal detoxification is the biosorption of heavy metal ions into bacterial cell walls, which is facilitated by the presence of teichoic acids and polysaccharides, which are helpful in attracting, and reducing oxidative stress, and sequestering heavy metals [1]. This biosorption process is then followed by bioaccumulation within the bacteria through the utilisation of cell membrane transition [1]. Further probiotics like *Bifidobacterium* actively transport or efflux heavy metals reducing circulating toxic heavy metal levels inside the organism exposed to contamination [1]. However, nowadays, genetically engineered microorganisms (GEMs) for heavy metal removal are popular due to their low cost, adaptability, and environmental friendliness. GEMs with high degradative potential are used in heavy metal bioremediation in plants, animals, groundwater, soil, and activated sludge. Two main methods are used to develop heavy metal-resistant GEMs: functional surface aggregates with a strong metal-binding ability for biosorption and metal ions carried into the cytoplasm and processed by storage mechanisms for improved intracellular bioaccumulation [2]. Therefore, the consequences of heavy metal toxicity are reversed inside the organism's body.

Potentially Effective Probiotic Strains (Traditional and Next Generation Probiotics) for Heavy Metals' Removal

According to the WHO (World Health Organization), probiotics are "live bacteria that, when supplied in suitable proportions, impart a health benefit to the host" [3]. Several probiotics have beneficial features such as the ability to bind to or resist heavy metals *in-vitro* that can be used for heavy metal detoxification and bioremediation (Fig. **1** and Table **1**). Previous studies have shown that probiotics have a strong ability to cling to the intestinal mucosa, to regulate the immune system, to tolerate gastrointestinal fluid, and to repress the growth of pathogens and to mitigate the heavy metal contamination [4 - 6]. Genera like *Lactobacillus, Lactococcus* and *Bifidobacterium*, *Escherichia coli*, some species of *Bacillus,* *Streptococcus, Enterococcus, Pediococcus,* and *Saccharomyces* yeast, which are effective in the treatment of several gastrointestinal problems and remediating heavy metal toxicity, are traditional probiotic strains that are marketed [7]. Several studies have reported the role of traditional and next-generation probiotics in removing heavy metal toxicity. For instance, *Lactobacillus reuteri* P16 and *L. plantarum* CCFM8661 against Pb [8, 9] toxicity, *L. plantarum* TW1-1 employed against Cr toxicity [10], *L. brevis* 23017 for Hg toxicity, and *B. cereus* and *L. plantarum* CCFM8610 against Cd toxicity [11, 12]. Through intestinal heavy metal sequestration and intestinal peristalsis, these strains can encourage the fecal elimination of heavy metals, lowering heavy metal absorption in the gut and correcting heavy metal-induced alterations to the gut microbiota.

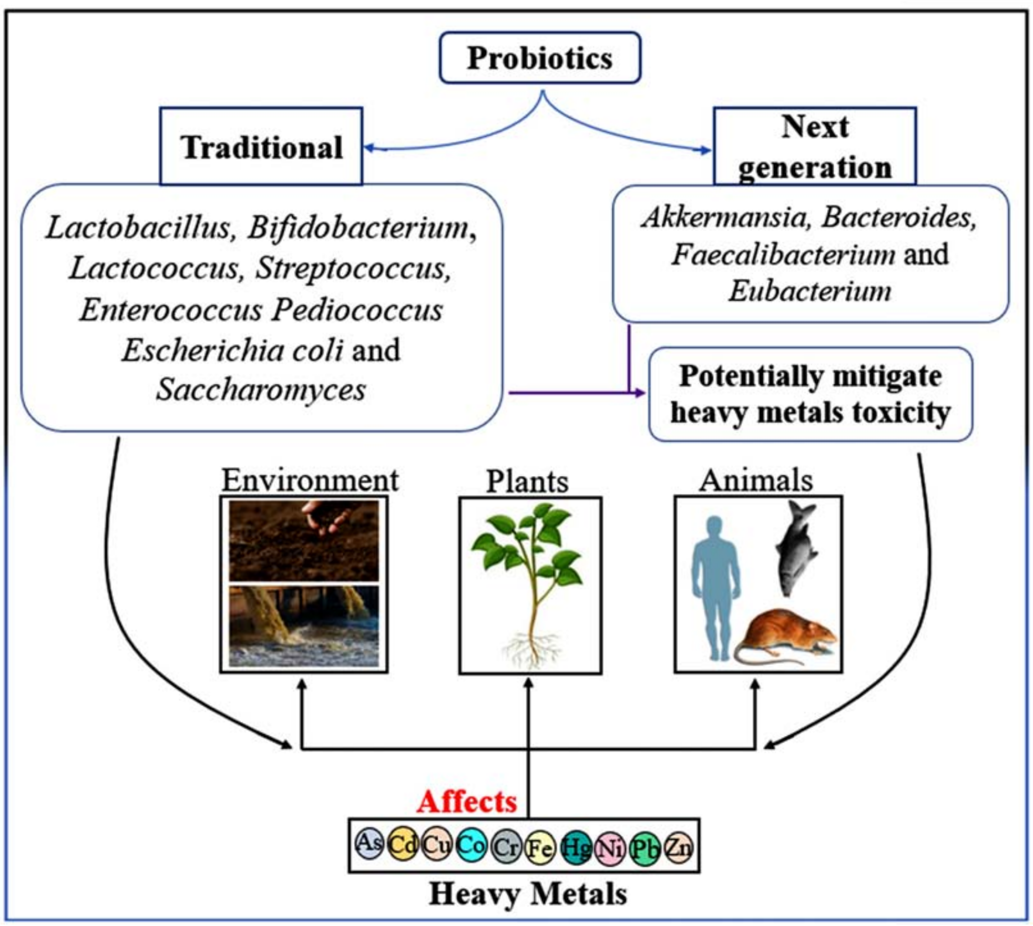

Fig. (1). Traditional and next-generation probiotics used for heavy metal removal.

CHAPTER 6

Plant Growth Promoting Bacteria as Promising Candidates for Heavy Metal Detoxification

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Abstract: Heavy metals are among the primary contaminants of the environment, which are released due to geological and anthropological activities. Although some heavy metals are vital for life processes, however, higher concentrations of certain metallic ions terribly disturb the equilibrium and remarkably become risky for human health. Owing to their toxicity and accumulation, the conventional means of their management are pricey, unfeasible, and trigger secondary pollution concerns. Hence, a novel, practical approach like bioremediation has gained importance where innate biological mechanisms in the microbes are utilized to degrade and detoxify metal ions. Being ubiquitous, eco-friendly, and cost-effective, the microorganisms alter soil characteristics including pH, valency, adsorption, chelation, and precipitation of pollutants. Moreover, they utilize direct and indirect mechanisms to suppress the toxic effects of metalloids and other combative in the environment. Also, rhizosphere-plant consortium in polluted soils plays a crucial role in improving plant tolerance by driving crucial nutrient cycles thereby, facilitating their survival in harsh circumstances. In addition to bacteria, fungi, and algae have also been utilized over the past years to nullify the danger caused by heavy metals. The present book chapter highlights the toxicity of heavy metals on plants and the mechanisms employed by plant growth-promoting bacteria to detoxify these heavy metals.

Keywords: Bioremediation, Heavy metals, Microbes, Plant growth-promoting bacteria, Rhizosphere-plant consortium.

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INTRODUCTION

Exponential growth in urbanization and industrialization has led to one of the major environmental pollutions known as metal pollution. The escalated concentrations of some of the inorganic elements pose a greater liability for human health and their surroundings owing to higher toxicity [1 - 4]. As compared to the biodegradable entities, these metalloids do not undergo chemical/ microbial degradation or be removed but are known to magnify into the major sinks such as soil or water over the years. Their bioaccumulation in organisms both on land and in water occurs through the food chains/ food webs eventually reaching the upper trophic levels [4 - 6]. Some of the well-known heavy elements include Lead (Pb), Mercury (Hg), Zinc (Zn), Iron (Fe), Cadmium (Cd), Cobalt (Co), Copper (Cu), Nickel (Ni), Arsenic (As), Chromium (Cr), and Selenium (Se). Though at lower concentrations, some of these elements- Fe, Zn, Cu, N, Mn, and Co are vital for the normal functioning and growth of living organisms, while others prove to be toxic. Thus, both the deficiency and sufficiency of heavy metals (HMs) can influence the plant systems [1, 7 - 9, 321].

In order to reduce metal pollution, various conventional methods have been adopted over the past years. These include physicochemical remedies such as excavation, electrochemical treatment, ion exchange, soil washing, in situ fixation, precipitation, adsorption, and evaporation technology [10 - 12]. However, these procedures are expensive, intrusive, demands high labour, are not apt for large-scale field applications, and raise serious concerns by disturbing both soil structure and its native microflora [6, 13 - 15]. Therefore, a more feasible, ecologically sound, non-invasive approach of bioremediation is being adopted for the restoration of a healthy environment free from heavy metal pollution [16 - 18].

Bioremediation is a surging, remarkable, cost-effective, and modest practice for restoring soils attenuated with heavy metals. It utilizes green plants, micro-fauna such as fungi, bacteria, yeast, and algae or their enzymes to restore the polluted grounds to their primary states [16, 19 - 21, 326]. The whole process solely relies on the native biological vigor, which in addition is dependent on several soil-related parameters including structure, pH, temperature, oxygen availability, moisture content, nature of contaminants, nutritional value, microbial heterogeneity, *etc* [16, 22, 23]. Detoxification *via* plant growth-promoting bacteria (PGPB) is the most crucial method that can alleviate heavy metal (HM) toxicity through a variety of processes including mobilizing/ immobilizing, uptaking, and transforming heavy metals and acclimatizing plants with the metal-polluted environment by producing siderophores, phytohormones, and antibiotics, and causing chelation, phosphate solubilization, biological nitrogen fixation and synthesis of lytic enzymes [2, 17, 24, 25, 322 - 326]. They can be free-living (communicate with plants under apt conditions), symbiotic (live in rhizospheric zones), or endophytic (form substantial associations with plant tissues/organs). They positively alter growth and productivity by producing plant growth regulators, driving essential nutrient cycles, and altering translocation and accumulation by modifying the photo-availability of HMs in attenuated soils [26 - 28]. Some commonly known genera of PGPB include: *Agrobacterium* spp., *Acinetobacter* spp., *Arthrobacter* spp., *Azospirillum* spp., *Azotobacter* spp., *Bacillus* spp., *Delfitia* spp., *Paenobacillus* spp., *Pantoea* spp., *Pseudomonas* spp., *Streptomyces* spp. and *Rhizobia* spp [28, 29].

Naturally, microbial-heavy metal detoxification in polluted spots is known as natural attenuation. However, depending on whether excavation is required or not, the process is mainly divided into two approaches- "*in-situ*" and "*ex-situ*" (Fig. 1). Of the two reclamation processes, *in-situ* (on-site) is usually preferred with minimal health risks, no site disturbance and transport of attenuated soil, with low cost as the soil is confined to their actual place throughout the process [18, 30, 328, 329]. It is further categorized into two types of remediation- intrinsic and engineered bioremediation depending upon the kind of microorganisms used. For intrinsic remediation, the metabolic activity of indigenous microbes is boosted while in the engineered process, specific genetically engineered microorganisms are used [31, 32]. In the case of *ex-situ* bioremediation, polluted soil, and water are excavated from the actual location and are further divided into solid-phase, slurry-phase, and vapour-phase systems (Fig. **1**) depending upon the type of contaminated samples [18, 32 - 34, 327, 328]. The current chapter summarizes the deleterious effects of heavy metals on plants and highlights the role and mechanisms followed by plant growth-promoting bacteria for the detoxification of heavy metals.

Sources of Heavy Metals

In the environment, weathering of rocks and parental chemicals normally occurs in trivial quantities (1000 mg/kg) and is rarely harmful [35, 36]. Most soils could build up heavy metals over the allowed average levels, significant enough to cause health concerns to people, flora and fauna, ecosystems, or other media in remote or urbanized settings [37, 38]. Various studies have recognized heavy metals as potential contaminants in the soil ecology for varied reasons; a) Heavy metals serve as soil pests because their rate of proliferation through synthetic phases is faster than those of natural sources; b) They are transported from mines to arbitrary ecological regions with increasing chances of direct exposure; c) Redundant products have higher heavy metal content than the receiving area; and d) In comparison to pedogenic or granular metallurgical soils, normal soils hold more movable and bioavailable heavy metals [39]. Therefore, heavy metals get

CHAPTER 7

Phytohormones and Heavy Metal Detoxification: Current Status and Future Perspectives

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Abstract: Escalating application of heavy metals (HMTs) due to diverse anthropogenesis is a staid problem. In contaminated soils plants are severely affected by HMTs contamination. Under such conditions, endurance of plants becomes difficult and overall vigor is highly affected under HMTs stress. Detoxification of HMTs in metal tainted soil is done by various biochemical and chemical methods. Plant hormones are signal molecules that are synthesized by plants and the main group of phytohormones includes auxins, gibberellins cytokinins, abscisic acid, brassinosteroids and jasmonic acid. It is interesting to note that using plant hormones to lessen the harmful effects of HMTs has become increasingly significant in recent years. Plant hormones are signal molecules that are synthesized in different parts of plants, which have a major impact on plant metabolism and play vital role in abiotic stress mitigation. Though, phytohormone level is altered under abiotic stress conditions, which cause plant growth and developmental inhibitions. Metal stress has also been reported to hamper the concentrations of phytohormones in plant tissues. Phytohormones act as signaling entities for mitigation of HMTs stress in plants, thus let them to regain their original growth and plasticity. The present chapter mainly focuses on the potential roles of phytohormones in recreating the mitigation of heavy metal toxicity in the environment. We will focus on the study of accumulation, uptake and translocation of heavy metals in plants and give different pathways of how different phytohormones interact with plant protection systems under heavy metal toxicity. The function of numerous genes, enzymes, proteins, and signaling compounds associated with HMTs tolerance by phytohormones and their regulation will be explored. Thus, this this chapter will cover the different aspects of HMTs toxicity tolerance in plants with the use of plant growth regulators for sustainable cultivation in the future prospects.

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Keywords: Abiotic stress, Environment, Heavy metals (HMTs), Phytohormones, Toxicity.

INTRODUCTION

Heavy metal stress has substantial influence on metabolism, plant viability, and sometimes on the ability of the plants to resist various effects of pathogens such as low crop productivity. To increase productivity extensive use of chemical fertilizers and artificial irrigation have been employed extensively, which is a common practice all over the world. Sometimes, such practices cause accumulation of metal in soil to such an extent that the land cannot support growth of any agricultural crop any more. The need of the hour is to find some other solution thatis technically simple and correct so that crop productivity can be increased without harming the environment. In this way use of plant growth regulators is simple and technically right way to make plants tolerant of heavy metal stress without harming ecology and environment. Phytohormones or plant hormones are naturally occurring organic substances that influence physiological functions in plants at very low concentrations [1]. In other words, phytohormones are growth regulators that regulate various cellular activities of plants [2]. Then [3] reported that seven categories of phytohormones, that is, Auxins, Cytokinins (CK), Gibberellins (GA), Abscisic acid (ABA), Ethylene (ETH), Brassinosteroids (BR) and Jasmonates (JA) have been identified so far. The first five phytohormones are sometimes referred to as the "classical" phytohormones, while the latter two are more additions to the growing phytohormonal family [2]. Among several heavy metals, four heavy metals Arsenic (As), Lead (Pb), Cadmium (Cd), and Mercury (Hg) are recognized as the major lethal metals based on their toxicity, occurrence, and most significantly, their revelation to plants and animals. Minute concentration of phytohormones play significant role in modulating the growth and development of plants [4]. Also, their role has been acknowledged in rendering plant HMTs stress tolerant therefore, this eco-friendly approach of using phytohormones against HMTs has been gaining greater importance [5].

The study [6] avowed that in response to abiotic stresses, it triggers signal transduction pathways and exogenous application of phytohormones is ensured to upregulate stress resistance in plants facing HMTs. The advancements in molecular studies such as mutant screening, expression profiling, microarray, genomics and proteomics have helped in looking deeper into mechanisms and pathways that phytohormones follow to shield plants against HMTs stress [7]. Still very little information is available in the stress management roles of phytohormones towards HMTs signaling pathways. In the present scenario, our chapter mainly focuses on the role of phytohormones auxins, cytokinins, gibberellins, ethylene, abscisic acid, brassinosteroids, and jasmonates in HMTs stress mitigation in plants (Fig. **1**).

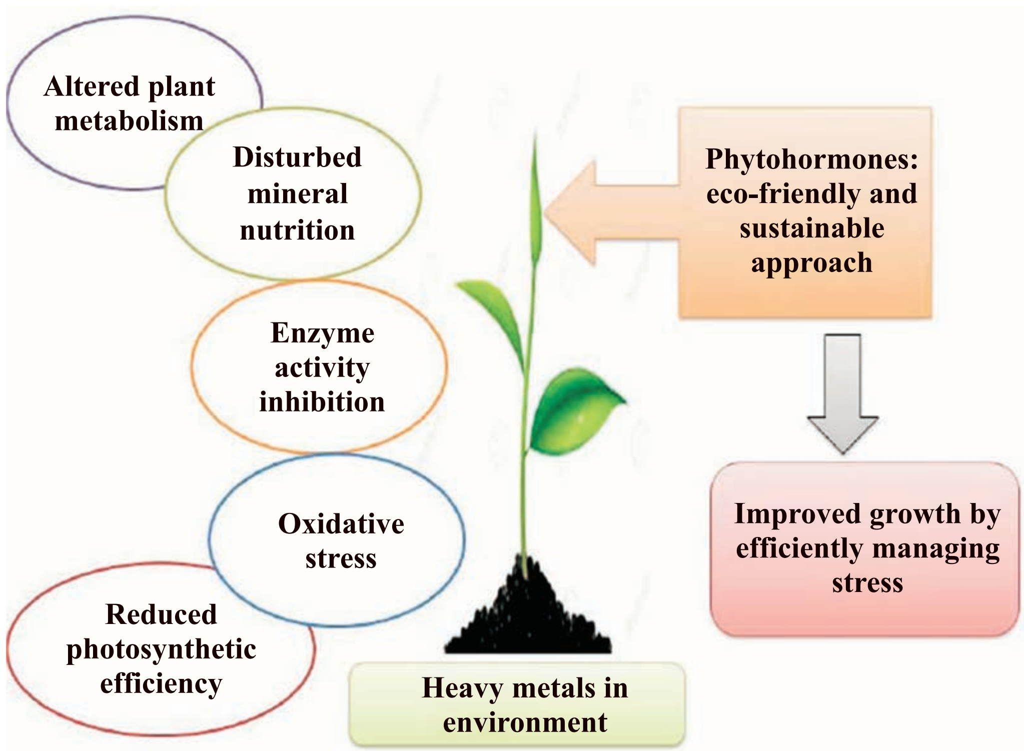

Fig. (1). Generalized mechanism of Heavy metals detoxification in plants by phytohormones.

Heavy Metals (HMTs) and their Toxicity in Plants and Environment

Arsenic (As) is a probably occurring metal which create staid healthiness to peoples across the world [8]. The contagion of As in underground water affects crop productivity and also accumulates in diverse plant tissues, grains and also contaminates food chain [9]. Different investigations have been carried out to explore the physiological and molecular means of As toxicity, accretion, detoxification, and tolerance in numerous plants including lettuce, rice, carrot and spinach [10]. **Lead(Pb)** is an abundantly distributed trace metal that exists in various forms in natural sources. It can affect plants and animals, by contaminations from leaded dust, fuels, old lead plumbing pipes, various industrial sites, or even old orchard sites in production where lead arsenate is used [11]. Pb^{2+} is non-biodegradable and its exposure is toxic to both flora and fauna [12]. **Cadmium (Cd)** is known as a heavy metal with phytotoxic nature, It is absorbed by the plants as it is soluble in water that is the main pathway for entry

CHAPTER 8

Microalgae-based Remediation of Heavy Metals Polluted Environment

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Abstract: Population explosion and rapid industrialization surge are posing a serious threat to plants, the human population, and the world's environment. These vigorous developmental prospects lead to the production of serious pollutants and higher concentrations of toxic heavy metals (HMs) in the environment. These toxic HMs are severely compromising the global environment, induce toxicity to the living systems, and cause the deterioration of water and land ecosystems globally. However, to minimize these toxic pollutants, certain remediation methods have been adopted to bring these pollutants to a minimum threatening level. Thus, remediation mechanisms like biological and non-biological methods are brought into consideration. Among these methods, biological methods like novel, phytoremediation techniques by employing "microalgae" are considered to be the most effective, inexpensive, easy to implement, and eco-friendly among all the recommended methods. Phytoremediation requires sunlight as energy input whereas it undergoes environmental reclamation such as nitrate, phosphate, and HMs removal from the wastewater sources as well as mediates HMs elimination from the soil. Microalgae act as bio-accumulators and further lead to HMs precipitation and fixation inside the algal tissues. This chapter reviews the application of different microalgae strains, their bioremediation strategies, and mechanisms adopted under HMs stress environments.

Keywords: Bio-accumulators, Biofilm, Biomagnification, Biosorption, Carcinogenic, Food chain, Heavy metals, Microalgae, Photo-bioreactors.

INTRODUCTION

Heavy metals (HMs) are among the natural constituents that are known to exist in the earth's crust and soil. However, HMs are defined by their density, or generally

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as an element with metallic properties and having an atomic weight ranging between 63.5 to 200.6, such as Copper (Cu), Lead (Pb), Silver (Ag), Iron (Fe), *etc* [1, 2].

Whereas, from an ecological point of view, HMs are those elements that are posing toxic impacts on the environment and are non-biodegradable, and highly toxic at low concentrations [3]. Among these HMs, some are considered to be micronutrients that are potentially required for the growth and development of plants such as Zinc (Zn), Cobalt (Co), Copper (Cu), Nickel (Ni), Manganese (Mn), *etc* [4]. However, extensive industrialization, urbanization, and false agricultural practices have globally increased the concentrations of these HMs in aquatic as well as terrestrial ecosystems [5, 6]. These rapid developments and technological innovations have further increased human contact with these toxic heavy metals through agriculture, and wastewater globally. These heavy metal residues exist in different chemical states in the environment and pose serious impacts on human health, plants, and the associated ecosystems [7]. Plants grown under HM-contaminated soils are found to have visible symptoms like chlorosis, stunted growth, root browning, and even death. Also, extensive concentrations are found to show hindrance in the normal functioning of the cellular, metabolic, and genetic potential of the affected plants [8]. In humans, these HMs can lead to multiple ill impacts from mutagenicity, bioaccumulating amplification, and carcinogenic and teratogenic effects. Also, after their entry into the human system, these HMs lead to arthralgia and headache, mental disorders, abnormal kidney, and liver functioning [9, 10]. In aquatic ecosystems, these HMs will flow through the wastewater and accumulate in oceans too. Their concentrations will lead to multiple detrimental impacts on aquatic organisms like fish. These aquatic organisms need good quality water for their survival and their contamination through different pollutants has existential pressure on these aquatic organisms. HMs also get their entry into humans through the aquatic food chains and accumulate along the different food chains [11]. Moreover, other health issues associated with the HMs accumulated in drinking water may lead to muscle atrophy and loss of appetite. Whereas, Cd toxicity may lead to bone mineral deficiency as well as kidney poisoning. Hexavalent Cd is well known to cause birth defects in newborn babies and other issues like diarrhoea and vomiting even at low doses [12 - 14]. However, in soil ecosystems, these HMs accumulate through multiple human activities like energy production, fuel production, mining, electroplating, agriculture, and wastewater sludge treatment. HMs like iron, arsenic, mercury, nickel, chromium, lead, cadmium, and zinc after reaching certain amounts get infiltrated into the groundwater and subsequently enter different food chains through crop plants, leading to the disruption of biological processes [15]. Furthermore, the elimination and treatment of HMs from polluted ecosystems is a challenge and thus requires an economical and efficient method to treat these pollutants. These methods may include physical, chemical, and biological methods to treat these ecosystems. Among these methods, biological methods have gained importance due to their eco-friendly and feasible nature with no secondary pollutants and thus gained importance in treating HMs [16, 17]. Microalgae have been introduced in order to eliminate these HMs from aquatic as well as terrestrial ecosystems. "Microalgae" are microscopic plants that are available in aquatic ecosystems and have been extensively exploited to treat sewage treatment plants since 1950 [15, 18]. However, microalgae have emerged as a potential bioremediation agent with promising solutions to clean up the HMs from the environment [19]. These microalgae cells are acclimatized to accumulate HMs, as well as act tolerant through ROS detoxification mechanisms under HMs toxicity and further undergo bioabsorption, chelation, and bioaccumulation of these HMs [20 - 22]. Also, the microalgal populations are efficient as they have a high capability of reproducing themself. High nutrient loads in the wastewater are the priority requirements for algal growth like nitrogen and phosphorous *etc*, whereas microalgal photosynthesis is triggered by CO_2 eliminated through wastes [23]. These microalgal populations are typically used as biosorbents to eliminate HMs, which undergo multiple steps from the biomass selection process, pre-treatment, and immobilization [24]. However, most of the time, a natural biofilm or mat is synthesized as a complex matrix that is typically made up of several microorganisms, including microalgae, bacteria, and fungal populations [25]. Microorganisms and microalgae upon colonizing the surface undergo secretions of multiple compounds such as polysaccharides, nucleic acids, and phospholipids and help to adhere to micronutrients [26]; thus, are efficient in the removal of organic pollutants, nutrients, pathogens, and HMs from the wastewater to get treated [27]. Therefore, microalgae-based heavy metals bioremediation in wastewater has been thoroughly discussed in this study and is considered to be a potential answer to address current environmental issues related to heavy metal contamination in aquatic ecosystems.

GENERAL OVERVIEW OF DIFFERENT HMS AND THEIR SOURCES

Heavy metals (As, Pb, Cd, Hg, Ni, Co, Cr, Zn, La, *etc*.) are generally considered non-essential, and toxic because they pose a health risk to the life of all living beings. These heavy metals get into the ecosystem through both natural and man-made processes. According to the findings of a large number of studies, natural sources of heavy metals in the environment are often less frequent as compared to anthropogenic activities [18]. 95% of Earth's crust is igneous rocks and 5% is sedimentary [28]. In general, basaltic ingenious rocks are abundant in a variety of heavy metals. These heavy metals in rocks can get into the soil environment through a variety of natural processes like erosion, leaching, surface winds, rain, weathering, biological, geological, terrestrial, and volcanic processes [29]. The

CHAPTER 9

Earthworm-mediated Remediation and Mitigation of Heavy Metals Toxicity in Plants

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Abstract: Earthworms are the ecosystem engineers that convert waste into vermicompost. The vermicompost is further utilized to improve the soil's organic composition, condition, and health. Recently, earthworms have been explored as an effective, efficient, and eco-friendly remediation approach called 'vermiremediation' to mitigate the toxic elements from the soil. The soil contains different types of essential or non-essential elements. The presence of these elements above threshold levels in the soil leads to its contamination. The major soil contaminants include xenobiotic compounds, agrochemicals, and heavy metals. The plants exposed to higher amounts of heavy metal-containing soils show symptoms of metal-induced phyto-toxicities that result from the loss of soil fertility, disturbance in nutrient uptake and translocation, and interruption in the regular physiological functions of affected plants. To overcome heavy metal-induced toxicities in plants and soils, the treatments of earthworms, either alone or in combination with PGPR or other soil amendments, are being tried. The present chapter is an attempt to compile information about phytoremediation and vermiremediation, distribution of earthworms in contaminated soils, remediation and amelioration of heavy metals by earthworms, and factors affecting bioaccumulation of heavy metals in earthworms.

Keywords: Bioaccumulation, Bioremediation, Phytoremediation, Phytotoxicity, Vermi-remediation.

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INTRODUCTION

Earthworms can help in the remediation of pollutants, potential toxicants, heavy metals, and contaminants in the soil [1]. Common soil contaminants that lead to degradation include organic materials, agrochemical residues, pesticides, fertilizers, and other xenobiotic compounds, including heavy metals. The heavy metal contamination poses risks to fertile soils and the health of nearby organisms [2]. These toxic metals reduce soil fertility and hinder photosynthesis and metabolic processes in plants, ultimately lowering crop yields. For remediation of contaminated soils, nano-biochar is being used because of its high hydrodynamic dispersivity and strong rhizospheric interactions [3]. However, its impact on the rhizosphere and biogeochemical behaviour makes it unsuitable for soil remediation due to environmental risks. Thus, biosafety guidelines are needed for the application of nano-biochar. The biochar and earthworms influence soil heavy metal bioavailability, with earthworm-mediated nitrification and gut digestion facilitating remobilization [4]. Rice-husk and sludge biochar immobilize heavy metals, while earthworm gut digestion enhances bioavailability and mobility. The epigeic species of earthworm, namely *Amynthas cortices* and an endogeic species of earthworm *Amynthas robustus* (*A. robustus*), have been found to restore soil properties by enhancing the bioavailability of heavy metals through interaction with soil [5]. Moreover, earthworms effectively absorb heavy metals from polluted soils, acting as pollution bioindicators and soil decontaminators, thereby enhancing organic matter decomposition and plant availability [6]. Hence, to clean up the contaminated soil ecosystem, earthworms can be used along with conventional methods [7]. Fig. (**1**) highlights the major useful roles of earthworms in heavy metal remediation.

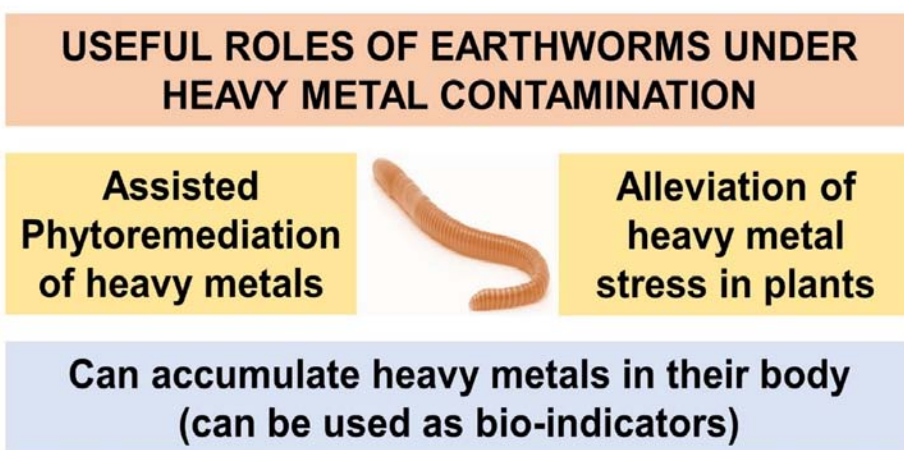

Fig. (1). Major useful roles of earthworms in heavy metal remediation.

As per existing physical remediation practices, polluted sites are restored through soil replacement and thermal desorption methods [8]. Chemical remediation approaches utilize chemical leaching and fixation, vitrifying technology, and electrokinetics remediation methods for soil cleanup. Also, the physicochemical approaches include techniques like soil encapsulation, immobilization techniques, electro-remediation, etc [9]. Soil properties, pH, acidity, and potential release of pollutants limit soil remediation methods. These methods are costly, time-consuming, and eco-friendly, making them unsuitable for large-scale pollution removal. Eco-friendly green technologies like plants, microbes, and earthworms may be more practical. *In situ* and *ex situ* bioremediation employs various biological organisms to eradicate soil toxicants, and it is an economically- and environmentally-sustainable remediation approach [10].

The biological remediation approach includes bioremediation, phytoremediation, plant growth regulators like brassinosteroids, microbes including Plant Growth Promoting Rhizobacteria (PGPR), integrated biological remediation methods, or the use of earthworms has emerged as a green and eco-friendly remediation method [2, 6, 7, 9, 11 - 17]. Several studies highlighted that the use of earthworms is beneficial to improve soil health and productivity [1, 2, 16, 18]. Earthworms can effectively remediate soil pollutants through a process called *vermiremediation*. This involves earthworms accumulating pollutants like heavy metals, crude oil, and non-recyclable and organic contaminants, which are then transformed and degraded by the soil [19]. This process can be used alone or in combination with other soil amendments or microorganisms [1]. The supplementation of phytoremediation, microorganisms, biochar, and surfactants helps in improving the potential of earthworms to remediate polluted soils. Recently, a study emphasized the use of *Eisenia fetida* (an earthworm) as a potential organism for the remediation of agricultural soils affected by sewage sludge [18]. Sewage sludge, used in agriculture, contains heavy metals, increasing soil concentrations. *E. fetida* can reduce these metals and improve soil fertility. Earthworm mucus and microbial symbionts release enzymes that activate biochar, reducing heavy metal levels and enhancing soil nutrients [20]. This hydrophobic interaction helps maintain soil fertility.

Earthworm-mediated activated biochar, known as *vermibiochar*, can effectively remediate heavy metals in textile mill dyeing sludge. This eco-friendly and cost-effective strategy uses *E. fetida* to convert dyeing sludge into nutrient-rich vermicompost [21]. The process enhances nutrient levels, nitrogen, phosphorus, and sodium levels. It also restores pH, Carbon-to-Nitrogen (C/N) ratio, and electrical conductivity, demonstrating that *E. fetida* has tremendous potential for waste sludge conversion into useful manure. The ability of earthworms to detoxify toxic pollutants from the soil into less harmful ones through bio-

CHAPTER 10

Mycorrhizae as an Effective Tool for Heavy Metal Detoxification

Kamini Devi[1], Tamanna Bhardwaj[1], Jaspreet Kour[1], Shalini Dhiman[1], Arun Dev Singh[1], Deepak Kumar[2], Roohi Sharma[2], Parkriti[2], Gurvarinder Kaur[3], Kanika Khanna[1,*], Puja Ohri[2], Geetika Sirhindi[3] and **Renu Bhardwaj[1]**

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Abstract: Heavy metal toxicity in the ecosystems is hazardous for living beings as it enters into the food chains through water and soil. The plants absorb the excessive toxic metal ions from the environment resulting in the disruption of various metabolic and physiological pathways in plants. The removal and stabilization of toxic metals from a plant's surroundings take place by Plant Growth-Promoting Rhizobacteria (PGPR), Endophytic Bacteria, and Arbuscular Mycorrhizal Fungi (AMF). AMF is used as a biological indicator for toxic metal pollution. Mycorrhizae use various mechanisms (avoidance and tolerance) for metal detoxification and help in maintaining the nutrition, growth, ecological processes and functioning, nutrient cycles, and diversity of plants. They have vesicles that act as vacuoles and store an excessive amount of toxic metal ions in them. About 80% of plant families showed a symbiotic connection with Mycorrhizal fungi. AMF produces 20% C (Carbon) from the plant and, in return, benefits the plant with more water uptake and other essential nutrients from the soil *via* their hyphal networks. This chapter presents the importance of Mycorrhizae-assisting phytoremediation by increasing the activities of defense enzymes, expanding the area of absorption, stimulating the expression of genes, and enhancing the chelation of toxic metal ions. This document also focuses on the toxic metal uptake, their amassing, and the mechanism associated with AMF-assisted phytoremediation of metalliferous soil.

Keywords: Arbuscular mycorrhizal fungi, Heavy metal, Phytoremediation.

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INTRODUCTION

Soil contains heavy metals in a variety of forms. They can exist as free ions, complexes, or compounds such as silicates, oxides, and hydroxides. They can also bind organic and inorganic substances. There are two ways, heavy metals can enter the roots once they are available for uptake. Heavy metal stress is an important stress that has adverse effects on plant health. High concentrations of heavy metals contradict normal plant metabolism pathways and other structural and functional processes in plants. The apoplastic pathway occurs first, followed by the symplastic pathway. Heavy metal ions present in the soil enter the plant epidermis through ion channels or as organic compounds formed after combining with chelates released by plant roots [1]. These metal-ligand complexes then enter the root epidermal layer. Heavy metals accumulate in the roots of non-hyperaccumulators because they lack the capacity to translocate heavy metals to aboveground parts [2]. The flow of metals into xylem vessels, which are involved in the transport of water and dissolved salts, causes the translocation of heavy metals from roots to shoots. The breakdown of metals is difficult; thus, when concentrations inside a plant surpass ideal levels, they have a negative direct and indirect impact on the plant [3, 4]. The replenishment of necessary nutrients at cation exchange sites in plants has an indirect, harmful impact. Furthermore, the detrimental effects of heavy metals on the development and function of soil microbes may indirectly affect plant growth [5]. For instance, a high metal content may result in fewer beneficial soil microbes, which may slow the decomposition of organic waste and reduce soil nutrients. The common mechanisms involved in HM stress alleviation by AMF include reduced uptake from the root or storage of HMS in fungal structures, improved nutrient uptake, maintenance of redox status, antioxidant system activation, reduced root-to-shoot translocation, and synthesis of organic acids, proline, and glomalin [6, 7]. Arbuscular mycorrhizal fungi (AMF) are an essential bioagent because they can significantly improve the efficiency of the terrestrial ecosystem by producing fungal structure networks such as arbuscules. Additionally, they facilitate the exchange of inorganic compounds and minerals necessary for plant growth, which in turn provides plants with a significant amount of strength. In addition, they function as biological filters for toxic HMs and contribute to the mitigation of their toxic effects. They do this by supporting the plant by increasing its water uptake, photosynthetic rate, and nutritional intake. This allows the plant to continue growing normally despite adverse conditions. Most of the time, HMs are stuck in the hyphae of fungi that live in a symbiotic relationship with plants. These fungi reduce the availability of heavy metals to plants by retaining them in the cell walls, vacuoles, or cytoplasm through chelation. This results in a reduction of the toxicity of metals to plants. When AMFs colonize the roots of higher vascular plants, the host plant can hold on to the HMs in its roots and external mycelium. Mycorrhizal plant roots have fungal vesicles that are similar to plant vacuoles, with a similar biological function, that is, sequestration or accumulation of harmful chemicals or toxic heavy metals. An increase in the number of enzymes in the soil, *e.g*., acid phosphatase, which enhances growth and nutrient uptake in plants, was also elevated by AMF under heavy metal stress. The cell wall is the main subcellular fraction that participates in heavy metal detoxification [8, 9]. AMF can boost the generation of phytochelatins [10]. Such peptides are produced by the enzyme phytochelatin synthase (PCS), which is induced by the presence of heavy metals using glutathione (GSH) as a substrate.

Uptake and Amassing of Heavy Metal Ions

The bioavailability and uptake of metal ions through roots initiate the uptake and accumulation of heavy metal ions. Roots take up heavy metals from the soil, which then translocate to other plant parts and get amassed in different regions of plant cells (Fig. **1**). Owing to the barrier effect, heavy metals frequently remain in roots [11]. The uptake and amassing of heavy metal ions in plant tissues depend on several factors, such as plant species, nutrient availability, organic matter, pH, temperature, moisture, and the most crucial factor: the presence of metals in the soil. The apoplastic pathway enables the soluble metal portion to pass across intracellular gaps without entering cells. In contrast, the symplastic pathway uses energy to transfer non-essential metals, such as Cd, Ni, and Pb through the cytoplasm [12]. Various plant species use diverse mechanisms to absorb various heavy metals, resulting in variations in their transport pathways. H+ ATPase/pumps are one of the pathways through which heavy metals enter the roots. They aid in maintaining a negative potential across the epidermal membrane of the roots. The uptake of heavy metal ions by plants involves other transporters, such as those belonging to the heavy metal ATPase (HMA), Zrt/Irt protein (ZIP), and natural resistance-associated macrophage protein (NRAMP) family [13]. Typically, root hairs increase the root surface area for heavy metal absorption, allowing ions to move swiftly inside the apoplast. Once absorbed by the roots, heavy metal ions exhibit apoplastic migration from the root epidermis to the cortex and are directly bonded to the carboxyl groups of mucilage uronic acid or to the polysaccharides in the rhizodermis. Because Casparian strips block the apoplastic pathway in the endodermis, these ions primarily build up in the root cells [14]. After passing through the endodermis, heavy metal ions move to the aboveground parts of the plant along the symplastic pathway through vascular tissues [15]. This phrase refers to all substances that must be carried from the roots to the leaves and vice versa through xylem tissues. It is an energy-intensive process that allows the entry of metals following the symplastic pathway. This is due to the Casparian strip of the endodermis, which prevents intracellular mobility during normal transport [16]. As a result, metals traveling through the apoplast

CHAPTER 11

Nanoremediation of Heavy-Metal Polluted Soils

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Abstract: Soil contamination with heavy metals poses a significant challenge to environmental sustainability and human health. Conventional remediation techniques, while effective to some extent, are often limited by high costs, lengthy processes, and site-specific challenges. Nanoremediation offers an advanced solution by utilizing nanoparticles' unique properties for efficient remediation. This chapter reviews advancements in nanoremediation, highlighting the diverse range of nanoparticles used for soil remediation, such as metal oxides, carbon-based materials, and biogenic materials. Sustainable approaches, including green and conventional synthesis methods, as well as nanobiotechnology strategies that integrate microbial and plant-based remediation, are emphasized. Further, mechanisms such as adsorption and redox transformations in nanoparticle-heavy metal interactions are explored alongside their potential impacts on soil ecosystems and microbial health. The chapter concludes with future prospects, advocating for scalable and cost-effective nanoremediation technologies and addressing regulatory considerations to ensure environmental restoration.

Keywords: Green Nanotechnology, Heavy metals, Nanoparticles, Remediation, Soil pollution.

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INTRODUCTION

Soil is an essential part of our ecosystem and fundamental for healthy crop production as it provides anchorage for roots, water and nutrient retention, and a habitat for microorganisms [1]. However, modern industrialization and the widespread application of chemical fertilizers in agricultural practices and other anthropogenic activities are progressively adding to the Heavy Metal (HM) pollution of soil, thereby degrading the soil quality and rendering it unsuitable for the growth of plants and microorganisms [2]. Contamination of soil with HMs has become a global concern due to their potential to accumulate at the various trophic levels of the food chain and, thus, pose a threat to human and environmental health [3]. Therefore, remediation of soil is an emerging challenge in the modern era. Numerous conventional methods have been employed to remove the pollutants from contaminated soil [4]. These include physical methods such as soil washing, vapour extraction, land farming, soil flushing, and chemical remediation by using chemical chelators, immobilization, oxidation, critical fluid extraction, *etc*. The use of conventional procedures is constrained by their high cost, laborious nature, risk of secondary pollutants, and need for excavation of contaminated soil [5]. In this context, bioremediation (the use of plants, microorganisms, and their products to remove pollutants) has been extensively studied and employed to remediate contaminated soils due to its cost-effective, eco-friendly, more efficient, and sustainable approach [6]. The advancements in nanotechnology have added new perspectives to the process of bioremediation of contaminated soil and water [7]. Nanotechnology has been recognised as a promising tool to remediate or restore contaminated soil efficiently by alleviating the toxicity of HM. Nanoremediation involves the use of nanoparticles (NPs), such as engineered and green synthesised NPs, to remove contaminants through various processes such as adsorption, reduction of toxic forms of HM into less toxic forms, photocatalysis precipitation, *etc*. Due to the unique physico-chemical properties, smaller particle size, enormous specific surface area, and high reactivity, NPsact as catalysts and possess the potential to lower the activation energy required for the reduction of compounds [8]. Lately, there have been numerous studies to synthesise and examine the effectiveness of various NPs in the remediation of HM-contaminated soils [9]. Among these, the most common are carbon-based and metal-based NPs such as Fe_3O_4, ZnO, TiO_2, zero-valent iron (nZVI) nanoparticles, and nanocomposites [5]. Diverse chemical methods are used to synthesise NPs, but these are burdened with the limitations of using toxic chemicals in the synthesis process and insufficient knowledge of the release of by-products and their possible toxic effects on human health and the environment [1]. Therefore, the research and industrial focus has been shifted to the green synthesis of NPs by using plants, microorganisms, and their products [10]. The greenly synthesised NPs are cost-effective, environmentally friendly, and more efficient in the remediation of pollutants [7].

Integration of nanotechnology with phytoremediation and microbial remediation has further enhanced the efficiency of bioremediation of pollutants in various environmental matrices [1]. For instance, the phytoextraction capacity of ryegrass (*Lolium perenne L.*) from lead-contaminated soil was enhanced with the use of NHAP (nano-hydroxyapatite) and NCB (Nano-Carbon Black) in a field experiment [11]. Singh and Lee also reported the increased Cd absorption by the *Glycine max* plant in the presence of TiO_2 NPs [12]. The role of NPs in promoting HM accumulation by plants is attributed to their ability to regulate cell wall permeability, heavy metal co-transportation, and transporter gene expression and to prevent the phytotoxicity of HM by scavenging free radicals and enhancing enzymatic antioxidant defense system in plants. However, there are concerns regarding the safe use of NPs in the remediation of contaminated soil and the fate of these NPs in the environment [13]. Thus, the impacts of various NMs on HM uptake and toxicity in various plant species still need to be explored for the large-scale application of nanoremediation. This chapter underscores the potential of nanotechnology in addressing heavy metal contamination in soils, emphasizing its integration with bioremediation techniques and underlying mechanisms. It also discusses environmental implications and challenges in scaling up nanoremediation technologies and sustainable solutions for heavy metal removal in contaminated soils.

Conventional Remediation Techniques and Associated Challenges

Various conventional remediation techniques like excavation, soil washing and stabilization, chemical reduction of reactive forms of HMs electrochemical methods, and bioremediation have been used to remediate the toxic heavy metals from the environment. Physical methods used to remediate the HM-contaminated soils include excavation, soil washing, and soil stabilization. Excavation, which is one of the quick and efficient methods, involves the physical removal of HM-contaminated soil from the site. Further, HM-polluted soils can be restored by washing with some specific wash solutions [14]. HMs can also be stabilized or fixed in soils by using certain immobilising agents, subsequently reducing their mobility and availability to biota [15]. To remediate the areas that are difficult to reach, such as deep soils or under-building soils, chemical methods, including chemisorption, ion exchange, precipitation, flotation, and electrochemical deposition *etc*., are used. These methods are based on the principle of conversion of reactive forms of HMs to stable forms by using some reducing agents that may be precipitated out at an alkaline pH of soil [16]. Many adsorption techniques are also used to adsorb the reactive forms of HMs on sorbents by non-covalent

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