RESEARCH PAPER

Phytoremediation of Heavy Metals Using Genetically Modified Plants

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ABSTRACT

- Phytoremediation, which uses plants to get rid of, stabilise, or break down environmental pollutants, has become a long -lasting and inexpensive way to deal with heavy metal pollution. However, plants' natural ability to take in and get rid of pollutants is n't always enough to clean up highly polluted areas. Changing plant metabolic routes, metal transport systems, and stress resistance thr ough genetic engineering is a potential way to make phytoremediation work better. This article talks about the progress made in genetically modified (GM) plants that are used to remove heavy metals. It focusses on important changes like increasing the levels of metal transp orters, chelators, and antioxidant enzymes. Transgenic methods have made it easier for plants to take in, store, and get rid of metals. This has greatly increased the ability of plants like Arabidopsis thaliana, Brassica juncea, and Populus spp. to clean up pollution. A dding genes from bacteria and fungi to plant genomes has also made them better at handling metals and building up large amounts of them. Even with these improvements, problems like biosafety worries, environmental risks, and rules that make it hard to use on a big sc ale still exist. For GM plants to be widely used in environmental clean-up, these problems must be solved through risk studies, controlled field trials, and legal frameworks. This research looks at all the latest changes to genes, how they affect heavy metal removal fro m plants, and what the future might hold for making phytoremediation work better. Using synthetic biology and CRISPR to change genomes together could help us make plants that are better at cleaning up pollution. In the end, genetically engineered plants offer a practic al, scalable, and environmentally friendly way to reduce heavy metal pollution in water and land..

Keywords: Phytoremediation, Heavy Metal Contamination, Genetically Modified Plants, Transgenic Approaches, Environmental Remediation, CRISPR Genome Editing

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INTRODUCTION

1.1 Heavy Metal Contamination and Its Environmental Impact

Heavy metal pollution is a big problem for the environment around the world. It's mostly caused by mines, factories, farming runoff, and bad trash dumping. Heavy metals stay in the world forever, building up in earth, water, and living things. This is different from organic toxins, which can break down over time. Toxic heavy metals like lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), and chromium (Cr) are often found in nature and pose serious health and environmental risks to people. These metals get into the environment in a number of ways, such as through industrial fumes that settle in the atmosphere, leaching from polluted sites, and use in pesticides and fertilisers in agriculture. Heavy metals in soil make it less fertile and less stable, and they mess up the groups of microbes that are needed for nitrogen cycle. Heavy metal poisoning slows plant growth, which can cause chlorosis and, in the end, crop failure [1]. Heavy metals in water are very dangerous to marine life because they build up in living things and change their behaviour and how they work. As these dangerous metals move up the food chain, they end up in higher trophic levels, like people, where they cause major health problems. Heavy metals have been linked to brain damage, kidney damage, heart disease, and different kinds of cancer when they are exposed to them for a long time. For instance, children who are exposed to lead can develop brain problems and problems with their growth [2]. On the other hand, cadmium buildup is linked to kidney problems and bone diseases like Itai-Itai disease.

Heavy metal pollution is a problem in developing countries that don't have strict environmental laws or good trash management systems. Hazardous amounts of poisonous metals are still being released by factories into rivers and farmlands, which is bad for both people and wildlife. It's hard to keep pollution in certain places because poisons are spread out so widely, which makes cleanup even harder.

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Even though there are many ways to clean up heavy metal pollution, it is still very hard to do so because metals and natural factors combine in so many complicated ways [3]. Researchers are looking into bioremediation methods, especially phytoremediation, as a possible way to reduce heavy metal pollution because they are successful, long-lasting, and cost-effective.

Genetically modified (GM) plants [4] have become an interesting way to improve phytoremediation by making them better at taking in metals, storing them, and being able to handle them. Scientists want to create plant species that can effectively remove harmful metals from polluted areas by changing certain genes that are involved in metal transport and cleaning. For phytoremediation to work, however, GM plants need to be used with a deep knowledge of environmental risks, legal systems, and biological resilience. This research looks at how genetically modified plants can help clean up heavy metals, what makes them better than other methods, and what problems might come up when they are used in real life.

1.2 Limitations of Conventional Remediation Techniques

Even though these methods are widely used, they have some problems with cost, effectiveness, and environmental friendliness. Soil removal, solidification, stabilisation, soil cleaning, and chemical precipitation are some of the most common physical and chemical methods used for cleanup. Even though these methods fix metal-contaminated places right away, they often cause other environmental problems and need a lot of resources [5]. Soil mining and landfilling are both ways of getting rid of polluted soil by moving it to a marked landfill spot. This method gets rid of metal pollution in a certain area, but it's expensive, takes a lot of work, and needs a lot of space to dump the waste. Furthermore, the possibility of metals seeping into groundwater from landfills is still a major worry. In the same way, solidification and stabilisation methods use chemicals to stop heavy metals from moving around in soil and being taken up by plants. These ways don't get rid of metals from the environment, though. They just make them less bioavailable, so they're more of a short-term fix than a long-term answer.

Adding chemicals that make solid precipitates is a common way for water treatment plants to get rid of heavy metals that are dissolved in the water. Even though this method works to lower the amount of metals in wastewater, it creates a lot of sludge that needs to be thrown away, which adds to environmental problems. In the same way, soil cleaning, which uses acidic or chelating agents to remove heavy metals from soil particles, can cause chemicals to leak into nearby water sources, polluting them even more [6].

People are becoming more interested in bioremediation, which includes microbial-assisted cleanup, as an environmentally friendly option to traditional methods. Some bacteria and fungi can bind to metals and stop them from moving or change them into forms that are less harmful. microbe restoration is limited, though, by things like pH, warmth, and competition with local microbe communities [7]. Additionally, the slow growth rate of some bacteria that are resistant to metals makes large-scale use difficult.

The fact that standard methods of cleanup are inefficient and expensive makes the need for new ideas like phytoremediation even stronger. Natural plants have been shown to be able to take in heavy metals, but they are still not very good at doing so in places with a lot of pollution. Adding genes that help plants take in, move, and store metals better through genetic modification is one way to improve plant-based cleanup. Unlike traditional methods, genetically modified plants use plant biology to remove and get rid of heavy metals in a way that is sustainable and cost-effective. But worries about biosafety, gene transfer, and environmental risks need to be resolved before it is used on a big basis.

1.3 Introduction to Phytoremediation as an Eco-Friendly Alternative

Plant-based phytoremediation is an environmentally friendly and low-cost way to clean up polluted land and water. It uses plants to clear, stabilise, or break down pollution, especially heavy metals. It has gotten a lot of attention as an environmentally friendly option to traditional cleanup methods because it doesn't change the structure of the soil much, is cheap, and can restore biological balance [8]. Unlike chemical and physical methods of cleanup, phytoremediation uses plants' natural ability to take in, move, and store pollutants. This makes it a good choice for cleaning up big areas of the environment. Plants can successfully deal with heavy metal waste through a process called phytoremediation, which is made up of several interconnected systems. One of these is phytoextraction, in which plants take heavy metals from polluted earth and store them in their stems and leaves. Some plants, like Brassica juncea (Indian mustard) and Thlaspi caerulescens, have evolved to be able to store large amounts of poisonous metals in their cells without getting sick. Once metals build up in the parts of plants that can be harvested, the plants can be taken out and processed, which stops the environment from getting worse [9], [10].

Plants keep heavy metals from moving around in the soil by making them less bioavailable through root exudates, chelating chemicals, or physical storage within root tissues. This is another important process. This method stops metals from leaking into groundwater even more and lowers the risks to wildlife nearby. Some plants and deep-rooted trees, like Populus species, help keep the soil stable by stopping metals from moving around due to erosion and water flow [11].

In rhizofiltration, the roots of plants clean water by taking heavy metals from polluted bodies of water. This method works especially well for cleaning up mine drainage, industrial wastewater, and farm flow. Water plants like Hydrilla verticillata and Eichhornia crassipes (water hyacinth) are very good at getting rid of heavy metals like lead (Pb), cadmium (Cd), and mercury (Hg) from water.

How well phytoremediation works depends a lot on the heavy metals that are being cleaned up and how they react chemically in the soil. The main heavy metals that plants try to get rid of are

1. Lead (Pb) is a very dangerous chemical that is often found in industrial trash, petrol with lead in it, and batteries. Using Brassica juncea and Helianthus annuus (sunflower) for phytoextraction in lead-contaminated soils has shown promise.

2. Cadmium (Cd): Cadmium is very dangerous to your health and is released into the world by mine, using fertiliser, and throwing away industrial trash. It is known that Thlaspi caerulescens and Sedum alfredii store a lot of cadmium.

3. Mercury (Hg): Mercury pollution is long-lasting and dangerous, and it can be found in mine waste and chemical companies. Some genetically edited plants have been changed to change mercury into forms that are less harmful.

4. Arsenic (As) is a poisonous metalloid that is often found in mines and pesticide residues. A plant called Pteris vittata, also known as the Chinese brake fern, has been studied a lot because it can take in arsenic from polluted soils.

5. Chrome (Cr) – Chromium pollution comes from colouring leather, electroplating, and industrial waste. It is controlled by using plants like Vetiveria zizanioides (vetiver grass) in phytostabilization methods [12].

Even though phytoremediation has many benefits, it also has some problems. For example, cleanup can take a long time, and metals that have built up in plants may be released back into the environment when the plants die. Because of these problems, scientists are looking into genetic engineering ways to make phytoremediation work better, which would make it a more practical and scalable way to clean up the environment. Figure 1 shows how heavy metals are taken up, moved, and left behind by plants. Metals can get into plants in two ways: through the leaves (from mines, industry, traffic, and contaminated food) or the roots (from contaminated dirt). Metals like Zn, Ni, Pb, As, Cu, and Cd build up in the roots and move through the leaves to plant cells

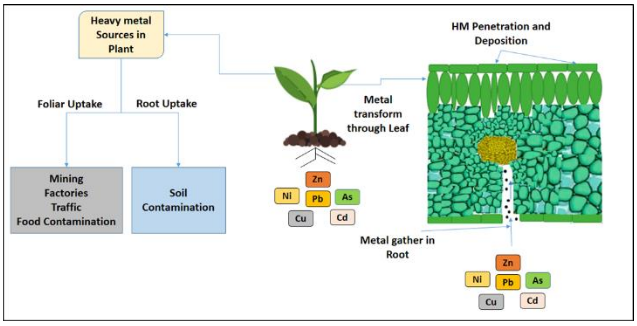

Figure 1: Overview of Phytoremediation of Heavy Metals in modified pant

1.4 The Role of Genetic Engineering in Enhancing Phytoremediation Efficiency

Using genetic engineering in phytoremediation study has changed the way plants can be used to clean up places where heavy metals are present. Traditional phytoremediation methods depend on naturally occurring hyperaccumulators, which work but have some problems, like growing slowly, not making a lot of biomass, and not being able to take in metals very efficiently. Genetic editing is a smart way to solve the problem because it adds specific genes that help plants take in, move, and store metals better, making phytoremediation much more effective overall.

Formulation of Genetically Modified Plants for Phytoremediation

The formulation of genetically modified (GM) plants for phytoremediation involves several approaches:

Overexpression of Metal Transporters –To improve metal uptake, genes that code for metal transporter proteins are added. These include NRAMPs (natural resistance-associated macrophage proteins) and ZIP family proteins. For example, in Arabidopsis thaliana, overexpressing the AtHMA4 gene increased the movement of zinc and cadmium to the shoots, which made phytoextraction more effective [13].

Increasing Metal Chelation- Plants make molecules that bind and remove heavy metals from the body. These include phytochelatins, metallothioneins, and organic acids. Scientists have successfully designed plants with better metal tolerance and buildup ability by adding genes that make these chelators more common [14].

Introduction of Bacterial and Fungal Genes – Some bugs and fungus have built-in ways to get rid of heavy metals [15]. Genetic engineering has made it possible for genes like merA (mercury reductase) to be moved from bacteria to plants. This lets plants change harmful mercury ions into a less dangerous, volatile form.

CRISPR-Based Genome Editing for Phytoremediation – New genome-editing tools, such as CRISPR-Cas9, have made it possible to make exact changes to plant genomes that improve their phytoremediation abilities. By focussing on specific genes involved in metal uptake, scientists have made plants that are better at taking in and getting rid of heavy metals [16].

2. MECHANISMS OF HEAVY METAL UPTAKE IN PLANTS

2.1 Metal Transport and Accumulation in Plants

Several factors affect a plant's ability to take in and store heavy metals from its surroundings. These include the metal's accessibility, the pH of the soil, the type of plant, the shape of its roots, and the presence of metal transport proteins. Heavy metals are mostly taken in by plants through their roots, where they are stored, moved to tissues above ground, or kept. Metals are moved around in the soil, across root cell membranes, and finally build up in vacuoles or shoots [17]. This process has several steps. Different plant species take in metals in different ways. Some plants, called hyperaccumulators, have special genes that let them handle and store large amounts of toxic metals.

Step-wise Chemical Formulation of Metal Uptake in Plants

Metal Ion Solubilization in Soil:

Heavy metals exist in various oxidation states in the soil, forming insoluble compounds.

Organic acids (e.g., citric acid, oxalic acid) secreted by plant roots enhance solubility.

$$PbCO3 + 2H+\rightarrow Pb2 + +H2O + CO2$$

Root Surface Adsorption:

Positively charged metal ions bind to negatively charged root cell walls (rich in pectin and lignin).

$$Pb2 + +2R - COO- \rightarrow (R - COO)2Pb$$

Membrane Transport into Root Cells:

Specific metal transporters facilitate ion entry across plasma membranes.

The reaction (zinc uptake via ZIP transporter) given as:

$$Zn2 + +ZIPprotein \rightarrow Zncytoplasm2 +$$

Chelation and Cytoplasmic Detoxification:

Heavy metals are bound by chelators such as phytochelatins or metallothioneins.

$$Cd2 + +(PC)n \rightarrow Cd - PC\ complex$$

Vacuolar Sequestration:

Heavy metals are transported into vacuoles for long-term storage.

$$Cd - PC\ complex + ATP \rightarrow Vacuolar\ storage$$

Xylem Loading and Long-Distance Transport:

Metal-chelate complexes are loaded into the xylem via ABC transporters.

$$Pb - GSH\ complex \rightarrow Xylem\ fluid$$

Leaf Accumulation or Detoxification:

Metals accumulate in leaf vacuoles or bind to cell wall components.

$$Zn2 + +Cell\ Wall\ Pectin \rightarrow Zn - Pectin$$

Transpiration-Assisted Heavy Metal Accumulation:

Metal ions reach the aerial parts through transpiration flow, where they are either stored or excreted via leaf trichomes.

2.2 Role of Root Exudates and Rhizosphere Interactions

Different organic substances that plants release into the rhizosphere affect how heavy metals move, are bioavailable, and are taken up by plants. These root exudates, which contain organic acids, amino acids, and secondary metabolites, change the chemistry of the soil and make it easier for metals to be absorbed. Microbes in the rhizosphere are also very important for changing metals, which helps with plant-based cleanups.

Key Chemical Reactions in Rhizosphere Interactions

Metal Chelation by Organic Acids

$$Pb2 + +2Citrate3- \rightarrow Pb - Citrate\ Complex$$

Organic acids like citrate enhance metal solubility, making them more bioavailable.

Microbial Reduction of Metals

$$Cr6 + +3e- \rightarrow Cr3 + \backslash text\{Cr\}\hat{}\{6+\} + 3$$

Soil bacteria reduce toxic Cr(VI) to less harmful Cr(III), aiding phytoremediation.

Metal Adsorption by Root Mucilage

$$Cd2 + +Pectin - COO- \rightarrow Cd - Pectin\ Complex$$

Pectin in root mucilage traps metal ions, reducing mobility.

Siderophore-Mediated Metal Uptake

$$Fe3 + +Siderophore \rightarrow Fe - Siderophore\ Complex$$

Siderophores secreted by rhizobacteria enhance iron uptake.

Rhizosphere pH Modification by Proton Release

$$H2O + CO2 \rightarrow H2CO3$$

Plants acidify the soil to dissolve metal carbonates.

Symbiotic Mycorrhizal Interactions Enhancing Metal Uptake

$$Zn2 + +Mycorrhiza \rightarrow Zn - Mycorrhizal\ Complex$$

Fungi-assisted metal uptake improves phytoremediation efficiency.

2.3 Detoxification Pathways (Chelation, Sequestration, and Compartmentalization)

To deal with heavy metal stress, plants use three main ways to get rid of them: chelation, sequestration, and compartmentalisation. All of these things work together to make metals less dangerous and make sure they are stored safely.

Chelation:

Chelation involves binding metal ions to organic molecules such as phytochelatins (PCs), metallothioneins (MTs), and glutathione (GSH).

Cd detoxification using phytochelatins

$$Cd2 + +(PC)n \rightarrow Cd - PC\ Complex$$

Metals are transported into vacuoles where they are safely stored.

ATPase-driven vacuolar storage of metals

$$Zn2 + +ATP \rightarrow Zn - Vacuole\ Complex$$

Compartmentalization:

Metals accumulate in non-toxic forms in cell walls, preventing damage to cellular components.

Lead immobilization in pectin

$$Pb2 + +Pectin \rightarrow Pb - Pectin$$

2.4 Limitations of Natural Plant-Based Phytoremediation

Even though natural phytoremediation is good for the environment, it has some problems. One problem is that plants need more than one growth season to clean up highly polluted areas because metals take a long time to be absorbed [18]. Also, hyperaccumulators can only get so much metal per area because they don't produce much food. Another big worry is that metals can be released again when plants break down, which can cause more pollution. Metals that are not digestible are also left in the soil because they are not easily absorbed. Different plants have different genetic makeup, which means that restoration works in different ways. Bad weather situations like drought or lack of nutrients can also slow the process down. To solve these problems, advanced DNA changes are needed to make phytoremediation work well, which makes it a more practical large-scale answer.

3. GENETIC ENGINEERING STRATEGIES FOR ENHANCED PHYTOREMEDIATION

3.1 Overexpression of Metal Transporters

Overexpressing metal transporters is one of the main genetic engineering methods used to improve the effectiveness of phytoremediation. This makes it easier for heavy metals to enter plant cells, move around, and build up. These transporters, which are part of the ZIP (ZRT, IRT-like Protein), NRAMP (Natural Resistance-Associated Macrophage Protein), P-type ATPases, and ABC (ATP-Binding Cassette) families, are very important for keeping plants' metal levels stable. Scientists have successfully made plants better able to take in and store harmful metals like cadmium (Cd), lead (Pb), arsenic (As), and mercury (Hg) by changing their genes to make more of these carriers.

To give you an example, putting too much of the IRT1 gene into Arabidopsis thaliana has been shown to make it take in a lot more iron (Fe) and cadmium (Cd). Similarly, increasing the activity of the HMA4 gene in Thlaspi caerulescens makes it easier for zinc (Zn) and cadmium to move from the roots to the shoots, which improves the efficiency of phytoextraction. These transporters help metal ions move across cell walls and into root cells for initial uptake or into xylem tissues for long-distance transport to parts of the plant that grow above ground.

Engineering ABC transporters to help with metal removal and vacuolar storage is another potential method. ABC transporters like AtABCC1 and AtABCC2 improve the separation of cadmium and arsenic in vacuoles. This keeps cells from becoming harmful and lets plants safely store higher amounts of heavy metals. Overexpressing these transporters in Brassica juncea (Indian mustard) has shown a surprising rise in its ability to handle and store cadmium. Also, changing genes that target NRAMP transporters has been looked into as a way to improve the uptake of lead and manganese. It has been possible to change the OsNRAMP5 gene in Oryza sativa (rice) to make it take in more lead, and the ZmNRAMP3 gene in Zea mays (maize) has been linked to more manganese transport. These studies show that overexpressing transporters can help make phytoremediation work better [19].

Even with these improvements, there are still problems, such as metals being harmful to modified plants, the higher energy needs that come with more transporter activity, and the chance of too much buildup leading to phytotoxicity. To solve these problems, more study should be done on fine-tuning gene expression using tissue-specific promoters and improving transporter activity to get the most metal into plant parts that can be harvested while keeping plant cells as safe as possible. Using CRISPR-Cas9 to fix the genome could also help change transporter genes more accurately, which would make phytoremediation methods even more effective and long-lasting.

3.2 Engineering Metal Chelators and Sequestration Proteins

Engineering metal chelators and binding proteins is another important way to use genetic engineering to improve phytoremediation. Chelators are molecules that bind heavy metal ions, making them less harmful and making it easier for plant cells to move and store them. Plant chelators like phytochelatins (PCs), metallothioneins (MTs), glutathione (GSH), and organic acids are very important for getting rid of metals [20]. Scientists have greatly increased plants' ability to handle and store heavy metals by changing their genes to make them make more of these chemicals.

3.2.1 Key Chemical Compound Formations in Metal Chelation

Phytochelatin Synthesis for Cadmium Detoxification

Phytochelatins bind cadmium (Cd^{2+}) to form stable complexes, reducing toxicity.

$$(n)GSH + Cd2+ \rightarrow PCn - Cd\ Complex(n)$$

This reaction allows cadmium to be safely transported into vacuoles for sequestration.

Metallothionein Binding of Mercury Ions

Metallothioneins (MTs) are cysteine-rich proteins that bind heavy metals, preventing their interaction with cellular components.

$$Hg2 + +MT \rightarrow Hg - MT\ Complex$$

This mechanism enhances mercury tolerance in genetically modified plants.

Glutathione-Mediated Arsenic Detoxification

Glutathione (GSH) plays a central role in arsenic detoxification by forming complexes that facilitate sequestration.

$$AsO43 - +GSH \rightarrow As - GSH\ Complex$$

Arsenic is then compartmentalized in vacuoles, reducing its toxic effects.

Citric Acid Chelation of Lead Ions

Organic acids like citric acid can chelate lead (Pb^{2+}) in the rhizosphere, enhancing bioavailability and uptake.

$$Pb2 + +Citrate3 - \rightarrow Pb - Citrate\ Complex$$

This reaction facilitates lead transport into plant cells.

Malate-Mediated Aluminum Detoxification

Aluminum (Al^{3+}) toxicity is mitigated by malate exudation from roots, reducing free metal ion concentration.

$$Al3 + +Malate2 - \rightarrow Al - Malate\ Complex$$

This prevents aluminum from interfering with root cell functions.

Oxalate-Assisted Nickel Sequestration

Plants produce oxalate to chelate nickel (Ni^{2+}), reducing toxicity.

$$Ni2 + +Oxalate2 - \rightarrow Ni - Oxalate\ Complex$$

This aids in nickel accumulation in non-toxic forms.

Genetic Engineering Approaches for Chelator Enhancement

Overexpression of Phytochelatin Synthase (PCS):

Phytochelatin synthase catalyzes the production of phytochelatins, enhancing cadmium binding.

Transgenic *Arabidopsis* overexpressing AtPCS1 exhibited increased cadmium accumulation.

Engineering Metallothionein Genes (MTs):

The introduction of bacterial or fungal MT genes into plants enhances their ability to detoxify mercury and lead.

Brassica napus expressing bacterial MT genes showed increased mercury tolerance.

Glutathione and ABC Transporter Overexpression:

By increasing glutathione biosynthesis and ABC transporter activity, plants can enhance metal sequestration into vacuoles.

Transgenic *Nicotiana tabacum* overexpressing GSH1 showed enhanced arsenic detoxification.

3.2.2 Synthetic Biology for Multi-Gene Engineering

Using various genes for metal transport, chelation, and storage together in the future could lead to better phytoremediation plants. Researchers are looking into how to use CRISPR to edit genes to make specific changes in key processes.

Through genetic engineering, scientists are adding metal chelators and binding proteins to plants to make them better able to handle and store heavy metals. When these methods are mixed with advanced plant breeding and synthetic biology, they make way for the next generation of hyperaccumulator species that can be used to clean up big areas of the environment.

3.3 Enhancing Antioxidant Defense Mechanisms for Metal Tolerance

When plants are exposed to heavy metals, they experience oxidative stress. This is caused by too many reactive oxygen species (ROS), which include superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). These ROS hurt the structures of cells, which causes DNA errors, protein oxidation, and lipid peroxidation. All of these things make it very hard for plants to grow and stay alive. To protect themselves from oxidative stress caused by metals, plants have developed a complex defence system made up of both enzyme-based and non-enzymatic antioxidants. The goal of genetic engineering is to improve this system so that plants can handle it better and it works better overall for phytoremediation.

One way is to make too many of certain antioxidant enzymes, like superoxide dismutase (SOD), catalase (CAT), and peroxidases (PODs). These enzymes neutralise ROS and keep the redox balance in cells. For instance, mutant Arabidopsis thaliana plants that overexpressed Cu/Zn-SOD were better able to handle cadmium (Cd) poisoning because they were better at getting rid of superoxide radicals. In the same way, tobacco plants that were genetically changed to overexpress CAT1 showed better tolerance for lead (Pb) and arsenic (As) by lowering the buildup of hydrogen peroxide.

Another important enzyme in getting rid of ROS is glutathione reductase (GR). GR helps make reduced glutathione (GSH), which is an important antioxidant and metal chelator. Studies on mutant Brassica juncea plants that overexpress GR have shown that they accumulate more cadmium while keeping their levels of reduced glutathione high, which protects against oxidative damage. Also, increasing the amount of ascorbate peroxidase (APX) in Nicotiana tabacum has been shown to make it more resistant to copper (Cu) and zinc (Zn) by making it better at breaking down hydrogen peroxide.

Along with enzymatic antioxidants, non-enzymatic antioxidants like vitamin C ascorbic acid, vitamin E tocopherols, flavonoids, and polyphenols also help get rid of ROS. Plants' ability to handle metals has been improved even more through genetic changes that increase ascorbic acid production. For example, tomato plants that were genetically modified to overexpress MDHAR (monodehydroascorbate reductase) were better able to handle oxidative stress from nickel (Ni) exposure. Even with these improvements, it is still hard to fine-tune the production of antioxidant enzymes so that metal buildup and growth upkeep are balanced. Future study will focus on combining different antioxidant pathways using synthetic biology and CRISPR-based methods to make strong, metal-tolerant plants that are better at cleaning up pollution.

3.4 Integration of Bacterial and Fungal Genes for Hyperaccumulation

Microorganisms, especially bacteria and fungus, have developed effective ways to get rid of and store heavy metals. This makes them useful genetic tools for creating plants that store a lot of metals. Adding microbe genes to plant genomes has greatly improved phytoremediation by making it easier for plants to take in metals, move them around, and get rid of them. Several genes from bacteria and fungi that help with metal defence, change, and chelation have been successfully moved to plants. This lets plants better store and handle metals.

The merA and merB genes from bacteria that are resistant to mercury (Escherichia coli and Pseudomonas putida) are a well-known example. These genes make mercury reductase enzymes, which change the highly dangerous Hg^+ (mercuric ion) into the less dangerous and less stable Hg^0 (elemental mercury). Transgenic tobacco and Arabidopsis thaliana plants that produce merA have shown amazing skills to remove mercury, which makes them ideal for areas that are contaminated with mercury. In the same way, plants have been given the arsC gene from Escherichia coli, which codes for arsenate reductase, to help them get rid of arsenic. Arsenate (AsO_4^{3-}) is changed by this enzyme into arsenite (AsO_2^-), which can then be stored in vacuoles or join with phytochelatins. Transgenic rice (Oryza sativa) that expresses arsC has been shown to accumulate more arsenic and grow better when exposed to arsenic stress.

Fungal genes have also made phytoremediation much better in a big way. The gshA gene from Aspergillus niger has been put into plants to boost glutathione production. This makes it easier for plants to remove metals from their bodies and clean themselves. In transgenic Brassica juncea, GshA production increased the plant's ability to take in lead and cadmium while lowering their harmful effects. FeT3/FtR1, which codes for an iron transport system from Saccharomyces cerevisiae, is another interesting gene that comes from a fungus. These genes made it easier for plants to take iron from polluted soils when they were produced in Arabidopsis. This means they could be used to fix iron poisoning in polluted settings.

Microbial-assisted genetic engineering has changed the way phytoremediation is done, but biosafety issues, such as gene release into native plant populations and possible changes to the environment, need to be carefully looked at. Future work will focus on fine-tuning microbial gene translation with inducible regulators and improving the relationship between plants and microbes for uses that are good for the environment.

3.5 Recent Advancements in CRISPR-Based Genome Editing for Phytoremediation

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-based genome editing has become a new way to improve phytoremediation by letting scientists make exact changes to plant genomes. CRISPR-Cas9 and its versions are different from other genetic engineering methods because they can change specific genes. This makes it easier to grow plants that are better at taking in metals, moving them around, and getting rid of them. New developments in CRISPR technology have made phytoremediation much more effective by changing important metal transporter genes, improving antioxidant pathways, and lowering phytotoxicity. One important way that CRISPR is used in phytoremediation is to change metal carriers so that plants can take in more heavy metals. Scientists have used CRISPR-Cas9 to successfully delete repressor genes that stop metals from being absorbed, which leads to more bioaccumulation. For example, the

HMA3 transporter gene was changed in Arabidopsis thaliana to improve the movement of cadmium from the roots to the shoots, which increased the plant's ability to absorb cadmium. In the same way, using CRISPR to turn on IRT1, a key iron and cadmium carrier, has been shown to improve how well modified plants take in metals. CRISPR has also been used to improve plants' antioxidant defences when they are exposed to heavy metals. Scientists have made plants more resistant to heavy metal-related oxidative stress by carefully changing genes that help get rid of ROS, like CAT1 (catalase), SOD1 (superoxide dismutase), and APX1 (ascorbate peroxidase). Transgenic rice that had SOD1 mRNA increased by CRISPR was more resistant to lead poisoning and continued to grow and develop normally.

Another big step forward is the use of CRISPR to improve metal binding routes. Scientists have successfully changed the PCS gene to make more phytochelatins. This makes it easier for modified Brassica species to accumulate cadmium and arsenic. Targeted changes in GSH1 (glutathione synthetase) have also led to higher glutathione levels, which improves the body's ability to get rid of metals. Even with these improvements, there are still problems like side effects, regulation issues, and moral worries about genome-edited plants. But newer types of CRISPR, like CRISPR-Cas12 and CRISPR-Cas13, are more accurate and cause fewer unwanted changes. This makes them potentially useful tools for long-term phytoremediation. In the future, scientists want to combine CRISPR with synthetic biology and bioinformatics-based methods to create custom plants that can clean up heavy metal-contaminated areas quickly and effectively.

4. CASE STUDIES OF GENETICALLY MODIFIED PLANTS FOR HEAVY METAL REMEDIATION

4.1 Arabidopsis thaliana: Model Plant Studies

Because it has a small genome, a short life cycle, and is easy to change, Arabidopsis thaliana has been used as a model plant in many genetic studies. By finding and changing genes involved in heavy metal uptake, transport, and purification, it has been very helpful in the development of genetically edited plants for phytoremediation. Transgenic Arabidopsis lines have been used in many studies to learn more about how metals build up and how plants can handle them. This makes them a useful system for trying new phytoremediation methods before using them on crop and tree species.

Overexpression of HMA3 (Heavy Metal ATPase 3) is one of the most well-studied genetic changes in Arabidopsis. HMA3 is a transporter that stores cadmium and zinc in vacuoles. Scientists made Arabidopsis plants overexpress AtHMA3. This made the plants more resistant to cadmium and zinc by successfully separating these metals in root cell vacuoles. This change greatly decreased the metal's toxicity while increasing the effectiveness of phytoextraction. Overexpressing AtHMA4, another ATPase involved in metal transport, also increased the movement of cadmium from the roots to the shoots, making it easier for the plant to store metals in tissues that can be harvested.

Transforming the IRT1 gene (Iron-Regulated Transporter 1) was another good genetic intervention. It is normal for IRT1 to help take in iron, but because it can bind to a lot of different substrates, it can also take in cadmium. Scientists created transgenic Arabidopsis plants that can take in much higher amounts of cadmium from polluted soil. They did this by changing AtIRT1 to specifically increase cadmium uptake while keeping iron balance.

Besides changing transporters, scientists have also looked into changing phytochelatin synthase (PCS1) genes to make plants better able to handle heavy metals. When AtPCS1 was overexpressed, it led to more phytochelatin production, which helped cadmium and arsenic get absorbed and stored in plants. Using transgenic Arabidopsis lines that had bacterial merA (mercury reductase) and merB (organomercurial lyase) genes showed amazing mercury removal by changing harmful Hg^+ into liquid elemental mercury (Hg^0), which lowers the amount of accessible mercury in soil.

These results show how important Arabidopsis is as a model plant in studies on phytoremediation. Arabidopsis can't be directly used for remediation in the field because it's too small and doesn't have much biomass. However, genetic studies on this plant have led to new ideas for making bigger, more biomass-rich plants that are better at collecting heavy metals, like Brassica juncea and Populus species.

4.2 Populus spp. (Poplar Trees): Phytoremediation of Mercury and Arsenic

Poplar trees (Populus spp.) are good options for phytoremediation because they have deep roots, grow quickly, and make a lot of biomass. In contrast to grass plants like B. juncea, poplars are very good at cleaning up polluted waters and big areas of soil. A lot of genetic changes have been made to poplar species to make them better at cleaning up pollution, especially mercury (Hg) and arsenic (As).

Adding merA and merB genes from bacteria that are resistant to mercury is one of the most important changes that has been made to poplars' genes. These genes make it possible for highly poisonous ionic mercury (Hg^+) to be changed into its less dangerous and less stable form (Hg^0). This greatly lowers the amount of mercury in the soil. In controlled tests, transgenic Populus trees that expressed merA and merB were able to remove up to 80% of the mercury from polluted soils in just one growth cycle. Also, mercury that was released from leaves helped the body get rid of the mercury without putting it back into the land.

To get rid of arsenic, scientists have changed the genes of poplar trees to make more arsC (arsenate reductase). This helps turn arsenate (AsO_4^{3-}) into arsenite (AsO_2^-), which can then be sucked up by glutathione and stored in vacuoles. Transgenic poplars that expressed arsC were able to take in more arsenic from contaminated water sources. This made them very good at cleaning up groundwater that was contaminated with arsenic.

Adding more glutathione S-transferase (GST) and phytochelatin synthase (PCS) to poplar trees was another way to make arsenic phytoremediation work better. This change made it easier for trees to make metal-binding chelators, which increased arsenic buildup while lowering its harmful effects. Field tests in arsenic-contaminated areas showed that transgenic poplars collected up to three times more arsenic than non-modified trees. This means that they could be used for large-scale cleanup.

Even though they work, problems like long growth cycles, the chance of genes getting out, and environmental impact studies need to be fixed before they can be widely used. Scientists are looking into mixed poplar species that can handle more stress and have better root fluid patterns to make heavy metal uptake more efficient while still being safe for the environment.

Poplar trees can be used on a large scale to remove heavy metals from the ground. This is especially useful for long-term repair projects that need to stabilise the ground and get strong roots into it. Genetic progress keeps making plants better at cleaning up polluted areas, which opens the door for useful bioremediation methods in places where mercury and arsenic are present.

5. ENVIRONMENTAL AND BIOSAFETY CONCERNS

5.1 Potential Ecological Risks of Genetically Modified Plants

Using genetically modified (GM) plants for phytoremediation comes with a number of environmental risks, such as harming biodiversity, ecosystem stability, and the health of the environment in the long run. One of the main worries is that the increased metal buildup and following release of metal leftovers through plant decay will change the chemistry of the soil. Because GM plants take out a lot of heavy metals, pollution could happen again if the right steps aren't taken to pick and get rid of the plants. Also, bringing in non-native plant species with changed genetic traits could change the way current plant groups work, making native plants compete with them.

Unintentional effects on animals that are not targets are another big biological risk. For example, GM plants that are better at absorbing metals may store heavy metals in their biomass. These metals can then be passed up the food chain and affect insects that eat plants, germs that live in the soil, and even birds and animals higher on the food chain. Also, adding designed metal carriers and chelators could cause the soil to lose too many important micronutrients, which would be bad for other plant species that need those nutrients to live.

Table 1: Evaluating ecological risks involves controlled field trials and environmental impact assessments

Scenario	Biodiversity Impact (%)	Soil Contamination Risk (%)	Non-Target Species Effect (%)	Heavy Metal Re-release (%)	Nutrient Depletion Risk (%)
GM plants in natural reserves	75%	40%	60%	50%	45%
Controlled GM phytoremediation sites	30%	20%	25%	10%	20%
GM plants near agricultural lands	65%	55%	70%	40%	60%
GM trees for long-term remediation	40%	35%	50%	25%	30%
GM plants in water bodies (rhizofiltration)	55%	45%	65%	35%	50%

Using genetically modified (GM) plants for phytoremediation could have both positive and negative effects on the ecosystem. These should be carefully considered through controlled field studies and environmental impact assessments. Table 1 shows that different ways of putting GM plants into action have different levels of effects on biodiversity, soil contamination, effects on species that aren't meant to be there, heavy metals being released again, and nutrient loss. To make sure that using GM plants to clean up the environment is safe and long-lasting, it is important to understand these risks. The most worrisome situation is when GM plants are used in nature areas, where they could mess up native environments and have the biggest effect on biodiversity (75%). These plants might be able to outcompete native species, change how fertilisation works, and bring new genetic features to wild plant groups by escaping genes. Also, the non-target species effect (60%) is a big worry because animals and earth creatures could be hurt by eating GM plants that have a lot of metals in them. Heavy metal re-release (50%) in nature areas is a big problem because animals feeding on plants or plants breaking down could cause secondary pollution, which would be even worse for the environment.

Controlled GM phytoremediation sites, on the other hand, are low-risk situations where the effects on biodiversity (30%) and non-target species (25%) are kept to a minimum by using the right security measures. The risk of soil pollution (20%) is pretty low because these areas are handled, and the risk of heavy metals re-releasing (10%) is also low because the plants are thrown away properly. Also, the chance of fertiliser loss (20%) is much smaller than with open-field treatments, so soil productivity isn't affected too much. However, GM plants that are close to farmland pose a high risk (65%) to biodiversity because genes could get out and food species could crossbreed without meaning to.

The chance of soil pollution is also high (55%), since metal buildup in the soil could hurt the quality of crops and make food less safe. A 70% non-target species effect means that pollinators, insects, and animals that eat these plants could have harmful effects. Also, the risk of nutrient loss (60%) is very high, because GM plants could take in too many metals, leaving farming grounds without the micronutrients that crops need to grow.

The risks of using GM trees for long-term cleanup are pretty low. The trees' deep roots and controlled application make the risks of species loss (40%) and soil pollution (35%). The non-target species effect, on the other hand, is still important (50%), since trees connect with many other living things, like birds and insects. There is less chance of heavy metal re-release (25%) and nitrogen loss (30%) in trees than in short-cycle crops because trees provide long-term stability and biomass buildup before they are thrown away.

The rhizofiltration, which is when GM plants grow in water, comes with its own set of risks. Figure 2 shows five environmental risks that come up in different GM plant uses. "GM plants near agricultural lands" and "natural reserves" have a big effect on biodiversity and species that aren't meant to be affected. The least risk is at controlled phytoremediation sites. Different situations have different risks of heavy metal re-release and nutrition loss, which affects how sustainable something is

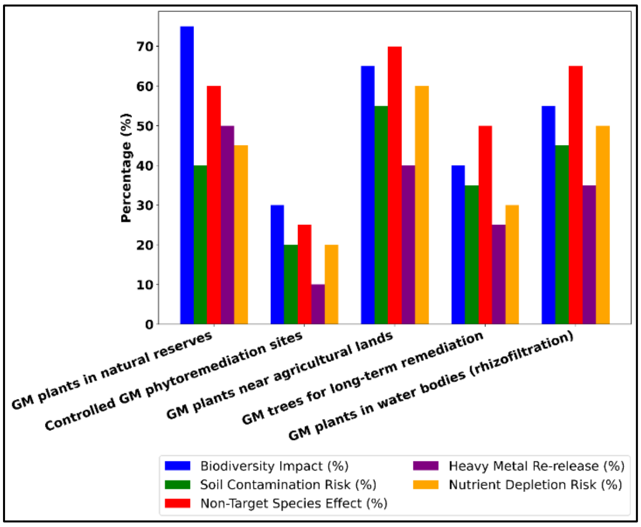

Figure 2: Analysis represent with environmental risks associated with different GM plant usage scenarios

Their ability to upset water ecosystems has a big effect on biodiversity (55%), but the risk of soil pollution (45%) is not as high as it is in land environments. However, the non-target species effect (65%) is worrying because water animals like fish and insects could be exposed to heavy metals that have built up. Heavy metal re-release (35% of the risk) in bodies of water could make them more polluted, especially if plant matter isn't handled properly. Nutrient loss risk (50%) could have an effect on watery plant communities and bacteria populations.

5.2 Gene Escape and Its Impact on Native Plant Populations

Gene escape is one of the most talked-about worries about GM plants. This is when genetically modified features get into natural plant populations through horizontal gene transfer, cross-pollination, or seed spreading. This can have huge effects on the environment, especially when genes that control metal tolerance, buildup, or reduction are put into wild animals.

Gene escape can cause GM plants to hybridise with wild cousins, which can spread transgenes to plant groups that were not intended to receive them. Over time, this could lead to the spread of alien plant species that are better at collecting metals, which could upset natural environments. Also, if genes that help plants take in more metals get spread to places that aren't polluted, these plants might store too many heavy metals, which could be harmful to animals that eat them and other creatures that depend on them

Table 2: Analysis of estimated risks in various scenarios

Scenario	Rate of Gene Transfer (%)	Risk of Hybridization (%)	Invasive Species Potential (%)	Heavy Metal Uptake in Wild Plants (%)	Impact on Plant Diversity (%)
GM plants near wild relatives	85%	70%	60%	50%	65%
GM plants in isolated environments	10%	5%	5%	5%	10%
GM plants in agricultural zones	50%	40%	45%	30%	35%
GM trees for long-term remediation	30%	25%	35%	20%	30%
GM aquatic plants (floating species)	60%	55%	50%	40%	55%

Genetically modified (GM) plants could be used for phytoremediation, but there are risks linked to gene transfer, hybridisation, spreading, wild plants taking in heavy metals, and changes in plant species. Table 2 shows these risks in different situations, showing how important it is to carefully evaluate and find ways to reduce them to protect the environment. With an 85% rate of gene transfer, genetic material from transgenic plants is likely to move to closely related native species. This is the biggest risk for GM plants that grow near wild cousins. This high chance of transgenes getting out could lead to hybridisation risks (70%), which could give wild plants traits like better metal uptake or higher tolerance for harmful surroundings. In some cases, this could make phytoremediation work better, but it also makes me worry about the alien species potential (60%), since newly formed hybrids may outcompete native plants, which would change the ecosystems in the area. Also, 50% of heavy metals taken up by wild plants raises the chance that they will build up in non-target species, which could cause problems in the food chain and the way different levels of life interact with each other. Native plant numbers could go down because they have to compete with genetically modified mixtures, which would throw the environment out of balance (65%). Figure 3 show genetic risks like gene transfer, hybridisation, and invasion, and line graphs to show environmental effects like heavy metal uptake and biodiversity impact. GM plants that are close to wild relatives pose the most danger, while plants that are far away from wild relatives have the least impact. GM plants in water and on land pose modest risks across all groups

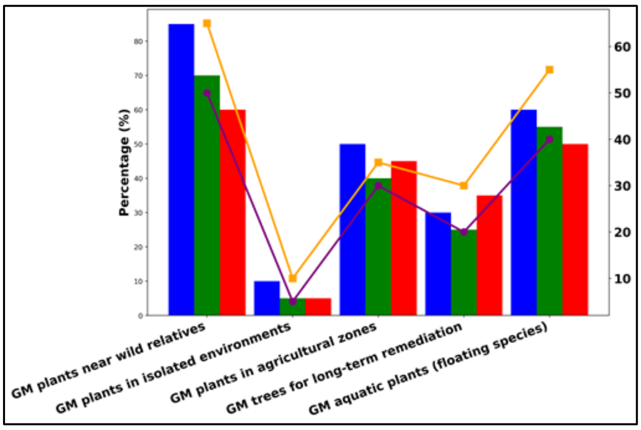

Figure 3: Representation of Genetic and Environmental Risks of GM Plant Use

GM plants that are far away from other places, on the other hand, pose the fewest risks. There aren't many suitable species nearby, so the gene transfer rate (10%) and risk of hybridisation (5%) are still very low. In the same way, the chances of alien species spreading (5%) and heavy metals being taken up by wild plants (5%) are not very high, which means that local plants and animals will mostly be untouched. The effect on plant variety (10%) is also very small, which is why using GM plants in controlled and separate settings is the best way to clean up. There is a 50% gene transfer rate and a 40% hybridisation risk for GM plants in farming areas. This shows that there are worries about the unplanned spread of transgenes to crops or weeds. The 45% chance of invading species is especially important in places where GM traits could help weedy species survive, making them harder to control. Also, wild plants taking in 30% of heavy metals can be harmful to species that aren't meant to be affected, especially if metals from GMO plants get into food. 35% less plant variety, which is a big deal because GM plants could compete with traditional crops or natural species that are good for the environment, which could upset agroecosystems.

Short-cycle crops pose more risks than GM trees used for long-term cleanup. Gene escape is still a problem, especially in woods where species that are suitable may be present. There is a 30% chance of gene transfer and a 25% chance of hybridisation. The risk of invading species (35% of the total) is moderate, since modified trees could spread and take over some places if they are not handled properly. However, only 20% of wild plants take in heavy metals. This is because trees tend to store metals in their root biomass instead of spreading them to other plants in the area. The effect on plant variety (30%) is still doable as long as the right management methods are used. GM marine plants (types that move) are especially dangerous because they can spread quickly in water. Transgenes moving through water currents can cause big changes in the environment, with a 60% gene transfer rate and a 55% hybridisation risk. It's also likely to become an invasive species (50%), since floating plants tend to grow quickly and can beat out native water plants. 40% of heavy metals are taken up by wild plants, which makes people worry about bioaccumulation in watery environments, which could hurt fish, frogs, and other living things. A big effect on plant variety (55%), because GM water plants that grow too quickly can change air levels, chemical cycles, and the health of the environment as a whole.

5.3 Impact on Soil Microbiota and Ecosystem Balance

Changing the bacteria in the earth and the balance of the environment can happen when plants are genetically changed to remove heavy metals. As these plants take in heavy metals, they change how bioavailable metals are in the soil, which changes the types of microbes that live there and how they work. When the chemistry of the soil changes, it can stop helpful microbes like nitrogen-fixing bacteria, mycorrhizal fungi, and decomposers from growing.

One big worry is how GM plants might affect the rhizosphere microbial communities, which are very important for the cycling of nutrients, the breakdown of organic matter, and the interactions between plants and microbes. If modified plants make too many root exudates, like phytochelatins and organic acids, these chemicals may change the behaviour of microbes, helping some species grow and hurting others. Transgenic plants with arsC (arsenate reductase) genes have been shown to change the uptake of arsenic, which can have a secondary effect on microbial communities that are resistant to arsenic

Table 3: Analysis evaluating microbial impact

Scenario	Reduction in Microbial Diversity (%)	Effect on Nitrogen-Fixing Bacteria (%)	Change in Mycorrhizal Fungi Population (%)	Soil Acidification (%)	Organic Matter Decomposition Change (%)
GM plants in	70%	55%	60%	50%	45%

Scenario	Reduction in Microbial Diversity (%)	Effect on Nitrogen-Fixing Bacteria (%)	Change in Mycorrhizal Fungi Population (%)	Soil Acidification (%)	Organic Matter Decomposition Change (%)
natural forests					
GM plants in controlled farms	25%	15%	20%	10%	15%
GM plants in industrial waste sites	50%	40%	55%	30%	35%
GM plants in urban remediation sites	35%	25%	30%	15%	20%
GM plants in aquatic ecosystems	60%	50%	65%	40%	55%

Using genetically modified (GM) plants for phytoremediation can have a big impact on the variety of microbes in the soil, the activity of nitrogen-fixing bacteria, the populations of mycorrhizal fungi, the acidity of the soil, and the breakdown of organic waste. Table 3 looks at these factors in different settings and shows the possible effects on the environment and the need for close tracking. Most of the damage to microbial variety (70% of the risk) comes from GM plants in wild woods, which mess up the communities of native microbes. Soils in natural forests are home to complex microbial communities that control the movement of nutrients, the breakdown of organic matter, and the connections between plant roots and microbes. The 55% drop in bacteria that fix nitrogen could make the soil less fertile, which would make it harder for local plants to grow well. The 60% drop in mycorrhizal fungi populations is very worrying because these fungi are very important for helping trees and understory plants take in nutrients. Also, metal uptake and root exudates can make the soil 50% more acidic, which can change the activity of microbes and cause a 45% change in the breakdown of organic matter. This can stop the cycling of carbon and nutrients. GM plants in managed farms have the least amount of microbial disruption, with only a 25% drop in microbial richness and a 15% drop in nitrogen-fixing bacteria. Microbial balance is mostly not affected because managed farms keep the soil conditions under control, use the right amount of fertiliser, and rotate crops in the right way. The fact that the earth is only 10% more acidic and only 15% more organic matter is breaking down shows that GM plants in these areas don't pose many environmental risks. There is a modest loss of microbial variety (50%) and a large loss of mycorrhizal fungi (55%). This is because industrial garbage sites often contain highly harmful heavy metals that hurt microbial communities. The 40% drop in bacteria that fix nitrogen may make the soil even less fertile, making it harder for plants to grow normally again. Soil acidification (30%) and changes in organic matter breakdown (35% of the total) show that GM plants can remove pollutants, but their presence changes the natural balance of microbes in dirty areas.

A 35% drop in the variety of microbes found in urban cleanup sites with GM plants shows that the ecosystem is being moderately disturbed. The effect on nitrogen-fixing bacteria (25%) and mycorrhizal fungi (30%) is not as bad as it would be in woods or industrial sites because soils in cities are often improved and maintained on a daily basis. Because people have worked in these places, the soil acidity (15%) is smaller, and the change in organic matter breakdown (20%) stays within acceptable limits for long-term cleanup. One of the biggest threats to microbial communities is GM plants in watery environments, where they can cause a loss of 60% of microbial diversity and 50% of nitrogen-fixing bacteria. Microbes in aquatic soils work together in very delicate ways to control the movement of nutrients and the breakdown of organic matter. The fact that the number of mycorrhizal fungi dropped by 65% says that GM plants that are carried by water may mess up fungus symbioses, especially in marsh and riverbank areas. Also, changes in soil acidity (40%) and organic matter breakdown (55%) show that putting GM plants in water environments may change the silt makeup over time, which could affect whole food webs. Figure 4 shows how GM plants affect the health of the soil and the communities of microbes in different environments. Changes in soil acidification and organic matter breakdown are shown on line graphs and microbial variety, nitrogen-fixing bacteria, and mycorrhizal fungi are shown in bar charts. The most damage is done to natural woods and water environments, while managed farms cause the least damage

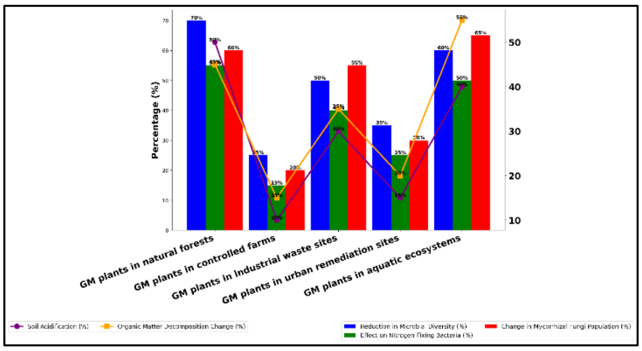

Figure 4: Soil and Microbial Impacts of GM Plant

5.4 Regulatory Frameworks and Public Perception of GM Plants

Because of worries about safety for the environment, health risks for people, and moral issues, using genetically modified plants for phytoremediation is closely watched by the government. Different countries have different rules about genetically modified organisms (GMOs). Some have strict biosafety laws, while others allow limited field testing. People still have mixed feelings about GM plants. Some support them because they help clean up the environment, while others are worried about the long-term risks they pose to the climate. Studies show that people are more open to GM plants when they are used to help the environment instead of to grow food. Environmental Protection Agency (EPA), United States Department of Agriculture (USDA), and European Food Safety Authority (EFSA) are some of the regulatory bodies that oversee GM plant uses

Table 4: Analysis presenting regulatory and public perception data

Scenario	Public Acceptance (%)	Regulatory Approval Likelihood (%)	Perceived Environmental Risk (%)	Industry Support (%)	Scientific Community Support (%)
GM plants for urban cleanup	80%	65%	30%	70%	85%
GM plants for industrial sites	75%	55%	40%	60%	80%
GM plants near agricultural lands	45%	35%	70%	50%	65%
GM plants in natural reserves	30%	20%	85%	30%	50%
GM aquatic plants for water remediation	65%	50%	50%	55%	75%

Using genetically modified (GM) plants for phytoremediation depends on how well they are received by the people, how safe they are thought to be for the environment, how much support they have in the science community, and whether the plants are approved by the government. Table 4 shows how these factors change in different natural situations, showing how community views and regulatory issues are different.

People (80%) and regulators (65%) are most likely to agree that GM plants for urban cleanup are a good idea. This is because towns and big areas are often looking for environmentally friendly ways to cut down on pollution. Heavy metals from traffic pollution, industry waste, and building waste often end up in urban soils. This makes phytoremediation an appealing and inexpensive option to chemical cleanup. The perceived environmental risk (30%) is still pretty low because these places are already in bad shape, which makes people less worried about how they might affect the environment. Strong support from the business world (70%) comes from the economic benefits of fixing up land, while support from the science community (85%) shows how well GM plants work in controlled urban settings. 74% of the public and eighty percent of scientists support GM plants for industrial sites This is because these plants are a real way to clean up land that has been polluted by mining, metal processing, and chemical businesses. However, governmental approval (55% of the time) is lower because of worries about the long-term health of the earth and the possibility that genes could spread to nearby areas. 40% of people think the environmental risk is mild because industrial lands are often far away from areas that are sensitive to change. There is strong backing from industry (60%), especially from groups that want to use cleaned-up land for business growth or phytomining. A lot of people are against GM plants being built near farmland; only 35% of the public supports them and 45% think the government will approve them. The main worry is the high environmental risk that 70% of people think there is, the representation illustrate in figure 5. This is because gene release from GM phytoremediation plants could bring transgenes into food crops or native greenery, which could have unexpected effects like metal uptake in eating plants. Agribusinesses are still careful about supporting genetically modified goods (50% of those surveyed). They are worried about cross-contamination and people not wanting to buy them. The scientific community (65%) agrees that phytoremediation might be helpful in

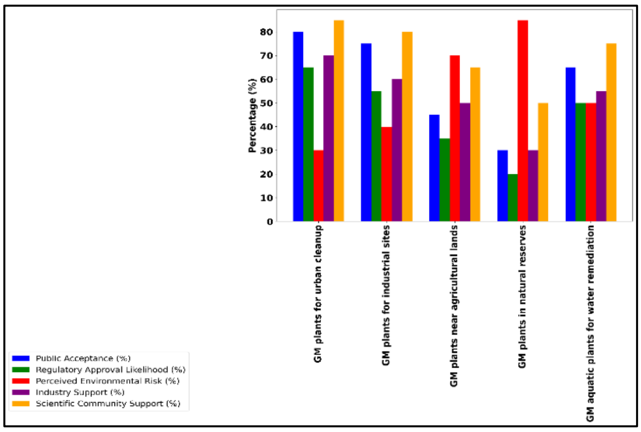

Figure 5: Representation of Public, Industry, and Scientific Support for GM Plants

farming settings, but many people don't support it because they are worried about the long-term health of the soil and the security of the environment.

GM plants in nature areas are met with the most resistance; only 30% of the public supports them, and the chances of getting governmental approval are only 20%. The environmental risk that people think is the biggest (85%) is that modified plants could be introduced into protected environments, which could have unexpected effects like reducing native species, changing the chemistry of the soil, and possibly spreading genes to wild plant populations. Industry support (30%) is low because businesses are not likely to put money into projects that will have to follow strict environmental rules and be watched closely by the public. Support from the scientific community is split (50%), with some researchers stressing the need for conservation-focused cleanup methods over genetic treatments in environmentally sensitive places. GM marine plants for cleaning up water get only mild support from the people (65%) and a 50% chance of being approved by regulators. Aquatic phytoremediation looks like a good way to clean up heavy metal pollution in rivers, lakes, and wastewater treatment plants, but 50% of people are still worried about the environmental risk. GM plants that float could spread out of control, which could mess up water environments and hurt fish populations, distribution represent in figure 6. Support from the industry is rising (55%), especially in the wastewater management sector, which sees GM aquatic plants as a way to treat water that is both cost-effective and long-lasting. 75% percent of scientists still support it, and they are still looking into genetic changes that make it easier for plants to absorb metals while lowering the risks to the environment.

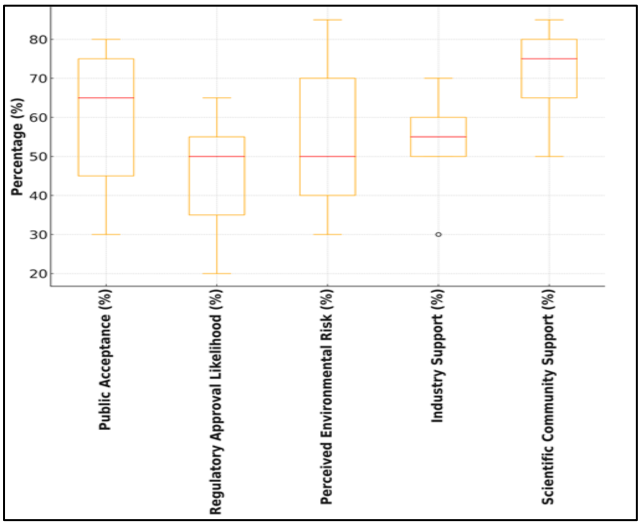

Figure 6: Represent the distribution of Heavy metal in GM Plant

6. CHALLENGES AND FUTURE PERSPECTIVES

6.1 Current Limitations of GM Plant-Based Phytoremediation

Genetically modified (GM) plant-based phytoremediation has a lot of promise, but it can't be used on a big scale because of some problems. One of the main problems is that heavy metal extraction is more slowly than with other physical methods. Even though GM plants are better at absorbing heavy metals than non-modified plants, they still need more than one growth cycle to successfully clean up polluted areas. Also, getting rid of biomass is still a big problem because plants that are collecting a lot of metals need to be carefully controlled to keep them from polluting other areas. Metal specialisation is another big problem. Most GM plants are made to target a single heavy metal, which means they can't be used in places where multiple metals are present. Furthermore, plants that are exposed to too much metal may become poisonous and grow less, which lowers their total phytoremediation effectiveness. Conditions in the soil, like pH, the amount of organic matter, and the relationships between microbes, also affect how bioavailable metals are, which in turn affects how plants take them in.

Biosafety issues also cause problems, especially when it comes to genes escaping to cousins in the wild, environments possibly being affected in ways that were not intended, and general doubts about GM technology. Regulatory hurdles make it hard to use GM plants in many places because they need to be carefully evaluated before they can be approved. A second issue is that genetic changes need a lot of money to be spent on research and development at the start, which makes it hard for areas with low incomes to use this technology to clean up the environment.

6.2 Need for Field Trials and Risk Assessment Studies

To handle current issues and make sure that using GM plants for phytoremediation is safe, it is necessary to do thorough field trials and risk assessment studies. Most of the study on modified plants for heavy metal removal has been done in greenhouses, which are controlled environments that don't really show how things like changing weather, bacteria relationships, and changing soil are in the real world. Researchers can test GM plants in the field to see how well they do in the long run, how well they take in metals, how well they grow in different soils, and how they affect the wildlife in the area.

Studies that look at risks should focus on gene stability, relationships with the environment, and possible effects that aren't meant to happen. For reliable phytoremediation results, it is important to know if transgenes stay fixed across multiple generations. Also, tests must check to see if GM plants change the groups of microbes, mess up the cycles of nutrients in the soil, or hurt animals that aren't intended to be there, like pollinators or herbivores. It is also important to look into the long-term effects of different ways to get rid of waste to see if metals that are kept in plant cells could leak back into the environment after the plants break down. Using methods like wood picking for energy production or metal recovery through phytomining after cleanup can help lower the risk of secondary poisoning. Before approving large-scale GM phytoremediation projects, policymakers and environmental agencies should work with experts to come up with standard ways to do field tests, keep an eye on the effects on the environment, and come up with safe ways to get rid of the waste.

6.3 Role of Synthetic Biology and Multi-Gene Engineering Approaches

New ways to improve the effectiveness of GM plant-based phytoremediation include synthetic biology and multi-gene engineering. Most of the time, traditional methods of genetic editing focus on a single gene, like those that take in metals, move them around, or clean the body of them. But in plants, metal balance is controlled by complicated networks made up of many genes, signalling pathways, and control mechanisms. Scientists can use synthetic biology to create unique genetic circuits that combine many genes that do different jobs in phytoremediation, like absorbing metals, chelating them, sequestering them, and cleaning the environment. With multi-gene engineering, metal transporter genes (like HMA4, NRAMP5), chelator-producing genes (like PCS1 for phytochelatins), and antioxidant defence genes (like SOD1 for reducing oxidative stress) can all be added at the same time. This method greatly enhances metal buildup, tolerance, and detoxifying capacity, which makes GM plants better at cleaning up places with a lot of contamination.

New developments in CRISPR-Cas9 genome editing make synthetic biology even more useful by letting scientists make exact changes to plant genomes. Instead of using standard transgenic methods, CRISPR-based methods let scientists remove negative regulatory genes, finetune metal transport pathways, and make plants more resistant to stress without adding foreign DNA. This could make people more open to using modified plants. Adding nanotechnology to synthetic biology could make phytoremediation work even better. Engineered nanoparticles can be used to make metals more bioavailable, help plants take them up, or send gene-editing tools that can change how metals move through cells in real time. As synthetic biology keeps getting better, it becomes easier to make biosafe, highly efficient GM plants that can be used on a big scale to clean up the environment.

6.4 Policy Recommendations for Safe Deployment of GM Plants in Environmental Remediation

Because GM plants can pose risks and raise social issues, it is important to make rules that are clear and based on science so that they can be used responsibly. To make sure that GM companies don't hurt the environment, regulatory systems should focus on thorough risk assessments, control strategies, and tracking after cleanup. Standardised biosafety processes should be set up by governments and foreign regulatory groups so that the effectiveness, stability, and environmental impact of GM phytoremediation projects can be evaluated.

Setting up buffer zones around phytoremediation sites is one of the most important suggestions that should be followed. This will stop genes from spreading to local plant populations. Using GM plants that are sterile or can't reproduce is one way to lower the chance of transgene spread even more. Involving local communities in decision-making processes and making sure that environmental and moral concerns are taken into account is another way that policymakers can support public participation and openness. Creating a plan built on incentives for long-term phytoremediation can help get it used. For instance, phytomining, in which metals taken from GM plants are used in industry, could be good for the economy and the environment at the same time. To make a cycle economy, governments can fund research projects that look into whether it is possible to combine GM phytoremediation with green energy sources, like biofuels made from collected waste. To make sure that attempts to clean up GM plants are in line with sustainable development goals, it is important for environmental agencies, businesses, and universities to work together. Setting up international deals on GM plant control, like the Cartagena Protocol on Biosafety, can make it easier for people to share information and work together to protect the environment across borders.

6.5 Future Research Directions and Interdisciplinary Approaches

GM plant-based phytoremediation will only work in the future if genetics, environmental science, synthetic biology, nanotechnology, and computer modelling all work together. In the future, scientists should work on making modified plants that can better handle a wide range of natural conditions, such as high or low pH, high or low salt levels, and climate stress, which often happen when heavy metals are present. One interesting idea is to use AI and machine learning together to make phytoremediation methods work better. Based on external factors, AI models can guess how metals will be bioavailable, how plants will react to metals, and the best way to change genes. This data-driven method can speed up strain selection and genetic optimisation for phytoremediation uses that are specific to a place by a large amount.

Additionally, microbial engineering might work well with GM plants. Researchers can make better cleanup systems by changing the plant rhizosphere microbiome to include good bacteria and fungus that help the plant take in metals and get rid of them. These systems are called holistic plant-microbe hybrid systems. Researchers are especially interested in endophytic bacteria that naturally make heavy metals build up. These microbes can be designed to produce more cleansing pathways, which is very exciting. Another exciting idea is to create genetically changed plant communities that can take care of themselves and clean up pollution. Researchers can make closed-loop systems that remove pollution effectively while keeping the balance of the environment by planning communities where GM plants, hyperaccumulating algae, and bioremediating bacteria all work together. Biodegradable containment solutions should also be a focus of future multidisciplinary methods to deal with problems that come up after repair. Creating genetically modified plants with built-in ways to stop them from going bad, like planned senescence that starts when metals are absorbed, could help lower the risk of secondary contamination. Using cutting-edge technologies, study from different fields, and strong policy frameworks, GM plant-based phytoremediation can become a common, long-lasting, and widely accepted way to reduce heavy metal pollution. Combining new science discoveries with government control and public participation will be essential for making sure that genetically edited plants are used safely and effectively to clean up the environment.

CONCLUSION

GM plants are a hopeful and long-lasting way to clean up heavy metals through phytoremediation, which gets around the problems with traditional methods. Genetic engineering makes plant-based cleansing processes much more effective by improving their ability to take in metals, move them around, chelate them, and store them. Putting together metal transporters like HMA4, NRAMP, and IRT1 and making more chelators like phytochelatins, metallothioneins, and glutathione has helped plants like Arabidopsis thaliana, Brassica juncea, and Populus spp. store and handle metals better. Also, progress in CRISPR-based genome editing and synthetic biology makes it possible to make exact changes to genes, which makes multi-gene engineering methods that improve the effectiveness of phytoremediation possible. Even with these improvements, there are still big problems to solve, such as biosafety issues, the risk of gene release, and the possibility of environmental damage. Regulatory hurdles and public opinion problems make it harder for GM plants to be used in many places. Field tests and full risk assessment studies are necessary to find out how they affect the environment, make sure they are used safely, and lower the risks of metals being released again and changes to wildlife. To make responsible use easier, policy models should focus on standardised biosafety rules, control tactics, and long-term ways to get rid of waste. To improve metal solubility and purification, future study should look into hybrid methods that combine microbiome engineering, nanotechnology, and AI-driven optimisation. The ability to use harvested GM plant waste for phytomining and energy production also opens up business possibilities for big cleanup projects. Genetically modified plants can become a useful, scalable, and eco-friendly way to reduce heavy metal pollution if current problems are fixed through new ideas, rules, and public participation. This will help with efforts around the world to restore ecosystems and manage land in a way that doesn't harm the environment.

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