

Article

Phytoremediation of Heavy-Metal-Contaminated Soils: Capacity of Amaranth Plants to Extract Cadmium from Nutrient-Poor, Acidic Substrates

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Abstract: Soil pollution is a threat to food security and ecological and human health. Cd is one of the most common pollutants in agricultural soil and, due its human toxicity, one of the most hazardous. Amaranth is a documented hyperaccumulator of Cd and other pollutants, and it is commonly grown in Asia and South America. A considerable amount of amaranth is grown in suboptimal conditions, including nutrient-poor acidic soils. The objective of this experimental study was to examine the capacity of *Amaranthus hypochondriacus* to extract Cd from a nutrient-poor, acidic substrate that was spiked with different concentrations of Cd (2 and 20 mg kg⁻¹ dw) during a period of 180 days. The plants grown in the substrate that was spiked with 20 mg Cd kg⁻¹ dw did not develop into mature plants, but the plants grown in substrate that was spiked with 2 mg Cd kg⁻¹ dw extracted a significant amount of Cd from the substrate by accumulating it into the above-ground biomass. The Cd levels varied from 113 to 176 mg kg⁻¹ in the stems at the four measuring points, and from 64 to 94 mg kg⁻¹ in the leaves. The concentrations in the plants increased with time and reached a maximal concentration of 176 ± 45 mg kg⁻¹ dw for stems and 94 ± 41 mg kg⁻¹ dw for leaves after 180 days. The mean bioaccumulation factor in the plants was 86 ± 15 after 90 days, 72 ± 12 after 120 days, 105 ± 37 after 150 days, and 99 ± 31 after 180 days, which confirms the previously reported capacity of *Amaranthus hypochondriacus* to hyperaccumulate Cd. *Amaranthus hypochondriacus* may, thus, be used to improve ecological and human health by remediating moderately Cd-polluted soils, even in nutrient-poor acidic soils.

Keywords: phytoremediation; amaranth; cadmium; soil pollution; nature-based solutions



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1. Introduction

Soil pollution of agricultural land is an ongoing process that requires urgent action since it is threatening food security and ecological and human health [1]. Despite its substantial effects on health, societies, and economies, soil pollution is largely overlooked by policymakers and the scientific community. Consequently, the available data about the scale and implications of the problem are often insufficient [1–3]. In agricultural soils, which comprise a considerable portion of the world’s polluted soils [2–4], the pollutants of most concern are inorganic elements such as heavy metals and metalloids, but persistent organic pollutants from the excessive use of agrochemicals are also important causes of pollution [4,5]. Soil pollution can typically not be directly assessed or visually perceived, and many activities (including food production) unwittingly occur on polluted land, with high risks for human exposure. The connection between soil pollution and health effects is difficult to recognise since the pollutants that end up in the food chain tend to cause chronic health risks rather than inducing sudden death. Millions of people live and grow food on

contaminated soil, often unsuspectingly of its health risks and without taking any measures to avoid exposure [6]. Soil pollution is, thus, one of the greatest sustainability challenges that our society faces today [7]. Inorganic pollutants may originate from anthropogenic processes such as applications of impure P fertiliser containing Cd, but geogenic sources such as weathering and volcanic eruptions are often equally important in terms of risks to human health [2–4,8]. Metals and metalloids are sometimes naturally present at toxic levels in the soil [9]. Since some edible plants accumulate pollutants in their biomass, toxic concentrations of pollutants may be found in the edible parts of plants grown in low-toxic soil [5]. In urban–industrial areas, polluted hotspots can generally be remediated by means of technologies that are based on excavation and ex situ treatment, but on agricultural soils, such resource-intensive, soil-disruptive methods are typically neither economically nor environmentally viable [5,10]. Due to access issues, a lack of economic incentives, and the spatial scale of many agricultural fields, cost-effective options are necessary for remediation to materialise [11–14]. Although considerably cheaper than most conventional remediation technologies, as a stand-alone technology, phytoremediation is often not lucrative enough to render it appealing for farmers, especially in economically vulnerable regions. In such cases, economic incentives such as the production of a marketable yield are crucial for remediation projects to materialise [5,15]. The use of phytoremediation, i.e., the use of plants to remediate polluted soil, sediment, or water, is a low-cost and resource-effective option that is typically a viable option for large-scale applications on agriculture soils. If combined with value-adding activities such as energy production, erosion control, or carbon sequestration, phytoremediation can be viable even in the most economically vulnerable regions [16,17].

Amaranth is a commonly grown plant in Asia and South America and is used mainly for its edible seeds, leaves, and stems. Many species of amaranth are documented hyperaccumulators of pollutants with a capacity to extract a broad spectrum of both organic and inorganic pollutants from the soil [18–21]. Since amaranth adapts to adverse growing conditions, a considerable amount of amaranth is grown in suboptimal conditions [22–24], including nutrient-poor, acidic, and polluted soil, which may render it vulnerable to metal pollution. Acidic soils are also associated with higher Cd bioavailability and solubility, which enhances its uptake by crops and, thus, poses a threat to food safety [25–27]. However, a higher Cd bioavailability is also beneficial for plant-assisted Cd removal, i.e., phytoextraction [5]. Although few phytoextraction studies focus specifically on acidic soils, Huang et al. [28], for example, demonstrated that phytoextraction could be an efficient way to remove Cd from acidic soils. Unfortunately, from a phytoremediation perspective, a low pH also tends to increase the damage that the Cd toxicity causes on plant physiological functions, i.e., water interactions, essential mineral uptake, and photosynthesis, ultimately leading to yield suppression [29]. *Amaranthus hypochondriacus*, the species that was used in this experiment, is known for being a high biomass producer, a fast grower, and easily cultivated [21], which are important success factors for large-scale phytoremediation operations. It has also been indicated as a particularly efficient phytoextractor of Cd in acidic soils [28]. Cd is one of the most commonly found inorganic pollutants in agricultural soil and, due its human toxicity, one of the most hazardous [30–32]. Exposure to low amounts can cause flu-like symptoms, and long-term exposure may cause respiratory damage, bone fractures, cancer, and renal failure [33,34]. Being an elemental pollutant, it cannot be degraded; it can only be removed from the soil through, e.g., phytoextraction or fixed through phytostabilisation.

The objective of this study was to examine the capacity of *Amaranthus hypochondriacus* to extract Cd from a nutrient-poor, acidic substrate (congruent with the suboptimal, nutrient-poor, acidic conditions in which a substantial amount of amaranth is grown) at four different stages of its life cycle. In this bench-scale, experimental study, we have investigated the bioaccumulation factor (BAF), i.e., the ratio of the concentration of a pollutant in an organism to the concentration of the pollutants in the surrounding environment, of Cd in *Amaranthus hypochondriacus* in a substrate that was spiked with different concentrations of Cd (2, 20 mg kg⁻¹ and control). The distribution of Cd between the major two above-

ground vegetative organs (leaves and stems) was assessed, as well as the variation in concentration depending on growth stages (day 90, 120, 150, and 180).

2. Materials and Methods

A bench-scale pot experiment was set up indoors at the laboratory of the research groups to determine the capacity of *Amaranthus hypochondriacus* to extract Cd from the substrate during a period of 180 days. In total, 36 pots were used: 12 pots that were spiked with 2 mg Cd kg⁻¹ dw, 12 pots that were spiked with 20 mg Cd kg⁻¹ dw, and 12 pots without additions of Cd (control).

2.1. Plant Growing Conditions

The substrate that was used in the experiment was prepared by mixing inert sand and commercial peat-based growth substrate from Lantmännen, Östersund, Sweden (with an organic matter content of 98% and a Corg content of 57%), at a ratio of 6:1 to obtain a mixture that would be congruent with the adverse growing conditions in nutrient-poor, acidic tropical soils in which a considerable amount of the global amaranth supply is grown [23–25]. The pH, EC, and SOM were analysed with standard methods by the commercial agronomy laboratory Agrilab in Uppsala, Sweden. Plant-available P, K, Mg, and Ca were measured via the ammonium lactate method, and the Cd content in the substrate was analysed as described in Section 2.2. Some chemical and physical properties of the substrate are presented in Table 1.

Table 1. Chemical and physical properties of the growth substrate before spiking.

pH (H ₂ O)	5.6
Organic Matter	4.1%
Sand	70%
Silt	22%
Clay	4%
Electrical Conductivity	23 mS/m
N (NO ₃ + NO ₂)	0.12 mg kg ⁻¹ dw
P-AL	0.14 mg kg ⁻¹ dw
K-AL	0.73 mg kg ⁻¹ dw
Mg-AL	0.86 mg kg ⁻¹ dw
Ca-AL	7.7 mg kg ⁻¹ dw
Cd (total)	0.01 mg kg ⁻¹ dw

Each pot was filled with 8 kg dw of the mixed substrate. The substrate was spiked with cadmium sulfate 8/3-hydrate (CdSO₄ *8/3H₂O) acquired from Sigma-Aldrich, Stockholm, Sweden. The powder was diluted in deionised water and sprayed onto the substrate during mixing to ensure even distribution within the matrix. The substrate was compacted to obtain a bulk density of 1.1. No fertiliser of any kind was applied to the substrate. Pots with a volume of 10 L were used, and the seeds were planted via direct seeding in the pot. After germination, the plants were thinned to a plant density of three plants per pot. Six 50 W LED lamps with a neutral colour temperature (4000 K) and a luminous flux of 3750 lm were used and distributed evenly at a height of 1 m above the plants. The plants were kept at a temperature of 24–26 °C and a relative air humidity of 40–50% and received 14 light hours per day. Approximately 200 mL of water was added to each pot every 4–5 days to keep the moisture level between 40% and 60% of field water capacity (FWC).

2.2. Sample Analysis

To assess the capacity of the plant to extract Cd from the substrate, plant samples were harvested at four intervals: 90, 120, 150, and 180 days after the planting day. At each sampling occasion, samples were collected and analysed in triplicate. Plant material from all plants of the same pot was combined to obtain a sufficient amount for the subsequent

quantification of Cd in plant tissue. These time intervals gave the opportunity to study the Cd content in the plant biomass during four different principal growth stages (according to the BBCH-scale): 90 days (stage 6, anthesis and axillary inflorescence), 120 days (stage 7, fruit and seed development), 150 days (stage 8, ripening), and 180 days post-seeding (stage 9 senescence). Plant material was taken from the stems and leaves of the plants. Roots and flowers and seeds were also collected, but the plant material was not present in sufficient amounts to be analysed. The plant material was dried in an oven at 105 °C to a constant weight and stored in a desiccator before it was crushed by using a mortar and pestle. The samples were subsequently sieved through a 600 µm sieve to prepare them for digestion. The samples were digested according to EPA 200.8. The digestion process involved additions of 5 mL of concentrated HNO₃ per 0.5 g sample, prior to warming for 45 min on a 90 °C hot plate. Water was continually added during the warming of the samples to compensate for evaporation. After 45 min, the temperature was raised to 140 °C, and the samples were evaporated to 1 mL. Finally, the samples were passed through a 47 mm Whatman filter and diluted to 25 mL with HNO₃ 1% *v/v*. Inductively coupled plasma mass spectrometry (ICP-MS) according to EN ISO 17294-2:2016 was then used to determine the concentration of cadmium that had been taken up by the plant samples.

2.3. Calculations and Data Analysis

The calculations of the Cd concentrations in the stems and leaves, based on the concentration in the extract, were performed according to the following formulas:

$$\text{Conversion formula : } C = y \text{ g}/20 \text{ mL} = 0.02 \times x/y \text{ mg Cd/g dw}$$

$$x = \text{g Cd per litre HNO}_3$$

$$y = \text{g dw plant}$$

$$\text{Bioaccumulation factor: } C_{\text{above ground biomass}}/C_{\text{substrate}}$$

The capacity of the plant to accumulate Cd from the substrate was determined by calculating the BAF [35]. The BAF was calculated based on the total cadmium added to the substrate, and the cadmium extracted from the plant material with the EPA 200.8 method. Analysis of variance (ANOVA) was used to test the statistical significance between the plants grown in the spiked substrate and control. All the values were expressed as means ± SD (standard deviation), and error bars in the figures indicate standard deviations of the three replicates.

3. Results

The plants grown in the substrate with 20 mg Cd kg⁻¹ dw did not develop according to the expected plant height. The plants grown in soil with 2 mg Cd kg⁻¹ dw had a similar plant development to that of the control group (although the seed production was hampered). The results for the mean concentration of Cd in the plants grown in a substrate that was spiked with the concentration 2 mg Cd kg⁻¹ dw compared to the non-spiked control are shown in Figure 1. The concentration of Cd was significantly higher (*p* < 0.05) in the plants grown in the substrate spiked with 2 mg Cd kg⁻¹ dw compared to the control plants. In total, 113 ± 14–176 ± 45 g Cd kg⁻¹ dw was found in the stems, and 64 ± 22–94 ± 41 g Cd kg⁻¹ dw was found in the leaves depending on the growth stage in the plant life cycle. The concentrations in the plants increased with time to reach a maximal concentration of 176 ± 45 mg kg⁻¹ dw in stems and 94 ± 41 mg kg⁻¹ dw in leaves after 180 days. The increasing trend, however, is not statistically significant.

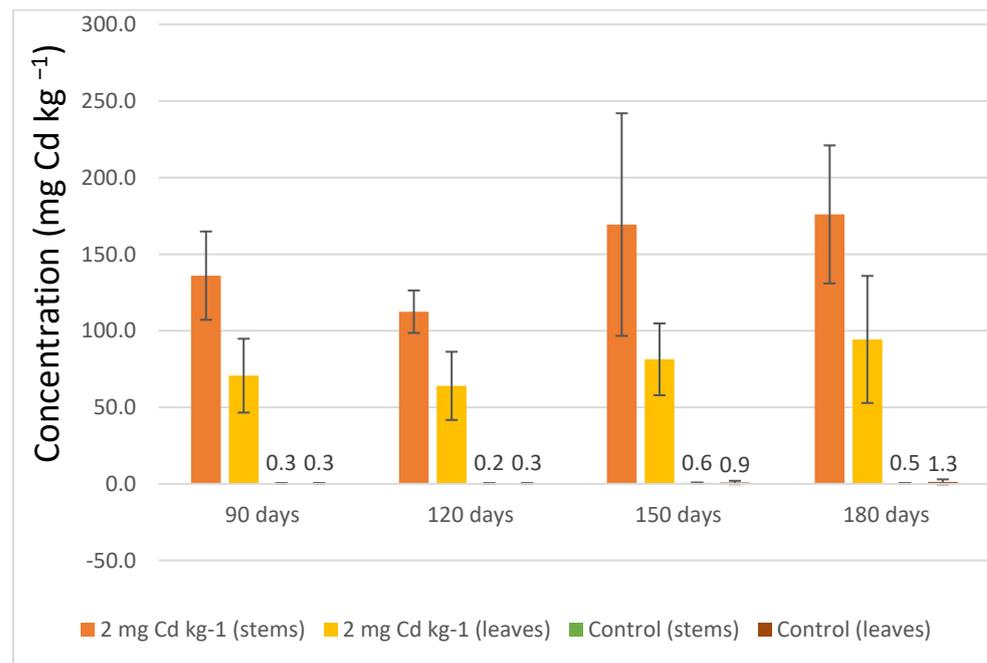


Figure 1. Cd concentration in stems and leaves in plants grown in the Cd-spiked substrate ($2 \text{ mg kg}^{-1} \text{ dw}$) and the non-spiked control. The plants grown in the control accumulated so little Cd that it is hardly visible in the diagram.

Bioaccumulation Factor (BAF)

The plants grown in the substrate with $2 \text{ mg Cd kg}^{-1} \text{ dw}$ substrate extracted a significant amount of Cd from the substrate by accumulating it into the above-ground biomass. The mean bioaccumulation factors were 86 ± 15 after 90 days, 72 ± 12 after 120 days, 105 ± 37 after 150 days and 99 ± 31 after 180 days (Figure 2).

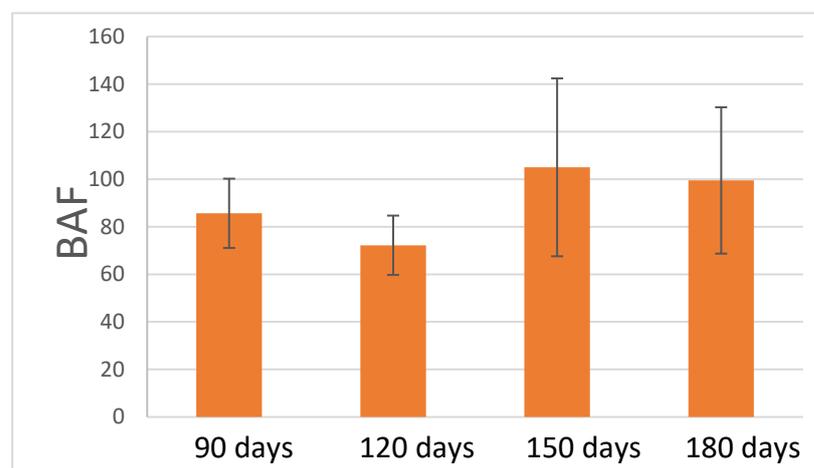


Figure 2. Bioaccumulation factors for plants grown in Cd-enriched substrate (2 mg kg^{-1}) over 180 days.

4. Discussion

The results from this experiment confirm the previously reported potential of *Amaranthus hypochondriacus* as a Cd-hyperaccumulator, i.e., a plant that can survive without suffering the phytotoxic effects of metalliferous soils and that can accumulate extraordinarily high amounts of heavy metals in the aerial organs [36,37]. This suggests that hyperaccumulators such as *Amaranthus hypochondriacus* may be used to improve ecological

and human health by removing Cd from agricultural soils, even in suboptimal conditions such as nutrient-poor, acidic soils. *Amaranthus hypochondriacus* hyperaccumulated Cd at moderate concentrations ($2 \text{ mg kg}^{-1} \text{ dw}$), but the plants grown at a concentration of $20 \text{ mg Cd kg}^{-1} \text{ dw}$ did not develop according to the expected plant height, suggesting that the growth was hampered by the combination of low nutrients, acidity, and the toxic effects of Cd. The poor plant development at the $20 \text{ mg Cd kg}^{-1} \text{ dw}$ concentration suggests that phytoremediation with amaranth is not a viable option in such conditions.

Although the development of the plants grown in the substrate that was contaminated with $2 \text{ mg Cd kg}^{-1} \text{ dw}$ did not produce seeds in commercially interesting quantities, they produced a considerable amount of biomass, and since they hyperaccumulated the Cd, they would still be interesting as phytoextraction in acidic, nutrient-poor soils. In this study (in which data for the roots and seeds are lacking), most of the Cd was found in the stems. This deviates from many other studies which found that most of the Cd was accumulated in leaves [37] or in the roots [21,38,39] of the amaranth. Under the conditions of this experiment, *Amaranthus hypochondriacus* was not able to develop healthy plants at $20 \text{ mg kg}^{-1} \text{ dw}$, but Li et al. [37] have shown that *Amaranthus hypochondriacus* can have a high capacity for Cd accumulation at concentrations as high as $90 \text{ mg Cd kg}^{-1} \text{ dw}$. In experiments with other species of amaranth that were carried out by, e.g., Fan and Wei [21] (*Amaranthus mangostanus* L.) and Zhang et al. [38] (*Amaranthus hybridus*), plants were able to grow in concentrations as high as $180 \text{ g kg}^{-1} \text{ dw}$. However those experiments were conducted in different types of nutrient-rich substrates with higher pH (Li et al. [37]: fertilised soil, pH 7.1; Fan and Wei [21]: fertilised soils at pH 6.3, 5.7, and 5.3; Zhang et al. [38]: fertilised soil, pH 6.3 and hydroponics solutions, pH 5.8).

Cadmium hyperaccumulation and phytotoxicity in amaranth depend on a number of parameters other than pH. Soil characteristics such as cation exchange capacity (CEC), soil organic matter, the species and cultivars used, agricultural practices, etc., all influence the capacity of amaranth to extract Cd from soil. Applications of fertiliser and sound agricultural technologies [37] have been reported to increase the efficiency of the Cd removal from soil with *Amaranthus hypochondriacus*, but this study suggests that *Amaranthus hypochondriacus* may be interesting as a candidate for phytoextraction even in suboptimal conditions such as nutrient-poor acidic soils.

The current trend is that the number of polluted sites that require urgent action doubles every 25 years [40]. The scarcity of soil resources as the human population continuously increases will inevitably force more farmers to cultivate in contaminated areas [41]. The development of effective, low-cost soil remediation strategies is, thus, an urgent matter, especially in developing countries where few affordable options exist for the remediation of the vast extensions of polluted agricultural land. Many farmers currently operate on polluted land, and the scarcity of soil resources as the human population continues to increase will inevitably force more farmers to cultivate in contaminated areas [5]. Hyperaccumulators such as *Amaranthus hypochondriacus* may be used to remove Cd from the soil, thus preventing vegetables, crops, and other products from absorbing them [5,18]. Phytoremediation is typically a slow process compared to costly methods that are based on heavy machinery, but in many marginalised regions, such as agricultural land in low-income countries or sparsely populated regions in industrialised countries, slower but more cost-efficient, higher-risk solutions may be the only economically feasible way that landowners will be willing to adopt [5,15]. A phytoremediation project can be sustainable only if the contaminated biomass is taken care of appropriately, but the post-harvest treatment protocol of phytoremediation projects has been largely overlooked by the scientific community [42–44]. Incineration, pyrolysis, and composting are the most common options, but gasification, direct disposal, liquid extraction, fibre production for sustainable eco-building, livestock bedding, oil production, and thermal-oxidation (solvolysis) have also been proposed as means to take care of the polluted biomass [40]. This study provides supplementary knowledge on Cd transfer and bioaccumulation of Cd in *Amaranthus hypochondriacus* in nutrient-poor acidic substrate. These data are valuable for predicting

the efficiency of phytoremediation projects and for determining in what conditions the phytoextraction of Cd with *Amaranthus hypochondriacus* may be a viable option. Field studies are necessary to confirm the results from this bench-scale experiment, and more bench-scale experiments in substrate with different pH and nutrient regimes would increase the understanding on the impact of pH and low nutrients on Cd phytotoxicity.

5. Conclusions

The capacity of *Amaranthus hypochondriacus* as a hyperaccumulator was confirmed in substrate that was spiked with 2 mg Cd kg⁻¹ dw. The plants extracted considerable amounts of Cd from the substrate by accumulating it into the above-ground biomass (stems and leaves). The mean bioaccumulation factor in the plants ranged from 72 ± 12 to 105 ± 37. However, the plants grown in substrate that was spiked with 20 mg Cd kg⁻¹ dw did not develop into mature plants, suggesting that they were hampered by the combination of low nutrients, acidity, and the toxic effects of Cd. The study suggests that hyperaccumulators such as *Amaranthus hypochondriacus* may be used to improve ecological and human health by removing Cd from agricultural soils, even in suboptimal conditions such as nutrient-poor, acidic soils. Field studies are necessary to confirm the results from this bench-scale experiment, but this study provides supplementary knowledge on the transfer and bioaccumulation of Cd in *Amaranthus hypochondriacus* in nutrient-poor, acidic substrates that is valuable for the development of strategies with the purpose of cleaning Cd-polluted agricultural soil.

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