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Bioavailability of Cd in *Plantago weldenii* and *Sonchus oleraceus* Plants: The Effects of a Humic and Fulvic Acids-Based Biostimulant

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Abstract: Cadmium (Cd) contamination poses a major threat to plants and human health, as well as to ecosystem services. Biostimulants provide a promising eco-friendly solution to enhance the phytoremediation of Cd-contaminated soils. We examined the influence of a humic and fulvic acids-based biostimulant on two plant species, e.g., *Plantago weldenii* and *Sonchus oleraceus* (common sowthistle), in a soil spiked with Cd at 45 mg kg^{-1}. The aim of this study was to determine whether a biostimulant can potentially affect Cd mobility in soil and absorption in plant tissues. We found that the biostimulant significantly decreased Cd bioavailability (recorded as DTPA extractability) in the soil where *Plantago* was grown from 17.57 to 13.12 mg kg^{-1}, probably due to the Cd immobilization effect of the added biostimulant. However, the biostimulant had the opposite effect in the soil where *S. oleraceus* was grown (Cd-DTPA significantly increased from 10.13 to 13.03 mg kg^{-1}). *S. oleraceus* was found to have accumulated higher Cd concentrations in its aerial parts, resulting in a soil-to-plant transfer value close to 1 and root-to-shoot translocation value well above 1. These two indices exhibited the potential of *S. oleraceus* to be used as hyperaccumulator in Cd-contaminated soils, while *P. weldenii* behaved rather as a Cd excluder. These findings highlight the complex dynamics of added biostimulants and Cd behavior in soil and plants. We recognize the need for further research so that the mechanisms dictating Cd behavior after biostimulant application can be better elucidated.

Keywords: heavy metal contamination; cadmium contamination; phytoremediation; soil pollution; common sowthistle; wild edible species

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1. Introduction

Soil deterioration due to the accumulation of potentially toxic elements (PTEs) is a threat to both crop production and the environment [1,2], while PTEs have become a great concern to food security and human health due to their high toxicity, stability, and non-biodegradability [3–5]. Among these PTEs, cadmium (Cd) is one of the most important pollutants, being a hazardous and carcinogenic element even at low concentrations [6].

Cd naturally occurs in the earth's crust as a result of rock weathering and volcanic eruption [7]. Cd is also capable of being bound to phosphorites and sulfides, resulting in increased accumulation in soils, due to the use of these rocks in the fertilizer industry [8]. Moreover, apart from fertilizers, its usage in industry, mining, and pesticides can elevate the Cd content in both soil and water [9]. Cadmium is a highly labile element in soil and can easily be assimilated by plants. The physicochemical properties of soil may significantly influence its fate and availability. For example, in acidic soil pH, Cd mobility is higher and can be released into groundwater, thus increasing the risk to the whole food chain [8]. Cadmium is more available in soils with low cation-exchange capacity (CEC) [10], leading to its leaching and/or uptake, whereas soil organic matter may increase Cd sorption, resulting

in reduced uptake by plant roots [11]. Cd toxicity in plants impacts their physiological functions and hormonal regulation [12,13]. Therefore, exposure to Cd leads to oxidative stress by producing reactive oxygen species (ROS), resulting in cell membrane damage and inhibition of plant development [14,15]. Therefore, effective alleviation strategies of Cd toxicity, such as phytoremediation, combined with novel soil amendments are essential to ensure sustainable agriculture practices [16,17].

Phytoremediation is an eco-friendly practice that relies on certain plant species that have the ability to restore and reclaim contaminated sites without damaging soil structure [18]. On the other hand, biostimulants are novel substances that have recently been used for enhancing the phytoremediation efficiency of contaminated soils [19]. It has been recognized that utilizing biostimulants in crop production increases plant vigor, crop yield, and resistance to biotic and abiotic stressors [16,20–22]. Recent studies have confirmed that these products can reduce the bioavailability of PTEs in soil and their toxic effects to plants [23–25]. However, the effect of biostimulants on Cd dynamics to soil and plants in contaminated soils has not been sufficiently elucidated. Humic substances-based biostimulants seem to assist phytoremediation by favoring plant growth and production, improving soil properties, and enhancing the nutrient acquisition and increasing the water efficiency of plants [26,27]. Moreover, humic acids are known as high molecular weight organic acids (HMWOA) that can influence the fate of PTEs (including Cd) at the soil–plant interface. These organic substances may alleviate the mobility of PTEs by forming chelating bonds and stable organometallic complexes with metallic ions in mineral structures, thereby mitigating accumulation by plants and transfer in the trophic chain [19]. Usage of HMWOAs has been reported to minimize the total concentrations of Pb and Cd [3]. However, Evangelou et al. [28] indicated the use of humic acids as an alternative chelating agent, as they can increase the solubility of cationic metals and absorption by roots. The opposite effect may be expected with low molecular weight fulvic acids, which may be able to formulate soluble organometallic complexes with PTEs like Cd, thus increasing their bioavailability.

Previous studies have primarily concentrated on biostimulant application in herbaceous plant species [29]. The phytoremediation strategies have used various hyperaccumulator species with high tolerance to different PTEs and high accumulation levels of PTEs aboveground, despite their small biomass production [19,30]. Among these species, wild edible plants have received more attention because of their adaptability to harsh environmental conditions, which could likely include exposure to high contents of Cd. For the remediation of PTE-contaminated soils, wild species have great potential, as they have resistance mechanisms to contaminants and they can uptake toxic elements [31]. *Sonchus oleraceus* L., commonly known as "common sowthistle", is an annual plant grown in various soil types and under diverse conditions [32]. It was reported that *S. oleraceus* has the ability to colonize areas with high concentrations of PTEs, including titanium (Ti) and manganese (Mn) [32]. This species has also been used for the phytoremediation of Pb [33] and Cd [34] in contaminated soils due to its elevated uptake and translocation in its aerial tissues [30]. On the other hand, *Plantago weldenii* is a wild edible perennial species that belongs to the family Plantaginaceae [35]. This herbaceous plant can be a potential halophyte crop since it can be grown under conditions of high salinity [35]. To the best of our knowledge, no information exists in the literature about the potential of *P. weldenii* to accumulate hazardous elements in its aerial tissues or to stabilize them via the root system. However, other related species from the family Plantaginaceae have demonstrated the ability to accumulate significant concentrations of metals, including aluminum (Al). Thus, they can be utilized in remediation strategies of contaminated soils [36].

The purpose of this study was to fill the research gap regarding the effects of biostimulant formulations based on humic and fulvic acids on the uptake and translocation of Cd in *S. oleraceus* L. and *P. weldenii* grown in pots. We also aimed to study the soil mobility and plant availability of added Cd in the two plant species and compare the metal's behavior with and without biostimulant application to soil. The obtained results could provide

knowledge about the beneficial use of biostimulants in alleviating stress from PTEs and the management of contaminated soil, which may be essential to a vast array of soil end-users.

2. Materials and Methods

2.1. Experimental Design

For the experiment, soil from the University Farm at Velestino (22.756 E, 39.395 N) was obtained. The soil was loamy (with sand 45% and clay 16%), with pH = 7.8, and had a Cd pseudo-total content of 0.67 mg kg^{-1}. Soil with pH 7.8 was selected in order to investigate the effects of higher pH levels on the phytotoxicity of Cd. This allowed us to examine the opposing impacts of elevated pH levels, complementing the previous report that the phytotoxicity of Cd increases under acidic soil conditions. The soil was weighed into 120 portions of 1 kg each and placed into 120 plastic bags. Sixty of them were spiked with $Cd(NO_3)_2$ so that the total added Cd concentration equaled 45 mg Cd per kg of soil. Into these bags, ca. 200 mL of water was added, the soil was thoroughly mixed, and the bags were left to equilibrate for a period of 3 months. During equilibration, water was added regularly to the bags to compensate for any moisture loss, and the bags were mixed multiple times in order to ensure a uniform distribution of added Cd. At the beginning of the equilibrium period, seeds of the two selected plants, *S. oleraceus* L. and *P. weldenii*, were sown in the same unamended soil as that used in the experiment. After 3 months, the soils were transferred into 2-L pots and before transplanting, the roots of plants (only 60 of 120 plants) were dipped in a solution of biostimulant product consisting of a balanced solution of humic and fulvic acids. The biostimulant was obtained from a raffinated extract of leonardite with a natural ratio of 30:70 fulvic acid:humic acid. Thus, the treatments of the experiment were a combination of 2 levels of Cd (a negative control with no added Cd and a positive control with 45 mg Cd kg^{-1} soil added) and 2 levels of biostimulant (unamended and amended), with 15 replicates per treatment, as follows: (a) Cd0B0 (no added Cd, no biostimulant); (b) Cd0B1 (no added Cd, with added biostimulant); (c) Cd1B0 (added Cd, no added biostimulant); and (d) Cd1B1 (both Cd and biostimulant were added). Each of these treatments was used for the two test plants, *S. oleraceus* and *P. weldenii*, resulting in a total of 120 pots (i.e., 2 levels of Cd × 2 levels of biostimulant × 2 plants × 15 replicates). Upon transplanting (considered as Day 1—beginning of the experiment, 4 November 2022 for *S. oleraceus* and 25 November 2022 for *P. weldenii*), the pots were placed in an unheated greenhouse. Plants were fertigated with nutrient solution that contained 200 mg L^{-1} of N-P-K according to their needs (approximately 150–250 mL of nutrient solution every 5 to 7 days). In the treatments where the biostimulant was added, on Days 5, 15, and 25, we added 100 mL of the biostimulant per pot (6 mL of biostimulant formulation in 6 L H_2O) by direct application to the roots. The experiment lasted until the flowering stage of the two plants, i.e., 53 days for *S. oleraceus* (harvest day 26 December 2022) and 90 days for *P. weldenii* (harvest day 23 February 2023).

2.2. Soil and Plant Analyses

2.2.1. Non-Destructive Analyses

Before harvest, measurements of certain plant growth characteristics were conducted, i.e., plant height and diameter of the rosette, across all experimental plants. The chlorophyll content in *S. oleraceus* leaves was measured using a portable chlorophyll meter (SPAD 502; Konica Minolta Inc., Tokyo, Japan), with measurements taken from a single leaf per plant. Also, the photosynthetic rate (in μmol CO_2 m^{-2} s^{-1}) was evaluated as photosynthetically active radiation (PAR), including the light intensity in the PAR range of 0, 400, 800, and 1200 w m^{-2} (Li-COR, Lincoln, NE, USA). Measurements were taken from a single leaf per plant, and the study included five plants for each treatment. Leaf samples for both analyses were obtained from the uppermost and fully developed leaves of plants. The photosynthetic rate and chlorophyll content were not measured in *P. weldenii* leaves, due to their small size.

2.2.2. Plant Growth Measurement and Chemical Analyses

On the harvesting day, the number of leaves and total fresh weight were measured across all plants of the experiment. The aerial biomass was cut, and after the fresh weight was recorded, plant tissues were washed with distilled H_2O and placed in paper bags. Then, soil samples were taken from each pot by obtaining 3 cores at different spots at the surface down to the depth of the pots, and the samples were mixed into one composite sample per pot and placed into paper bags, which were taken to the laboratory. While still fresh, *S. oleraceus* leaves were measured for total leaf area (cm^2) using the LI-3100C Area Meter (LI-COR Biosciences; Hellamco S.A, Athens, Greece). Moreover, specific leaf area (SLA; expressed in $m^2\ kg^{-1}$) was calculated using the following equation: SLA = total leaf area/dry weight of leaves. After that, leaves were placed back into the paper bags. The roots were then carefully extracted by delicately washing off soil particles. The roots were then washed with distilled H_2O and placed in paper bags. All plant material (aerial and roots) was then placed into an oven at 70 °C until no further weight loss was recorded. The dry weight was then recorded.

As for the soil samples, after being air-dried, the organic matter was measured by wet oxidation (Walkely and Black method), and the pH was measured at 1:2.5 H_2O. The bioavailable Cd levels were extracted with diethylenetriaminepentaacetic acid-$CaCl_2$ (DTPA) solution at a 1-to-2 ratio. All measurements followed the methodology described by Rowell [37]. Plant samples, including the aerial parts and roots, were extracted for Cd with dry ashing. Precisely, 0.5 g of plant tissues was ashed at 500 °C for 5 h and extracted with 10 mL 20% HCl. Soil and plant samples were analyzed for Cd using atomic absorption spectrometry (Perkin Elmer A3300, Analytical Instruments S.A., Athens, Greece).

2.2.3. Indices for Cd Transfer and Translocation

Based on the primary data, the following indices were calculated as per Antoniadis et al. [38]:

$$\text{TC (transfer coefficient)} = (\text{concentration of Cd in plant})/(\text{total concentration of Cd in soil}) \quad (1)$$

$$\text{TF (translocation factor)} = (\text{concentration of Cd in aboveground})/(\text{concentration of Cd in roots}) \quad (2)$$

2.3. Data Quality Control and Statistical Analysis

In order to address data quality control, Cd recovery was measured in the Cd-added treatments with aqua regia, recording the pseudo-total concentration (digestion at 140 °C for 5 h with 3:1 concentrated HCl:HNO_3). The recovery was 90–95%. In all extraction batches, blanks were included in order to account for any in-house contamination. Also, reference materials of plant and soil were tested in order to account for analytical precision; the recovery of analyzed Cd was satisfactory and ranged from 92% to 105%. All measurements were performed in triplicate and the acceptable coefficient of variation was less than 15%. Statistical analysis was performed using IBM SPSS Statistics 26. One-way analysis of variance (ANOVA) was conducted for each species, and post hoc analysis according to Duncan's multiple range test was also employed. The significance of differences among treatments was decided at a level of $p < 0.05$.

3. Results

3.1. Soil Properties

The two measured soil properties, pH and organic matter (OM), did not show any statistically significant changes in the studied treatments (Table 1). As for the pH in the unamended soil (Cd0B0) with both *P. weldenii* and *S. oleraceus* test plants, there were slightly and non-significantly lower values (8.58 and 8.46, respectively) compared to the Cd-spiked soil (Cd1B0 and Cd1B1). The same was true for the soil pH, where no significant differences

were observed. Regarding organic matter, no differences were observed in the soils from both test plants. The OM percentage for *P. weldenii* ranged from 1.86% to 2.23%, whereas the OM of *S. oleraceus* ranged from 1.99% to 2.28%. Despite the variations observed between treatments, no significant differences were indicated for either plant.

Table 1. Soil properties (pH and organic matter—OM) as affected by Cd and biostimulant addition in the soils where *Plantago weldenii* and *Sonchus oleraceus* were grown.

		P. weldenii	***S. oleraceus***
pH	Cd0B0	8.58 ^{a}	8.46 ^{a}
	Cd0B1	8.65 ^{a}	8.54 ^{a}
	Cd1B0	8.67 ^{a}	8.51 ^{a}
	Cd1B1	8.69 ^{a}	8.53 ^{a}
	Significance	$p = 0.084$ ^{NS}	$p = 0.112$ ^{NS}
OM (%)	Cd0B0	2.11 ^{a}	1.99 ^{a}
	Cd0B1	2.23 ^{a}	2.28 ^{a}
	Cd1B0	2.18 ^{a}	2.12 ^{a}
	Cd1B1	1.86 ^{a}	2.25 ^{a}
	Significance	$p = 0.377$ ^{NS}	$p = 0.097$ ^{NS}

Values are the mean ± standard error (n = 15 replicates). Different letters indicate significant differences among means in columns according to the post hoc Duncan's multiple range test at $p < 0.05$. Treatments are as follows: Cd0B0 = no Cd, no biostimulant; Cd0B1 = no Cd, but biostimulant added; Cd1B0 = Cd added, but no biostimulant; Cd1B1 = both Cd and biostimulant added. ^{NS} = Non-significant.

3.2. Plant Growth and Physiological Parameters

The rosette diameter in *P. weldenii* increased significantly as compared to the control (Cd0B0) in the treatments with added biostimulant, from 17.9 cm at Cd0B0 to 19.7 cm at Cd0B1 and 21.3 cm at Cd1B1 (Table 2). However, no significant difference was observed between Cd0B0 and Cd1B0. In the Cd-spiked treatments, the application of the biostimulant favored only the fresh biomass of the aerial parts (increased from 6.87 g at Cd1B0 to 9.78 g at Cd1B1). Also, the leaf number significantly increased from 24.6 at Cd0B0 to 30.4 at Cd0B1, whereas no significant difference was recorded between the non-spiked soils, regardless of biostimulant treatment. The application of biostimulant in the non-spiked treatment resulted in significantly higher values of dry weight for both the aboveground parts (1.20 g) and roots (0.49 g), while the rest of the treatments did not differ significantly from one another. Similarly, the number of leaves was the highest for the same treatment, with Cd0B1 being significantly different only from the control treatment (Cd0B0).

On the other hand, in *S. oleraceus* the rosette diameter was the lowest at Cd1B1 (28.0 cm) and it significantly increased to 32.5 only in the case of Cd0B1 (Table 2). Regarding the aerial biomass (both fresh and dry), no significant differences were recorded, whereas the root biomass had the highest value at Cd0B1 (0.9 g), which was significantly different from the rest of the treatments. Also, there were significant differences in the number of leaves, with Cd0B0 having the highest value of 15.2, which was significantly different from that at Cd0B1 and Cd1B0.

As shown in Table 3, no significant differences were recorded in the case of the leaf area of *S. oleraceus*, with values ranging from 279 to 289 across the treatments (measurements were not performed in *P. weldenii* due to the morphology of the leaves). However, the specific leaf area (expressed in $cm^2\ kg^{-1}$ plant) had its maximum value at Cd0B0 (30.1 $cm^2\ kg^{-1}$), a value that was significantly different only from Cd0B1 (26.3 $cm^2\ kg^{-1}$). As for the total chlorophyll content (SPAD index), the highest value was recorded at Cd0B0, a value that was significantly different from that at Cd1B0 and Cd1B1 (15.45 and 13.17, respectively).

Table 2. Plant growth parameters of *Plantago weldenii* and *Sonchus oleraceus* as affected by Cd and biostimulant addition in the soils: rosette diameter, fresh leaf weight (FW), dry leaf weight (DW), root dry weight, and number of leaves per plant.

		P. weldenii	***S. oleraceus***
Rosette diameter (cm)	Cd0B0	17.9 ^{a}	30.5 ^{ab}
	Cd0B1	19.7 ^{b}	32.5 ^{b}
	Cd1B0	18.9 ^{ab}	29.7 ^{ab}
	Cd1B1	21.3 ^{c}	28.0 ^{a}
	Significance	$p < 0.001$ ***	$p = 0.077$ ^{NS}
Aboveground FW (g)	Cd0B0	6.28 ^{a}	9.35 ^{a}
	Cd0B1	7.36 ^{a}	9.41 ^{a}
	Cd1B0	6.87 ^{a}	9.63 ^{a}
	Cd1B1	9.78 ^{b}	10.03 ^{a}
	Significance	$p < 0.001$ ***	$p = 0.744$ ^{NS}
Aboveground DW (g)	Cd0B0	0.76 ^{a}	0. ^{a}
	Cd0B1	1.20 ^{b}	1.07 ^{a}
	Cd1B0	0.83 ^{a}	1.01 ^{a}
	Cd1B1	0.95 ^{a}	1.04 ^{a}
	Significance	$p < 0.005$ **	$p = 0.487$ ^{NS}
Roots (g)	Cd0B0	0.20 ^{a}	0.66 ^{ab}
	Cd0B1	0.49 ^{b}	0.91 ^{c}
	Cd1B0	0.25 ^{a}	0.71 ^{b}
	Cd1B1	0.28 ^{a}	0.56 ^{a}
	Significance	$p < 0.001$ ***	$p < 0.001$ ***
Number of leaves	Cd0B0	24.6 ^{a}	15.2 ^{b}
	Cd0B1	30.4 ^{b}	13.7 ^{a}
	Cd1B0	27.1 ^{ab}	13.2 ^{a}
	Cd1B1	29.2 ^{ab}	14.3 ^{ab}
	Significance	$p = 0.171$ ^{NS}	$p = 0.023$ *

Values are the mean ± standard error (n = 15 replicates). Different letters indicate significant differences among means in columns according to the post hoc Duncan's multiple range test at $p < 0.05$. Treatments are as follows: Cd0B0 = no Cd, no biostimulant; Cd0B1 = no Cd, but biostimulant added; Cd1B0 = Cd added, but no biostimulant; Cd1B1 = both Cd and biostimulant added. * Significant at $p < 0.05$. ** Significant at $p < 0.01$. *** Significant at $p < 0.001$. ^{NS}, non-significant.

Table 3. Plant growth parameters (leaf area, specific leaf area, total chlorophyll concentration) as affected by Cd and biostimulant application in *Sonchus oleraceus*.

		S. oleraceus
Leaf area (cm^2)	Cd0B0	283 ^{a}
	Cd0B1	279 ^{a}
	Cd1B0	279 ^{a}
	Cd1B1	296 ^{a}
	Significance	$p = 0.797$ ^{NS}
Specific leaf area (m^2 kg^{-1})	Cd0B0	30.1 ^{b}
	Cd0B1	26.3 ^{a}
	Cd1B0	27.8 ^{ab}
	Cd1B1	28.5 ^{ab}
	Significance	$p = 0.032$ *

Table 3. *Cont.*

		S. oleraceus
Specific leaf area (m^2 kg^{-1})	Cd0B0	30.1 [b]
	Cd0B1	26.3 [a]
	Cd1B0	27.8 [ab]
	Cd1B1	28.5 [ab]
	Significance	$p = 0.032$ *
Total chlorophyll concentration	Cd0B0	15.45 [b]
	Cd0B1	13.47 [ab]
	Cd1B0	13.17 [a]
	Cd1B1	12.15 [a]
	Significance	$p = 0.024$ *

Values are the mean ± standard error (n = 15 replicates). Different letters indicate significant differences among means in columns according to the post hoc Duncan's multiple range test at $p < 0.05$. Treatments are as follows: Cd0B0 = no Cd, no biostimulant; Cd0B1 = no Cd, but biostimulant added; Cd1B0 = Cd added, but no biostimulant; Cd1B1 = both Cd and biostimulant added. * Significant at $p < 0.05$. [NS] = Non-significant.

The photosynthetic rates in *S. oleraceus* plants are presented in Table 4. Thus, it appears that the photosynthetic rate generally increased with high light intensity. Although the only significant difference was observed at 0 w m^{-2}, Cd0B0 had a significantly higher photosynthetic rate (−0.41) compared to Cd0B1 (−1.14).

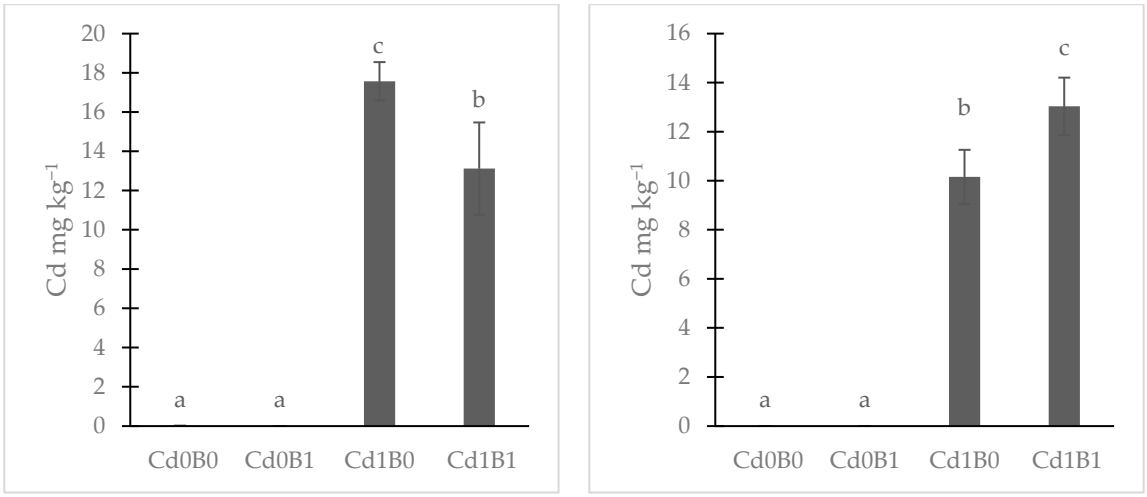
Table 4. Photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) evaluated as photosynthetically active radiation (PAR), including the light intensity in the PAR range of 0, 400, 800, and 1200 w m^{-2} as affected by Cd and biostimulant application in *Sonchus oleraceus*.

		PAR (0 w m^{-2})	**PAR (400 w m^{-2})**	**PAR (800 w m^{-2})**	**PAR (1200 w m^{-2})**
Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	Cd0B0	−0.41 [b]	6.43 [a]	7.94 [a]	8.72 [a]
	Cd0B1	−1.14 [a]	8.18 [ab]	9.44 [ab]	10.06 [ab]
	Cd1B0	−0.82 [ab]	8.25 [ab]	9.76 [ab]	9.45 [ab]
	Cd1B1	−0.57 [b]	10.27 [b]	11.96 [b]	13.14 [b]
	Significance	$p = 0.027$ *	$p = 0.047$ *	$p = 0.077$ [NS]	$p = 0.096$ [NS]

Values are the mean ± standard error (n = 5 replicates). Different letters indicate significant differences among means in columns according to the post hoc Duncan's multiple range test at $p < 0.05$. Treatments are as follows: Cd0B0 = no Cd, no biostimulant; Cd0B1 = no Cd, but biostimulant added; Cd1B0 = Cd added, but no biostimulant; Cd1B1 = both Cd and biostimulant added. * Significant at $p < 0.05$. NS = Non-significant.

3.3. Bioavailable Concentration of Cd in Soil

The bioavailable fraction of Cd in soil, estimated with DTPA, showed that in the soils where *P. weldenii* was grown, DTPA-extractable Cd was 17.6 mg kg^{-1} at Cd1B0 and it significantly decreased to 13.1 mg kg^{-1} at Cd1B1 (Figure 1a). Conversely, in the soils with *S. oleraceus*, DTPA-extractable Cd at Cd1B0 was 10.16 mg kg^{-1} and it significantly increased to 13.03 mg kg^{-1} in the case of Cd1B1 (Figure 1b). In the treatments with no added Cd (i.e., Cd0B0 and Cd0B1), no DTPA-extractable Cd was recorded, as expected (Figure 1a,b).



(**a**) (**b**)

Figure 1. Bioavailable concentration of Cd in (**a**) *Plantago weldenii* and (**b**) *Sonchus oleraceus*. Values are the mean and error bars represent the standard error (n = 15 replicates). Different letters indicate significant differences among means according to the post hoc Duncan's multiple range test at $p < 0.05$. Treatments are as follows: Cd0B0 = no Cd, no biostimulant; Cd0B1 = no Cd, but biostimulant added; Cd1B0 = Cd added, but no biostimulant; Cd1B1 = both Cd and biostimulant added.

3.4. Cd Concentration in Plant Tissues

The Cd concentration in *P. weldenii* roots was significantly higher at Cd1B0 (27.48 mg kg^{-1}) and Cd1B1 (31.11 mg kg^{-1}), with the two treatments having no significant difference, while they both differed significantly from Cd0B0 and Cd0B1 (both with Cd below detection). The aboveground concentration was 0.12 mg kg^{-1} at Cd1B0 and it was elevated to 0.54 mg kg^{-1} at Cd1B1, with the two values exhibiting no significant difference. As for *S. oleraceus*, the Cd concentrations in the aerial biomass were significantly higher at Cd1B0 and Cd1B1 (8.29 mg kg^{-1} and 9.16 mg kg^{-1}, respectively) compared to those in the non-spiked soil (Cd0B0 and Cd0B1), without a significant difference between them. In the case of the roots, the Cd concentrations were 3.43 mg kg^{-1} at Cd1B0 and 2.74 mg kg^{-1} at Cd1B1, with no significant difference between them, while both of them differed from plants grown in the non-spiked treatments (Table 5).

Table 5. Cd concentration in shoots (expressed both in mg kg^{-1} dry weight and mg kg^{-1} fresh weight) and roots (mg kg^{-1} dry weight) in *Plantago weldenii* and *Sonchus oleraceus* plants as affected by Cd and biostimulant application.

		P. weldenii	***S. oleraceus***
Shoots (dry weight)	Cd0B0	0 ^{a}	0 ^{a}
	Cd0B1	0.11 ^{ab}	0 ^{a}
	Cd1B0	0.12 ^{ab}	8.29 ^{b}
	Cd1B1	0.54 ^{b}	9.16 ^{b}
	Significance	$p = 0.046$ *	$p < 0.001$ ***
Shoots (fresh weight)	Cd0B0	0 ^{a}	0.01 ^{a}
	Cd0B1	0.018 ^{ab}	0 ^{a}
	Cd1B0	0.015 ^{ab}	0.87 ^{b}
	Cd1B1	0.052 ^{b}	0.95 ^{b}
	Significance	$p = 0.046$ *	$p < 0.001$ ***

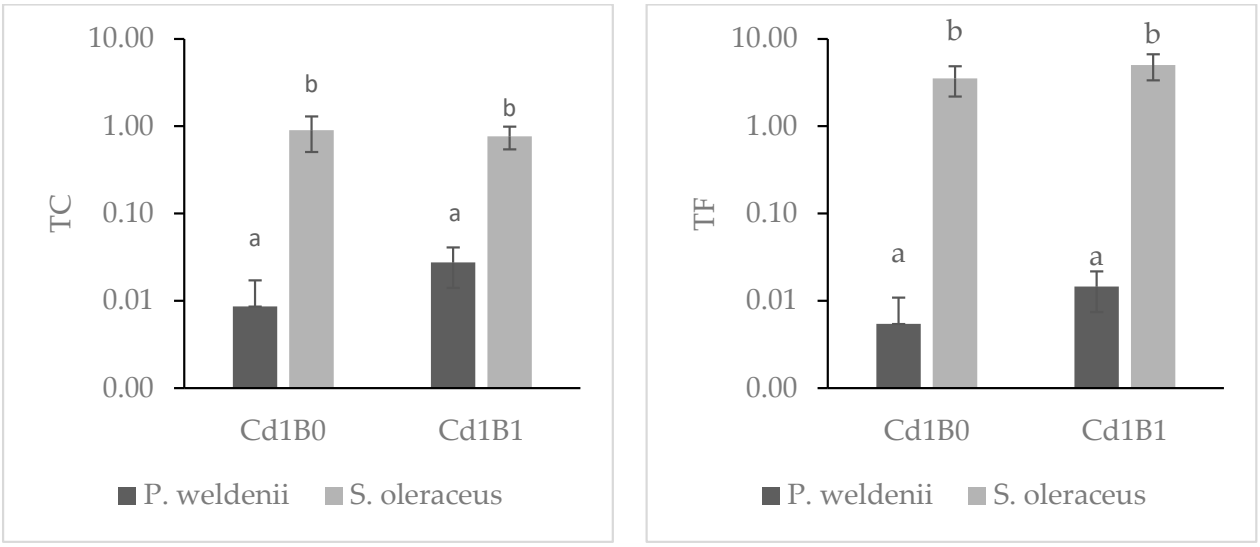
Table 5. *Cont.*

		P. weldenii	*S. oleraceus*
Roots	Cd0B0	0 [a]	0 [a]
	Cd0B1	0 [a]	0 [a]
	Cd1B0	27.48 [b]	3.43 [b]
	Cd1B1	31.11 [b]	2.74 [b]
	Significance	$p < 0.001$ ***	$p < 0.001$ ***

Values are the mean ± standard error (n = 5 replicates). Different letters indicate significant differences among means in columns according to the post hoc Duncan's multiple range test at $p < 0.05$. Treatments are as follows: Cd0B0 = no Cd, no biostimulant; Cd0B1 = no Cd, but biostimulant added; Cd1B0 = Cd added, but no biostimulant; Cd1B1 = both Cd and biostimulant added. * Significant at $p < 0.05$. *** Significant at $p < 0.001$.

3.5. Indices for the Transfer and Translocation of Cd

The transfer of Cd from soil to plant (TC) was close to 1 at Cd1B0 for *S. oleraceus* (0.90) and reached 0.77 at Cd1B1, with both values exhibiting no significant difference. On the other hand, the Cd TC of *P. weldenii* was much lower, with values equal to 0.0086 and 0.027 at Cd1B0 and Cd1B1, respectively (Figure 2). As for the root-to-shoot translocation (TF), it was well above 1 for *S. oleraceus*, i.e., 3.53 at Cd1B0 and 5.01 at Cd1B1, while for *P. weldenii*, the respective TF values were 0.005 and 0.015 for Cd1B0 and Cd1B1.



(**a**) (**b**)

Figure 2. (**a**) Transfer coefficient in *Plantago weldenii* and *Sonchus oleraceus* plants. (**b**) Translocation factor in *Plantago weldenii* and *Sonchus oleraceus*. Values are the mean and error bars represent the standard error (n = 15 replicates). Different letters indicate significant differences among means according to the post hoc Duncan's multiple range test at $p < 0.05$. Note the logarithmic scale in the y-axes. Treatments are as follows: Cd1B0 = Cd added, but no biostimulant; Cd1B1 = both Cd and biostimulant added.

4. Discussion

Regarding pH and OM, no statistically significant differences were observed among any treatments in the soils where both plants were grown. Cd is known to interact with colloidal particles and other soil components. The electrostatic forces between Cd cations and the negatively-charged colloidal surfaces increase at higher pH [39]. Adding the biostimulant could have influenced the pH. However, any such effects were rather insignificant. Our results indicate that the added biostimulant increased root growth in both plants in the control treatment. The humic substances might be responsible for this effect because of their ability to enhance H^+ ATPase activity in the plasma membranes in roots [27]. This

activation may be related to the increase in lateral hair growth [40,41]. Certain studies have detected in different plant species that the application of humic substances led to an increase in the lateral root surface area and root length [25,42–45]. Regarding the Cd-spiked treatments, in *P. weldenii* the application of biostimulant resulted in an increase in aboveground biomass. Similar results were noted by Canal et al. [46] in *Brassica napus* L., which was found to thrive in a soil laden with Cd. After adding humic acids, increases in shoots and roots were observed. No major changes were found with regards to the leaf area in *S. oleraceus*—only in the specific leaf area at Cd0B1, where there was a slight decrease compared to the control. This was rather expected since there were increases in the surface area and root weight, while at the same time, the aboveground biomass was unchanged. It is possible that the addition of humic substances promoted the growth of the underground biomass, leading to a change in the distribution of plant resources, an effect that likely impacted leaf morphology [47,48]. *S. oleraceus* growing under Cd stress had a lower content of chlorophyll compared to the control treatment. This finding was consistent with reports from other studies. Zhang et al. [49] found that the chlorophyll content of tobacco leaves was inhibited by Cd stress. Likewise, Szopinski et al. [50] reported a significant decrease in chlorophyll concentrations in *Arabidopsis arenosa* and *Arabidopsis halleri* when the plants were subjected to a solution containing 100 μmol L^{-1} Cd (equal to 11.24 mg L^{-1}). The presence of Cd can cause oxidative stress by substituting Mg^{2+} in chlorophyll and redistributing its content [51]. However, in our study, the addition of Cd did not seem to negatively impact the growth of either species, despite many studies that indicated the opposite [52–55]. This could be due to the fact that plants have the capacity to develop various mechanisms to limit Cd toxicity [38,56]. Indeed, many reports have pointed out that *S. oleraceus* is highly tolerant to Cd as well as other toxic metals, e.g., Pb, as exhibited by the fact that it was actually found to have increased its biomass when exposed to these metals [30,57,58]. As for *P. weldenii*, there is rather limited evidence available for its tolerance towards PTEs. However, in different species within the family Plantaginaceae, there are related studies showing their resistance to contaminants [59,60]. It is noteworthy that Cd concentrations per fresh weight in *S. oleraceus* well exceeded the regulation limit of 0.2 mg kg^{-1} of category 3.2.15 ("leaf vegetables") of the Directive 86/278/EC, while those in *P. weldenii* did not, even when grown in such soil spiked with Cd at 45 mg kg^{-1}. This finding, combined with *P. weldenii* TC and TF (discussed later), shows that this species behaves rather as a Cd excluder. The opposite seems to be the case for *S. oleraceus*, which shows the potential to have the behavior of a hyperaccumulator. It is also noteworthy that biostimulant addition at Cd1B1 to the soil grown with *P. weldenii* led to a lower concentration of plant Cd. Regarding the bioavailable soil Cd, it was not detected at Cd0B0 and Cd0B1 due to the low total Cd content in the control soil. In the soil grown with *P. weldenii*, we found lower DTPA-extractable Cd concentrations, which may be attributed to the ability of humic substances to form stable complexes with metal ions (mainly cations such as Cd), as they contain phenolic and carboxyl groups capable of retaining Cd and removing it from the soil solution [61,62]. Similar findings were reported in other studies indicating a decrease in bioavailability after the use of humic substances [63,64]. On the contrary, in the soil grown with *S. oleraceus*, we found an increase in the levels of Cd extracted with DTPA. It is well known that organic acids can dissolve cationic metals and enhance their availability [28,65]. Many studies have reported that exogenous humic substances have complicated interactions with PTEs and can enhance their bioavailability in soil. For instance, Perez-Esteban et al. [66] reported that the addition of these substances led to increased extractability of Zn and Cu from contaminated soils. This suggests an interesting trend of the Cd dynamics in soil being influenced in different ways although the same treatments have been imposed: in the soil grown with *P. weldenii*, there was a decreasing trend with biostimulant addition, while in the soil with *S. oleraceus*, there was an increase. Although the soil geochemical mechanisms explained earlier do apply, the effect of the certain plant in each case can hardly be overlooked. In other words, the two diametrically opposite trends in Cd dynamics with added biostimulant seems

to indicate that the particular plants extended an influence to these soil mechanisms to some degree. Such an effect must certainly be related to the excluder (i.e., *P. weldenii*) vs. hyperaccumulator (i.e., seemingly in the case of *S. oleraceus*) dipole—with the former assisting in Cd phytostabilization through the exudation of Cd-stabilizing siderophore substances, while the latter facilitates Cd release via the exudation of low molecular weight organic substances that tend to increase Cd mobility.

The Cd indices used in this work functioned as indicators to categorize the plants as potential excluders or hyperaccumulators. The TC is an indicator of a plant's ability to take up and accumulate an element from the soil. There is no universally accepted threshold for indicating a hyperaccumulator; however, any value close to, or higher than, unity provides a strong indication. Our data showed that the TC values of both test plants were lower than 1, although those of *S. oleraceus* were orders of magnitude higher than those of *P. weldenii* and very close to 1. This means that *S. oleraceus* is potentially a more efficient accumulator of Cd than *P. weldenii.* The TF is another accumulation indicator, which is used to describe the translocation of an element from the root system to the aboveground parts. The picture of the two plants was similar for TF (i.e., values much higher than 1 for *S. oleraceus*, and values several orders of magnitude lower for *P. weldenii*). These findings, coupled by the noteworthy results of the fresh weight Cd content (lower for *P. weldenii* than the regulation limit of 0.2 mg kg^{-1} fresh weight, while much higher for *S. oleraceus*) provides strong evidence for the difference in behavior between the two plants, as indicated earlier. A greater Cd concentration in the roots than in the aerial parts (i.e., lower TF) indicates a plant's strategy (in our case, *P. weldenii*) to phytostabilize contaminants, thus reducing potential damage due to toxicity [67]. Specifically, it has been reported that *Plantago* spp. can accumulate significant amounts of Cd in their roots. Specifically, Nadgórska et al. [60] exposed *P. lanceolata* and *P. major* to different Cd concentrations (0.4, 6.0, 11.7, and 20.1 mg kg^{-1}) and reported a Cd accumulation in the dry weight of roots of 10.7, 13.1, 9.7, and 7.9 mg kg^{-1}, respectively, for *P. lanceolata*, and an accumulation of 6.1, 4.0, 5.7, and 5.3 mg kg^{-1}, respectively, for *P. major*. Moreover, the TF values were 1.51, 0.88, 0.87, and 0.84, respectively, for *P. lanceolata* and significantly lower for *P. major* (0.92, 0.78, 0.54, and 0.39, respectively). Similar results were obtained by Abe et al. [68] when *P. virginita* and *P. major* were grown in a soil contaminated with 3 mg Cd kg^{-1}. The TF values were shown to be as low as 0.03 and 0.07, respectively, for the two species, similar to our findings. Likewise, Tinkov et al. [69] collected three *P. weldenii* species from the Ural River and found that *P. major*, *P. lanceolata*, and *P. maxima* accumulated Cd mainly in the roots. Especially, *P. major* accumulated an amount of 0.5 mg kg^{-1} dry weight. These findings indicate that *P. weldenii* plants have a common feature of being capable of phytostabilizing soil Cd in or through their root system. Contrary to this behavior, the *S. oleraceus* TF values of well over 1 were indicative of a typical hyperaccumulator [38]. Samadi et al. [70] also found that *S. oleraceus* had the ability to accumulate higher Cd concentrations in shoots than in roots, and this concentration was 5.3-fold higher than the mean concentration of Cd in soil (also indicating a TC of higher than 1). Furthermore, Abe et al. [68] conducted work in a sandy loam containing 3 mg kg^{-1} Cd and reported that *S. oleraceus* accumulated 25.4 and 14.2 mg kg^{-1} Cd in the shoots and roots, respectively, resulting in a TF of 1.78.

5. Conclusions

P. weldenii and *S. oleraceus* displayed distinct responses to both Cd stress and soil amendment with a biostimulant including humic and fulvic acids. *P. weldenii* demonstrated typical Cd exclusion behavior, whereas *S. oleraceus* exhibited typical hyperaccumulator activity. These two behaviors were judged by their soil-to-plant transfer (TC) and root-to-shoot translocation (TF) values (TC close to, and TF much higher than, 1 for *S. oleraceus* vs. these indices being orders of magnitude lower for *P. weldenii*). This was also indicated by the fact that, although both plants were exposed to 45 mg kg^{-1} spiked Cd, *P. weldenii* exhibited a Cd fresh weight content lower than the regulatory limit for "leaf vegetables" of

0.2 mg kg^{-1}. On the other hand, *S. oleraceus* displayed a much higher Cd fresh weight content, in line with its characteristic hyperaccumulator behavior. The impact of biostimulant addition demonstrated divergent effects on the two test plants. Specifically, the application of biostimulant in *P. weldenii* exhibited a stabilization role for Cd, with a decrease in DTPA-extractable Cd in soil. On the other hand, in *S. oleraceus*, the addition of biostimulant led to the augmentation of Cd-DTPA levels, indicating a remarkable influence of this plant in assisting Cd release from soil. Due to the controlled environment of a pot experiment and the time constraints of a single growing season, the complexity and generalizability of the results may not have been fully captured. However, our research provides valuable insight into the existing literature. As these contrasting effects are rather novel and noteworthy, we recognize the necessity for further future research in an effort to elucidate the behavior of certain plants in influencing the geochemical dynamics of certain PTEs, like Cd, especially when biostimulants are applied to soil. In addition, conducting similar experiments in the field may provide insights into the ecological relevance of our findings and may allow extrapolation of our findings to field conditions where Cd toxicity naturally occurs. Finally, extending the study to multiple growing seasons and all plant growth stages, including the flowering stage, is necessary to determine the accumulation and translocation of contaminants in plant tissues throughout their life cycle.

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