REVIEW



Phytoremediation: a transgenic perspective in omics era

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Abstract Phytoremediation is an environmental safety strategy that might serve as a viable preventative approach to reduce soil contamination in a costeffective manner. Using plants to remediate pollution from the environment is referred to as phytoremediation. In the past few decades, plants have undergone genetic manipulation to overcome inherent limitations by using genetically modified plants. This review illustrates the eco-friendly process of cleaning the environment using transgenic strategies combined with omics technologies. Herbicides tolerance and phytoremediation abilities have been established in genetically modified plants. Transgenic plants have eliminated the pesticides atrazine and metolachlor from the soil. To expand the application of genetically engineered plants for phytoremediation process,

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Department of Genetic Engineering and Biotechnology, Jashore University of Science and Technology, Jashore 7408, Bangladesh it is essential to test strategies in the field and have contingency planning. Omics techniques were used for understanding various genetic, hormonal, and metabolic pathways responsible for phytoremediation in soil. Transcriptomics and metabolomics provide useful information as resources to understand the mechanisms behind phytoremediation. This review aims to highlight the integration of transgenic strategies and omics technologies to enhance phytoremediation efficiency, emphasizing the need for field testing and comprehensive planning for successful implementation.

Abbreviations

- ACC 1-Amino-cyclo-propane-1-carboxylate ATP Adenosine triphosphate AMF Arbuscular mycorrhizal fungus ArsC Arsenate reductase DNA Deoxyribonucleic acid EDTA Ethylene-diamine-tetra-acetic-acid HMs Heavy metals Mercuric-ion-reductase merA ADNT Nitroaromatic dynamite amino di-nitrotoluenes Organomercurial lyase merB
- PETN Pentaerythritol tetranitrate reductase
- PGPR Plant growth-promoting rhizobacteria

RDX	Royal-Demolition-Explosive
TOM	Toluene o-monooxygenase gene
TIM	Transgenic-Indian-mustard
TCE	Trichloroethylene
TNT	Trinitrotoluene
FRT	Fippase recognition target

Introduction

Enormous mining, farming, industrial, and military operations discharge many harmful substances into the environment (Devi et al. 2021; Spanier and Zviely 2022; Srivastava 2022). Heavy metals and organic contaminants harm plants, animals, and humans (Elgarahy et al. 2021; Elvevoll et al. 2022; Gupta et al. 2022; Rajendran et al. 2022). Strategies such as physicochemical extraction, soil cleaning, excavation, immobilization, and landfilling-expensive but ineffective-can be used to rehabilitate polluted areas (Rajendran et al. 2021). Frequently, these actions result in the extinction of natural ecosystems and leave unattractive scars on the environment (Barnett 2022; King 2022). In recent years, using plants to remove metal or organic pollution has gained popularity and established the concept of phytoremediation worldwide (Ozyigit et al. 2021; Sarma et al. 2021). With the help of plants, so-called phytoremediation, a low-cost method, uses plants to stabilize, transform, or eliminate pollutants like organic pollution from water, sediments, and soils (Yan et al. 2020). Recently, emphasis has been placed on the uses of plants to purify contaminated soil and water supplies (Kumar et al. 2022a, b; Ogundola et al. 2022). In recent years, nanomaterials have become a new bioremediation technique in agricultural soil (Palani et al. 2021; Rana et al. 2022). It's crucial to create innovative methods for cleaning up vulnerable areas due to the global problem of organic pollution, which includes pesticides, medications, petroleum compounds, and other xenobiotics (Rahman et al. 2022). Remediation of polluted places involves a variety of biological, physical, and chemical techniques (Abhilash et al. 2009).

The manifestation of a foreign gene is driven by the random integration of a gene organized into a cell or tissue-specific promoter fragment in transgenic approaches (Palani et al. 2021). Recombinant DNA technology makes genetic engineering much more conventional (Martin et al. 2020; Sharma et al. 2022). Numerous toxins, such as metals, herbicides, explosives, and oil, can be cleaned up with transgenic plants (Anjum et al. 2022; Kumar 2022; Rathour et al. 2022). Using plant-based bioremediation, it is widely acknowledged as an efficient and successful technique to remove soil contaminants, including organic compounds and heavy metals. This procedure was initially created to remove heavy metals (HMs) from polluted soil, but it has since shown effective in removing additional organic contaminants such as explosives, polyaromatic hydrocarbons, and chlorinated solvents (Queiroz et al. 2022; Shukla et al. 2019). In addition to eliminating soil toxins, there are several phytoremediation processes, such as synthetic breakdown (Van Aken 2008). Access to gene sequences from many organisms is necessary to use transgenics in phytoremediation. When a typical plant is exposed to a pollutant, these sequences may improve, control, or dramatically alter its behavior, allowing it to adapt to the contamination. Identifying particular genes that are either elevated or expressed erratically becomes extremely important in this scenario (Pan et al. 2019). These investigations helped identify prospective genes later examined for usage in transgenic animals (Aken and Doty 2009). Despite this, most metal-hyperaccumulating species have little potential for successful phytoremediation because of things like stunted development, high levels of biogas emissions, and a propensity to be strongly connected with a specific environment.

On the other hand, an effective phytoremediation plant should have genetic characteristics that promote both survival and metal hyperaccumulation in tissues above ground. In addition, it should develop its biomass vigorously and quickly, ideally scaring off herbivores to stop metalloids from entering the food chain (Pan et al. 2019). A potential applicant should also be prone to genetic modification (Kotrba et al. 2009). Using phytoremediation, it has been possible to eliminate atrazine, chlorpyrifos, metolachlor, and other chlorinated substances. For example, rice (Oryza sativa) plants can be grown under human supervision in dry and non-dry environments (Huang et al. 2021; Mridha et al. 2022). As a result, rice plants are thought to be good candidates for soil and stream water phytoremediation. It may be possible to use plants with high P450 actions as a phytoremediation approach for a particular xenobiotic (Malik et al.

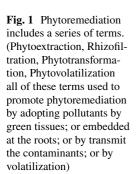
2022; Mishra et al. 2020). For pesticide phytoremediation, it has been observed by experimenting with transgenic rice plants that express the cytochrome P450 gene (Kawahigashi et al. 2008). The potential for xenobiotic remediation of transgenic plants would be increased and integrated crucial processes in the assimilation of routes for the deterioration of resistant imitational compounds (Francova et al. 2001; Land et al. 2020). When plants were demonstrated to digest pesticides in the 1940s, the idea of xenobiotics degradation was born. Ever since, proteomics, genomes, and metabolomics fields have significantly advanced our understanding of how to enhance or modify plants. The primary goal of this study was to explain the transgenic methods, such as post-genomic and genomic endeavors for phytoremediation to acquire the detoxification of contaminants as well as an ecofriendly environment.

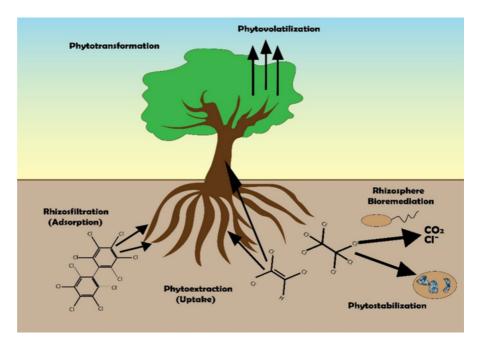
Phytoremediation

The use of phytoremediation appears to be a viable way to clean up polluted soil that is full of harmful compounds. An important finding is that plant roots have a remarkable capacity to extract both necessary and unnecessary components from the soil (Zaheer et al. 2022). This corrective strategy



includes a variety of subsets, such as Rhizofiltration, Phytoextraction, and Phytostabilization (Dubchak and Bondar 2019; Nedjimi 2021; Wani et al. 2023) (Fig. 1). Phytoextraction uses plants with the ability to absorb metals from the soil to their top sections, where they can be managed further using the appropriate techniques (Land et al. 2020). Rhixofiltration uses plant roots as filters to collect, precipitate, and absorb hazardous metals from contaminated effluents (Awa and Hadibarata 2020). However, Phytostabilization refers to the method by which plants immobilize and stabilize soil contaminants (Awa and Hadibarata 2020). Plants absorb volatile substances from the soil, like mercury, and then release them into the atmosphere through their leaves. This process is known as phytovolatilization. Plants get an advantage over microorganisms, which depend on sun energy for living, thanks to this procedure that uses it. The range of skills displayed by plants is extensive (Land et al. 2020). It is crucial to carefully choose plants with robust extraction, hyperaccumulation, and stability potential (Dou et al. 2022). Figure 1 shows how contaminants in soil and groundwater can be taken up by a plant's green tissues (phytoextraction) or retained at the roots (Rhizofiltration) (Fig. 1). While some contaminants can volatilize into the atmosphere (Phytovolatilization) or be incorporated into the soil itself through rhizosphere bioremediation (Fig. 1),





a process driven by microorganisms residing in the root zone, plant enzymes facilitate the transformation and stabilization of contaminants within plant tissues (Phytotransformation) (Fig. 1) (Aken and Doty 2009). The process of soil contaminants being taken by plants, changed into less dangerous volatile forms, and then released into the atmosphere through their leaves is known as phytovolatilization. The destruction of organic poisons by plant metabolic processes and enzymatic compounds produced by plants is referred to as Phytotransformation, or phytodegradation. Rhizofiltration broadens the scope of phytoremediation by using plant roots to filter and clean contaminated groundwater and surface water, efficiently getting rid of extra nutrients or dangerous compounds (Kristanti et al. 2021). In order to remove heavy metals from soil and water, phytoextraction of plants is an essential step. Due to their high density, these metals are dangerous to organisms, even at very low doses. Heavy metallic elements in the soil can be held in place with the help of Phytostabilization. Through actions like root-zone precipitation or absorption onto the root surfaces, plant roots reduce the movement of pollutants by collecting them.

Factors affecting phytoremediation

Plants may be used in a variety of ways to clean up polluted places. Various phytotechnology has previously been implemented, each utilizing various plants or plant features (Latif et al. 2023). Some of the beneficial plant traits used for cleanups include a rapid growth rate, higher biomass production rate, toughness, and pollution endurance. Furthermore, biological activities such as plant-microbe interactions might impact the efficacy of remediation (Jan et al. 2015). Because it collects more metals than the surrounding sediment, the rhizosphere of plants may have an impact on metal mobility. The presence of Halimione portulacoides resulted in a significant rise in dissolved phase metal concentrations (Almeida-Neto et al., 2008). The absorption of Cd^{2+} and modulation of Plasma membranes of root cells containing Ca²⁺ transporters or channels were influenced by different root apexes of Suede salsa. For example, the Cd^{2+} inflow was largest in the rhizosphere at the root tip. These findings might provide a theoretical foundation for enhancing Cd²⁺ pollution phytoremediation. Soil factors have long been known to influence metal mobility and Phyto availability, and consequently plant metal absorption (Liu et al. 2013). The bioaccessibility of metals for plant uptake is influenced by the pH concentration of soil.

Heavy metal concentrations in soil solution may usually be increased by lowering the pH of the soil. Metal accumulation and translocation capabilities, as well as phytoremediation efficiency, vary with species. Monocotyledons and dicotyledons, for example, both store Cu²⁺ in their roots, whereas monocotyledons primarily accumulate Pb²⁺. Chelating compounds are crucial in the elimination of harmful metals from the body through washing. Ethylenediamine-tetra-acetic-acid (EDTA) and ethylenediamine-succinic acid are two common chelating agents. The use of innovative biodegradable and non-toxic organic chelators as organic solvents might enhance the evacuation of metals from corrupted soils (Ullmann et al. 2013). Due to improved Cd^{2+} absorption efficiency and increased translocation of Cd²⁺ to the shoots, the addition of EDTA resulted in a twofold rise in Shoot Cd²⁺ concentration. As a result, Spartina kali appears to be a suitable species for Cd²⁺ phytoremediation. In (Fig. 5), factors that impact phytoremediation are displayed.

Hyperaccumulator plants for phytoremediation

Many plants, including Leucaena, poplar, parrot fescue, feather, ryegrass, Indian rice, mustard, and others, have been used to study the phytoremediation of xenobiotic pollutants. Poplar trees make an excellent potential employee for phytoremediation, according to several lines of evidence, since they produce a lot of biomass, have a lot of root depth, and are resistant to both pollutants that are organic and inorganic (Yan et al. 2020). The potential plants for the development of genetically modified plants must be open to genetic modification by Agrobacterium or bombardment with particles. Crop plants like rice produce herbicide tolerance because they photodegrade the herbicide, reducing herbicide transmission to the food chain. However, for ethical considerations, it's best to avoid using human DNA in transgenic plants for agriculture (Eapen et al. 2007). The plant can be considered an ideal plant for phytoremediation, which exhibits the following potential characteristics: (1) the ability to

collect metals, (2) tolerance to metals accumulating concentration, (3) high biomass and quick growth; (4) a deeply rooted system with many branches; (5) simple harvest ability plants that might be used in genetic engineering. An increased nutrient plant that can be grown for a short or extended period (trees) should be used for phytoremediation and should have a built-in phytoremediation capability. Plants that are candidates for genetic change should be friendly. Some hyperaccumulators have a lot of biomasses. Indian mustard (B. indices) is one of the plants for which regeneration techniques have previously been created. Sunflower (Helianthus annuus), yellow mustard (Taraxacum officinal), and tomato (Lycopersicon esculentum) most of those viable plants are agricultural ones that, when harvested, provide food. They are used for phytoremediation; neither people nor animals should consume them. Non-crop plants with high biomass, such as Nicotine species, can be used instead of crop plants, which herbivores find unpleasant (Divekar et al. 2022).

Molecular mechanisms of phytoremediation in plants

Some genes derived from fungi, plants, or bacteria that play a major role in the sequestration and degradation of environmental contaminants (such as HMs) were announced into bear plant species.

Two useful strategies for hyperaccumulation HMs have been established. a) increased expression of the HMs hyper-accumulation-related genes, b) overview of genes of interest from other living things like fungi, bacteria, or other plants (He et al. 2015).

Mechanisms of genes involved in HMs detoxification

Enzymes named mercuric-ion-reductase (*merA*) and organomercurial lyase (*MerB*) are complicated to augment the Hg²⁺ (Mercury) decontamination (Jha 2020). *MerB* protonolysis convert Hg²⁺ organic form into a less toxic ionic form, and Hg²⁺ and Hg²⁺ were volatilized from Hg⁰ by *merA* reduction (He et al. 2015). It is established that gene *SbMT*-2, obtained from *Salicornia brachiate* deliberates Zn²⁺, Cd²⁺, and Cu²⁺ tolerance and controls ROS hunting in transgenic *N. tabacum* (Bizily et al. 2003). A macrophage protein called *Nramp* was found to play a major role in Cd²⁺ buildup in transgenic *A. thaliana* by overexpression of the *SaNramp6* gene isolated from *Sedum alfredo. OsMTP1* gene from *O. sativa* cv. IR64 combined with transgenic tobacco strangely upgraded its (*O. sativa* cv. IR64) Cd²⁺ accumulation (Ye et al. 2020). Many genes exhibit the major function of phytoremediation of HMs, such as, *AtACR2* genes extract from *A. thaliana*, which promote As⁵⁺ decontamination in modified tobacco plants (Table 1) (Gupta et al. 2021).

Mechanisms of protein transporters involved in HMs detoxification

It has been found that protein transporters have a significant ability to detoxify HMs. VIT transporter; COPT5-transporter, H⁺-ATPase; H⁺/Na⁺ antiporter exhibit major role in HMs detoxification (Zhang et al. 2018). Under Cd²⁺ stress, Transporters such as P1Btype ATPase (HMA4 and HMA2) were overregulated in Sedum plumbizinciocla hyper-accumulator (Crassulaceae) (Peng et al. 2017). Investigation indicates that the reaction of the tomato to various levels of CdCl₂ and Cd²⁺ contents where they were divided into vacuoles was primarily because of the high protein activity of the NRAMP3 transporter (Meena et al. 2018). Glutathione, metal lothioneins, and phytochelatins play a major part in heavy metals (HMs) decontamination by chelating harmful metals and converting them to vacuoles with low or high molecular weight (Tan et al. 2019). Gene overexpression of some enzymes involved in the phytochelatin biosynthetic pathway, such as glutathione synthase and phytochelatin synthase, contributed to increased tolerance and detoxification of HMs (Shukla et al. 2013). Overexpression of Arabidopsis ATP sulfurylase genes and their transfer into Medicago sativa enhanced its tolerance against Cd^{2+} (Kumar et al. 2019).

Omics as source of genetic manipulation

Phytoremediation can be improved by manipulating DNA and transforming genomes—to develop new genes that cannot be found naturally, which increases the remediation mechanism of species (Nedjimi 2021). In this modern age, the main research field in biotechnology is playing with genes to come up with great ideas on how to develop phytoremediation

Name of bacteria	Name of target plant	Name of genes	Name of enzymes expressed	Valuable effects	
Caenorhabditis elegans	Nicotiana tabacum	AtPCS1 and CePCS	Phytochelatin synthase	Increase As accumulation	
Escherichia coli	Poplar	ECS genes	γ-glutamylcysteine syn- thetase	Increase Cd fluidity and purification	
Saccharomyces cerevisiae	Arabidopsis thaliana	Gsh1 and AsPCS1	Glutathione synthase and phytochelatin synthase	Increase Cd and As toler- ance	
Pseudomonas fluorescens	Sedum alfredii	SaNramp1	Iron-regulated transporter	Increase Zn uptake	
Saccharomyces cerevisiae	aromyces cerevisiae Nicotiana tabacum		Metallothionein trans- porter	Increase Cd and Zn accu- mulation	
Streptococcus thermo- philus	Beta vulgaris	STGCS-GS	γ-glutamylcysteine syn- thetase and glutathione synthetase	Increase Cd, Cu, and Zn tolerance	
Bacillus subtilis	Brassica juncea	Bse-4	Phytochelatin synthase	Increase tolerance and accumulation of Cd and Pb	
Agrobacterium tumefa- ciens	Tomato	AtGST	Glutathione S-transferase	Enhanced detoxification of heavy metals like Cd	
Pseudomonas putida	Rice (Oryza sativa)	pptA	Phytase	Increase phosphate solubi- lization and As accumu- lation	
Rhizobium meliloti	Soybean (Glycine max)	nodABC	Nodulation factors	Improved heavy metal uptake and nitrogen fixation	
Serratia marcescens	Maize (Zea mays)	smtA	Metallothionein	Enhanced Cd and Zn toler- ance and accumulation	
Klebsiella oxytoca	Sunflower (Helianthus annuus)	<i>copA</i> and <i>copB</i>	Copper-binding proteins	Increased Cu uptake and tolerance	

Table 1 Expression and impact of some genes situated into plants for HMs tolerance

Here is the updated Table with studies involving various genes related to heavy metals in vegetables, fruits, crop plants, and model plants

concepts by creating genome-manipulated species (Nedjimi 2021). Overexpression of manipulated genes can overcome the pressure caused by contaminants and influence phytoremediation procedures (Liu et al. 2020). The prime focus of this genomic manipulated strategy is to buy plants with a high tolerance for, ability to store, or ability to degrade HMs (He et al. 2015).

Omics for phytoremediation

In bioremediation, many plants play a significant role in natural ways or by the accumulation of pollutants, microbial inspiration, stabilization, and volatilization. With the introduction of new omics approaches, the genetics behindhand phytoremediation came to light because it uncovered the molecular variations between seemingly alike individuals (Mansoor et al. 2022).

"OMICS" study under the term of phytoremediation chiefly showed plants' response to metallic contaminants. This explains that plants exhibit a significant role in endorsing organic-degrading microorganisms, indicating their useful function in the degradation, volatilization, and aggregation of organic molecules (Brentner et al. 2010).

There are two different ways to apply omics— (1). Descriptive omics gathers a huge database of participating mechanisms of relevance and a biological community's observation. And secondly, (2). manipulative omics: using vast amounts of chemical and biological tools worrying counting oxidoreductases, applying fertilizer, altering the physical properties of the soil, etc., to produce new, changed biological systems that enable beneficial species to thrive and reduce harmful contaminants (Rosaler 2015).

Transcriptomics

Transcriptomics can be used to genes in *B. juncea* that identify genes in *B. juncea*, which are involved in the phytoremediation of chromium and sulfur from polluted environments (Schiavon et al. 2012). Developing plants with mercury tolerance was placed miserably when bacterial genes, which transform mercury, were programmed and considered (Barkay et al. 2003). Similar to this, three chromosomal areas were identified by Zinc QTL analysis that account for 42% of the diversity in plant zinc tolerance resistant *A. lyrate* and *A. halleri* inbreeds descendants (Mansoor et al. 2022).

Metagenomic

By using SIP (stable isotope probing) coupledmetagenomics submission to polluted soil to eliminate impurities such as diesel, hydrocarbons, and mineral oil in a manner independent of culture, the identification of strains that can metabolize hydrocarbons has been accomplished (Rodgers-Vieira et al. 2015). The hydrocarbon degradation role of root exudations has been demonstrated via substrates with SIP-labeled (Thijs et al. 2017). Rhizosphere plants and contaminated soils were also thought to contain plasmid DNA, which and is an important site for the exchange of plasmids (Chen et al. 2003). Compared to the overall genome, data from the sequencing of plasmid DNA from public waste-water treatments showed a disproportionately high concentration of heavy-metal-resistance genes. The same study also identified the role of genes in plasmids involved in the breakdown and conversion of organic compounds (Balcom et al. 2016).

Proteomics

The study of proteomics has been utilized to decipher plant responses and pinpoint the genes that cause stress brought on by pollutants. For instance, the use of proteomics to classify alterations in Populus brought about by Cd^{2+} was found to have an impact on the plant proteome both directly and indirectly, and that the reaction of the plant varied between tissues in the cambium and the leaves. Reduced metabolic rates as a result of systemic toxicity can be attributed to the indirect effect (Durand et al. 2010).

When combined with conventional methods, integrated omics may aid in identifying the phytoremediation properties of meta organisms which is targetable, such as by analyzing root transudation designs to target phantom characteristics or by examining the relationships between a meta-organism's activity, expression, and translation properties through microbiome studies. Similarly, interactome investigations may provide previously undiscovered data on how microbes promote and inhibit plant growth (Bell et al. 2014).

Future research may use a combination of various approaches (Table 2) to provide a more thorough understanding of the involved gene, metabolic, and protein networks, as well as their interactions with plants and ecological variables. Even though "omics" studies are very cost-effective, high throughput screening is ultimately required to support the data in addition to the separation of key microbial features, which helps identify, even though it may not be naturally dominant, the microbiological behavior that must be addressed in phytoremediation (Blomberg 2011).

Use of transgenic plant for phytoremediation

The initial phase, which aimed to lessen the prevalence of commercially available genetically modified plants in agriculture, ran into difficulties because the harm caused by beneficial organisms and the decreased need for specific pesticides, such as in plants that produce Bt toxin, were present. Beyond these challenges, however, transgenic plants show promise. They have demonstrated their ability to digest several environmental contaminants, including explosives, chlorinated solvents, and phenolics, according to research investigations (Abhilash et al. 2009). The expression of genes taking part in the metabolism, exploitation, or transportation of certain contaminants in engineered plants acts to improve the efficacy of phytoremediation (Yan et al. 2020). Genes from Agrobacterium tumefaciens-mediated plants may be easily transformed into potential plant species. Because large, rapidly growing plants are much

Name of strategies	Advantages	Limitations
Genomic approach	Fully developing aquatic plant genomics can enhance phytore- The use of this technology is constrained by the incomplete mediation by enabling detailed research on specific proteins, genome sequences of many important aquatic plants. Add genes, or biological processes in water plants	The use of this technology is constrained by the incomplete genome sequences of many important aquatic plants. Addi- tionally, this approach cannot be used as a standalone tool
Proteomics approach	The proteomics approach enhances molecular understand- ing and provides insights into handling phytoremediation capacity. It's useful for screening specific genes to create stress-tolerant cultivars	The main obstacle is the paucity of genomic data for many plants, whose complete genome sequences are still pending. A comprehensive proteome reference map is essential
Metabolomics approach	This method is powerful for identifying stress-response metabolites and potential pathways to improve phytoreme- diation. It provides information on new metabolic routes connected to existing metabolic networks	Following metabolic pathways and identifying potential targets is laborious. There are information gaps about detoxification pathways in plants whose genomes are not yet fully synchro- nized and investigated
Transgenics approach (Transgenic plants, Genetically Modified Plants, and CRISPR Technology)	This method has broad applications and has been used extensively. Transgenic plants show increased remediation efficiency, lower costs, and decreased need for expensive chemicals. Eco-friendly and low-maintenance. CRISPR systems can enhance bioaccumulation, complexation, vola- tilization, and degradation processes	Producing transgenic plants is laborious. There is a risk of invasion into indigenous plant ecosystems due to the high vigor and capacity to spread. Expressing certain genes could increase plant sensitivity or toxicity, affecting detoxification and potentially leading to plant deterioration. CRISPR trans- formation in aquatic plants may result in genomic incongrui- ties, and only 20% to 50% of transformed colonies may have the desired mutation

more effective for phytoremediation and transformation techniques (Gomes et al. 2019; Mohanty et al. 2022).

Use of transgenic plants for organic pollutants remediation

The efficiency of phytoremediation for nitro aromatics has advanced significantly as a result of the use of transgenic plants. Due to their phytotoxicity, nitro aromatic explosives make phytoremediation difficult and frequently restrict the process to non-transgenic plants. The ability of plants to combat these pollutants has improved thanks to the introduction of bacterial genes that break down nitro aromatics into plants. Tri-nitrotoluene (TNT) (Fig. 2) can only be broken down by an enzyme called pentaerythritol tetranitrate reductase (PETN) by turning it into innocuous compounds. Figure 2 shows how this change occurred. By integrating the bacterial genes nfsl and xplA/B, phytoremediation of organic pollutants, including those with phytotoxic qualities like TNT and Royal-Demolition-Explosive (RDX), can be greatly improved. This enhancement enables plants to efficiently remove harmful pollutants. Figure 2 illustrates the comprehensive phytoremediation of organic contaminants.

The ability of the gene *CYP2E1* to absorb and degrade tri-chloroethylene (TCE) and other small-scale volatile contaminants is demonstrated in Fig. 2. The elimination of benzene and TCE, two volatile chemicals, from the air has enhanced due to the *CYP2E1* gene's inclusion. Furthermore, plants that express the human *CYP2B6* gene or gamma-gluta-mylcysteine synthases have demonstrated the ability to degrade a variety of herbicides (Jha 2020). Transgenic plants have been able to degrade phytotoxic pollutants without ingesting them by secreting detoxifying enzymes, including lactase-1 and haloalkane dehalogenase.

Bioremediation of metal pollutants by using transgenic plants

The goal of soil remediation seems to be to remove metal from a large sample (soil) and transfer it to a lesser quantity of organic tissue (plant tissue) for disposal and harvest. Because metals cannot be digested or transformed into less dangerous forms (Table 3). Harmful metal elevated plants that move the metal to the shoots from roots and compartmentalize or modify it for volatilization, which is required for phytoremediation (Ozyigit et al. 2021; Sharma et al. 2021). The use of transgenic approaches enhanced

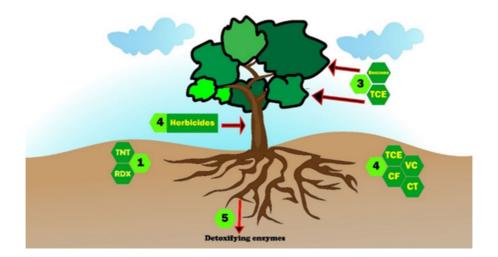


Fig. 2 Improving phytoremediation of organic pollutants by using transgenic approaches. (TNT stands for Tri-nitrotoluene; RDX stands for Royal-Demolition-Explosive; TCE stands for Tri-chloro-Ethylene; VC stands for Vinyl-Chloride; CT stands for Carbon Tetrachloride) TNT can be degraded by PETN (Pentaerythritol tetranitrate reductase enzyme. TNT and RDX exhibit phytotoxic effects which enables the plants to eliminate organic pollutants. The overall organic pollutants phytoremediation has been demonstrated in this figure)

Genes	Products	Targets	Effects
Atrazine chloro- hydrolase	Pseudomonas sp.	N. tabacum	Dechlorinates atrazine to hydroxy atrazine
MT2	Metallothionine	N. tabacum	increased CD tolerance in seedlings
CUP1	Metallothionine	Yeast	No Cd tolerance but a higher Cu content compared to the control
CYP1Y1	Homo sapiens	Oryza sativa	increased metabolism of norflurazon and chlortoluron
SAT	Serine acetyltransferase	Arabidopsis	Boost in shoot Ni sensitivity of 5 times
Laccase	C. versicolor	N. tabacum	Cleaning up after bisphenol A

Table 3 Phytoextraction performance of transgenic plants with increased gene expression

the phytoremediation of hazardous metals. The outcome, if indeed the genes were repurposed into different plant species with high biomass and wide root systems, such as poplar and willow, significant heavy metal removal should be feasible efforts are being made to improve selenium (Se^{2–}) phytoremediation (DalCorso et al. 2019; Ozyigit et al. 2021).

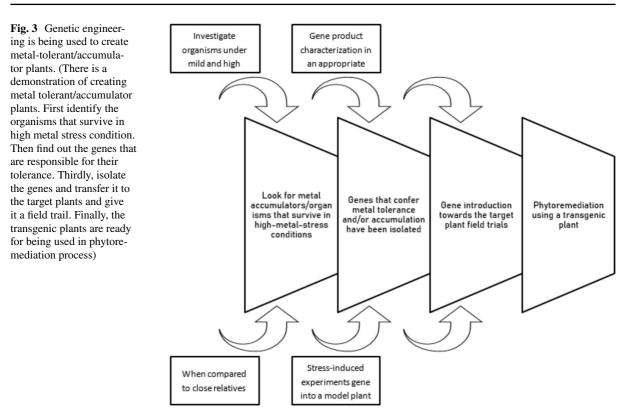
Many genes are associated with metal absorption and transportation, and transferring one of these genes through genetic engineering into suitable plants might be an approach to improving phytoremediation features (Agnihotri and Seth 2019; Kumar et al. 2022a, b; Ozyigit et al. 2021; Raza et al. 2021). Depending on the technique, transgenic plants with modifications to accumulate large quantities of obtainable metal portions can be created. By transfer of desirable genes, metal absorption, translocation, sequestration, and intracellular targeting will be improved. Metal chelators can be produced by genetically engineered plants, which will boost the capacity of the plants to absorb metals. According to traditional genetic research, it's hard to find those genes that can control metal tolerance because they are rare (Ent et al. 2015). Appropriate transgenes for phytoremediation can be created (Fig. 3) by transplanting genes using hyperaccumulators or other sources. The following are some of the conceivable fields of genetic modification.

Metallothioneins, phytochelatins, and metal chelators

Metallothionein is a high-cysteine protein that helps maintain metal homeostasis and defend against heavy metal toxicity, DNA damage, and oxidative stress. Several plant species have had their metallothionein genes cloned and introduced. Greater Cd^{2+} tolerance was seen in plants when the MT-2 gene was transferred to oil seed rape or tobacco, while increased Cu²⁺ accumulation was seen when the pea MT gene was transferred to Arabidopsis thaliana (Turchi et al. 2012). Modified gene from Brassica juncea was shown to derive more Cr^{6+} , Cd^{2+} , Zn^{2+} Pb^{2+} , and Cu^{2+} than untamed plants when several phytochelatin production-related enzymes were abundantly expressed. Upregulation of two enzymes known as transgenic-Indian-mustard (TIM) with higher amounts of phytochelatins and glutathione as a result of G-glutamyl cysteine synthesis or glutathione synthetase showed boosted Cd²⁺ accumulation and tolerance (Gupta and Reddy 2019). Plants with increased Al³⁺ tolerance were produced when the citrate synthase gene was expressed. In their roots, these plants created up to ten times the amount of citrate and discharged it. Genes for nicotinamide-aminotransferase are transferred to an iron chelator to be overproduced in rice. In unsound soils, the genetically engineered plants produced phyto siderophores and grew more quickly (Divekar et al. 2022).

Metallic carriers

Hereditary modification of metallic carriers is believed to buildup of metal allowances in plants from *A. thaliana* was introduced to tobacco and reflected in increased Cd²⁺, Mn²⁺, and Ca²⁺ accumulation (Nahar et al. 2017). Increased Ni²⁺ acceptance was achieved via transferring the calmodulinbinding protein-encoding *NtCBP4* carrier gene (Virdi et al. 2015). Tobacco plants' mineral content increased 1.5 times when the ferric reductasecoding yeast *FRE1* and *FRE2* genes were added (Divekar et al. 2022).



Changing the mechanisms of cellular oxidative stress

Upregulation of peroxidase and glutathione-S-transferase increased Al^{3+} tolerance, indicating that oxidative stress-related enzymes can modify metal tolerance (Gaafar et al. 2022). 1-aminocyclopropane and 1-carboxylic acid deaminase overexpression increased metal buildup (Naing et al. 2021).

Rhizoremediation of organic xenobiotics using transgenic plants

One of the most promising approaches for advancing phytoremediation technologies is the introduction of transgenic post-flooding systems that target plat root systems to enhance rhizosphere emissions and target toxicant metabolites suitable for pollutant uptake (Kawahigashi 2009). The advantage of this technique is that the pollutants can be broken down in the rhizosphere by releasing enzymes rather than by the plants taking up the toxins to detoxify them (Kawahigashi 2009). The rhizosphere is the part of the soil that is affected by root activity immediately adjacent to a root. This occurs whenever a root tips penetrate the soil then disappears as the root dies and rots (Beidler and Pritchard 2017). There are some enzymes (Fig. 4) that have a potential impact on improving rhizosphere zone cleanup procedure by transgenic plants (Fig. 5).

Phyto-reduction of Mercury (Mg²⁺) using transgenic plants

All living things are hazardous to Mg²⁺and its compounds. Pathogens have evolved strategies for settling in mercury-polluted environments, and a gene operon encoding biochemical detoxifying carriers and enzymes has been identified as Mg²⁺resistance (mer) (Boyd and Barkay 2012). There are three plant species Liriodendron tulipifera, Nicotiana tabacum, and A. thaliana, genetically altered plants possessing MerA and MerB genes, demonstrating that modified plants could flourish in the vicinity of deadly quantities of Mg^{2+} (Christakis et al. 2021). The bacterial merA DNA sequence was changed to increase the appearance of mer genes in plants by lowering the protein-coding region's GC contents in a 9% block and adding plant-governing components (Christakis et al. 2021). The novel gene construct (merA)

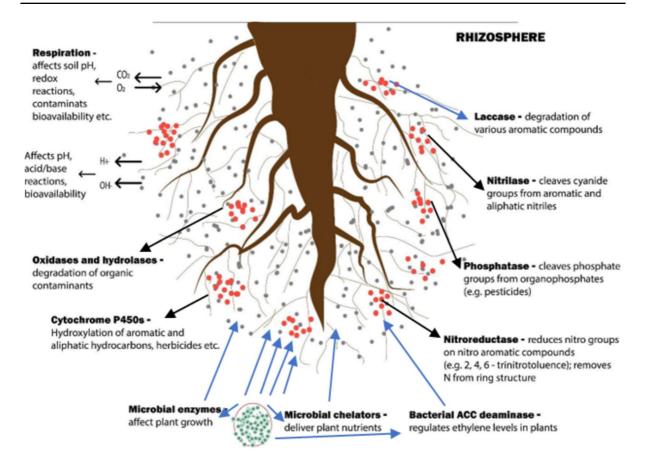


Fig. 4 Microbiological and enzymatic processes responsible for improved rhizosphere zone cleaning. (Potential enzymes like laccase, nitrilase, phosphatase, nitroreductase, oxidases, hydrolases, cytochrome P450s etc. have useful impact on rhizosphere remediation procedure by degrading aromatic

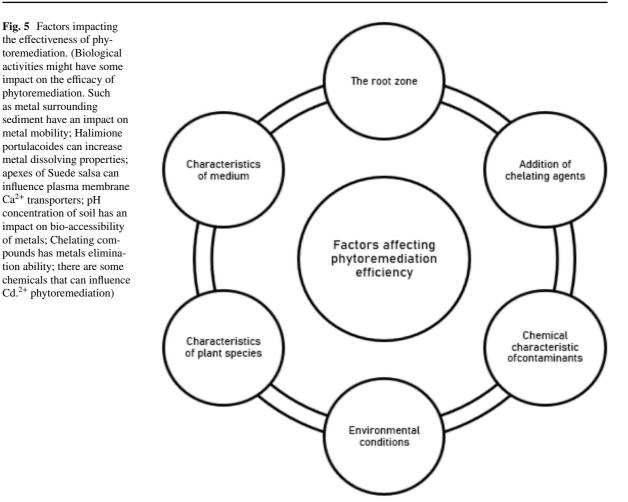
provided tolerance to 50 mm Hg(II) when transplanted to *A. thaliana* and tobacco, suggesting that plants with the *merA* gene enzymatically degrade Hg(II) and fade away Hg(0). When transgenic trees were cultivated on soil with 40 ppm Hg(II), they produced more bioenergy. It has been established that methylmercury lyase targeting inside cells improves its particular biological detoxification of Mg²⁺in plants (Kumar et al. 2017).

Selenium (Se²⁻) tolerance using transgenic plants

 Se^{2-} is a prominent contaminant in the environment, and higher levels of the Se^{2-} requirement can cause hazardous consequences (He et al. 2018). The oxidized form of Se^{2-} , termed selenate or selenite, is compounds; cleaves cyanide group from aromatic and aliphatic nitriles; elimination phosphate group from organophosphates; reduce nitro groups from nitro aromatic compounds; affect on plant growth; regulates ethylene levels in plants etc.)

simple for plants to extract, yet inorganic versions like selenide or atomic Se^{2-} are more challenging to get ATP sulfurylase-upregulating transgenic plants produced three times as much Se^{2-} per plant and even had four times the enzyme activities of wild-type plants. The transgenics grew faster and more quickly than the natural type and had greater Se^{2-} resistance (Bawa and Anilakumar 2013). Using a bacterial-glutathione reductase, the chloroplast and cytoplasm of Indian cultivars were similarly altered. Both forms of modified plants outgrew natural-type seedlings on agar substrate spiked with dangerous amounts of selenite or selenate (Li et al. 2022).

 Se^{2-} is harmful at high concentrations because it substitutes sulfur in proteins. There is research where Mammalian-selenocysteine-lyase is recognized in



Arabidopsis, which guides Se^{2-} where it wouldn't interrupt protein synthesis (Harris et al. 2014). Because Se^{2-} poisoning is assumed to be caused using selenocysteine as a component of proteins (Kolbert et al. 2019). This technique involves breaking down selenocysteine and free Se^{2-} being released. Genetically modified plants expressing cytosolic-selenocysteine-lyase showed increased Se^{2-} resistance (Doty 2008).

Arsenic (As⁵⁺) tolerance using transgenic plants

 As^{5+} is a highly poisonous metalloid contaminant and exceedingly harmful to human health (Huda et al. 2022). It is established that there are certain microbes whose genes can reduce and oxidize As^{5+} toxicity and play a major part in the reduction and immobilization of As^{5+} pollutants from agricultural soil (Huda et al. 2022; Khanom et al. 2022) Creation of modified Arabidopsis plants that can move the oxyanion-arsenate to the surface, convert it to arsenate $((AsO_4)^{-3})$, and then isolate it in thiol-peptide clusters. AsO_4)⁻³reductase (ArsC) and glutathione-coupled electrochemical reductions of AsO_4)⁻³in plenty of deadly AsO_4)⁻³ are catalyzed by the ArsC gene in E. coli. Arabidopsis plants altered with the ArsC gene from an SRSIp (light-induced-soybean-rubiscopromoter) produced high levels of ArsC protein was oversensitive to AsO_4)⁻³ and was present in the leaves although not in the root system. In comparison to control plants, the E. coli gene that is expressed by the Arabidopsis plants producing glutamyl-cysteinesynthetase with the actin activator was only mildly resistant to As^{5+} . Plants addressing ACT 2p/g ECS and SRSIp/ArsC demonstrated higher tolerance to As⁵⁺. These modified plants gathered 4–17-fold higher initial weight shoots and 2–threefold additional As^{5+} per gram problems, which equated to natural plants or genetically engineered plants conveying *ArsC* or *g-ECS* alone.

Herbicide degradation using transgenic plants

Mammalian P450s mediate herbicide metabolism in the liver. Transgenic rice and potato plants have been used to produce mammalian cytochrome P450 genes to detoxify herbicides. Due to enhanced metabolism by the inserted P450 enzymes, compared to non-transgenic rice plants, rice plants modified with genes expressing humans CYP1A1, CYP2B6, and/or CYP2C19 are much more herbicide tolerant. Atrazine and simazine levels in hydroponic solutions are decreased in transgenic rice plants expressing CYP1A1, demonstrating herbicide-tolerant for chlorotoluron, atrazine, quizalofop-ethyl, diuron, and other herbicides. In the presence of chloroacetanilide herbicides, transgenic rice harboring CYP2B6 germinates effectively. Atrazine is a systemic s-triazine pesticide that inhibits photosynthesis. Metolachlor, an herbicide, an herbicide and chloroacetanilide, stop plants from producing long-chain fatty acids. These herbicides are used extensively worldwide. Throughout the globe, plants with transgenic rice have demonstrated phytoremediation efficacy in the presence of somewhat large-scale investigations, atrazine and metolachlor were effective. Rice plants were shown in stainless steel pots with soil that contained 4.2 mM atrazine. Metolachlor at 2.9 mM. Transgenic and nontransgenic mice were both employed. Over a month, the plants exhibited good development. With plants, the remnant atrazine in the soil was 70.1% of that in soil devoid of plants, against 70.1% in the soil with desired plants. The percentage of the plants nontransgenic was 93.2% (Kawahigashi 2009).

Improved explosives remediation using transgenic plants

Environmental concerns about contaminated substances due to extensive military actions are frequent (Fernandez-Lopez et al. 2022). Contamination occurs mostly during manufacturing, using, loading, storing, and disposing of products (Van Aken 2009). Nitrate esters, nitroaromatics, and nitroimines are the three primary types of explosives (Rylott and Bruce 2009). Glycerol-trinitrate and PETN are the most common nitrate esters. Nitroaromatic dynamite amino di-nitrotoluenes (ADNT), diamino nitrotoluene, and nitrobenzene have a nitro group-filled aromatic ring. N-nitro groups are found in nitro amines. RDX is currently the most often used heavy offensive weapon in the military, which is utilized by a variety of organizations. Because of their presence in the environment, even in low quantities, they constitute a risk of explosion as well as a threat to the health of people and natural systems. Because of their poisonous and mutagenic effects, even at low concentrations, their natural range endangers organic systems and human health (Alengebawy et al. 2021). In the existence of various climatic conditions, most explosives resist natural lessening processes, including biodegradation, hydrolysis, and volatilization, which leads to their persistence in surface and groundwater.

Furthermore, PETNr-expressing tobacco plants germinated and grew normally on a solid medium bearing 1 mM GTN, a dose that might render nontransgenic plants unusable (Aduse Poku et al. 2020). Pentaerythritol dinitrate is created when PETNr progressively reduces a couple of PETN's multiple nitro groups, subsequently oxidizing to dialdehyde (Aduse Poku et al. 2020). PETNr has also been shown to act against nitroaromatics in later experiments. Compared to transgenic trees, non-transgenic plants were intelligent enough to absorb more TNT from soil and liquid culture (Van Dillewijn et al. 2008).

In A. thaliana, two of the uridine diphosphates are abundantly expressed, and uridine glycosyltransferases genes resulted in upregulation seedlings treated in liquid culture with TNT showed higher conjugate synthesis and improved root development, according to recent research (Gandia-Herrero et al., 2008). The insertion of fresh foreign genes that produce proteins that participate in different detoxifying processes is often used to genetically modify plants for improved phytoremediation capacities (Jan et al. 2015). Microorganisms are heterotrophic creatures with the catalytic mechanisms needed for mineralizing organic molecules. A plant's metabolic capacity can be supplemented by microbial and mammalian catabolic genes (Eapen et al. 2007).

Strategies for reducing risks while using transgenic plants for phytoremediation

Various protective studies have been conducted on the potential and perceived risks associated with using transgenic plants for agriculture, and some characteristics of transgenic plants for phytoremediation must also be considered (Gunarathne et al. 2019). Unlike transgenic crops meant for use with either humans or animals, phytoremediation plants do not have to worry about food safety, allergenicity, or labeling, and application of insect, herbicide, and viral tolerance genes does not show to be a possibility at this time (Ghimire et al. 2023). As a result, the major danger is gene transfer from farmed plants to wild cousins, which would need to be monitored. It has been demonstrated that plants made by genetic engineering are more tolerant of dangerous metalloids. The possibility of some natural flora alteration through long-distance cross-pollination, the risk of favored plant invasion, and the possible loss of variety should all be considered. Creation of a modified plant threat assessment concept for heavy metals removal (Kumar 2021). Since these facilities would be in remote commercial areas instead of rural areas, the authors emphasized that the dangers of metals entering through designed accumulators would be negligible. When a plant's flowering stage is interrupted before harvest for phytoextraction, there is a much-reduced chance of inadvertent pollination or seed dispersal among relatives. Combining the relevant gene with a companion gene that is beneficial or neutral in agricultural settings but damaging or lethal in the wild is another strategy for reducing or eliminating gene flow. Another option is to utilize the crelox bacteriophage or yeast FLP-FRT recombination systems to precisely delete a gene resistant to antibiotics derived from a plant chromosome. The undesired gene and the recombinase genes cre and/or FLP should be introduced into the chromosome, bordered by *loxP* and/or *FRT* sequences (Houdebine 2014). Site-specific deletion at loxP or FRT sites is done upon a biochemically driven activator's stimulation of the recombinase gene expression. The Zn^{2+} and Cd^{2+} levels in the transgenics were much greater. Transgenics must undergo extensive field testing to assess their function in a natural environment. Enhanced exposure risk to wild species and, as a result, to people is one of the probable dangers associated with such transgenics. The risk of wild animals ingesting metal can be reduced by enclosing the area with an appropriate fence and using nonpalatable plants. So far, no transgenic plants have been employed commercially for phytoremediation. The risks related to plants that volatilize mercury reveal that they constitute little environmental impact (Gworek et al. 2020). Concerns about transgenic plants emitting volatile Se have been studied, and it has been determined that there is no major concern (Kos et al. 2009). Additionally, there is extremely little likelihood of genes escaping from modified plants.

Future prospects

Researchers have advocated the use of phytoremediation as an environmentally benign strategy for polluted site remediation. Nonetheless, the process is impeded by the hyperaccumulator plant's poor growth rate, which is caused by limited biomass output. Modern technologies, such as genetic engineering or recombinant technology, can help plants produce more biomass and improve their capacity to absorb, decompose, or tolerate different contaminants in soils and aquatic habitats. The use of nanoparticles in phytoremediation is mediated by transgenic plants, as well as the obstacles (Rana et al. 2022). Lower costs, the development of recyclable metal-rich plant waste, application to a wide variety of harmful metals, minimum environmental disturbance, and public acceptability are all advantages of metal-accumulating plants to remove metals from polluted soils. Plants with improved metal-absorption capabilities might be used to boost agricultural yield in locations where metal levels are low or as enriched food and feed (Rai et al. 2019). Despite these benefits, phytoextraction is still a new technique, and significant efforts are required to this environmentally favorable technology fully. Textile dye phytoremediation is a relatively recent approach to textile wastewater treatment. Nowadays, various laboratory-size study methodologies have been used.

Conclusions

Phytoremediation has acquired widespread recognition as a novel and promising method, and it is now a hot topic when studying plant biology. Many plants have already been named as viable applicants for bioremediation. Rice plants are believed to be good candidates for soil and stream water phytoremediation. A molecular knowledge of metalloid mobilization, uptake, transport, and installation in hyperaccumulating plants is being worked on, which might lead to identifying candidate genes for phytoremediation and identifying additional physiological demands. A variety of strategies have been created to reduce or eliminate environmental contamination and to replant damaged soil. Different steps involved in phytoremediation are phytoextraction, phytostabilization, rhizosphere bioremediation, rhizofiltration, phytovolatilization, etc. Another method for improving plant performance for phytoremediation is to use plant-associated microorganisms. Rhizobacteria that promote plant development have been found to have a lot of promise for improving phytoremediation efficiency. Additional advancements will include broadsubstrate, natural, or manufactured catabolic genes, such as mammalian cytochrome P450 or fungal peroxidase, enabling the simultaneous cleanup of several contaminants, such as those present in polluted soil. Candidate genes/proteins for phytoremediation may also be found using proteome and DNA array technologies. Plants have been genetically engineered during the last decade to overcome natural limits in plant detoxifying capacity in a technique comparable to the production of transgenic crops. Different factors affect phytoremediation efficiency, such as characteristics of the medium, characteristics of plant species, environmental conditions, etc. From an environmental perspective, the first modified species used for phytoremediation applications are cultivated plants that express genes implicated in pesticide biodegradation. In practice, a single strategy is neither practicable nor adequate to effectively clean up heavy metal-polluted soil. In the years ahead, highly efficient and thorough phytoremediation will require a combination of methods, including genetically engineered, microbe-assisted, and chelate-assisted phytoremediation.

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Authors contributions M.M.R. oversaw the entire project, and all authors approved the final manuscript. A.A.M. and

M.M.R. conceptualized and planned the study, carried out the analysis, wrote the manuscript, and created the graphs and illustrations. M.A.H., M.R., M.R.R., S.T.R., M.L.K., M.K.B., and M.M.R. contributed to the writing and critical revision of the manuscript.

Declarations

Competing interests The authors declare no competing interests.

Conflict of interest Each author declares that they have no conflicts of interest.

Ethical approval There is no ongoing study using human subjects or animals in research.

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