



Review

Phytoremediation of Cadmium Polluted Soils: Current Status and Approaches for Enhancing

Mirel Subašić^{1,2}, Dunja Šamec³ , Alisa Selović¹ and Erna Karalija^{4,*}

¹ Department for Chemistry, Faculty of Science, University of Sarajevo, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina; m.subasic@sfsa.unsa.ba (M.S.); alisa.selovic@pmf.unsa.ba (A.S.)

² Faculty of Forestry, University of Sarajevo, Zagrebačka 20, 71000 Sarajevo, Bosnia and Herzegovina

³ Department of Food Technology, University Center Koprivnica, University North, Trg dr. Žarka Dolinara 1, 48000 Koprivnica, Croatia; dsamec@unin.hr

⁴ Department for Biology, Faculty of Science, University of Sarajevo, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina

* Correspondence: erna.k@pmf.unsa.ba

Abstract: Cadmium (Cd) is a heavy metal present in atmosphere, rocks, sediments, and soils without a known role in plants. It is relatively mobile and can easily enter from soil into groundwater and contaminate the food chain. Its presence in food in excess amounts may cause severe conditions in humans, therefore prevention of cadmium entering the food chain and its removal from contaminated soils are important steps in preserving public health. In the last several years, several approaches for Cd remediation have been proposed, such as the use of soil amendments or biological systems for reduction of Cd contamination. One of the approaches is phytoremediation, which involves the use of plants for soil clean-up. In this review we summarized current data on the use of different plants in phytoremediation of Cd as well as information about different approaches which have been used to enhance phytoremediation. This includes data on the increasing metal bioavailability in the soil, plant biomass, and plant accumulation capacity as well as seed priming as a promising novel approach for phytoremediation enhancing.

Keywords: heavy metal tolerance; phytoremediation enhancement; hyperaccumulating plants; metallophytes



Citation: Subašić, M.; Šamec, D.; Selović, A.; Karalija, E.

Phytoremediation of Cadmium Polluted Soils: Current Status and Approaches for Enhancing. *Soil Syst.* **2022**, *6*, 3. <https://doi.org/10.3390/soilsystems6010003>

Academic Editors: Edgar Pinto and Mallavarapu Megharaj

Received: 17 November 2021

Accepted: 31 December 2021

Published: 4 January 2022

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1. Introduction

Cadmium (Cd) is a non-essential metal with an unknown role in plants and toxic effects on plants and animals. It is naturally present in the atmosphere, sedimentary rocks, and soils. Major natural sources of Cd contamination are the result of geological weathering of rocks [1] and anthropogenic sources that include application of agrochemicals, or pollution of soils by disposal or reuse of industrial or urban wastes [2]. Cd may be produced as part of industry processes, such as Zn smelting, and historically, it has found uses in batteries, semiconductors, electroplating, and stabilizers [3]. Phosphatic (P) fertilizers are a major source of Cd in agricultural systems, and increased Cd content in soil was observed in countries where P fertilizer is used extensively [3]. In unpolluted soils, Cd occurs at concentrations of 0.01 to 1 mg/kg with a worldwide mean of 0.36 mg/kg (reviewed by [2]). It is one of the heavy metals with relatively high mobility (depending on many factors) in the environment and may be faster released from the soil into groundwater than other heavy metals [2]. From the soil, it can be relatively easily transferred into vegetative cover entering the food chain [4]. Since 1972, Cd has been recognized as a food contaminant, and if administered in high amounts, it may cause renal failure, bone demineralization and increased cancer risk [5]. At birth, Cd is not present in the human body, but it accumulates with age, mainly in the kidneys and liver [3]. Cd-contaminated food is the main source of Cd exposure in the general population, but some specific groups such as smokers, workers

in Cd industries, or people with high industrial or environmental exposure have increased risk for negative impacts of Cd [5].

Pollution of soils and groundwater with Cd is a global problem. Different approaches have been proposed for prevention of Cd contamination or its remediation. Wastewater cleaning, control of Cd levels in landfills and mines, and reduction of the use of Cd-contaminated phosphate fertilizers may help reduce soil and water contamination. Various approaches could be used to remove Cd from the soil and to prevent food chain contamination. One of the proposed approaches is soil washing with chemicals, where different amendments have been proposed for agricultural use [6–8]. In addition, different microbes such as bacteria, fungi, algae, and plants may be potentially useful for removing Cd from soil [9–11]. In this review, we will examine the use of phytoremediation, a plant-based approach that is economically feasible, eco-friendly that has attracted great attention for the past several years [12–15]. In this work, we focused on summarizing the data about current aspects of Cd phytoremediation, which plants have a potential to be used in phytoremediation, and what different approaches can be used to enhance Cd phytoremediation.

2. Behaviour of Cadmium in the Soil and Uptake by Plants

Cd (II) is a highly toxic, soil-persistent, primary heavy metal contaminant [16], relatively easily absorbed by plant roots by which it can contaminate the food chain and consequently bioaccumulate in the human body, expressing its toxic effects. There are several factors that can affect uptake of Cd by plants, pH is one of the most prominent ones since adsorptive capacity of soils for Cd triples for each pH unit increase within the interval 4–7 [17]. Cd is relatively water soluble under acidic conditions, with limited solubility in carbonate forms (CdCO_3) and neutral solubility in alkaline soils [18]. Besides pH, other soil factors can also affect Cd solubility, such as organic matter content, cation exchange capacity and concentration of other cations. Organic matter bounds Cd and converts it into an organically bound fraction, reducing its bioavailability [19]. Replacement of structural magnesium cation (Mg) with Cd is an important mechanism in cation sorption, affecting cation exchange capacity (reviewed by [18]).

Cd is a soft Lewis acid promoting formation of strong complex ions with S^{2-} , HS^- , halide ions and organic sulphides and thiols. The presence of inorganic and organic ligands in the soil solution may decrease soil adsorption by formation of dissolved complexes. Cadmium belongs to the group of metals that interact more with low molecular weight organic matter with an order of affinity as follows: $\text{Cu}^{2+} > \text{Cd}^{2+} > \text{Fe}^{2+} > \text{Pb}^{2+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+} > \text{Zn}^{2+}$ [20]. Organic acids that are dominated by carboxyl groups facilitate complexation of Cd when present in large concentrations, with a magnitude of Cd solubilisation in the following order: fumaric > citric > oxalic > acetic \approx succinic acid [21]. The presence of some anions in the soils influences sorption behaviour of Cd, e.g., Cl^- and NO_3^- restrain Cd sorption due to the formation of soluble inorganic complexes, while H_2PO_4^- and HSO_4^- enhance Cd sorption due to surface precipitation [22]. Because of similar geochemical behaviour, Zn is the most efficient Cd competitor for sorption sites, but the limiting pH for Cd mobility is 6.5, higher than that of Zn (5.5–6) and other heavy metals (Ni 5.5; Cu 4.5; Cr 4.0; Pb < 4), reducing the competition between Cd and other minerals [23].

Absorption of Cd from the soil and its (re)distribution between roots and shoots is a highly regulated process where several key players are involved: metal transporters of the root cell plasma membrane, xylem and phloem loading/unloading and leaf/shoot sequestration and detoxification. Plants absorb cadmium through the roots, and there are several factors that can affect availability of cadmium to plants, such as above-mentioned pH, the rhizosphere, and organic acids [24]. In nature, Cd can exist in different forms such as $\text{Cd}(\text{OH})_2$, CdCO_3 , CdSO_4 or as a precipitate in the form of arsenates, chromates, sulphides, etc. For plants, Cd uptake can happen at pH 6–7 in forms such as CdCl^- , CdHCO_3^+ , CdCO_3^+ and CdCl_n [25].

Generally, uptake of any metal consists of two stages: (1) apoplastic adsorption, a rapid process, where metal ions accumulate in root apoplast due to electrostatic interactions between positively charged metal cations and deprotonated, negatively charged carboxyl groups (dissociation of carboxylic acid due to pH increase) and (2) symplastic uptake which is correlated to metabolic activity and a much slower process (reviewed by [24]). For cadmium to enter the root cells (symplast), it needs to cross the cell membrane, which is facilitated through the presence of various channels and metal transporters. Metal transporters that participate in Cd transport are: ZIP (ZRT and IRT-like proteins) transporter family which can transport metals from extracellular space to cytoplasm; OPT (oligopeptide transporter) family, such as YSL (yellow stripe-like) transporters that transport metal–nicotinamide complexes through the plant cell membrane and can transport Cd complexes; NRAMP (natural resistance-associated macrophage protein), proton-coupled metal ion transporters [26]. There are some reports that Cd could enter the cell also through Ca^{2+} channels [27] and that Cd can block Ca^{2+} channels of the cytoplasmic side of vacuolar membrane [28].

At the level of the plasma membrane within the root cells, H^+ and HCO_3^- are dissociated from H_2CO_3 in the process of respiration, followed by rapid exchange of H^+ with Cd^{2+} resulting in adsorption of Cd^{2+} on the cell surface preparing Cd for apoplast absorption pathway [29]. Cd then enters the cell through ion channels for Fe^{2+} , Zn^{2+} and Ca^{2+} , additionally plants can secrete low molecular compounds (e.g., mugineic acid, malic acid etc.), enhancing the availability of the ions and forming metal chelates [30] which are subsequently absorbed by the plant.

After Cd crosses the membrane of the root, metal ions are transported from the symplast to xylem and this process is regulated by several factors. Once the metal enters the plant cell, it can be accumulated to a certain level, depending on the plant tolerance, and the rate of accumulation can depend upon the affinity of the chelating molecules (such as nicotianamine, glutathione, and proline), and selectivity and presence of the transporters. Excess Cd is removed from the cytosol to preserve plant activities, and this is achieved by chelation and compartmentalisation in vacuole or plant cell walls [31].

Chelating agents (phytochelatins), vacuolar sequestration and apoplectic barriers, as well as loading activity to the xylem [32,33], where high cation exchange capacity of xylem cell walls controls metal ion transport [24], are major factors that affect Cd xylem loading. In hyperaccumulating plants where high concentration of Cd accumulate in the cells, plants employ different mechanisms of detoxification such as of Cd vacuolar sequestration, Cd chelation (binding Cd to S-containing ligands—phytochelatins, glutathione and metallothionines—cysteine-rich, metal-binding proteins) to alleviate Cd toxicity [34,35]. Phytochelatins are involved in metal inactivation and accumulation mechanisms while metallothionines' role is restrained to cytosol, and they play small or no roles in the accumulation of Cd [24].

If the metal is transported via phloem, it must be ligated to nicotianamine, glutathione (GSH) or phytochelatins (PCs) [36,37], and PCs have high affinity to Cd binding [36]. It is considered that Cd is loaded into the phloem in the form of Cd-thiolate complexes where stability of Cd-S bond minimizes the toxicity [38], and xylem to phloem transfer plays a key role in Cd transport in plants [39]. Movement of metals from the root through xylem is coordinated with sulphur and acetate ligands and ability to load Cd into xylem parenchyma cells is dependent upon the activity of transport proteins. Cd^{2+} uptake from the xylem to shoot symplast is governed by the activity of heavy metal transporters embedded in shoot cell membrane [29]. There are still many aspects of Cd transport and accumulation that remain unclear, especially in plants with different levels of Cd tolerance, resistance and accumulation capacity, further clarification of those mechanisms will lead to more efficient use of hyperaccumulating plants in the processes of phytoremediation.

3. Toxic Effects of Cd on Plants

Toxic effects of Cd are evident in different metabolic and physiological plant processes, with different degrees of severity depending on how resistant the plant is to Cd. Even at low dosages, Cd can cause leaf chlorosis, necrotic lesions, destruction of chloroplast structures, water stress, inhibition of root elongation, impaired gas exchange, wilting, and it can affect uptake of macro- and micronutrients [40]. Once Cd enters plant cells, elicitation of free radicals leads to outbursts of reactive oxygen species (ROS) initiating apoptosis [41]. Plants must counteract these toxic effects by a variety of mechanisms starting from the first point of Cd entrance—the root membrane [42]. Plants transport Cd in the form of metal-organic complexes and in the rhizosphere, Cd often competes with several essential metal ions and Cd absorption can lead to iron deficiency in plants exposed to Cd toxicity [43].

Since root is the first contact point between the plant and Cd it often gets damaged due to oxidation of membrane proteins/thiols, inhibition of protein pumps or simply by altered membrane fluidity reviewed by [42]. Once Cd reaches plant leaves it is sequestered in vacuoles to reduce its toxic effects on photosynthesis and other processes, or it is detoxified by chelating compounds (glutathione, phytochelatins, metallothioneins and other cysteine-rich membrane proteins), reviewed by [42]. In other cases, plants can prevent Cd absorption by excretion of root exudates including carboxylic acid (citric, malic) and histidine [44]. Correlation between synthesis of citric, malic, and oxalic acid and phenolic acids and Cd tolerance has been reported in *Silene sendtneri* [45].

Level of cadmium that is toxic for plants varies among plant species based on ecotypes, cultivars etc. [46]. Some species are sensitive to low Cd concentrations, while other species are highly tolerant and can accumulate high concentrations of Cd in their shoots ($>100 \text{ mg Cd kg}^{-1}$) and are considered as Cd hyperaccumulators [47,48]. There are several species classified as Cd hyperaccumulators with high bioconcentration and translocation factors and enhance accumulation of Cd in shoots ($>2000 \text{ mg Cd kg}^{-1}$), such as *Arabidopsis thaliana*, *Chromolaena odorata* [49], *Chara aculeata*, *Nitella opaca* [50] and *Silene sendtneri* [45]. Since hyperaccumulating plants do not show toxic symptoms, such as shoot biomass decrease, plants with high translocation factor (ratio of metal concentration in the shoot to that in the root) are suitable for phytomining (re-extraction of metals from plant biomass) [51].

In contrast, non-accumulating and non-tolerant plant species are susceptible to toxic effects of Cd and severity of toxic symptoms varies in relation to degree of soil contamination, plant species, ecotypes, cultivars, soil composition etc. [24]. Cd can affect plant growth, biomass production, photosynthesis and carbon assimilation, mineral uptake and translocation, development of reproductive tissues, and many other processes in non-tolerant plants.

In the context of Cd effects on plant growth, most prominent effect is reduction of root length and dry mass which is related to decrease of mitotic activity in root meristems under Cd stress [51,52]. In shoots most prominent effects of Cd toxicity can be seen through changes in leaves such as chlorosis, desiccation, necrosis, and stunting [53]. Cd affects photosynthetic apparatus influencing complex II and two photosystems (PSI and PSII) [54] altering the chloroplast ultrastructure. Cadmium disrupts enzymes involved in the Calvin cycle, decreasing photosynthetic rate. Cd can displace Ca^{2+} ions in oxygen-evolving complexes and Mg^{2+} in chlorophyll pigments and decrease chlorophyll and carotenoid content [54,55].

Cadmium interferes with absorption of minerals such as zinc, iron, calcium, manganese, magnesium, copper, silicon, and potassium [56,57]. It is considered that the effect of Cd on mineral absorption is a result of molecular competition between Cd and other cations in channels for essential metal uptake from soil to root leading to deficiency of essential elements [44]. One of the minerals highly affected is iron, where deficiency of iron is one of the main reasons for toxicity of Cd in leaves [58]. The citrate transporter responsible for xylem loading of iron and its translocation is downregulated by Cd [59], causing Fe deficiency. Other metals using the same transport mechanism such as Cu, Al, Cr and Ni show similar decrease under Cd toxicity. For alkaline earth ions, Mn, and Zn

(Ca-like metals) competitive inhibition of translocation is also present suggesting that Cd can use the same translocation system as Ca [24].

Plants resistance to heavy metals depends on different levels of defence, and the first line of defence is the epidermal layer in the roots with the root tip and root hairs as the most important plant part for the absorbance of Cd²⁺ ions from the soil [29]. In the cell, Cd²⁺ can induce oxidative stress inducing production of reactive oxygen species (ROS) indirectly by blocking cysteine groups in enzymes, by competitive binding to Ca²⁺ binding motifs in calmodulin and water-splitting complex of photosystem II [60]. Cd²⁺ can also induce increased production of oxygen radicals at complex III [61]. Plants respond to increased ROS generation by activation of antioxidant response. In hyperaccumulating plants, this is often regulated by changes in gene expression and related to heavy metal resistance genes presence. Antioxidant is any class of compounds that can protect cells from damage caused by exposure to ROS. Series of antioxidant enzymes comprise enzymatic antioxidant plant system including superoxide dismutase (SOD), catalases (CAT), ascorbate peroxidases (APX) and guaiacol peroxidase (SOD), numerous plant metabolites can also serve as antioxidants in detoxification of heavy metals building a non-enzymatic antioxidant response [62]. The antioxidant role of amino acids has been confirmed, suggesting a different contribution to antioxidant response, e.g., proline in Cd stress reduces formation of free radicals and enhances levels of GSH [63]. Other metabolites, such as organic acid, bind directly to metals and are involved in sequestration of metals. Citrate has high affinity for Cd and Fe, while malate binds to Zn [64]. Confirmations for accumulation of phenolic compounds as a defensive mechanism against heavy metals has been reported for several plants and different metals: maize under aluminium [65] and under Cd exposure [66], *Silene sendtneri* under Cd stress [45].

4. Phytoremediation of Cadmium from Polluted Soils

Phytoremediation is considered an eco-friendly remediation of soil, often called green remediation [67]. Basic mechanism of phytoremediation is based on the use of fast-growing plants to eliminate toxic contaminants in the soil or water [68]. Depending on the mechanism of heavy metal elimination from the soil, phytoremediation can be divided into five types: phytostabilisation, phytostimulation, phytotransformation, phytofiltration and phytoextraction [67,69].

Phytostabilisation refers to the process in which the plant reduces the mobility and bioavailability of heavy metals and reduces their leakage into the ground water consequently decreasing the contamination of the food chain [70]. Process of the metal mobilization reduction includes immobilization of the metal (chemical or physical) by the plant roots and fixation of the metal with different soil amendments [43]. In this method, plants have a role in reduction of water percolation limiting the contact with heavy metals to decrease movement of contaminants [71]. It can be employed for clean-up of Cd from the soil, among other pollutants [72]. For Cd, several species could be used for phytostabilisation such as *Virola surinamensis* [73]; *Miscanthus x giganteus* [74], oats, white mustard [75]. Most of the plants that have potential for phytostabilisation are plants that are considered as hypertolerant plants or heavy metal excluders (such as *Commelina communis*, *Thlaspi arvense* and others) (Table 1). This method can reduce the availability of metals to the plant, but the method does not actually remove the metals and it is considered more as a management strategy rather than elimination strategy [76] which is the main disadvantage of this technique [1]. List of plants that have shown potential for the use in phytostabilisation in the past 10 years are listed in Table 1.

Phytostimulation, often called rhizo-degradation, refers to the process of degradation of organic pollutants in rhizosphere with enhanced microbial activity [77]. Root exudes stimulate microbial activity by providing nutrients for their growth and in return microbes convert toxic to non-toxic chemicals. It is not suitable for Cd soil remediation. Phytotransformation or phytodegradation refers to breakdown of organic compounds either in plant metabolism or by plant enzymes and it is not related to microbial community [78]. Plants

can degrade organic compounds, Hg and Se and I in this manner and during this process they release volatile compounds into the atmosphere (phytovolatilization) [79].

Phytofiltration exploits plant roots for remediation of soil surface, ground water and wastewater in cases of lower heavy metal contamination [77]. During filtration, contaminants are absorbed or precipitated (due to excretion of root exudates and change in pH) [80], and this method can be used for extraction of Cd, Cr, Cu and Zn. There are reports for several plants with potential of Cd phytofiltration such as *Limnicharis flava* [81], *Arunda donax* [82]. Additionally list of plants that have potential to be used in phytofiltration is given in Table 1. Main disadvantage is that any contaminant below rooting depth is not extracted and it is a time-consuming technique.

Phytoextraction/phytoaccumulation exploits fast growing plants for removal of heavy metals from the contaminated soil or water [66,83] through absorption and accumulation of contaminants in the plant. Heavy metals are removed by roots and transported to upper plant parts [84], harvested subsequently and used for biomining/phytomining (metal recovery) [85]. Phytoextraction includes elimination of heavy metals by absorption, translocation, and accumulation of the metals in hyperaccumulating plants families (Scrophulariaceae, Lamiaceae, Asteraceae, Euphorbiaceae and Brassicaceae). Plants that accumulate more than: 100 mg/kg for Se and Cd; 300 mg/kg for Cr, Co and Cu; 1000 mg/kg for As, Pb and Ni; 10,000 mg/kg for Mn; 3000 mg/kg for Zn are considered as a hyperaccumulating plants [86]. The metal hyperaccumulation should be achieved while maintaining the growth [86] for the plant to be usable in phytoextraction purposes. In cases that there is no suitable plant for the phytoextraction, some chelating agents could be added to the soil (EDTA, citric acid, proline etc.) to increase the solubility and availability of the pollutants and facilitate the phytoextraction process [1].

The technique is best when contamination levels are low to medium, since in highly polluted soils, even hyperaccumulating plants can have severely impaired growth minimising the success rate of phytoextraction [70]. Currently, several plants are identified as Cd hyperaccumulating plants such as: *Celosia argentea* [87], *Cassia alata* [88], *Vigna unguiculata*, *Solanum melonaena*, *Momordica charantia* [89], *Nicotiana tabacum*, *Kummerowia striata* [90], *Swietenia macrophylla* [91], and *Silene sendtneri* [45].

There are several benefits of this method including improvement of the soil for future plant colonisation [92], it is environmentally friendly method, and it is an affordable and cost-effective technique for soil remediation [93]. A main disadvantage is the limitations of plants capacity for accumulation and plants sensitivity to soil contamination levels [94,95]. Plants usually accumulate only one metal and may be highly sensitive to presence of other contaminants [94].

Table 1. The list of the plants which have shown potential for application in phytoremediation and their rate of cadmium accumulation (Table summarizes data for the past 5 years according to the data available on Web of Science (WoS)).

Plant Species	Cd Concentration in the Plant (mg/kg)	Plant Part Where Cd Is Accumulated	Recommended for
<i>Aerva sanguinolenta</i> [95]	186	roots	phytostabilisation
<i>Amaranthus hybridus</i> [96]	242	shoots	phytoextraction
<i>Amaranthus hypochondriacus</i> [87]	217	leaf	phytoextraction
<i>Amaranthus mangostanus</i> [97]	102–604	shoots, roots, leaf	phytoextraction phytostabilisation
<i>Arabidopsis halleri</i> [98,99]	228–5722	shoots, roots	phytoextraction phytostabilisation
<i>Arabis gemmifera</i> [100]	1810	leaf	phytoextraction
<i>Arabis paniculate</i> [101]	1662–8670	leaves, roots	phytoextraction, phytostabilisation
<i>Arabis yokoscense</i> [100]	685	leaves	phytoextraction
<i>Atriplex halimus</i> [102]	217–606	shoot, roots	phytoextraction phytostabilisation

Table 1. Cont.

Plant Species	Cd Concentration in the Plant (mg/kg)	Plant Part Where Cd Is Accumulated	Recommended for
<i>Azolla pinnata</i> [103]	740	whole plant	phytoextraction
<i>Beta vulgaris</i> [104]	314.17–4547.9	shoots, roots	phytoextractin phytostabilisation
<i>Bidens pilosa</i> [105]	400	leaf	phytoextraction
<i>Brachiaria mutica</i> [106]	186	shoots	phytoextraction
<i>Brachiaria</i> sp. [95]	137.3–647	shoot, roots	phytoextraction phytostabilisation
<i>Cosmos. bipinnata</i> [107]	112.62	shoots	phytoextraction
<i>Chromolaena. odorata</i> [108]	>100	shoots	phytoextraction
<i>Calendula calypso</i> [109]	165	roots	phytostabilisation
<i>Callisia fragrans</i> [110]	>101	shoots	phytoextraction
<i>Carthamus tinctorius</i> [111]	148.1–236.6	leaves	phytoextraction
<i>Cassia alata</i> [85]	159	roots	phytostabilisation
<i>Celosia Argentea</i> [87,112]	121–236	leaves, roots	phytoextraction phytostabilisation
<i>Chlorophytum comosum</i> [110]	>100	shoots	phytoextraction
<i>Chromolaena odorata</i> [49]	102–1440	leaves, roots	phytoextraction phytostabilisation
<i>Desmostachya bipinnata</i> [106]	312	shoots	phytoextraction
<i>Eucalyptus camaldulensis</i> [113]	10.5	roots	phytostabilisation
<i>Eleusine indica</i> [95]	150	roots	phytostabilisation
<i>Eucalyptus camaldulensis</i> [113]		leaves	phytostabilisation
<i>Eucalyptus globulus</i> [114]	5.11	roots	phytostabilisation
<i>Glycine max</i> [115]	74.8–290	leaves	phytoextraction
<i>Gynura pseudochina</i> [95,116]	457.7	shoots	phytoextraction
<i>Helianthus annuus</i> [117]	65.7	shoots, roots	phytoextraction, phytostabilisation
<i>Helianthus tuberosus</i> [118]	328.77–2167.9	leaves, roots	phytoextraction phytostabilisation
<i>Hydrocotyle sibthorpioides</i> [119]	128.5	shoots	phytoextraction
<i>Impatiens violaeiflora</i> [95]	212,3	shoots	phytoextraction
<i>Imperata cylindrica</i> [95]	133,2	roots	phytostabilisation
<i>Iris lacteal</i>	121	shoots	phytoextraction
<i>Iris tectorum</i>	171	shoots	phytoextraction
<i>Justicia procumbens</i> [95]	548	shoots	phytoextraction
<i>Lantana camara</i> [16]	>100	shoots	phytoextraction
<i>Leptochloa fusca</i> [106]	245	shoots	phytoextraction
<i>Lolium multiflorum</i> [115]	106.83	leaves	phytoextraction
<i>Lonicera japonica</i> [120]	402.96	shoots	phytoextraction
<i>Lycopersicon esculentum</i> [121]	130–174	shoots	phytoextraction
<i>Microsorium pteropus</i> [122]	>400 mg/kg	root, stem, leaves	phytoextraction
<i>Macleaya cordata</i> [123]	163.39	roots	phytostabilisation
<i>Malva rotundifolia</i> [124]	900	shoots	phytoextraction
<i>Nicotiana</i> sp. [125]	271.5	leaves	phytoextraction
<i>Nicotiana tabacum</i> [90]	314.6	shoots	phytoextraction
<i>Phytolacca acinosa</i> [87]	110	leaves	phytoextraction
<i>Phytolacca americana</i> [126]	188.4	shoots	phytoextraction

Table 1. Cont.

Plant Species	Cd Concentration in the Plant (mg/kg)	Plant Part Where Cd Is Accumulated	Recommended for
<i>Picris divaricata</i> [127]	585	shoots	phytoextraction
<i>Pistia stratiotes</i> [128]	248	shoots	phytoextraction
<i>Populus nigra</i> [129]	2070	shoots	phytoextraction
<i>Potamogeton pectinatus</i> [130]	422	shoots	phytoextraction
<i>Prosopis laevigata</i> [131]	8176	shoots	phytoextraction
<i>Pteris vittata</i> [132]	216.5	shoots	phytoextraction
<i>Pterocypsela laciniata</i> [133]	207.97	shoots	phytoextraction
<i>Rorippa globosa</i> [134]	150	shoots	phytoextraction
<i>Sedum plumbizincicola</i> [135]	152.93	shoots	phytoextraction
<i>Sida rhombifolia</i> [124]	225.31	roots	phytostabilisation
<i>Sedum alfredii</i> [136]	9000	leaves	phytoextraction
<i>Sedum plumbizincicola</i> [124]	139	shoots	phytoextraction
<i>Siegesbeckia orientalis</i> [137]	193	shoots	phytoextraction
<i>Silene sendtneri</i> [45]	2156	shoots	phytoextraction
<i>Silene vulgaris</i> [138]	203–750	Roots	Phytostabilisation
<i>Solanum lycopersicum</i> [115]	133.45	leaves	phytoextraction
<i>Solanum nigrum</i> [104]	100.6–2021.7	shoots, roots	phytoextraction phytostabilisation
<i>Sphagneticola calendulacea</i> [139]	>100	shoots	phytoextraction
<i>Sporobolus arabicus</i> [106]	171	shoots	phytoextraction
<i>Tagetes. erecta</i> [140]	166.07	shoots	phytoextraction
<i>Tagetes. patula</i> [141]	231.72–601.45	shoots	phytoextraction
<i>Taraxacum ohwianum</i> [142]	181.39	shoots	phytoextraction
<i>Turnip landraces</i> [143]	139.7	leaves	phytoextraction
<i>Vetiveria zizanioides</i> [144,145]	263–2232	Roots	phytostabilisation
<i>Viola baoshanensis</i> [132]	2310	shoot	phytoextraction

5. Approaches for Enhancing Cadmium Phytoremediation

Phytoremediation can be enhanced using different methods and techniques that could be generally grouped in: (1) techniques employed to enhance phytoremediation through amelioration of the soil (mostly used for phytoextraction techniques) and (2) enhancement of plant performance/tolerance/accumulation properties. Some examples of phytoremediation enhancement treatments are shown at Table 2.

5.1. Enhancement of Phytoremediation by Soil Amelioration

The mobility of Cd in the soil can be affected by supplementation of: (a) certain chemicals and surfactants such as ethylenediaminetetraacetic acid (EDTA) [146] alone or in combination with biochar [147], urea [148], ethylenediamine disuccinic acid (EDDS) [149], citric acid [150] or reduction of pH in the soil [151,152] by addition of acids or acid-producing fertilisers [153] and (b) by addition of biological enhancers (bacteria, fungi, intercropping).

The most-known chemical chelator is EDTA, which increases the concentration of water-soluble Cd, promotes its uptake, and facilitates its transfer to shoots [148]. Conversely, EDTA has a complex relationship with pH when small concentrations of EDTA are added to the soil (the number of extracted cations is dependent on pH). Additionally, EDTA efficiency is related to soil type, and in Ca-rich soil EDTA is rapidly consumed by dissolution of calcite [153]. Addition of EDTA can increase the bioavailability of heavy metals, but it also can affect the soil microorganisms, contaminate groundwater and due to slow decomposition, it can cause ground water pollution [154]. Organic acids, due to their

biodegradability, can be used as less hazardous chelators with lower possibility of ground water contamination. Citric acid, if added in smaller dosages, can be an efficient mobiliser of Cd facilitating phytoextraction [155], and enhancing effects on the uptake of Cd are probably a result of ameliorative effect of citric acid. Changes in root structure and shape and activation of ATPases in root plasma membrane changing the transport of ions increasing Cd symplastic and apoplastic uptake have been recorded in plants grown in the soil ameliorated with citric acids. Humic acids can be used for enhancement of phytoextraction by soil supplementation. These acids are not water soluble in acidic conditions, but under higher pH, they are extractable and soluble. Their carboxyl and OH functional groups enable them to play a role in the transport, bioavailability, and solubility of heavy metals [156]. In the context of the sustainable increase of bioavailability, acidified manure can be applied to increase phytoextraction efficiency [157].

A widely used enhancement of Cd accumulation is bioaugmentation of soils with cadmium-resistant bacteria. There are many studies reporting the potential of microorganisms for phytoremediation through their effect on Cd bioavailability [158,159]. For phytoremediation, we use bacteria that can transform metals into soluble and bioavailable forms through production of siderophores and these bacteria are classified as plant-growth promoting bacteria (PGPB). Such activity has been recorded for several groups of bacteria: *Pseudomonas* sp., *Microbacterium* sp., *Bacillus* sp., *Rahnella* sp., *Burkholderia* sp. and *Enterobacter* sp. [148,160]. Some of the identified bacteria are resistant to Cd and can be used for improvement of tolerance as well as accumulation capacities of hyperaccumulating plants to further phytoremediation efficiency. *Micrococcus* sp. MU1 and *Klebsiella* sp. BAM1, cadmium-resistant PGPBs promote root elongation of *Helianthus annuus* in cadmium-contaminated soil through stimulation of the indole-3-acetic acid (IAA) synthesis. Increase of Cd accumulation was achieved after adding *Klebsiella* to the soil 4 weeks after plant cultivation in Cd-contaminated soils [161,162]. Change in pH in the rhizosphere was induced by addition of cadmium-resistant *Enterobacter* sp. FM-1 resulting in an increase of cadmium content in aerial parts of *Centella asiatica* up to 160% [163]. Successful increase of cadmium accumulation in roots and shoots of *Zea mays* was achieved by soil augmentation with cadmium-resistant *Micrococcus* sp. TISTR2221 [164]. One problem in bioaugmentation is that some bacteria have growth problems in cadmium polluted soils, in those cases biostimulation which includes addition of mineral nutrients to the soil together with bacteria, can enable the bacterial growth regardless of Cd contamination. Stimulation of phytoextraction of Cd by biostimulated bioaugmentation with *Sphingobium* sp. SA2 was recorded for *Glycine max* [165].

5.2. Enhanced Phytoremediation by Increasing Plant Capacities

One of the simplest approaches of phytoremediation enhancement is to increase plant biomass production and subsequently enhance Cd phytoremediation rates [166], which can be achieved through soil amendments or by plant changes directly. Seed pre-treatments (priming) before sowing can increase seedling vigour and increase biomass production.

Seed priming is a controlled rehydration (imbibition) of seeds for induction of metabolic activity without radicle emergence, followed by seed drying and re-imbibition prior to sowing. It is widely used for improvement of seed vigour, enhancement of germination and achieving germination uniformity, especially under stress conditions. Due to commencement of re-hydration, so-called "pre-germinative metabolism" is triggered which includes cellular processes of de novo nucleic acid and protein synthesis, accumulation of phospholipids and sterols, DNA repair and activation of antioxidant mechanisms. The potential of plant priming in abiotic stress tolerance has been extensively investigating using different types of molecules added exogenously to plant organs (roots, leaves etc.) with a result of enhanced tolerance of abiotic stress [167], there are only few papers concerning how seed priming affects tolerance levels, and what is the mechanism of plants memory of "primed" state in seeds. Seed priming with different agents (water, proline, salicylic acid,

silicic acid) increased biomass production in *Silene sendtneri* under Cd stress by increasing tolerance levels [45].

One of the most studied molecules in plant priming is plant hormone salicylic acid (SA). This hormone plays a pivotal role in many metabolic processes including antioxidant response under different abiotic stressors [168,169]. It has been recorded that seed priming and plant priming with salicylic acid can affect the plant antioxidant status resulting in increased tolerance levels towards heavy metal exposure [170,171]. Exogenously applied salicylic acid can enhance plant tolerance to heavy metals and increase phytoremediation efficiency [172]. One other molecule that can be used as a priming agent is proline. Proline is considered as a stress marker in plants subjected to abiotic stress [173]. Using proline, seed priming can imprint seeds for defence against various abiotic stressors including heavy metals [45,66]. It is still unknown in which way seed priming induces changes in metabolism that is memorised and transferred to growing plants under heavy metal stress [174]. Main advantage of this method is how easy it is to perform seed priming, the method often uses simple steps such as hydropriming (pre-treatment of seeds with water), and it is considered an eco-friendly method of seed performance improvement. A main disadvantage lays in sensitive timing of priming, since emergence of radicle must be avoided, and time of priming needs to be adjusted to ensure that metabolic processes are initiated but radicle is not emerged.

Genetic engineering of plants can be utilised for production of more advanced, more efficient, more robust hyperaccumulators. Candidate plants for genetic engineering are usually plants with high biomass production and existing capacity for heavy metal accumulation. Genetic engineering can also be employed for induction of gene overexpression such as glutamylcystein synthetase enhancing heavy metal accumulation [175]. *Brassica juncea* transgenic plant has gsh1 gene from *Escherichia coli* and it synthesises higher concentrations of phytochelatin, glutathione and nonprotein thiols and displays increased heavy metal tolerance [174]. Incorporation of gene for nicotinamine synthase responsible for synthesis of metal chelating amino acid, HcNAS1 from *Hordeum vulgare* into *Arabidopsis* can stimulate heavy metal accumulation [176]. Similarly, incorporation of the metallothionein gene IIMt2a gene from *Iris lactea* var. *chinensis* incorporated in *Arabidopsis* genome resulted in higher tolerance of Cd [177].

Beside incorporation of new genes into the plant genome, through genetic engineering, overexpression of different genes responsible for enhanced heavy metal, increase in tolerance and accumulation can be achieved. Overexpression of metal transport proteins in plants can induce enhanced metal accumulation in roots (phytostabilisation) or in the shoots (phytoextraction) can be achieved. Additionally, manipulation of genes for phytochelatin (phytochelatin synthetase and c-glutamyl cysteine synthetase) can result with enhanced heavy metal tolerance, such as higher Cd accumulation in transgenic tobacco (*Nicotiana glauca* and *Nicotiana tabacum*) [178]. In the past few years, numerous studies have shown plants overexpressing metallothioneins transgenes with demonstrated improvement of heavy metal tolerance [179]. Overexpression of trans genes responsible for antioxidant plant response (superoxide dismutase, ascorbate peroxidase, catalase, and glutathione S-transferase) in some cases impaired the morphological and physiological plant parameters [176].

A newly taken approach in genetic engineering for improvement of heavy metal tolerance is gene silencing. This process includes a process in which small RNA molecules suppress gene expression and translation of target mRNA [180]. This technique can be employed in crops to ensure that no heavy metals are accumulated in plants and by silencing phytochelatin synthase gene Cd levels in grains were drastically decreased. Conversely, by silencing gene encoding root-localized Cd-transporter *OsNRAMP5* enhanced Cd translocation to the shoots was achieved [181]. The most prominent disadvantage of gene manipulation and genetic transformation is low acceptance by the public, and there is still fear from GMO plants and the process of introduction of such plants in open fields is long and complex.

Table 2. Some examples of phytoremediation enhancement treatments.

Method	Used Enhancer	Plant Species	Result
Chemical chelators	ethylenegluatarotriacetic acid (EDTA); sodium dodecyl sulfate (SDS) [112]	<i>Calendula officinalis</i> ; <i>Althea rosea</i>	Significant increase of Cd accumulation in <i>A. rosea</i>
	SDS EDTA [182]	<i>Calendula officinalis</i>	Efficient chemical enhancement of Cd phytoremediation
	Citric acid, ethylenediamine disuccinic acid (EDDS), EDTA [149]	<i>Ricinus communis</i>	Low effectiveness great risks due to toxicology and environmental persistence
	[N, N]-bis glutamic acid (GLDA), nitrilotriacetic acid (NTA), [S, S]- EDDS, and citric acid (CA) [183]	<i>Amaranthus hypochondriacus</i>	Combination of chelators effective for enhancement of Cd phytoremediation
	EDTA [149]	<i>Lolium perenne</i>	Increased heavy metal absorption
	Biochar and EDTA [147]	<i>Brassica juncea</i>	Enhanced heavy metal tolerance
	EDTA [184]	<i>Pelargonium hortoum</i>	Increased biomass, increased accumulation of heavy metals
	EDTA [185]	<i>Sedum aizoon</i> <i>Suaeda salsa</i>	Enhanced efficiency of Cd removal
	EDTA [186]	<i>bamboo</i>	Increased absorption of heavy metals
	EDTA [187]	<i>Datura stamonium</i>	Enhanced phytoremediation of Cd
EDTA, EDDS [183]	<i>Amaranthus hypochondriacus</i>	Enhanced accumulation of heavy metals	
Electro-phytoremediation	Application of low voltage direct current to electrodes in the soil [188]	<i>Solanum tuberosum</i> . Var. Kuras	Increase of Cd accumulation in plant roots
	DC electric fields [189]	<i>Eucalyptus globulus</i>	Increase of phytoremediation capacity
	DC electric fields [190]	<i>Eucalyptus globulus</i>	Increased uptake of heavy metals
Bioagumentation	<i>Micrococcus</i> sp., <i>Pseudomonas</i> sp. <i>Arthrobacter</i> sp. [191]	<i>Glycine max</i>	Increased Cd uptake
	<i>Lactococcus</i> , <i>Raoultella</i> , <i>Bacillus</i> , <i>Acinetobacter</i> , <i>Gluconacetobacter</i> , <i>Dyella</i> [192]	<i>Phragmites australis</i>	Enhanced phytoremediation
	<i>Cyanobacteria</i> [193]	<i>Portulacea oleracea</i>	Enhanced phytoremediation of heavy metals
	<i>Rhizobacteria</i> [194]	<i>Scirpus grossus</i>	Enhanced phytoremediation of pollutants
	<i>Vibrio alginolyticus</i> [195]	<i>Scirpus grossus</i> <i>Thypha angustifolia</i>	Enhanced removal of heavy metals from soil
<i>Enterobacter</i> sp. FM-1 [196]	<i>Polygonum hydropiper</i> <i>Polygonum lapathifolium</i>	Enhanced Cd phytoextraction	

Table 2. Cont.

Method		Used Enhancer	Plant Species	Result
Soil supplementation	Bioaugmentation	<i>Kluyvera intermedia</i> , <i>Klebsiella oxytoca</i> , <i>Citrobacter murlinae</i> [197]	<i>Sorghum bicolor</i>	Enhanced phytoremediation
		<i>Simplicillium chinense</i> QD10 [150]	<i>Phragmites communis</i>	Significant removal of acid-extractable and reducible metals in soils and the increase of Cd accumulation in <i>P. communis</i>
		<i>Funneliformis mosseae</i> and <i>Rhizophagus intraradices</i> , β -cyclodextrin [198]	<i>Solanum nigrum</i>	Combination of fungi and surfactant effective enhancement of phytoremediation
Plant enhancement	Plant growth regulators	Indole-3-acetic acid (IAA), gibberellin A3 (GA ₃) and 6-Benzylaminopurine (6-BA) [189]	<i>Brassica juncea</i>	Significant increase of shoot uptake of Cd after IAA treatment
		IAA, GA ₃ , 6-BA, 24-epibrassinolide (EBL) [199]	<i>Brassica juncea</i>	Enhanced phytoremediation of Cd
		GA ₃ [200]	<i>Luffa acutangula</i>	Improved phytoremediation of pollutants
		GA ₃ , IAA, [201]	<i>Dysphania ambrosioides</i>	Improved Cd phytoextraction
		Salicylic acid [199]	<i>Impatiens balsamina</i>	Enhanced phytoremediation of pollutants
	Seed priming	Sound waves of frequency 200, 300, 400, 500, and 1000 Hz [202]	<i>Festuca arundinacea</i>	Increase of Cd extraction ability in positive correlation with sound frequency
		Proline [66]	<i>Zea mais</i>	Increased tolerance of Cd
		Proline, salicylic and silicic acid [45]	<i>Silene sendtneri</i>	Increased tolerance and accumulation of Cd in shoots (enhanced phytoremediation)
	Putrescin [203]	<i>Coriandrum sativum</i>	Enhanced phytoextraction of Cd	

In search of the most effective phytoremediation, there is much work to perform in combining different treatments, supplements, and processes to obtain higher efficiency of hyperaccumulating plants. Often, a combination of plant growth regulators (such as salicylic acid) and bioaugmentation with rhizobacteria is used for enhancement of phytoremediation with promising results [172,204].

6. Conclusions

Cadmium is a serious soil contaminant posing a threat to human health through contamination of the food chain since it can be easily absorbed by plants growing on agricultural land that is heavily contaminated by Cd (through application of fertilizers). Unfortunately, current global climate change is making this metal more dangerous, affecting its mobility in soil, and causing cadmium leakage to underground freshwater reservoirs. All this places Cd on top of the list of soil contaminants that need remediation. One of the approaches for Cd remediation is the use of phytoremediation (especially phytoextraction), which represents an eco-friendly, economical, and simple method for heavy metal removal from polluted soils. There is only a relatively small number of plant species that are considered as Cd hyperaccumulators that could be used effectively for this purpose, and there is a constant search for new hyperaccumulating species as well as methods for improvement of phytoremediation. The latest eco-friendly improvement method is seed priming, representing a safe method for enhancement of plant tolerance and accumulation capacities without disrupting soil properties, while increasing the rate of soil clean-up. The next step in the phytoremediation process can be in succeeding of soil clean-up through use of primed plants in intercropping systems, thus cleaning the soil while crops are being grown and ensuring there is no disruption in crop growth. In addition, future research should be focused on finding a solution for more efficient soil clean-up, including investigation of possible inter-cropping systems of hyperaccumulating plants and crops. Such systems would have the benefit of simultaneous soil remediation.

Author Contributions: Conceptualization, E.K. and D.Š.; writing—original draft preparation, M.S., D.Š., A.S. and E.K.; writing—review and editing, E.K. and D.Š.; visualization, E.K.; supervision, E.K.; project administration, D.Š. All authors have read and agreed to the published version of the manuscript.

Funding: D.Š. research is supported by the Grant for Scientific Research and Artistic Work from the University North under no. UNIN-BIOTEH-21-1-1.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Additional data are available upon request.

Conflicts of Interest: The authors declare no conflict of interest.

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