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Approaches for alleviating heavy metal stress

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Abstract

Anthropogenic activities have led an augment in heavy metal contamination in water and soil throughout the past few decades. Increased crop plant exposure to heavy metals reduces production and growth, which affects agricultural production's future viability. Therefore, there is an urgent requirement for various strategies for mitigation of heavy metal stress. Among them, silicon supplementation presents a promising role in mitigating heavy metal stress. Numerous types of microorganisms, including fungi and bacteria are used as effective natural bioactive stimulants in the rhizosphere to help plants stressed by heavy metals. Nanoparticles (NPs) are useful for reducing heavy metal stress because of their distinct physiochemical characteristics. Plants can be used as hyper-accumulators of heavy metal ions, which is beneficial both economically and practically. Plant genetic modification techniques are used to increase phytoremediation's potential. The use of these strategies may prove to be economical and sustainable method for heavy metal detoxification.

Keywords: Heavy metal stress, phytoremediation, nanoparticles, transgenic plants, microorganisms

1. Introduction

Human health, wildlife, and agricultural productivity are all seriously threatened by heavy metal contamination. Heavy metals are defined as elements with an atomic mass more than 20 and a specific gravity higher than 5 (Rascio & Navari-Izzo, 2011) ^[39]. Plant development and function depend on a few heavy metals. Molybdenum, copper, nickel, manganese, zinc, and iron are some examples of heavy metals that are necessary for the development and growth of plants. They aid in the resilience of plants against abiotic and biotic stressors, inhibit fungal penetration, and reduce insect resistance (Adrees *et al.*, 2015) ^[1]. However, elevated concentrations of heavy metals are toxic for plants as this causes heavy metal stress in them. Heavy metals including nickel, mercury, arsenic, chromium, lead, cadmium copper, and cobalt accumulate in soil and the environment due to human activities including smelting, sewage disposal, fertilizer applications, and industrial waste. One of the biggest issues facing modern crop cultivation is the buildup of excessive concentrations of heavy metals in water and soil. The amount of these hazardous metals that have contaminated agricultural soils has grown during the past ten years (Ashraf *et al.*, 2019) ^[6]. Active oxygen species (AOS) are produced in response to elevated heavy metal concentrations. AOS induce apoptosis, oxidative stress, disruption of cell membrane and structure, cytoplasmic enzyme inhibition and cellular organelle damage (Adrees *et al.*, 2015) ^[1]. The primary metabolic processes of plants are impacted, including metabolism of nitrogen, photosynthetic process, and the water and nutrients uptake. Furthermore, heavy metals cause AOS, which upset the redox state of the cell (Iqbal *et al.*, 2015) ^[20]. There have also been reports of decreased root and shoot development, disruption in respiration, and a reduction in chlorophyll production due to grana structural disarray (Austruy *et al.*, 2014) ^[7]. Millions of people's access to food is negatively impacted by heavy metal-contaminated water, air, and soil because it stunts crop growth and lowers agricultural yields (Su *et al.*, 2014) ^[47]. Therefore, the demand for innovative and eco-safe techniques to get rid of these harmful heavy metals is growing. Plant tolerance to the toxicity of heavy metals can be strengthened by silicon. It has advantageous effects on detoxification that can be attributed to both the external and internal mechanism of plants (Bhat *et al.*, 2019) ^[8]. Microorganisms can boost phytoremediation efficacy by solubilizing heavy metals and synthesizing phytohormones in soil polluted with heavy metals (Xie *et al.*, 2021) ^[54]. NPs possess distinct characteristics and hold promise in mitigating the heavy metals' deleterious impact on plants by adsorption of these metals and triggering antioxidant mechanism of plants (Konate *et al.*, 2017) ^[24].

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Native plants' application in phytoremediation is an emerging technology that is seen to be one of the greatest ways to treat soil polluted with metals given its extensive variety of uses, affordability, and aesthetic appeal. Phytoremediation appears to be a workable way to remove metals from contaminated areas although most common plants absorb metals minimally. Consequently, the goal of genetically modified plants is to increase their metal accumulation capacity (Ozyigit *et al.*, 2021) [36]. This review describes the various strategies that have helped to decrease the harm that heavy metal stress causes to plants.

2. Approaches for alleviation of heavy metal stress

2.1 Silicon mediated approach

Silicon is regarded as a quasi-essential element because of its many advantages for plants, particularly amidst of biotic and abiotic stress (Rasoolizadeh *et al.*, 2018) [40]. Most commonly accepted mechanisms adopted by silicon for mitigating the heavy metal stress involve co-precipitation of metals, immobilization of toxic metals in the soil, chelation of metal ions, compartmentation, activation of both enzymatic and non-enzymatic antioxidants, structural changes in plant tissues, and modification of molecular responses at the plant level (Bhat *et al.*, 2019) [8]. The immobilization was brought on by either an increase in soil pH or a change in metal speciation in the soil solution as a result of the formation of silicate complexes. Si-rich supplements were observed to raise soil pH in rice from 4.0 to 5.0–6.4 and reduce heavy metal phyto-availability by at least 60%, which further inhibited metal uptake in rice (Gu *et al.*, 2011) [19]. Application of silicon decreased the levels of cadmium in brown rice by 11.45~51.85% in the acidic purple soil (pH = 4.77, soil total cadmium content 0.413 mg kg⁻¹), which was mildly contaminated with cadmium, and by 26.93~43.77% in the purple calcareous paddy soil (pH = 7.77), which had comparable levels of cadmium pollution. Silicon application mostly prevented cadmium translocation from stem to the rice grain or from the root to the stem, thereby reducing the amount of cadmium accumulated in rice grains. A number of processes including adsorption, compartmentalization, chelation and apoplastic barriers may be responsible for the elevated levels of cadmium maintained in the roots (Li *et al.*, 2020) [29]. Silicon inclusion in culture media containing cadmium reduced the harmful effects of cadmium on wheat plant leaves by elevating the activities of catalase, superoxide dismutase, peroxidase and contents of ascorbic acid, and glutathione (Rahman *et al.*, 2021) [38]. Silicon supplementation in rice has been shown to drastically downregulate the expression of the genes encoding heavy metal transporters (OsHMA2 and OsHMA3) and upregulate the genes involved for Si transport (OsLSi1 and OsLSi2) (Kim *et al.*, 2014) [23].

2.2 Microorganisms

Microorganisms use a wide range of harmful substances as energy sources for growth and development through their unique metabolic processes of respiration, fermentation, and co-metabolism. Because of their enzymes that particularly break down heavy metals, they have evolved unique mechanisms to maintain normal states and tolerance for heavy metals, allowing them to adapt many of the hazardous metals found in the ecosystem (Priya *et al.*, 2022) [37]. It is well recognised that plant-associated microbes, particularly plant

growth-promoting rhizobacteria, are essential for both boosting plant development and for remediating soils from organic and metal contaminants through a variety of methods (El-Meihy *et al.*, 2019) [14]. Plant metallothionein, superoxide dismutase, ascorbate peroxidase, and phytochelatin synthase genes are expressed more in plants when bacterial strains and fungus resistant to arsenic is inoculated in soil. This is because these microorganisms can produce indoleacetic acid and siderophores and can mediate phosphate solubilization. Indoleacetic acid lessens the stress brought on by heavy metal and arsenic contamination in soil by directly encouraging plant cell division and elongation (Soto *et al.*, 2019) [45]. AR6 strain alleviates heavy metal stress in plant by exopolysaccharide production. The cationic charged chromium (VI) can get readily entrapped by anionic charged bacterial exopolysaccharide molecules. This reduces chromium (VI) availability and mobility in the rhizosphere and gives the host plant a stress-free environment (Karthik *et al.*, 2017) [21].

2.3 NPs mediated approach

There has been a notable surge in interest in recent years for heavy metal pollution due to the fast rising quantities of these metals in agricultural soil. There are plenty of evidence which shows that stress caused by heavy metal has an effect on the plant's morpho-physiological and biochemical parameters. NPs possess the capacity to be a powerful tool in the management of heavy metal stress because of their distinct physiochemical characteristics. They employ many mechanisms to mitigate the effects of stress caused by heavy metals on the plants. NPs lower the amount of bioavailable heavy metal content in the soil (Moharem *et al.*, 2019) [34]. The mobility and bioavailability of heavy metals in the soil can be reduced by NPs through the absorption and transformation of heavy metals by them. Cadmium and other heavy metals' mobility in the soil were reduced by magnetite NPs (Sebastian *et al.*, 2019) [43]. Additionally, certain NPs can enhance the characteristics of the soil. For example, hydroxyapatite NPs can increase the soil pH and release phosphate, which lessens the negative impacts of heavy metals in the soil (Cui *et al.*, 2018) [11].

Plant roots have many protein and ion transporters in the root cell's plasma membrane that may transport heavy metals, concurrently, apoplastic barriers might not be an ideal means of reducing the harm brought on by stress due to heavy metals (Cao *et al.*, 2020) [10]. However, strengthening of the extracellular barrier of plants prevent heavy metals stress. The majority of NPs build up in the cell walls where they attach to heavy metals to create complexes that prevent them from being accessed. These complexes get adsorbed to the surface of the cell which prevents heavy metal from migrating through plants and lowers their biological activity. Additionally, to lessen the harm that heavy metal stress causes to plants, organic acids found in plant roots and leaves have the ability to chelate heavy metals (Wang *et al.*, 2021) [51]. It is shown that NPs increase the synthesis of structural protective agents; for instance exogenous silicon-NPs, enhanced the production of organic acids and reduced the harm that cadmium caused to plants (Cui *et al.*, 2017) [12]. Furthermore, NPs also control the expression of the heavy metal transport genes in plants. A silicon-NP hydrosol can reduce the toxicity caused by cadmium in rice by reducing its uptake (Wang *et al.*, 2015) [50]. NPs are able to boost the

capacity of plant antioxidant systems in addition to improving the physiological processes (Wang *et al.*, 2020) ^[53]. By increasing the levels of antioxidant enzymes and nitrate reductase activity, foliar-applied zinc oxide NPs on tomato plants exposed to cadmium metals reduced its negative impacts and enhanced plant development and growth (Faizan *et al.*, 2021) ^[15]. In rice grown hydroponically (CdCl₂ 50 µM), foliar spray of Cerium oxide NPs (200 mg/L) successfully boosted the antioxidant defense system and reduced the accumulation of cadmium (Wang *et al.*, 2019) ^[52]. De Sousa *et al.*, (2019) ^[13] discovered that by triggering the antioxidant defense mechanisms, silicon NPs (4 mg/kg) reduced the toxicity of aluminium to maize.

Lian *et al.*, (2020) ^[30] demonstrated that foliar application of titanium dioxide NPs enhanced biomass and prevented maize's absorption of cadmium. Additionally, they discovered that applying titanium dioxide NPs foliarly improved glutathione-S-transferase and superoxide dismutase activities as well as upregulated aspartame acid, galactose, alanine, and other metabolic pathways in order to mitigate the damage of maize from cadmium stress. It has been demonstrated that applying nanoscale zero-valent iron to sunflowers can decrease the buildup of heavy metal, boost superoxide dismutase and peroxidase activities in plant leaves, and encourage plant development (Micháľková *et al.*, 2017) ^[33].

2.4 Phyto-remediation

Phytoremediation is considered an effective, efficient, aesthetically appealing, economical, and eco-friendly method for eliminating potentially dangerous metals from the environment. Plants absorb contaminants via the root system during phytoremediation, and subsequently advance those pollutants into the parts of their bodies that are growing above ground (Ashraf *et al.*, 2018, Sharma *et al.*, 2015) ^[5, 44]. It is possible to employ both in situ and ex situ remediation in a phytoremediation procedure. Because in-situ application minimizes the risk to the surrounding environment by reducing the multiplicity of contaminants in water and airborne waste, it is employed more frequently. Phytoremediation can treat multiple types of pollutants on-site, negating the requirement for a disposal location. By halting soil erosion and leaching, it also restricts the spread of contamination (Sová *et al.*, 2009) ^[46]. The biggest benefit of this method is that it is far less expensive to clean up than other traditional remediation procedures (Gerhardt *et al.*, 2017) ^[16]. Due to the lack of specialized workers and the supplies needed, phytoremediation is a comparatively simple process. This can be used to clean up big areas where other traditional methods have shown to be very expensive and inefficient (Leguizamo *et al.*, 2017) ^[28].

For phytoremediation to be an environmentally viable method, plants must possess the following characteristics: high biomass yield, native and rapid growth rate, high heavy metal absorption, capacity to transfer metals in aboveground plant components, and ability to withstand metal toxicity (Arslan *et al.*, 2017, Burges *et al.*, 2018) ^[4, 9]. The aquatic ecosystem is an innovative and affordable cleaning technique for phytoremediation of a large polluted area. Notably, aquatic plants are the greatest choice for the absorption of contaminants via their roots and shoots because of their vast root systems, which can function as a natural absorbent in the phytoremediation for heavy metals and toxins (Ali *et al.*, 2020) ^[2]. Common accumulator plants for cleaning up

of water that is polluted include water hyacinth, water lettuce, and duckweed (Kristanti & Hadibarata, 2023) ^[26].

Duckweed is the smallest and fastest-growing plant species on Earth (Ali *et al.*, 2020) ^[2]. In accordance with the results of a recent finding by Rezanía *et al.*, (2016) ^[42], duckweed is frequently utilized to extract the nutrients and heavy metals discharged from agricultural and residential wastewaters. *Lemna gibba* was shown to be a hyperaccumulator plant using data from yet another investigation since it outperformed *Azolla caroliniana* and *Salvinia minima*. *Lemna minor* may also collect elevated levels of uranium, arsenic, magnesium, zinc, copper, nickel, and zinc. *Wolffia globosa* can tolerate 400 mg of arsenic per kilogram of dry weight with remarkable tolerance (Ansari *et al.*, 2020) ^[3].

2.5 Transgenic plants

One unique way to increase the efficacy of phytoremediation is to apply transgenic plants to the process. According to Koźmińska *et al.*, (2018) ^[25], Specific genes present in transgenic plants facilitate the absorption, metabolism, and accumulation of specific heavy metals. A well-established transformation process, a high biomass production, and adaptation to the local and target environments are the attributes that make a plant appropriate for phytoremediation engineering. Plant breeding-based cultivar selection offers a more workable and acceptable method, enabling crop production in environments with significant heavy metal bioavailability. Metal-safe cultivars may be produced using both conventional and innovative breeding techniques. Nonetheless, traditional breeding techniques have developed pollution safe-cultivars with some degree of success. Since this procedure is expensive, time-consuming, and requires a great deal of work (Grant *et al.*, 2008) ^[18]. A recent breakthrough in biotechnology has hastened the progress in pollution safe-cultivars breeding with the application of technologies including allele pyramiding, genome-wide association mapping, gene editing, allele discovery, genome mutation, genome selection and molecular marker-assisted selection (Oladzad-Abbasabadi *et al.*, 2018) ^[35].

According to a study by Kidwai *et al.*, (2019) ^[22], arsenate and arsenite stress consistently enhanced the level of class III peroxidase (OsPRX38) in rice. By controlling the synthesis of lignin, which serves as an apoplast barrier to prevent arsenate from entering root cells and reduces its accumulation in transgenic plant, OsPRX38 overexpression in transgenic rice greatly enhanced arsenic tolerance. In transgenic tobacco, the co-expression of the wheat-gene encoding the vacuolar proton pumps (V-PPase proton pump) and sodium/proton antiporter (NHX antiporter) reduces copper toxicity (Gouiaa & Khoudi, 2019) ^[17]. To create the transgenic tobacco lines, the phytochekatin gene "*PPH6HIS*" was synthetically created. Transgenic tobacco with *PPH6HIS* gene expression has a higher capacity for cadmium accumulation and is more resistant to cadmium's detrimental effects on plants (Vershina *et al.*, 2022) ^[49].

2.6 Gene editing

A further effective technique for tailoring a target genome to produce distinctive phenotypes in plants that accumulate little metal is gene editing (Mao *et al.*, 2019) ^[32]. This technique is currently used extensively to minimize plant metal absorption and improve food safety, in response to concerns about the consequences of rising heavy metal contamination on food

safety (Ma *et al.*, 2018) [31]. Gene editing uses sequence-specific nucleases, such as transcription activator-like effector nucleases, zinc finger nucleases, and primarily clustered regularly interspaced short palindromic repeats nucleases (CRISPR/Cas9) (Razzaq *et al.*, 2019) [41]. By altering the plant's genetic code with molecular scissors to eliminate high-metal-accumulating attributes or add low-metal-accumulating features, these methods produce "custom crops" (Lassoued *et al.*, 2019) [27]. For example, the CRISPR/Cas9 based genome alteration in the metal transporter gene - *OsNRAMP5* led to minimal cadmium buildup in indica rice yield (Tang *et al.*, 2017) [48].

3. Conclusion

Heavy metals accumulation in soil and plants' uptake of them cause disturbances to physiological and biochemical processes. Application of silicon, microorganisms, nanoparticles and use of hyper accumulating and transgenic plants are potential, beneficial and sustainable approaches, that has drawn a lot of interest for their ability to decrease soil toxicity from heavy metals and improve plant growth that is impacted by heavy metal stress.

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