Cadmium Hyperaccumulation and Translocation in *Impatiens Glandulifera*: From Foe to Friend?

Stephanie Coakley, Gary Cahill, Anne-Marie Enright, Brian O'Rourke and Carloalberto Petti *

Department of Science and Health, EnviroCORE, Institute of Technology Carlow, Kilkenny Road,
R93 V960 Carlow, Ireland; stephanie.coakley@itcarlow.ie (S.C.); gary.cahill@itcarlow.ie (G.C.);
anne-marie.enright@itcarlow.ie (A.-M.E.); brian.orourke@itcarlow.ie (B.O.)
* Correspondence: carloalberto.petti@itcarlow.ie; Tel.: +35-359-917-5595

check for
updates

Abstract: The use of phytoremediation to sustainably recover areas contaminated by toxic heavy metals such as cadmium (Cd) has been made feasible since the discovery of hyperaccumulator plants. This study examines the potential of the invasive *Impatiens glandulifera* for phytoremediation propensity of Cd. In these experiments, the plants were exposed to and tested for Cd accumulation; the propensity to accumulate other heavy metals, such as Zinc, was not investigated. The efficacy of phytoaccumulation was assessed over two trials (Cd concentrations of 20 mg/kg to 150 mg/kg) via examination of bioconcentration factor (BCF), translocation factor (TF), and total removal (TR). Exposure to Cd levels of up to 150 mg/kg in the trials did not affect the biomass of the plants compared to the control. *Impatiens glandulifera* accumulated cadmium at a rate of 276 to 1562 mg/kgin stems, with BCFs, TFs, and TRs of 64.6 to 236.4, 0.2 to 1.2, and 3.6 to 29.2 mg Cd, respectively. In vitro germination revealed unprecedented germination ability, demonstrating the remarkable hypertolerance of *I. glandulifera*, with no significant difference in the germination of seedlings exposed to 1000 mg/kg Cd compared to the control. This study also examined the localization of Cd in plant tissues via a histochemical assay using dithizone. The results presented herein suggest that *I. glandulifera* can act as a hyperaccumulator of Cd for phytoremediation.

Keywords: phytoremediation; heavy metal; bioconcentration factor (BCF); translocation factor (TF); total removal (TR)

1. Introduction

Heavy metal contamination resulting from anthropogenic activities is a global environmental concern that poses severe risk to the health of both plants and animals [1]. Cadmium is one of the most important, toxic, and widely distributed pollutants in our environment. Naturally occurring levels of Cd are typically <1 mg/kg, but over two centuries of industrialization has led to cadmium levels of over 1000 mg/kg in some geographical regions [2–5]. Anthropogenic sources of Cd include water discharge from industrial plants, mining and smelting, sewage sludge, and disposal of materials containing heavy metals, e.g., electronics, rechargeable batteries, fertilizers, steel, and pigments [1,3,6,7]. In Europe, phosphate fertilizers contribute to Cd levels, with Ireland and the U.K. possessing some of the highest Cd levels in soil and sediments. The upper limit for Cd levels in soil of 20 mg/kg can be exceeded for several kilometers surrounding mines or non-ferrous smelters (see Figure 1).
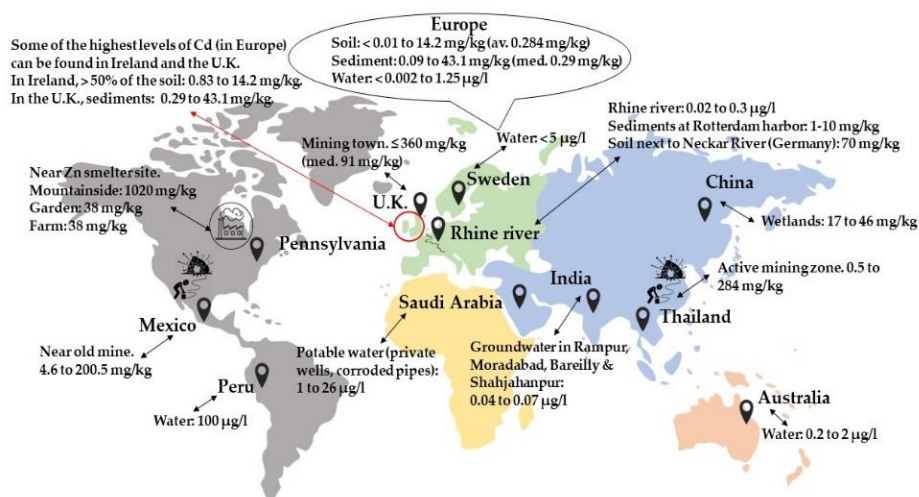
Figure 1. Cadmium (Cd) concentrations in soils, sediments, and water. Average European values are included in the text bubble, while sites with elevated levels of Cd (due to anthropogenic activities) are denoted by location pins. Mining and smelting are largely responsible for some of the most heavily polluted regions [4,5,8–14].

Cadmium is a non-essential element that can be toxic—and in some cases, lethal—to plants at concentrations as low as 2.5 mg/kg. Cadmium can also reduce plant biomass, the numbers of flowers or fruits, chlorophyll content, and the ability to uptake essential plant nutrients [2,3]. Cadmium is highly mobile, as it has similar physiochemical properties to essential micronutrients such as zinc, enabling it to be readily taken up by plants [4,15,16]. Phytoextraction is a form of phytoremediation where pollutants are removed from the soil using plants that can accumulate and bioconcentrate pollutants in its tissues, thereby remediating the soil [17,18]. Phytoremediation is a sustainable, efficient, and cost-effective means of rectifying and restoring soil and water to their natural conditions [4,19]. The efficacy of phytoremediation is dependent on the properties of the plant, the bioavailability of the heavy metal(s), and the characteristics of the soil [4,6,20]. The most suitable phytoremediation plants are fast growing with high biomass production that can tolerate and accumulate high levels of the pollutant. Ideally, these plants ought to be easy to grow and harvest and be resistant to disease and pests [6,16,18,21]. In general, phytoaccumulators show an exponential relationship between the heavy metal concentration in their tissues and the concentration of heavy metal in the soil they are grown in [3,20,22].

The term hyperaccumulator was first coined by Brooks and collaborators [23] to describe plants that are hypertolerant and can accumulate elevated concentrations of heavy metals in their aboveground tissues at levels that far exceed those present in the soil. Hyperaccumulator plants possess higher tolerance and accumulation abilities, enhancing the efficacy of phytoremediation [19]. There are reports of over 400 species of hyperaccumulators, though many of these, such as the well-known Cd hyperaccumulator *Thlaspi caerulescens*, have low biomass or are slow growing [6,22]. Cadmium hyperaccumulators must be capable of accumulating 100 mg/kg of Cd in its shoots, which is approximately 100 times the level of Cd that would be found in the tissues of a non-hyperaccumulator species [21,24–26]. Hyperaccumulators should also have a bioconcentration factor and a translocation factor with values greater than unity. The bioconcentration factor (BCF) indicates the efficacy of the plant to accumulate the pollutant in its tissues. The BCF is calculated as the ratio of concentration of the pollutant in the harvested plant to that of the soil [25,26]. The translocation factor represents the plant's ability to translocate the pollutant from the roots to the aerial parts of the plant; it is calculated as the ratio of the concentration of the pollutant in the aerial parts of the plant to the root concentration [26,27]. These factors are utilized to help identify new potential hyperaccumulator species.

Plants vary not only in their tolerance and their accumulation of Cd but in how they partition Cd in their various organs and cells [2,17,28]. The varied subcellular distribution of Cd in different organs was

attributed the responsibility for the high accumulation capacity of *Impatiens walleriana* [17,29]. Plants have evolved various mechanisms to tolerate and mitigate the toxicity of cadmium [7,15]. For example, many plants such as *Zea mays* and *I. walleriana* compartmentalize Cd in the cell wall of stems as a mechanism of tolerance [7,28–30]. Many of the most effective hyperaccumulators are food crops—for example, *Brassica juncea, Helianthus annuus,* and *Z. mays* [26]. However, some ornamental species have been identified as promising sources of new potential phytoremediators [24,25]. For example, recent research has highlighted *I. walleriana,* the common busy lizzie, as a promising hyperaccumulator of Cd, capable of tolerating up to 120 mg/kg of Cd [20,31,32]. *Impatiens glandulifera* was originally introduced as an ornamental species and is now a widespread invasive across Europe and North America. This invasive has been observed along several polluted rivers in the United Kingdom, suggesting a tolerance for the toxicity of environmental pollutants resulting from industry [33]. *Impatiens glandulifera* displays a high level of phenotypic plasticity, potentially enhancing its ability to adapt to new environments and produce high biomass in a wide range of environmental conditions [34–37]. Additionally, *I. glandulifera* is considered to be a "transformer", with the ability to alter the ecosystem it invades [38,39]. *Impatiens glandulifera* is the tallest annual invasive in Europe, reaching up to 3 m. This tall plant is fast growing with shallow roots, which makes it easy to harvest [33]. Furthermore, other invasive species such as *Eichhornia crassipes*, *Prosopis glandulosa,* and *Ipomoea carnea* have been identified to have potential use in phytoremediation [40–42].

Considering the species traits in conjunction with the findings of *I. walleriana* being a hyperaccumulator, the potential of *I. glandulifera* to tolerate, accumulate, and translocate Cd was hereby investigated. This study is relevant to both Ireland and the U.K. in particular, considering the aforementioned higher levels of Cd and the widespread invasion of *I. glandulifera*.

2. Materials and Methods

2.1. Localization of Cadmium in I. glandulifera

Hydroponic cultures of *I. glandulifera* were obtained by growing pre-germinated seedlings of *I. glandulifera* in a modified Hoagland nutrient solution, which did not contain Na-EDTA [43,44]. The modified solution contained only the macronutrients and was adopted from Lombi et al. [45]. Three treatments were utilized: 0 mg/L Cd (control), 1 mg/L Cd, and 10 mg/L Cd. Twelve seedlings were used in each treatment. Aqueous solutions of CdPO4 were utilized to supplement the two Cd treatments 48 hours after being introduced to the modified Hoagland nutrient solution. The seedlings were allowed to grow for seven days. Dithizone (Sigma-Aldrich) was employed to stain Cd in hand-prepared sections of the roots, the stems, and the leaves of the seedlings. The dithizone staining method was adapted from Clabeaux et al. [46]. Images were captured using a Nikon Eclipse 80i microscope and a Nikon Digital Sight DS-SMc camera.

2.2. Soil Characteristics

For the Cd accumulation soils, a homogenized mixture of top soil (Westland Top Soil) and compost (Westland mulitpurpose compost with added John Innes) was employed. Basic soil characteristics were determined after the soil was air-dried, ground, and sieved. Soil characterization included soil texture [47], soil pH [48], organic carbon [49], total nitrogen, reactive phosphorus, potassium, and cation exchange capacity [50]. Total nitrogen [51], reactive phosphorus (molybdovanadate method) [52] and potassium (tetraphenylborate method) [53] were completed using HACH working procedures and materials on a HACH Lange DR 6000 Spectrophotometer.

2.3. Cadmium Trials

A homogenized mixture of top soil (Westland Top Soil) and compost (Westland multipurpose compost with added John Innes) was employed to plant seedlings (circa two months old) of *I. glandulifera* in a temperature-controlled glasshouse at a 22 ± 2 °C constant temperature in the Institute of Technology

Carlow, Ireland. A 16 h light/8 h dark cycle was employed with a light intensity of 75 ± 5 Klx maintained for the 12 light hours. Pots were irrigated twice a day with circa 100 mL of water. Three seedlings were potted in 1 kg (±50 g) of the soil/compost (dry weight) in polyethylene pots. Two trials were conducted with four treatments per trial. There were five pots containing a total of 15 plants per treatment in both trials, giving a total of 60 plants per trial. The first treatment from each trial acted as the control and was not spiked with Cd.

Trial One: This trial was utilized as a preliminary estimation of Cd tolerance; therefore, only primary plant organs (*i.e.*, roots, stems, and leaves) were examined for Cd accumulation over a nine week trial. A week after the seedlings were planted, treatments 2–4 were spiked with aqueous solutions of $CdPO_4$. Treatment two was initially spiked to 10 mg/kg Cd, treatment three to 20 mg/kg, and treatment four to 50 mg/kg. Initially, the concentrations of 10 mg/kg and 20 mg/kg were chosen, as these are the lower and the upper threshold values for cadmium, and 50 mg/kg (Cd-50) was chosen to represent heavily contaminated soil. However, no injury symptoms were apparent, and the growth of the plants in the spiked soil was comparable to that of the controls. Therefore, the pots in treatments two and three received a second spike, bringing the final soil concentration to 20 mg/kg (Cd-20) and 40 mg/kg (Cd-40). Trial one was harvested (roots, stems, and leaves) after nine weeks.

Trial Two: Stemming from the outcomes of trial one, the second trial was completed using higher concentrations of Cd in order to examine the extent of the tolerance of *I. glandulifera* to Cd. Concentrations reflecting heavily polluted regions were chosen: 60, 90, and 150 mg/kg (Cd-60, Cd-90, and Cd-150, respectively). In trial two, the ability of *I. glandulifera* to translocate and accumulate Cd in its flowers was investigated in addition to the primary organs (roots, stems, and leaves). In order to collect sufficient material to assess the levels of Cd in the flowers, the duration of this trial was extended, and plant materials (roots, stems, leaves, and flowers) were harvested after 11 weeks. Similar to the first trial, a week after the seedlings were planted, the soil was spiked with aqueous solutions of Cd. The Cd levels were raised to the desired concentrations gradually over the space of a week.

2.4. Cadmium Analysis

The harvested plant material was divided into roots, stems (further divided into nodes and internodes), leaves, and flowers. This material was dried overnight at 65 °C and ground using a Micro-Mill Grinder before acid digestion. The biomass (dry weight, DW) of the plant was recorded. Plant material (0.5 g) was digested in 10 mL of 65% (*w/v*) nitric acid and 30% (*w/v*) hydrogen peroxide (4:1). The samples were incubated overnight at room temperature and were then incubated at 60 °C for two hours (open vessel digestion). The digested samples were diluted to 50 mL with deionized water and filtered using filter paper (Whatman Grade 1) and cellulose acetate syringe filters (VWR). The soil was sieved and dried overnight at 65 °C before acid digestion in 10 mL of 37% (*w/v*) hydrochloric acid and 65% (*w/v*) nitric acid (3:1). The soil samples were incubated overnight and then filtered twice using filter paper (Whatman Grade 1) and once using cellulose acetate syringe filters. The levels of cadmium in soil, roots, nodes, internodes, leaves, and flowers were measured using atomic absorption spectrometry (AAS). A 55AA Atomic Absorption Spectrometer (Agilent Technologies) was used in flame mode (air/acetylene) with a coded hollow cathode Cd lamp (Wavelength 228.8 nm, Agilent). The software utilized during AAS analysis was the SpectrAA Version 5.4 (Agilent 55AA). All chemicals were sourced from Scientific and Chemical Supplies.

2.5. Effect of Cadmium on Germination

2.5.1. In Vitro Seed Germination

To test the effect of Cd on the germination of seeds of *I. glandulifera,* the following concentrations were utilized: 0, 1000, 10,000, 20,000, 40,000, and 50,000 mg/kg. The Cd was delivered in 1 mL of deionized water (i.e., 0, 1, 10, 20, 40, and 50 mg/mL of $CdPO_4$). This experiment was replicated twice, and both trials were conducted at 5 °C under non-sterile conditions. Seeds of *I. glandulifera* require

a cold-wet stratification period to break the dormancy of the seeds. Optimum seed germination for *I. glandulifera* occurs at 5 °C [54].

2.5.2. Pollen Germination

To determine if the pollen of *I. glandulifera* could germinate when exposed to Cd, samples of pollen were taken from each of the treatments in trial one and two and were tested for the ability to germinate (in vitro) using liquid pollen germination media, as described in Hussein [55]. After 20 min, the pollen solution was stained using a modified Alexander's stain [56]. Images were captured using a Nikon Eclipse 80i microscope and a Nikon Digital Sight DS-SMc camera.

2.6. Statistical Analysis

The biomasses (g DW) of the plants (roots, stems, leaves, and total) across the different Cd treatments were assessed using one-way ANOVAs on SPSS. Trials one and two were assessed separately. Roots, stems, leaves, and total biomass were assessed independently using one-way ANOVAs. In trial two, the biomass of the flowers across the Cd treatments was also assessed using a one-way ANOVA on SPSS. Similarly, one-way ANOVAs were also utilized to compare Cd accumulation (roots, stems, and leaves) across treatments. The Cd accumulation in the flowers of trial two was also assessed using a one-way ANOVA on SPSS. One-way ANOVAs were employed to compare the total removal of Cd across treatments (roots, stems, leaves, and total). Trials one and two were assessed independently for both Cd accumulation and total removal of Cd. A one-way ANOVA was utilized to assess the total removal by the flowers harvested from trial two. Pearsons correlation coefficient was employed to assess the interaction between the biomass (g DW) of the plants (roots, stems, leaves, and total) and the total removal of Cd. The significance of the germination success of *I. glandulifera* was assessed using one-way ANOVA to compare each Cd treatment to the control. All analyses were completed on SPSS, and statistical significance was defined as α value of ≤ 0.05.

3. Results and Discussion

3.1. Localization of Cadmium

Cadmium was visible at both concentrations (1 mg/L and 10 mg/L) in the roots, the stems, and the leaves, clearly showing the ability of *I. glandulifera* to translocate and accumulate Cd in all parts of the plant (Figure 2 presents images from seedlings exposed to 10 mg/kg). Indeed, Cd was visible after only one hour, showcasing the ability of *I. glandulifera* to rapidly translocate this heavy metal. Dithizone complexed Cd to form a brilliant red with larger precipitates of Cd staining dark red/black.

Cadmium was highly concentrated in both primary and secondary roots of *I. glandulifera;* an abundance of large Cd precipitates were visible (Figure 2, R1 to R3).

Cadmium could be seen in the cells throughout the stems (Figure 2, S1), and it appeared more concentrated in two regions, the first of which comprised the epidermis and the collenchyma directly underneath the epidermis, as seen by the red pigmentation and the larger red/black precipitates and indicated with arrows in Figure 2, S1 to S3. The second region Cd accumulated quite strongly in was the sclerenchyma fibers surrounding the xylem vessels (Figure 2, S3, indicated by arrow at the center of the image), as was expected, as Cd was mobilized via xylem transportation [16,57].

In the leaves, Cd was primarily located in the cells of the petiole (Figure 2, L1 and L3) and the veins. Cadmium accumulation could clearly be seen as bright red pigmentation in leaf epidermal cells (Figure 2, L2), despite the fact that the chlorophyll made visualization of Cd in the leaves difficult. However, other phytoaccumulators are known to store Cd in the epidermal cells of the leaves at concentrations four times higher than that of mesophyll cells, presumably to avoid damaging photosynthesis apparatus in the leaf [57].

Most plants compartmentalize Cd in the cell wall or the vacuole of the roots as a method of tolerating the toxicity of the heavy metal, i.e., to sequester it from the root cytoplasm, preventing

translocation to the shoots and thereby protecting photosynthesis apparatus [17,57]. In *I. glandulifera*, the Cd was compartmentalized primarily in the cell wall and was translocated and stored in all plant parts—similar to *I. walleriana* and other phytoaccumulator plants [28,31].
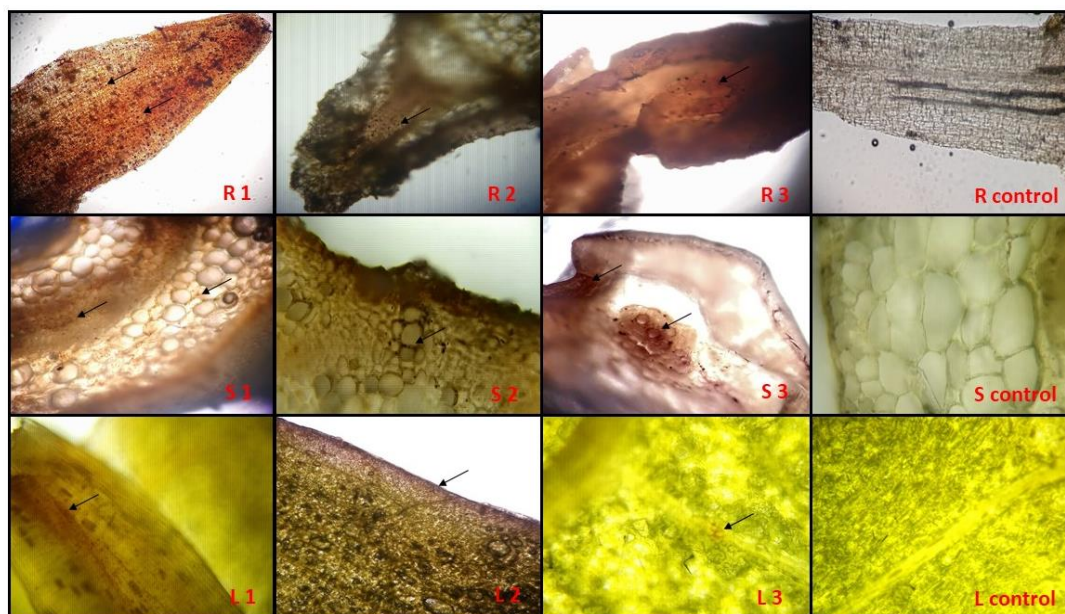

Figure 2. Visualization of Cd in the roots, the stems, and the leaves of *I. glandulifera* seedlings exposed to 10 mg/L Cd for seven days. R1 to R3 show Cd deposits in the roots, S1 to S3 show Cd in the stems, and L1 to L3 show the leaves. Images R control, S control, and L control are control tissues for roots, stems, and leaves, respectively (these samples were stained with dithizone but not exposed to Cd).

3.2. Cadmium Trials

3.2.1. Soil Characteristics

The soil utilized was acidic (pH of 5.8) and had a relatively high cation exchange capacity (see Table 1). The soil was classified as silt loam with low organic carbon content. The Cd concentration of the unspiked soil was found to be 0.66 mg/kg.

Table 1. Characteristics of the soil utilized in bioaccumulation trials.

Soil Properties	Result
Texture	Silt Loam
Sand	320 mg/kg
Silt	640 mg/kg
Clay	40 mg/kg
pH (soil:water = 1:1)	5.8
Organic Carbon (%)	2.7 mg/kg
Total Nitrogen	74 mg/kg
Reactive Phosphorus	39.3 mg/kg
Potassium	29.4 mg/kg
Cation Exchange Capacity	21.7 meq/100 g
Cd concentration	0.66 mg/kg

Average of 3 replicates.

3.2.2. Plant Growth

Impatiens glandulifera displayed a high tolerance for Cd, with plants growing up to circa 1.6 m, even when exposed to 150 mg/kg of Cd. Unlike other phytoaccumulators such as *H. annuus*, *Brassica*

napus, or *I. walleriana*, even the highest concentration of Cd did not impact the height of the plants (data not shown) or the biomass (DW) produced by the plants [20,58,59]. The highest stem biomass recorded was 10.9 ± 9.3 g, which was higher than *T. caerulescens* (up to 7.6 g; [60]) and significantly higher than *I. walleriana* (up to 4.3 g; [20]). There was no significant difference in the plant biomass of the any of the treatments compared with the control ($p > 0.05$, ANOVA). Notably, plants growing in Cd-50 had the highest biomass, achieving a total biomass (DW) of 15 ± 10 g (Tables 2 and 3). No outward injury symptoms (e.g., yellow and withered leaves) were observed for any of the plants. Similarly, no signs of outward toxicity were evident for *I. walleriana*, though *H. annus* showed visual symptoms of toxicity (light white/yellow spots on leaves) after almost six weeks when exposed to concentrations of up to 15 mg/kg Cd [61]. The maintenance of high biomass is considered to be a mechanism of tolerating the toxicity of heavy metals [62]. Indeed, the tall stems of *I. glandulifera* provide a large surface area over which to accumulate Cd. Though hyperaccumulators such as *Arabidopsis halleri* and *Thlapsi caerulescens* possess very high tolerance for Cd, the use of these species is limited due to the limited range of environmental conditions in which they can thrive [63]. As *I. glandulifera* is an invasive, it may be possible to cultivate this species in a broader range of environmental conditions.

Table 2. Accumulated concentration, biomass (dry weight, DW), total removal (TR), translocation factor (TF), and bioconcentration factor (BCF) of *Impatiens glandulifera* in Trial 1.

Cadmium Treatments		Cadmium Concentration (mg/kg)	Biomass (g)	TR (mg/plant)	TF *	BCF **
Control	Roots	30 ±11.1 a	1.42 ± 0.6 a	0.05 ± 0.03 a		
	Stems	32.7 ± 11.3a	8.4 ± 7 a	0.3 ± 1.7 a		
	Leaves	46.2 ±32.7 a	2.5 ± 1 a	0.1 ± 0.1 a		
	Total		**10.2 ± 8.8 a**	**0.4 ± 0.2 a**	2.6	8.3
20 mg/kg	Roots	822 ± 465 b	1.5 ± 0.6 a	1.2 ± 0.8 b		
	Stems	277 ± 102 b	7.5 ± 3.4 a	1.9 ± 0.8 b		
	Leaves	208 ± 75 b	2.5 ± 0.7 a	0.5 ± 0.3 b		
	Total		**11.5 ± 4.1 a**	**3.6 ± 1.5 b**	0.7	64
40 mg/kg	Roots	1030 ± 375 b	1.3 ± 0.6 a	1.4 ± 0.7 b		
	Stems	470 ± 200 c	4.8 ± 2.5 a	2.3 ± 1.1 bc		
	Leaves	325 ± 213 b	2.1 ± 0.7 a	0.7 ± 0.4 b		
	Total		**8.2 ± 3.5 a**	**4.3 ± 1.8 b**	0.8	45
50 mg/kg	Roots	868 ± 158 b	1.7 ± 0.8 a	3.1 ± 5 b		
	Stems	426 ± 225 c	10.9 ± 9.3 a	4 ± 3.4 c		
	Leaves	193 ± 35 ab	2.4 ± 1.1 a	1.6 ± 3.1 b		
	Total		**15 ± 10 a**	**8.7 ± 7.8 c**	1.2	54

Cadmium concentration, biomass, and TR presented as average values ± SD. TF * = [Cd] in aerial parts of plant/[Cd] in roots, BCF ** = [Cd] in harvested plant/[Cd] in soil. The biomass, the accumulation, and the total recovery for stems, roots, leaves, and total plant were analyzed independently. The same lowercase letter indicates no significant difference between Cd treatments for the same organ ($p < 0.05$).

Table 3. Accumulated concentration, biomass (DW), TR, TF, and BCF of *Impatiens glandulifera* in Trial 2.

Cadmium Treatments		Cadmium Concentration (mg/kg)	Biomass (g)	TR (mg/plant)	TF *	BCF **
Control	Roots	57.2 ± 19.5 a	1.2 ± 0.49 a	0.07 ± 0.03 a		
	Stems	44.6 ± 13.8 a	5.1 ± 2.5 a	0.3 ± 4.7 a		
	Leaves	55.2 ± 19.7 a	0.9 ± 0.7 a	0.06 ± 0.06 a		
	Flowers	0 a	0.23 ± 0.2 a	0 a		
	Total		**8.2 ± 3.8 a**	**0.4 ± 0.2 a**	1.7	11.9

Table 3. *Cont.*

Cadmium Treatments		Cadmium Concentration (mg/kg)	Biomass (g)	TR (mg/plant)	TF *	BCF **
60 mg/kg	Roots	7089 ± 3098 b	1.2 ± 0.3 a	8.3 ± 3.3 b		
	Stems	1099 ± 447 b	7 ± 2 b	7 ± 2.7 b		
	Leaves	915 ± 357 b	0.9 ± 0.6 a	0.8 ± 0.5 b		
	Flowers	98 ± 40 b	0.2 ± 2 a	0.07 ± 0.04 b		
	Total		**9.2 ± 2.7 a**	**16.1 ± 5 b**	0.3	206
90 mg/kg	Roots	14,361 ± 6164 c	1.3 ± 0.5 a	18.9 ± 9.5 c		
	Stems	1537 ± 426 c	6.7 ± 1.8 ab	9.3 ± 3.6 c		
	Leaves	825 ± 297 b	1.2 ± 0.8 a	1.01 ± 0.7 b		
	Flowers	129 ± 36 b	0.32 ± 0.3 a	0.21 ± 0.09 c		
	Total		**9.3 ± 2.5 a**	**29.2 ± 11.9 c**	0.2	236
150 mg/kg	Roots	13,000 ± 4717 c	1.3 ± 0.5 a	16.2 ± 8.1 c		
	Stems	1562 ± 572 c	6.2 ± 3 ab	8.9 ± 4.6 bc		
	Leaves	1052 ± 416 b	0.9 ± 0.7 a	1.05 ± 1.04 b		
	Flowers	136 ± 88 b	0.26 ± 0.2 a	0.13 ± 0.1 bc		
	Total		**8.4 ± 4 a**	**26 ± 11.9 c**	0.2	134

Cadmium concentration, biomass, and TR presented as average values ± SD. TF * = [Cd] in aerial parts of plant/[Cd] in roots, BCF ** = [Cd] in harvested plant/[Cd] in soil. The biomass, the accumulation, and the total recovery for stems, roots, leaves, and total plant were analyzed independently. The same lowercase letter indicates no significant difference between Cd treatments for the same organ ($p < 0.05$).

3.2.3. Cadmium Accumulation

Impatiens glandulifera displayed a similar pattern of accumulation as seen in *I. walleriana*, whereby Cd accumulation increased with increasing soil Cd concentrations (up to Cd-90, see Tables 2 and 3) and accumulated in decreasing order of root > stem > leaf [17]. This trend has been observed in other, non-related species (such as soybeans), and it is common that the roots contain the highest levels of Cd, with fruits/seeds containing the least [64]. The highest Cd concentration was observed in the roots of plants in Cd-90 (14,361 ± 6164 mg/kg, Table 2). The roots of *I. glandulifera* were able to hyperaccumulate impressive concentrations of Cd (822 to 14,361 mg/kg Cd) far exceeding those observed in *I. walleriana* (29.5 to 1900 mg/kg Cd; [20,31]) and other phytoaccumulator species, e.g., *Tagetes patula* (273.77 mg/kg) and *Eichhornia crassipes* (6103 mg/kg); see Sun et al. [65] and Zhu et al. [66], respectively. There was no difference observed in accumulation by nodes and internodes, and therefore these results were averaged and presented as stems. The highest stem concentration of Cd was observed in plants from Cd-150 (1562 ± 572 mg/kg). The Cd concentration in the stems of plants across all treatments far exceeded the 100 mg/kg requirement for Cd hyperaccumulators [22,67]. Though lower than the values of the roots or the stems, the leaves still contained high levels of cadmium, ranging from 193 ± 35 to 1052 ± 416 mg/kg. Interestingly, phytoaccumulators such as *Cannabis sativa* and *Hibiscus cannabinus* accumulate very high levels of Cd in the leaves [60,65]. Indeed, the highest levels of Cd found in *C. sativa* are in the leaves—contrary to the pattern of accumulation displayed here by *I. glandulifera*. In one study, *H. cannabinus* was cultivated in such a way as to collect the leaves as they fell from the plant during the growing season. This consideration may be worthwhile for future *I. glandulifera* plant trials (particularly field trials) to prevent Cd leaching back into the environment [63–65]. The flowers of *I. glandulifera* accumulated Cd up to 136 mg/kg (see Table 3). The presence of Cd in the flowers supports the efficient translocation and accumulation observed throughout the plant. However, the presence of Cd in the flowers also lends the recommendation to harvest the plants before they set flower to avoid interaction with pollinators.

While there was a large degree of variation in the accumulation of Cd, this has been observed in other species such as *T. caerulescens*, *Chamaecrista fasciculata*, poplar clones, and *I. walleriana* [2,27,68,69]. Such a variation can be attributed to a number of variables. For example, the experimental design of these trials placed three plants per pot, and one of these three plants typically grew larger and accumulated higher concentrations. Overall, there was a positive correlation between the biomass (g DW) of the plant and the amount of Cd it accumulated. This correlation, however, was only significant for the stems (Pearsons's correlation coefficient of 0.297, *p* < 0.01, see Figure 3).

Though the plants utilized were grown from seeds collected from a single population of *I. glandulifera*, the diversity of this species resulting from numerous introduction events and high phenotypic variation (e.g., floral pigmentation, degree of branching) could potentially impact its ability to phytoaccumulate. There are known geographical variations in the phytoaccumulation capacity of *T. caerulescens,* and different clones of poplar clones were seen to vary "remarkably" in their tolerance and their accumulation of Cd [27,66]. Indeed, the white floral morph of *I. glandulifera* accumulated more Cd and had a higher biomass in Cd-60 than the purple morph [white morph: 11 g (DW), 39 mg Cd total removal. Purple morph: 8 g (DW), 26 mg Cd total removal]. Due to the uneven number of white and purple flowered plants, statistical significance could not be assessed.
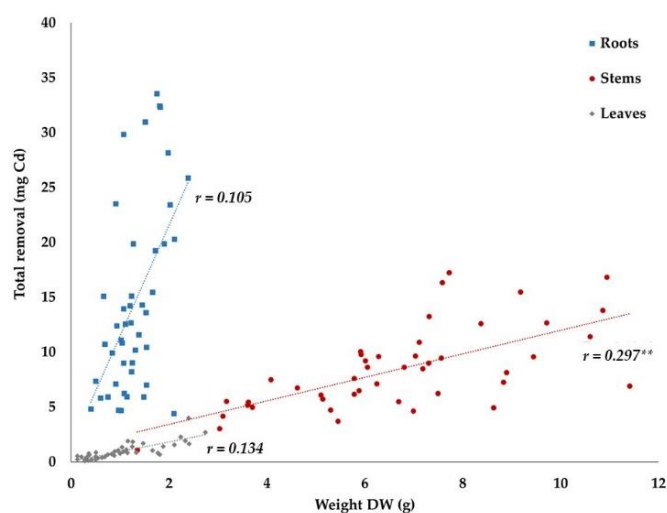

Figure 3. Correlation between the biomass of the plant and the amount of Cd removed by the plant. Spearman's correlation revealed a positive correlation for all plant tissues with a significant correlation for stems. Pearson's correlation coefficient (*r*), ** denotes an alpha value of <0.001.

3.2.4. Bioconcentration Factor and Translocation Factor

The bioconcentration factor, which indicates the efficacy of the plant to accumulate the pollutant in its tissues, was largely greater than unity across all treatments, ranging from 45 to 236 (see Figure 4). Similar to *I. glandulifera,* other phytoaccumulator species have attained BCFs of up to 100 [70]. Impressively, the roots of the floating macrophyte *Azolla pinnata*, which is especially good at bioaccumulating Cd when exposed to levels less than 1 mg/kg, were reported to have a BCF of 24,000 [63]. The translocation factor, which indicates the plant's ability to translocate the pollutant from the roots to the aerial parts of the plant, for *I. glandulifera* was low—only plants in Cd-50 achieved a TF value greater than unity (see Table 2). However, though TF is a useful measure, it cannot be used in isolation to describe hyperaccumulation [71]. In fact, the aerial parts of *I. glandulifera* accumulated high levels of Cd, and many times, the 100 mg/kg requirement and up to 70% of the total Cd removed by the plants was located in the aerial parts of the plant (Figure 5B).
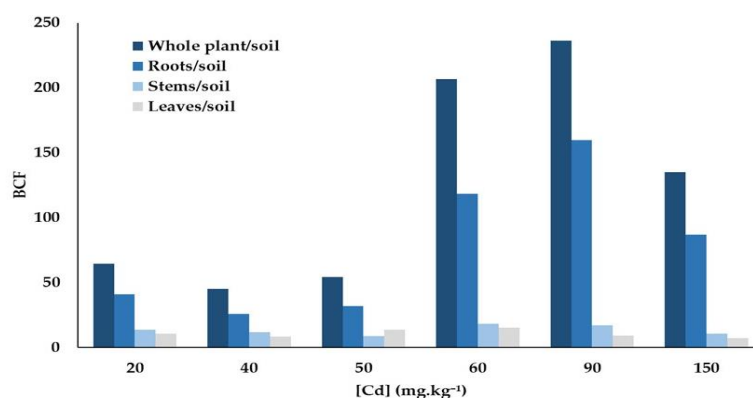
Figure 4. Bioconcentration factors for *I. glandulifera* across all Cd concentrations. The concentration of Cd in all parts of the plant exceed that of the soil.
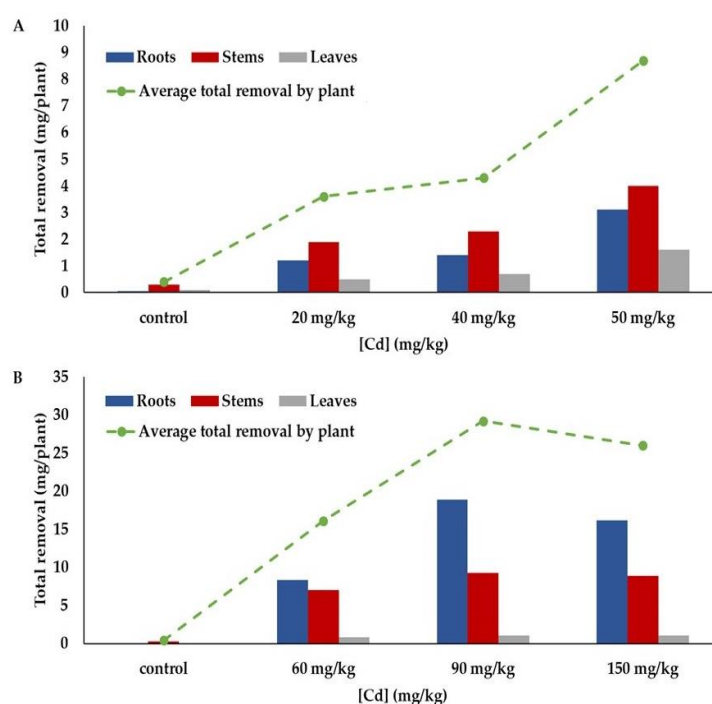

Figure 5. Total removal of Cd per *I. glandulifera* plant. Trial one is presented in A, and trial two is presented in B. The amount of Cd removed by the roots, the stems, and the leaves of both trials are represented in the bar charts. A linear increase was observed in the total amount of Cd accumulated by a whole plant in trial 1 (**A**) and up to 90 mg/kg Cd in trial two (**B**). Values represent average (*n* = 15). Statistical analyses of total removal for trials one and two can be found in Tables 2 and 3, respectively.

Interestingly, plants of *I. glandulifera* grown in the control soil achieved BCF and TF values of above unity. Though the BCF value was considerably lower for the control than the treatments, the highest TF value was attained by the control plants. Although the control soil was not spiked with Cd, it was found nonetheless to contain very marginal levels of Cd (0.66 mg/kg of Cd). Though many phytoaccumulator plants such as *Brassica juncea* or Panicum virgatum are not effective at removing Cd when soil levels are low, there are many species that show great potential to be used to bioaccumulate Cd in soils with levels as low as 0.33 mg/kg. These species include *T. caerulescens*, *Hibiscus cannabinus*, *Azolla pinnata*, *Lemna minor*, Vertiever grass, rice, maize, and sugar beet [63,64,70,72]. Indeed, even *H. annus* grown in control soil and not treated with Cd was found to accumulate Cd, though at a lesser extent than the Cd treatments [61].

Interestingly, some plants have been observed to release exudates, which increase the solubility of heavy metals, facilitating their uptake even at low concentrations [73]. Considering that *I. glandulifera* has been shown to release allelochemicals that impact both fungi and insects, future research may consider investigating if *I. glandulifera* and other potential phytoaccumulator invasive plants utilize such a strategy [74,75].

Though the control plants did not achieve the requisite 100 mg/kg Cd in their shoots, the BCF and the TF factors were both above unity, and an average total recovery of 0.4 mg/plant of Cd was attained. This property (accumulating in the presence of low levels of Cd) is as intriguing as the high tolerance of *I. glandulifera* towards the toxicity of Cd. These results warrant further investigation, and future trials ought to assess the accumulation of Cd at both low and even higher levels.

This research, through pot trials, enables an initial assessment of tolerance and suitability for bioremediation. However, the capacity of *I. glandulifera* to accumulate Cd of non-spiked soil highlights the importance of field trials to truly establish the feasibility of utilizing *I. glandulifera* as a phytoremediator. The efficacy of *T. caerulescens* is known to vary significantly in the field, as accumulation is influenced by a number of factors [68]. Therefore, field trials are necessary to allow conclusions to be made regarding the efficacy and the applicability of *I. glandulifera* as a phytoremediator.

3.2.5. Total Removal

In trial one, there was a linear increase in the total removal of Cd (Figure 5A); this linear trend was observed in trial two up to Cd-90 (Figure 5B). The total Cd removed by a single plant exposed to Cd across the two trials varied between 3.6 ± 1.5 mg to 29.2 ± 11.9 mg (Figure 5). In comparison, the largest amount of Cd removed by a single plant of *I. walleriana* (grown in 80 mg/kg Cd) was 3.4 mg [20]. The largest amount of Cd was located in the stems of plants from the treatments ranging from Cd-20 to Cd-60 (Figure 5A) and in the roots of plants from treatments ranging from Cd-90 and Cd-150 (Figure 5B). The Cd located in the stems of Cd-20 to Cd-60 plants represented between 43 to 53% of the total Cd removed by the plant. Whereas for Cd-90 and Cd-150 plants, the level of Cd found in the roots was approximately twice the level of that found in the stems of the plant and represented more than 60% of the total Cd removed by the plant (Figure 5). The ability to translocate Cd to the aboveground parts is critical for efficacious phytoremediation. However, species with high tolerance and BCF values that cannot effectively translocate Cd to the stems are often useful for phytostabilization [63,76].

In trial two, the total removal of Cd did not vary significantly after 60 mg/kg (see Table 3 and Figure 5B). In future research, it would be interesting to establish the extent of tolerance of *I. glandulifera* for Cd, and indeed other heavy metals, in order to examine the concentration at which maximum efficacy of accumulation and translocation occurs. It is important to note that the results herein presented are from two accumulation trials of different concentrations and durations. This research provides a strong indication that *I. glandulifera* has a high tolerance for Cd and appears to be a very promising potential hyperaccumulator of Cd. Nonetheless, further experimental work is required before concluding that *I. glandulifera* is a Cd hyperaccumulator. Future research should consider taking into account the duration of accumulation. *Impatiens glandulifera* appears to uptake Cd rapidly (see visualization of Cd), and it is important to examine the optimum duration of accumulation trials, which is likely to differ depending on environmental conditions.

3.3. Germination

3.3.1. In Vitro Seed Germination

One of the limiting factors of phytoremediation is that germination in heavily contaminated soil is typically poor, as seedlings of many plants are often more sensitive to metal pollution than mature plants [4]. *Impatiens glandulifera* was found to possess an unprecedented level of hypertolerance for the toxicity of Cd, with seeds germinating in the presence of up to 40 mg/mL of Cd under vitro conditions (equivalent to 40,000 mg/kg Cd, see Figure 6). Indeed, there was no significant difference

in the germination of seeds exposed to 1 mg/mL (equivalent to 1000 mg/kg) compared to the control (0 mg/mL, $p < 0.05$). Though there was a statistically significant decrease in germination passed this concentration (44%, 18%, 8%, and 0% for 10, 20, 40, and 50 mg/mL, respectively, $p < 0.05$), noteworthy is the ability for the seeds to retain a 44% germination rate at 10 mg/mL (equivalent to 10,000 mg/kg). This suggests that *I. glandulifera* could be utilized in even the most heavily polluted regions as a phytoaccumulator. Given that each *I. glandulifera* plant can produce up to 2500 seeds, this fast growing annual could be grown with ease to rapidly decrease Cd levels [33]. The invasive grass *Cynodon dactylon* was also shown to germinate when exposed to high levels of Cd (30% of seeds germinated when exposed to 200 mg/kg Cd [4]), indicating the high metal tolerance of some invasive plant species and their potential application in phytoremediation.
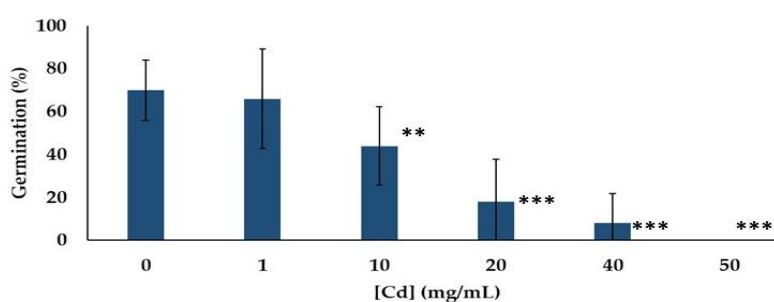
Figure 6. Germination of *I. glandulifera* seedlings in the presence of Cd. A significant decrease in germination was observed from 10 mg/mL Cd. Significance was established as alpha value of * ≤0.05, ** ≤0.01, *** ≤0.001. Average values ($n = 50$), ± standard deviation.

3.3.2. Pollen Germination

Pollen has been suggested as a useful bioindicator for heavy metal contamination, as pollen has been observed to be more sensitive to pollutants than vegetative parts of the plant. Indeed, it has been observed that the germination of pollen of several species is strongly inhibited by low levels of Cd [7,77]. Therefore, the ability of pollen to germinate was established for samples taken from *I. glandulifera* plants (control plants and Cd-20 to Cd-150 plants). No difference in the ability to germinate was observed, with pollen germinating across all treatments. This indicates that even levels of Cd as high as 150 mg/kg do not impact the ability of this species to reproduce, highlighting the strong tolerance of *I. glandulifera* towards Cd.

4. Conclusions

An ideal hyperaccumulator ought to be fast growing, easy to harvest, show resistance to pests and disease and tolerance towards toxicity, and possess the ability to rapidly uptake, translocate, and bioconcentrate the toxin [6,16,44,70]. *Impatiens glandulifera* is a fast-growing plant resistant to pests and disease outside of its native range in the Himalayas [78]. This research indicates that *I. glandulifera* possesses a high tolerance of Cd [plants grew to 1.6 m with no sign of toxicity, and seeds germinated (in vitro) in the presence of 1000 mg/kg Cd] and is therefore deserving of future research to ascertain if this species can be classified as a hyperaccumulator. *Impatiens glandulifera* accumulated and translocated Cd in exceedingly high concentrations, removing up to 29 mg of Cd per plant. If *I. glandulifera* can replicate these results in field trials, this invasive may prove to be very beneficial—even in the face of its potentially limiting factors (invasive status, annual flowering plant, shallow roots). The fast growth and the apparent ability to rapidly uptake Cd may facilitate effective phytoaccumulation in a relatively short period, enabling the plants to be removed before setting seed and spreading. Though it is preferable for hyperaccumulators to have deep roots (e.g., poplar and willow), the shallow roots of *I. glandulifera* make it feasible to easily harvest the entire plant—something that is not typically possible—enabling the recovery of all the accumulated Cd [6,70,79].

Impatiens walleriana was also found to accumulate mercury, though at a lower capacity than Cd. Therefore, there may be potential for *I. glandulifera* to act as a phytoaccumulator for other heavy metals [67]. More trials are needed to further assess the capacity of *I. glandulifera* to tolerate and accumulate Cd and other heavy metals. In particular, bioaccumulation trials in the field using naturally contaminated soil will be an important next step in determining the full potential of *I. glandulifera*.

References

1. Liu, Y.; Yang, Y.; Li, C.; Ni, X.; Ma, W.; Wei, H. Assessing soil metal levels in an industrial environment of northwest china and the phytoremediation potential of its native plants. *Sustainability* **2018**, *10*, 2686. [CrossRef]

2. Henson, T.M.; Cory, W.; Rutter, M.T. Extensive Variation in Cadmium Tolerance and Accumulation among Populations of Chamaecrista fasciculate. *PLoS ONE* **2013**, *8*, e63200. [CrossRef] [PubMed]

3. Li, J.-T.; Baker, A.J.M.; Ye, Z.-H.; Wang, H.-B.; Shu, W.-S. Phytoextraction of Cd-contaminated soils: Current status and future challenges. *Crit. Rev. Environ. Sci. Technol.* **2012**, *42*, 2113–2152. [CrossRef] [PubMed]

4. Del Rosario Delgado-Caballero, M.; Alarcón-Herrera, M.T.; Valles-Aragón, M.C.; Melgoza-Castilo, A.; Ojeda-Barrios, D.L.; Leyva-Chávez, A. Germination of Bouteloua dactyloides and Cynodon dactylon in a multi-polluted soil. *Sustainability* **2017**, *9*, 81. [CrossRef]

5. Science Communication Unit, University of the West of England, Bristol. Science for Environment Policy In-depth Report: Soil Contamination: Impacts on Human Health. Report produced for the European Commission DG Environment, September 2013. Available online: https://ec.europa.eu/search/?queryText=Soil+Contamination%3A+Impacts+on+Human+Health&query_source=ENVSCPOL&filterSource=ENVSCPOL&swlang=en&more_options_language=en&more_options_f_formats=&more_options_date= (accessed on 12 September 2019).

6. Ali, H.; Khan, E.; Sajad, M.A. Phytoremediation of heavy metals- Concepts and applications. *Chemosphere* **2013**, *91*, 869–881. [CrossRef] [PubMed]

7. Xiong, Z.-T.; Peng, Y.-H. Response of pollen germination and tube growth to cadmium with special reference to low concentration exposure. *Ecotoxicol. Environ. Saf.* **2001**, *48*, 51–55. [CrossRef] [PubMed]

8. Brown, S.L.; Chaney, R.L.; Angle, J.S.; Baker, A.J.M. Phytoremediation potential of Thlaspi caerulescens and bladder campion for zinc- and cadmium-contaminated soil. *J. Environ. Qual.* **1994**, *23*, 1151–1157. [CrossRef]

9. Pan, J.; Plant, J.A.; Voulvoulis, N. Cadmium levels in Europe: Implications for human health. *Environ. Geochem. Health* **2010**, *32*, 1–12. [CrossRef] [PubMed]

10. Simmons, R.W.; Pongsakul, P.; Saiyasitpanich, D.; Klinphoklap, S. Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice grain downstream of a zinc mineralized area in Thailand: Implication for public health. *Environ. Geochem Health* **2005**, *27*, 501–511. [CrossRef]

11. World Health Organisation, Geneva. Cadmium in Drinking-Water. Background Document for Development of WHO Guidelines for Drinking-Water Quality. 2011. Available online: https://www.who.int/water_sanitation_health/dwq/chemicals/cadmium.pdf (accessed on 12 September 2019).

12. World Health Organisation, Geneva. Cadmium-Environmental Aspects. 1992. Environmental Health Criteria 135. Available online: http://www.inchem.org/documents/ehc/ehc/ehc134.htm (accessed on 12 September 2019).

13. Idrees, N.; Tabassum, B.; Abd_Allah, E.F.; Hashem, A.; Sarah, R.; Hashim, M. Groundwater contamination with cadmium concentrations in some West U.P. Regions, India. *Saudi J. Biol. Sci.* **2018**, *25*, 1365–1368. [CrossRef]

14. Deng, H.; Ye, Z.H.; Wong, M.H. Accumulation of lead, zinc, copper and cadmium by 12 wetland plant species thriving in metal-contaminated sites in China. *Environ. Pollut.* **2004**, *132*, 29–40. [CrossRef] [PubMed]

15. Meng, H.; Hua, S.; Shamsi, I.H.; Jilani, G.; Li, Y.; Jiang, L. Cadmium-induced stress on the seed germination and seedling growth of *Brassica napus* L., and its alleviation through exogenous plant growth regulators. *Plant Growth Regul.* **2009**, *58*, 47–59. [CrossRef]

16. Ghosh, M.; Singh, S.P. A review on phytoremediation of heavy metals and utilization of it's by it's products. *Asian J. Energ. Environ.* **2005**, *6*, 214–231.

17. Lai, H.-Y. Subcellular distribution and chemical forms of cadmium in *Impatiens walleriana* in relation to it phytoextraction potential. *Chemosphere* **2015**, *138*, 370–376. [CrossRef] [PubMed]

18. Padmavathiamma, P.K.; Li, L.Y. Phytoremediation technology: Hyper-accumulation metals in plants. *Water Air Soil Pollut.* **2007**, *184*, 105–126. [CrossRef]

19. Dixit, R.; Wasiullah; Malaviya, D.; Pandiyan, K.; Singh, U.B.; Sahu, A.; Shukla, R.; Singh, B.P.; Rai, J.P.; Sharma, P.K.; et al. Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes. *Sustainability* **2015**, *7*, 2189–2212. [CrossRef]

20. Wei, J.-L.; Lai, H.-Y.; Chen, Z.-S. Chelator effects on bioconcentration and translocation of cadmium by hyperaccumulators, *Tagetes patula* and *Impatiens walleriana*. *Ecotoxicol. Environ. Saf.* **2012**, *84*, 173–178. [CrossRef] [PubMed]

21. Yoon, J.; Cao, X.; Zhou, Q.; Ma, L.Q. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Sci. Total Environ.* **2006**, *368*, 456–464. [CrossRef]

22. Baker, A.J.M.; McGrath, S.P.; Reeves, R.D.; Smith, J.A.C. Metal hyperaccumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of polluted soils. In *Phytoremediation of Contaminated Soil and Water*; Bañuelos, T.N., Ed.; CRC Press: Boca Raton, FL, USA, 2001; pp. 85–107.

23. Brooks, R.R.; Lee, J.; Reeves, R.D.; Jaffrre, T. Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *J. Geochem. Explor.* **1977**, *7*, 49–57. [CrossRef]

24. Liu, J.-N.; Zhou, Q.-X.; Sun, T.; Ma, L.Q.; Wang, S. Growth responses of three ornamental plants to Cd-Pb stress and their metal accumulation characteristics. *J. Hazard. Mater.* **2008**, *151*, 261–267. [CrossRef]

25. Wu, M.; Luo, Q.; Liu, S.; Zhao, Y.; Long, Y.; Pan, Y. Screening ornamental plants to identify potential Cd hyperaccumulators for bioremediation. *Ecotoxicol. Environ. Saf.* **2018**, *162*, 35–41. [CrossRef] [PubMed]

26. Zhuang, P.; Yang, Q.W.; Wang, H.B.; Shu, W.S. Phytoextraction of heavy metals by eight plant species in the field. *Water Air Soil Pollut.* **2007**, *184*, 235–242. [CrossRef]

27. Zacchini, M.; Pietrini, F.; Mugnozza, G.S.; Iori, V.; Pietrosanti, L.; Massacci, A. Metal tolerance, accumulation and translocation in poplar and willow clones treated with cadmium in hydroponics. *Water Air Soil Pollut.* **2009**, *197*, 23–24. [CrossRef]

28. Akhter, M.F.; Omelon, C.R.; Gordon, R.A.; Moser, D.; Macfie, S. Chemical speciation of cadmium in the roots of barley and lettuce. *Environ. Exper. Bot.* **2014**, *43*, 10–196. [CrossRef]

29. Lai, H.-Y. Effects of leaf area and transpiration rate on accumulation and compartmentalization of cadmium in *Impatiens walleriana*. *Water Air Soil Pollut.* **2014**, *226*, 2246. [CrossRef]

30. Boominathan, R.; Doran, P.M. Cadmium tolerance and antioxidative defences in hairy roots of the cadmium hyperaccumulator, *Thlaspi caerulescens*. *Biotechnol. Bioeng.* **2002**, *83*, 158–167. [CrossRef] [PubMed]

31. Lai, H.-Y.; Cai, M.-C. Effects of extended growth periods on subcellular distribution, chemical forms, and the translocation of cadmium in *Impatiens walleriana*. *Int. J. Phytoremediation.* **2016**, *18*, 228–234. [CrossRef] [PubMed]

32. Lin, C.-C.; Lai, H.-Y.; Chen, Z.-S. Bioavailability assessment and accumulation by five garden flower species grown in artificially cadmium-contaminated soils. *Int. J. Phytoremediation* **2010**, *12*, 454–467. [CrossRef] [PubMed]

33. Beerling, D.J.; Perrins, J.M. *Impatiens glandulifera* Royle (Impatiens roylei Walp.). *J. Ecol.* **1993**, *81*, 367–382. [CrossRef]

34. Clements, D.R.; Feenstra, K.R.; Jones, K.; Staniforth, R. The biology of invasive alien plants in Canada. 9. *Impatiens glandulifera* Royle. *Can. J. Plant Sci.* **2008**, *88*, 403–417. [CrossRef]

35. Skálová, H.; Havlíčková, V.; Pyšek, P. Seedling traits, plasticity and local differentiation as strategies of invasive species of *Impatiens* in central Europe. *Ann. Bot.* **2012**, *110*, 1429–1438. [CrossRef] [PubMed]

36. Sakai, A.K.; Allendorf, F.W.; Holt, J.S.; Lodge, D.M.; Molofsky, J.; With, K.A.; Baughman, S.; Cabin, R.J.; Cohen, J.E.; Ellstrand, N.C.; et al. The population biology of invasive species. *Annu. Rev. Ecol. Syst.* **2001**, *32*, 305–332. [CrossRef]

37. Vervoort, A.; Cawoy, V.; Jacquemart, A.-L. Comparative reproductive biology in co-occurring invasive and non-invasive Impatiens species. *Int. J. Plant Sci.* **2011**, *172*, 366–377. [CrossRef]

38. Hejda, M.; Pyšek, P. What is the impact of *Impatiens glandulifera* on species diversity of invaded riparian vegetation? *Biol. Conserv.* **2006**, *132*, 143–152. [CrossRef]

39. Tanner, R.A.; Gange, A.C. The impact of two non-native plant species on native flora performance: Potential implications for habitat restoration. *Plant. Ecol.* **2013**, *214*, 423–434. [CrossRef]

40. Eid, E.M.; Shaltout, K.H.; Moghanm, F.S.; Youssef, M.S.G.; El-Mohsnawy, E.; Haroun, S.A. Bioaccumulation and translocation of nine heavy metals by *Eichhornia crassipes* in Nile Delta, Egypt: Perspectives for phytoremediation. *Int. J. Phytoremediation* **2019**, *21*, 1–10. [CrossRef] [PubMed]

41. Abbas, A.M.; Abd-Elmabod, S.K.; El-ashry, S.M.; Soliman, W.S.; El-Tayeh, N.; Castillo, J.M. Capability of the invasive tree *Proposis glandulosa* Torr. to remediate soil treated with sewage sludge. *Sustainability* **2019**, *11*, 2711. [CrossRef]

42. Padley, V.C. Invasive species based efficient green technology for phytoremediation of fly ash deposits. *J. Geochem. Explor.* **2013**, *123*, 13–18. [CrossRef]

43. Hoagland, D.R.; Snyder, W.C. Nutrition of strawberry plant under controlled conditions. *J. Am. Soc Hortic Sci.* **1933**, *30*, 288–294.

44. Wang, A.S.; Angle, J.S.; Chaney, R.L.; Delorme, T.A.; Reeves, R.D. Soil pH effects on uptake of Cd and Zn by *Thlaspi caerulescens*. *Plant Soil* **2006**, *281*, 325–337. [CrossRef]

45. Lombi, E.; Zhao, F.J.; Dunham, S.J.; McGrath, S.P. Phytoremediation of heavy metal-contaminated soils: Natural hyperaccumulation versus chemically enhanced phytoextraction. *J. Environ. Qual.* **2001**, *30*, 1919–1926. [CrossRef] [PubMed]

46. Clabeaux, B.L.; Navarro, D.A.G.; Aga, D.S.; Bisson, M.A. Cd tolerance and accumulation in the aquatic macrophyte, *Chara australis*: Potential use for charophytes in phytoremediation. *Environ. Sci. Technol.* **2011**, *45*, 5332–5338. [CrossRef] [PubMed]

47. Shirazi, M.A.; Boersma, L. A unifying quantitative analysis of soil texture. *SSSA* **1984**, *48*, 142–147. [CrossRef]

48. Thomas, G.W. Soil pH and soil acidity. In *Methods of Soil Analysis, Part 3, Chemical Methods*, 1st ed.; Page, D.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E., Eds.; Soil Science Society of America, Inc.: Madison, WI, USA, 1996; pp. 475–490.

49. Jackson, M.L. Soil Chemical Analysis. *J. Plant Nutr. Soil Sci.* **1958**, *85*, 251–252.

50. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]

51. HACH, Laton Total Nitrogen Cuvette Test 20–100 mg/L TNb, 25 Tests. Available online: https://ie.hach.com/asset-get.download.jsa?id=25593604089 (accessed on 12 September 2019).

52. HACH, Phosphorus, Reactive (Orthophosphate) Molybdovanadate Method 8114, Test 'N Tube™ Vials. Available online: https://ie.hach.com/asset-get.download.jsa?id=7639983833 (accessed on 12 September 2019).

53. HACH, Potassium, Tetraphenylborate Method 8049, Spectrophotometer, Powder Pillows. Available online: https://ie.hach.com/asset-get.download.jsa?id=7639983843 (accessed on 12 September 2019).

54. Perglová, I.; Pergl, J.; Skálová, H.; Moravcová, L.; Jarošík, V.; Pyšek, P. Differences in germination and seedling establishment of alien and native Impatiens species. *Preslia* **2009**, *81*, 357–375.

55. Hussein, N. In vitro manipulation of *Impatiens glandulifera* pollen for transporting extracellular substances to the embryo sac. *Curr. Res. J. Biol. Sci.* **2014**, *6*, 66–70. [CrossRef]

56. Peterson, R.; Slovin, J.P.; Chen, C. A simplified method for differential staining of aborted and non-aborted pollen grains. *Int. J. Plant Biol.* **2010**, *1*, 66–69. [CrossRef]

57. Milner, M.J.; Kochian, L.V. Investigating heavy-metal hyperaccumulation using *Thlaspi caerulescens* as a model system. *Ann. Bot.* **2008**, *102*, 3–13. [CrossRef]

58. Selvam, A.; Wong, J.W.C. Phytochelatin systhesis and cadmium uptake of *Brassica napus*. *Environ. Technol.* **2008**, *29*, 765–773. [CrossRef]

59. Alaboudi, K.A.; Ahmed, B.; Brodie, G. Phytoremediation of Pb and Cd contaminated soils by using sunflower (*Helianthus annuus*) plant. *AOAS* **2018**, *63*, 123–127. [CrossRef]

60. Epelde, L.; Becerril, J.M.; Kowalchuk, G.A.; Deng, Y.; Zhou, J.; Garbisu, C. Impact of metal pollution and *Thlaspi caerulescens* growth soil microbial communities. *Appl. Environ. Microbiol.* **2010**, *76*, 7843–7853. [CrossRef] [PubMed]

61. Mani, D.; Sharma, B.; Kumar, C. Phytoaccumulation, interaction, toxicity and remediation of cadmium from Helianthus annuus L. (sunflower). *Bull. Environ. Contam. Toxicol.* **2007**, *79*, 71–79. [CrossRef] [PubMed]

62. Pietrini, F.; Zacchini, M.; Iori, V.; Pietrosanti, L.; Bianconi, D.; Massacci, A. Screening of poplar clones for cadmium phytoremediation using photosynthesis, biomass and cadmium content analyses. *Int. J. Phytoremediation* **2010**, *12*, 105–120. [CrossRef] [PubMed]

63. Mahajan, P.; Kaushal, J. Role of phytoremediation in reducing cadmium toxicity in soil and water. *J. Toxicol.* **2018**, *2018*, 1–16. [CrossRef]

64. Alkorta, I.; Hernández-Allica, J.; Becerril, J.M.; Amezaga, I.; Albizu, I.; Garbisu, C. Recent findings on the photoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead and arsenic. *Rev. Environ. Sci. Biotechnol.* **2004**, *3*, 71–90. [CrossRef]

65. Baker, A.J.M.; Brooks, R.R. Terrestrial higher plants which hyperaccumulate metallic elements—A review of their distribution, ecology and phytochemistry. *Biorecovery* **1989**, *1*, 81–126.

66. Sun, R.; Sun, Q.; Wang, R.; Cao, L. Cadmium accumulation and main rhizosphere characteristics of seven French marigold (Tagetes patula L.) cultivars. *Int. J. Phytoremediation* **2018**, *20*, 1171–1178. [CrossRef]

67. Zhu, Y.L.; Zayed, A.M.; Qian, J.-H.; de Souza, M.; Terry, N. Phytoaccumulation of trace elements by wetland plants: II. Water hyacinth. *J. Environ. Qual.* **1999**, *28*, 339–344. [CrossRef]

68. Zhao, F.J.; Lombi, E.; McGrath, S.P. Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens*. *Plant Soil* **2003**, *249*, 37–43. [CrossRef]

69. Pant, P.; Allen, M.; Tansel, B. Mercury Uptake and Translocation in *Impatiens walleriana* plants grown in the contaminated soil from oak ridge. *Int. J. Phytoremediation* **2010**, *13*, 168–176. [CrossRef]

70. Sun, Y.; Zhou, Q.; Wang, L.; Liu, W. Cadmium tolerance and accumulation characteristics of Bidens pilosa L. as a potential Cd-hyperaccumulator. *J. Hazard. Mater.* **2009**, *161*, 808–814. [CrossRef] [PubMed]

71. Van der Ent, A.; Baker, A.J.M.; Reeves, R.D.; Pollard, A.J.; Schat, H. Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. *Plant. Soil* **2013**, *362*, 319–334. [CrossRef]

72. Shrestha, P.; Bellitürk, K.; Görres, J.H. Phytoremediation of heavy metal-contaminated soil by switchgrass: A comparative study utilizing different composts and coir fiber on pollution remediation, plant productivity, and nutrient leaching. *IJERPH* **2019**, *16*, 1261. [CrossRef] [PubMed]

73. Ishikawa, S.; Noriharu, A.E.; Murakami, M.; Wagatsuma, T. Is *Brassica juncea* a suitable plant for phytoremediation of cadmium in soils with moderately low cadmium contamination?—Possibility of using other plant species for Cd-phytoextraction. *J. Soil Sci. Plant Nutr.* **2006**, *52*, 32–42. [CrossRef]

74. Ruckli, R.; Rusterholz, H.-P.; Baur, B. Invasion of *Impatiens glandulifera* affects terrestrial gastropods by altering microclimate. *Acta Oecol.* **2013**, *47*, 16–23. [CrossRef]

75. Ruckli, R.; Rusterholz, H.-P.; Baur, B. Invasion of an annual exotic plant into deciduous forests suppresses arbuscular mycorrhiza symbiosis and reduces performance of sycamore maple saplings. *Forest Ecol. Manag.* **2014**, *318*, 285–293. [CrossRef]

76. Nirola, R.; Megharaj, M.; Palanisami, T.; Aryal, R.; Venkateswarlu, K.; Naidu, R. Evaluation of metal uptake factors of native trees colonizing an abandoned copper mine—A quest for phytostabilization. *J. Sustain. Min.* **2015**, *14*, 115–123. [CrossRef]

77. Conti, M.E.; Botrè, F. Honeybees and their products as potential bioindicators of heavy metals contamination. *Environ. Monit. Assess.* **2001**, *69*, 267–282. [CrossRef]

78. Tanner, R.A. A review on the potential for the biological control of the invasive weed, *Impatiens glandulifera* in Europe. In *Plant Invasions: Human Perception, Ecological Impacts and Management*; Tokarska-Guzik, T., Brock, J.H., Brundu, G., Child, L., Daehler, C.C., Pyšek, P., Eds.; Backhuys Publishers: Leiden, The Netherlands, 2008; pp. 343–354.

79. Seo, B.-H.; Kim, H.S.; Kuppusamy, S.; Kim, K.-H.; Kim, K.-R. Enhanced nitrogen and phosphorus removal by woody plants with deep-planting technique for the potential environmental management of carcass burial sites. *Sustainability* **2017**, *9*, 155. [CrossRef]