Phytoremediation of Cadmium

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Cadmium (Cd) is one of the most toxic metals in the environment, and has noxious effects on plant growth and production. Cd-accumulating plants showed reduced growth and productivity. Therefore, remediation of this non-essential and toxic pollutant is a prerequisite. Plant-based phytoremediation methodology is considered as one a secure, environmentally friendly, and cost-effective approach for toxic metal remediation. Phytoremediating plants transport and accumulate Cd inside their roots, shoots, leaves, and vacuoles. Phytoremediation of Cd-contaminated sites through hyperaccumulator plants proves a ground-breaking and profitable choice to combat the contaminants.

Keywords: heavy metals ; phytoremediation ; antioxidant defense system ; genetic engineering ; microbes ; metallothionein ; omics ; phytochelatins

1. Introduction

Cadmium (Cd) is a non-essential element for plants and humans but is present in many soils in excessive amounts [1][2]. When it enters into the food chain, it poses a major threat to the living biota. The control of Cd accumulation in plants is complicated by the fact that most of the essential nutrient transporters, such as copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn), also facilitate Cd uptake [2]. Cd stress alters plant growth, as evident from a reduced dry matter yield and leaf area, and stunted growth [3][4][5][6]. Cd affects plant growth at both the morphological and physiological level [7]. At the whole plant level, Cd toxicity includes leaf chlorosis, a delay in the growth rate, and inhibition of respiration and photosynthesis [8], increased oxidative damage, and decreased nutrient uptake ability [9].

Generally, Cd occurs in sedimentary rocks (0.3 mg kg^{-1}), lithosphere (0.2 mg kg^{-1}), and soil (0.53 mg kg^{-1}) [10]. Cd enrichment in soil occurs from both anthropogenic and natural sources [11]. Geologically weathering of rocks is the major natural source of Cd contaminants [12][13], while primary anthropogenic sources of Cd include agrochemicals, manufacturing, vehicular emission, irrigation wastewater, smelting, and mining [14][15]. Moreover, improper and uncontrolled waste disposable practices, sea spry, windblown dust, forest fires, and volcanic eruption also increase the Cd level in soil [12][13][14].

Apart from this, Cd toxicity has been reported to damage human physiology by various means, such as Cd-contaminated water and food. For example, Cd exposure influences the human male reproductive organs/system and deteriorates spermatogenesis and semen quality, especially sperm motility and hormonal synthesis/release. Based on experimental and human studies, it also impairs female reproduction and the reproductive hormonal balance and affects menstrual cycles [16][17]. In animals, experimental studies revealed that Cd and Cd compounds (referred to as Cd) by multiple routes of exposure prompt benign and malignant tumor formation at various sites in many species of experimental animals [18]. Besides, environmental Cd contact can cause pancreatic cancer in animals [19].

Efficient and economical remediation of contaminated urban and agriculture land is a pressing need for sustainable agriculture development prospects. Different methods, like biological, chemical, and physical, have been used for the remediation of heavy metal contaminants from soil. Some of them face limitations due to mechanical limitations, logistical problems, time, and cost. Soil remediation techniques include physical, chemical, and biological remediation; electrokinetics; and phytoremediation. Physical remediation includes both the soil high replacement method and thermal desorption method. In the soil replacement method, clean soil is used for partial and full replacement of contaminated soil [20][21]. Importing new soil dilutes the contaminated soil; however, this practice is only useful for small-scale severely contaminated soil.

Chemical remediation is a mechanical process used for leaching the contaminated soil by using liquids enriched with solvents, freshwater, and chelating agents [20]. Researchers found that ethylenediaminetetraacetic acid (EDTA) is an effective chelating agent for soil washing. Recent studies have shown that biosurfactants, such as sophorolipids, saponin, and rhamnolipids, can efficiently remove Cd from contaminated soils [22]. According to Juwarkar et al. [23], more than 92%

of Cd was removed with 0.1% di-rhamnolipid. In chemical oxidation, oxidants, such as Fe^{2+}-activated peroxymonosulfate, are used to degrade and oxidized the contaminant particles [24]. Bioremediation is the use of microorganisms, plants, and microbial or plant enzymes to treat the contaminated soil through natural biodegradation. Some Cd-removing microorganisms include *Aspergillus niger* (fungus) [25], *Pleurotus ostreatus* [26], *Spergilus versicolor* [27], *Fomitopsis pinicola*, *Pseudomonas aeruginosa*, *Streptomyces*, and *Bacillus* [28]. Electrokinetics is another technique to remediate heavy metals from soil by using an electrical current [29][30]. In this context, Shen et al. [31] reported an accumulation of 99% of total Cd at the cathode after remediating soil with approaching anode electro-kinetics. Similarly, Li et al. [32] reported a removal of 97.32% of the total Cd through electroosmotic and electromigration, which was positively correlated with the electric voltage. Recently, it has become the best technique and seems to be a promising alternative to conventional approaches.

Phytoremediation is a cost-effective and eco-friendly technique for remediating soils. Notably, phytoremediating plants uptake and accumulate Cd inside their roots, shoots, leaves, and vacuoles. Still, it takes a long time to provide fruitful results because phytoremediation is still under the investigation and progress phase, and several technical barriers have to be overcome. In the present review, we illustrate the recent advancements in the physiological, biochemical, and molecular mechanisms associated with Cd phytoremediation. Additionally, the potential of omics and genetic engineering approaches are outlined for the efficient remediation of a Cd-contaminated environment.

2. Plant Responses to Cadmium Toxicity

The toxic effects of Cd on plant growth and metabolism differ among plant species [3]. The Cd concentration in plants is a direct function of its presence in the soil. An increase in Cd concentration in the growth medium led to a subsequent rise in its accumulation in different parts of the plants [33][34]. It might alter the plant growth and metabolism even if present in a minute amount [33][35]. Cd application in basil seeds delayed the germination period from 4.66 to around 7–10 days [5]. Several other studies have reported high Cd accumulation in wheat seedling roots compared to shoots [36][37].

In some studies, Cd toxicity is linked with the low dry matter accumulation in roots [4], turning them black [38], and leads to a reduction in lateral root growth [39]. It has further been associated with root development with the mature apoplastic pathway, enhanced porosity, and few root tips per surface area in rice plants [40]. In the genus *Citrus*, seedlings wilted and turned yellow, followed by eventual death under cadmium chloride ($CdCl_2$) treatment [41]. In contrast, tomato plants were reported to endure short-term exposure to high (250 µM) $CdCl_2$ concentrations [42]. Increased exposure to Cd in carrots and radish significantly inhibited the development of radicals due to increased Cd accumulation in roots [43]. Moreover, a considerably high metal concentration was found in parsley seedlings under Cd stress, but the plants did not show any visual stress symptoms [44].

The morphological, biochemical, and physiological effects on plant growth are more pronounced with a high concentration of Cd [39]. An evaluation of the morpho-physiological growth parameters of tomatoes showed that at high Cd concentrations, the root-shoot growth decreased at a relative rate, which could be attributed to the lesser water content in the seedlings due to reduced imbibition. The literature suggests that Cd stress decreased the root-shoot length in wheat [45], peas [46], *Corchorus capsularis* [47], and *Suaeda glauca* [48]. Further, it has been documented to reduce the dry weight in wheat [49], maize [50], and tomatoes [51].

Wu et al. [52] described an increase in the net photosynthesis rate by Cd application, which translated to an increase in the net biomass. On the contrary, Cd toxicity has been reported to inhibit plant growth by decreasing the water-use efficiency (WUE) and the net rate of photosynthesis [53]. A significant reduction in the leaf area and dry mass in female *Populus cathayana* under Cd stress has been observed [54]. In contrast, growth inhibition and a disturbance in photosynthetic performance has been reported in Cd-stressed tomato [55] and cucumber [56]. Cd exposure has further been reported to decrease the stomatal conductance and net rate of photosynthesis in rapeseed [57].

Cd toxicity also affects the plasma membrane of plants, which could be attributed to electrolyte leakage [58], and membrane proteins like H-ATPase inhibition [42]. It has been known to affect the DNA repair system [59]; thus, the stability of the genomic template was distinctly reduced in *Phaseolus vulgaris* [60] and peas [61] in response to the direct application of Cd.

Reactive oxygen species (ROS) generation in response to Cd-induced oxidative stress affects the electron transport and leaks electrons to molecular oxygen [62]. Cd was found to be toxic for peanuts at a higher dosage, marked by the production and accumulation of ROS in the cytosol. Moreover, it damaged the integrity and selective transport system of plasma membranes, leading to metal transport in cells [63]. Furthermore, the overproduction of ROS in wheat seedlings upon Cd exposure, marked by an increase in the hydrogen peroxide (H_2O_2) content and malondialdehyde (MDA) level, was linked to genotoxicity [64]. Being sessile, plants try to elude its harmful effects by adopting various defense mechanisms, which include antioxidant activation and other mechanisms of metal homeostasis [65]. In response, plants have developed enzymatic and non-enzymatic antioxidant mechanisms. Increased activities of catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), and peroxidase (POD) were found against increased Cd stress in *Brassica juncea* [66]. In another study, the glutamate-mediated alleviation of Cd toxicity reduced ROS-induced membrane lipid peroxidation, metal uptake, and translocation to rice shoots, and improved the chlorophyll biosynthesis [67].

3. Phytoremediation Processes and Their Salient Features

Phytoremediation refers to the biological cleaning of the environment (soil, water, and air) by plants. Plants make a symbiotic association with microorganisms, which helps in the remediation of the soil, particularly from heavy metals and organic pollutants. Phytoremediation is generally considered as a green technology because of its excellent decontamination ability of heavy metals with a minimum influx of secondary waste to the environment. Alternatively, phytoremediation is highly acceptable among the general public due to its ease of application, low cost, and environmentally friendly nature [1][2]. However, hampered growth activities, such as reduced biomass and increased sensitivity to Cd, were observed in the plants involved in phytoremediation processes [6].

Phytoremediation involves various processes, such as phytoextraction, phytoaccumulation, phytovolatilization, phytostabilization, and phytotransformation. The phytoextraction and phytoaccumulation processes work in association. For instance, during phytoextraction, plants uptake heavy metals, such as Cd, Zn, nickel (Ni), chromium (Cr), and other minerals and nutrients from the soil. After this, these elements accumulate in the shoots and leaves with the help of the phytoaccumulation mechanism [6]. Many plants species have been reported previously for their high accumulation capacity; these are potential candidates for phytoremediation.

In Cd phytoremediation, plants are often used to absorb or translocate Cd into harvestable plant parts. Plants have evolved many diverse adaptations to maintain normal growth even under high Cd-contaminated soils, which also includes detoxification mechanisms [68]. The Cd concentration in plant parts shows the following trend: root > stem > leaves [69]. Many techniques are being used to increase the efficiency of Cd phytoremediation (**Table 1**).

Table 1. Types of phytoremediation approaches and their specific methods. Abbreviations are explained in the text.

Types	Process	Mechanism	Plants	References
Phytoextraction/ Phytoaccumulation	**Bioaugmentation-assisted phytoextraction**	**Combined with mycorrhiza**	***Suaeda salsa* and *Trichoderma asperellum***	[70]
	Chelated-assisted phytoextraction	**Chelates like EDTA, SDS, and EGTA**	***Fagopyrum esculentum***	[71]
	Phytomining	**Phytoextraction for commercial use, like silver (Ag), Ni**	***Alyssum murale, Odontarrhena chalcidica***	[72]
Phyostabilization	**Organic fertilizers, biochar**	**Immobilization of Cd by using biomolecules**	***Virola surinamensis*, *Boehmeria nivea***	[73][74]
Phytofiltration	**Biosorption**	**Metals are absorbed bound in cells, used for phytoremediation**	***Lythrum salicaria***	[75]
	Rhizofiltration	**Metals are absorbed and bound on only roots**	***Micranthemum umbrosum***	[76]
	Blastofiltration	**Metals are absorbed and bound on only seedlings**	***Moringa Oliefera, Cucumis melo, Abelmoschus esculentus, Ricinus communis***	[77][78]
	Caulofiltration	**Metals are bond and absorbed on excised plant**	***Berkheya coddii***	[79]

Types	Process	Mechanism	Plants	References
Phytostimulation	**Fungi, bacteria**	**Phytoremediation with the intervention of microorganisms in different terms to remediate soil with organic pollutants**	***Rumex K-1* (*Rumex patientia* × *R. timschmicus*) *Viola baoshanesis. Vertiveria zizanioides***	**[80][81]**

3.1. Phytoextraction

This technique is used to absorb inorganic and organic contaminants through the stem and roots. Plants that are already growing in the ecosystem should be chosen for this technique. After harvest, they are exposed to another method known as composition, or burned in an incinerator [82]. Hyperaccumulator families, such as *Scrophulariaceae*, *Lamiaceae*, *Asteraceae*, *Euphorbiaceae,* and *Brassicaceae*, are essential for this technique. Moreover, some particular plant species, like *Celosia argentea* [83], *Salix mucronata* [84], *Cassia alata* [85], *Vigna unguiculata*, *Solanum melonaena*, *Momordica charantia* [86], *Nicotiana tabacum*, *Kummerowia striata* [87], and *Swietenia macrophylla* [88], may be used as potential plant choices to increase the process of Cd phytoextraction. Moreover, a sub-division of phytoextraction, known as chelate-assisted phytoextraction, is also used as a possible solution for metals that have no hyperaccumulator species. Several amino polycarboxylic acid and chelating agents have been applied to soil to increase the solubility of trace elements. For instance, EDTA-assisted phytoextraction of Cd was preferred by Farid et al. [89]. Similarly, citric acid was used as a chelating agent to increase the Cd uptake ability of jute mallow (*Corchorus olitorius*) [90].

Phytoextraction helps to reduce metalloid toxicity by improving substrate geochemistry for future colonization of native plants [91]. It is an effective, affordable, environmentally friendly, and potentially cost-effective technique for remediating soils [92]. Despite the generally agreed advantages of phytoextraction, there are some disadvantages, such as the time required for the remediation of highly contaminated soils may be decades [93], and a limitation for mine waste applications [94]. Mostly hyperaccumulator plants have developed the capacity to accumulate only one metal and may be sensitive to the presence of other elements [93].

3.2. Phytostabilization

There has been a progressing shift from phytoextraction to phytostabilization. Phytostabilization is the ability of plants to store and immobilized heavy metals by binding with biomolecules; this process prevents metal transport, and converts them into less toxic substances [95]. Most of the plants growing on contaminated soils are not hyperaccumulators but work as excluders. An excluder transforms the metals and metalloids into a less toxic mobile form without extracting them from the soil and accumulates these compounds in roots by absorption or precipitation within the rhizosphere [96]. Recently, promising results of *Virola surinamensis* for Cd phytostabilization have been documented [97]. Likewise, *Miscanthus* x *giganteus* [73], and oats and white mustard [98] also have phytostabilization potential for Cd. In another example, the putative role of Fe-Si-Ca, organic fertilizers, and coconut shell biochar has been reported to enhance the phytostabilization ability of *Boehmeria nivea* L. for Cd [99].

Phytostabilization is one emerging ecofriendly phytotechnology, which immobilizes the environmental toxins [100]. Roots take part in phytostabilization, so the metal availability is reduced to the plants, thus reducing the exposure to the other tropic level of the environment [101]. At the same time, the major disadvantage is the fact that pollutant remains in the soil or in the root system, generally in the rhizosphere [74].

3.3. Phytofiltration

Phytofiltration is categorized as rhizofiltration that includes blastofiltration (use seedlings) and caulofilteration (use of excised plant) (**Table 1**) [102]. Rhizofiltration is the remediation of water in which roots effectively absorb contaminates [103]. In rhizofiltration, contaminant clings or assimilates to the roots, and can be transported to the plants. This method is mostly used to sterilize underground wastes or polluted water. Mostly radioactive substances or metals are removed by this method. Abhilash et al. [104] used the phytofiltration technique to increase the Cd uptake from water by using *Limnicharis flava* as an experimental plant. Islam et al. [76] reported the phytofiltration capability of *Micranthemum umbrosum* to remove Cd and arsenic (As) from a hydroponic system. In another experiment, the rhizofiltration potential of *Arunda donax* for Zn and Cd removal, it and recommended the use of the rhizofilteration technique for Cd elimination [105].

It is a cost-effective technique, and plants act as solar-driven pumps to extract the contaminants from the environment [103][76]. However, any contaminant below the rooting depth is not extracted. It is a time-consuming technique and will not suffice for the extraction of both organic and metal contaminants [76][106].

3.4. Phtytostimulation

Phytostimulation is a technique used to boost the process of phytoremediation by stimulating the root-released compounds to enhance microbial activities. These exudates enhance microbial growth by fulfilling their nutrient requirements. This process is being used in rhizoremediation technologies. It is a low-cost technique for Cd removal and other organic compounds [107]. Another method is the addition of resistant microbial inoculants into the soil, which can cause the accumulation of heavy metals, including Cd [108].

It is a more effective technique for converting toxic contaminants into non-toxic chemicals. Both in situ and ex situ practices can be done with low-cost treatments [109]. Microbes are able to help limit the growth of plant pathogens and increase nitrogen (N) fixation [110]. However, it is a more time-consuming technique, and the use of volatile and biodegradable compounds ex situ is not an easy practice. The process is sensitive to the level of toxicity in soil, and in some cases, incomplete breakdown of the organic compounds is observed. Moreover, well-controlled monitoring is required for this technique [109].

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