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Original Research

Enhancing Phytoremediation Efficiency Using Regulated Deficit Irrigation

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Abstract

In this study, *Beta vulgaris* L. var. cicla was grown in cadmium-contaminated soil in a greenhouse. Regulated deficit irrigation was applied using three different irrigation levels (T1: 300 L, T2: 200 L, T3: 100 L per block during each irrigation event during the organogenesis stage; T1 was the control) to examine the effects on phytoremediation efficiency. According to the experimental results, the regulated deficit irrigation treatment (T2) decreased the *Beta vulgaris* L. var. cicla shoot biomass by 15.8%, increased the Cd concentration in the shoots by 23%, and maintained a constant root-shoot ratio. By contrast, T3 decreased the *Beta vulgaris* L. var. cicla shoot biomass by 33.0%, decreased the Cd concentration in shoots by 9.8%, and increased the root-shoot ratio by 62.8%. The Cd remediation potential efficiency (PE) of treatment T2 was 5.42 g ha^{-1} – i.e., 39.7% higher than that of T1 and 61.8% higher than that of T3. This study indicated that regulated deficit irrigation can be used to enhance Cd phytoremediation and save water, but should be applied in a suitable way.

Keywords: cadmium, phytoremediation efficiency, regulated deficit irrigation, water conservation

Introduction

Excessive cadmium (Cd) in soil can reduce soil productivity and subsequently result in economic losses in agricultural production. Cadmium is highly toxic to both animals and plants [1-3], even at relatively low concentrations [4]. In addition, unlike organic pollutants, Cd can persist in soils for a long time [5], resulting in extensive damage. Industrial and agricultural activities are the major sources of Cd enrichment in soils [6-7]. Over the past five decades, global Cd emissions reached approximately 22,000 t [8]. In China, 1/6 of cultivated land is contaminated by heavy metals, of which approximately 1/4 is polluted by Cd [9].

Among all soil remediation methods, phytoremediation is considered a cheap and sustainable technology [8], and it can preserve natural biodiversity while reducing pollution [10]. To enhance phytoremediation efficiency, plant growth regulators [11-13], bacteria, and fungi [10, 14, 15] have been used mainly to enhance the biomass of the phytoremediation plants, as Cd accumulated by plants is strongly related to plant dry biomass [16] (i.e., a higher biomass can result in higher phytoremediation efficiency). However, in northwestern China, a well-known arid and semi-arid area, the lack of irrigation water could be the major factor that limits the biomass of phytoremediation plants, and the high cost of irrigation would increase the cost of phytoremediation. Therefore, an approach that increases phytoremediation

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efficiency without requiring high amounts of irrigation water is needed.

In general, the yield and nutrient uptake of a plant decreases under drought stress [17-19]. However, because the concept of regulated deficit irrigation was developed only in the 1970s, drought stress was still considered to have only a negative effect on plant biomass accumulation. This concept has successfully been used to maintain yield as high as possible while reducing the amount of irrigation [20]. The use of regulated deficit irrigation indicates that suitable drought stress during the plant organogenesis stage may enhance shoot biomass or at least not result in a considerable decrease, and as a result increase phytoremediation efficiency. To the best of our knowledge, data pertaining to the use of drought to enhance phytoremediation efficiency are limited.

Thus, to test the hypothesis that drought stress can promote Cd phytoremediation efficiency and reduce water use, a field study with three different irrigation treatments was carried out at a Cd-contaminated site. This experimental site was located in northwestern China — a well-known semi-arid area with an annual precipitation of approximately 500 mm. There are numerous reports of soil pollution caused by heavy metals in this area [21-23]. The phytoremediation plant chosen in this study was Swiss chard (*Beta vulgaris* L. *var. cicla*), which is reported to be sensitive to soil Cd [24-26]. The results from this study will be meaningful for soil phytoremediation in arid areas.

Experimental Procedures

Experimental Site

The experiment was conducted in a greenhouse located in Yangling, Shaanxi Province (N34º17′28″, E108º00′27″), where half of the agricultural irrigation water was underground [27]. Soil in the greenhouse was polluted by Cd, and the pollution status was reported in Tang et al. [16, 23]. The soil was classified as loess soil, and some of the physical and chemical properties are listed in Table 1. The soil total Cd concentration was 1.87 mg kg^{-1}; DTPA-Cd was 0.36 mg kg^{-1}. The experimental field was located in a semi-humid area with an average annual precipitation of approximately 600 mm and an average annual evaporation of approximately 900 mm.

Experimental Layout

The experiment consisted of one factor, i.e., irrigation (T1: apply 300 L per block each time, T2: apply 200 L per block each time, T3: apply 100 L per block each time; T1 was the control), with a randomized block design and three replications. The study was carried out from April to July 2016, and the test period spanned the 2^{nd} to 6^{th} week after the chard was transplanted from the seedling nursery into each plot, i.e., the organogenesis stage of the chard. During time-out of test period, all the chard in plots were irrigated according to treatment T1. Each plot was 2 m wide and 5 m long. Field ridges (0.5 m wide) were used to separate the plots.

The chard was cultured according to the following method:

A) Chard seeds were sown in soil next to the experimental plots. The cultivation area was covered with polyethylene plastic film to maintain moisture before germination. When necessary, water was sprayed to maintain the soil water holding capacity at 60%.
B) When the seedlings matured and reached the four-leaf stage, they were transplanted into the plots. Before transplanting, the surface soil of the nine plots was well ploughed using a machine to ensure uniformity. The chard planting distance and row spacing were 40 cm and 30 cm, respectively.
C) One week after chard transplanting, the 9 plots were randomly divided into three groups and irrigated according to the treatment designation, i.e., T1, T2, or T3. Irrigation was applied during the test period at 17:00 on May 17, May 24, May 31, June 7, and June 14. The irrigation method was flooding. No fertilizer was applied before or during the growth period in order to exclude the effect of fertilizer on chard Cd uptake.

Determining Leaf Chlorophyll Content

Ten chard plants were randomly selected from each plot to determine the leaf chlorophyll content. The chlorophyll content of all mature leaves was measured using a SPAD-502 chlorophyll meter, and the average value was calculated.

Sampling and Sampling Pre-Treatment

Three chard plants were randomly selected from each plot, and the entire plants were carefully harvested by hand. Each chard plant was cleaned carefully using distilled water and high purity water to remove surface dirt. After air-drying, each sample plant was divided into shoots and roots and then dried at 80ºC to a constant weight. The fresh and dry biomass of each plant was measured. The dried samples were crushed using a stainless-steel plant tissue grinder (LD-Y500A, Shanghai, China). All samples were treated similarly.

After sampling, the aboveground parts of the remaining chard plants in each plot were harvested using a knife and were weighed to calculate the yield.

Cd Analysis

Cadmium was analyzed following the method published by Tang et al. [16]. A crushed plant sample (0.5 g) was added to a quartz container and mixed with 9 ml HNO_3 (GR) and 3 ml $HClO_4$ (GR) and then digested

Table 1. Surface soil properties of the experiment field (0-20 cm); data shown as mean ±SD, (n = 3).

Parameter	Units	Value
Soil type		Heavy loam
pH (water: soil = 5:1)		7.83±0.1
maximum field capacity	%	23±1
soil bulk density	g cm^{-3}	1.23±0.03
soil organic matter	g kg^{-1}	36.1±1.2
total nitrogen	g kg^{-1}	1.72±0.06
Olsen phosphate	mg kg^{-1}	302.6±8.52
available potassium	mg kg^{-1}	721.6±14.39
Cd	mg kg^{-1}	1.87±0.07
Available Cd[a]	mg kg^{-1}	0.36±0.03

[a]Note: extracted for 2 h at 25ºC with DTPA (DTPA-TEA-$CaCl_2$, pH7.3) as buffer solution

at 160ºC until almost dry. The digested liquid was made up to a volume of 25 ml using high-purity water. The Cd concentration in the liquid was detected using a flame atomic absorption spectrophotometer (AAS) (Z-2000, Hitachi, Japan) at a wavelength of 228.8 mm. Standard material, GBW-10015, was purchased from a standard material center in Beijing, China, to control the analytical quality. The recovery rate was 95±5%. The standard substance was made from spinach and can be used in the environmental analysis of leafy vegetables.

Cd uptake Capacity

To assess the Cd uptake capacity of the chard, the total Cd that was phyto-accumulated by a single chard plant (PU_{Cd}) and the chard Cd remediation potential efficiency (PE) were calculated according to Eq. (1-2) [26].

$$PUCd = [Cd]shoots\ (\mu g\ g^{-1}) \times BMshoots\ (g) + [Cd]roots\ (\mu g\ g^{-1}) \times BMroots \quad (1)$$

$$PE = [Cd]shoots\ (mg\ kg^{-1}) \times yield\ (kg\ ha^{-1}) \quad (2)$$

The extraction coefficient (EC), translocation factor (TF), and bioaccumulation factor (BAF) were calculated according to Eq. (3)-(5), respectively, in order to assess the effect of the irrigation treatments on the chard phytoremediation capacity [15, 28].

$$EC = \frac{[Cd]_{plant}\ mg\ kg^{-1}}{[Cd]_{soil}\ mg\ kg^{-1}} \quad (3)$$

$$TF = \frac{[Cd]_{shoots}\ mg\ kg^{-1}}{[Cd]_{roots}\ mg\ kg^{-1}} \quad (4)$$

$$BAF = \frac{[Cd]_{plant}\ mg\ kg^{-1}}{[Cd]_{available\ in\ soil} mg\ kg^{-1}} \quad (5)$$

Data Analysis

Data were statistically analyzed using the SigmaPlot 12.5 package (Systat Software Inc.) and Excel 2010. One-way analysis of variance and multiple comparisons (Duncan's Method) were used to compare the significance of the differences between groups ($\alpha = 0.05$).

Results and Discussion

Effect of regulated deficit irrigation on chard growth

The chard growth condition was based on characteristics such as biomass, root/shoot ratio, and the leaf chlorophyll content. Table 2 shows the individual biomass and root/shoot ratios of Swiss chard plants. It can be observed that in treatments T1, T2, and T3, the individual plant fresh biomass values were 639, 466, and 403 g, respectively, with corresponding dry biomass values of 43.6, 36.7, and 29.2 g. Significant differences were observed among the three treatments with respect to the fresh and dry biomass of individual Swiss chard plants.

The root-shoot ratios were 2.0, 2.3, and 3.0 based on fresh weight and 4.3, 4.3, and 7.0 based on dry weight in T1, T2, and T3, respectively. The root-shoot ratios of T1 and T2 were not significantly different, whereas the root-shoot ratio of T3 was significantly higher than that of T1 and T2. This indicates that chard plants in the T2

Table 2. Chard biomass and root/shoot ratios under three regulated deficit irrigation treatments (T1: 300 L, T2: 200 L, T3: 100 L per block during each irrigation event during the organogenesis stage; T1 was the control); FW: fresh weight, DW: dry weight (n = 15, $\alpha = 0.05$).

Treatments	Shoots			Roots			Root/shoot ratio	
	FW g^{-1}	DW g^{-1}	DW/FW %	FW g^{-1}	DW g^{-1}	DW/FW %	FW%	DW%
T1	639±12a	43.6±4.9a	6.6±0.2b	12.7±1.48a	1.8±0.2a	14.1±0. 4a	2.0±0.3b	4.3±1.0b
T2	466±17b	36.7±3.0b	8.1±0.1a	10.8±0.94a	1.6±0.4a	15.2±5.1a	2.3±0.3b	4.3±0.6b
T3	403±13c	29.2±0.3c	7.5±0. 5ab	12.0±1.05a	2.0±0.3a	17.1±0.7a	3.0±0.3a	7.0±1.2a

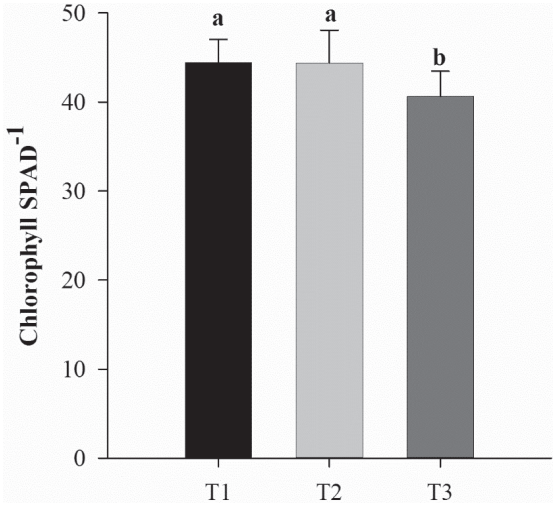

Fig. 1. Chlorophyll content in chard leaves under three regulated deficit irrigation treatments (T1: 300 L, T2: 200 L, T3: 100 L per block during each irrigation event during the organogenesis stage; T1 was the control); error bars stand for standard deviation (n = 9, α = 0.05).

treatment could adapt to drought stress compared with the T3 treatment. The root-shoot ratio is an indicator of the proportion of plant material contained in the root versus the shoot. Hoffmann [29] observed that chard maintained an almost constant root-to-leaf ratio, whereas the transpiration coefficient changed after irrigation at 100% and 30% of the required water supply. This could explain why the chard in the T1 and T2 treatments had very similar fresh and dry root-shoot ratios. This result indicates that although the irrigation frequency declined from T1 to T2, the proportion of material allocated to roots and shoots did not change. However, the chard in the T3 treatment was severely affected by the decline of the irrigation frequency. The results indicated that the material transport from the root to the shoot was restricted; consequently, the chard plants in this treatment exhibited the highest root-shoot ratio.

The leaf chlorophyll content measured in the three treatments supports the results presented above (Fig. 1). The leaf chlorophyll content is the main indicator of photosynthesis; under severe drought stress, the leaf chlorophyll content decreases [19]. To compare the effect of the three treatments on the chard leaf chlorophyll content, the leaf chlorophyll was measured using a SPAD-502 chlorophyll meter; these data are shown in Fig. 1. From a statistical point of view, the leaf chlorophyll content of T1 was equal to that of T2 but significantly different from that of T3. This means that in this field experiment, when the irrigation supply changed from T1 to T2, the chlorophyll content of the chard leaves did not change significantly. However, when the irrigation supply further decreased to T3, the chlorophyll content decreased dramatically. Iron is required for the formation of chlorophyll, and the results indicated that in the T2 treatment, drought stress did not negatively affect the transport of iron from the root to the shoot.

Effect of Regulated Deficit Irrigation on Cd Uptake

The Cd content of chard shoots and roots is shown in Table 3. The shoot Cd contents in T1, T2, and T3 were 1.12, 1.37, and 1.01 mg/kg, respectively. The T2 shoot Cd content was 23% and 37% higher than that measured in T1 and T3, respectively. However, there was no significant difference in the shoot Cd content between T2 and T1 or between T1 and T3. This indicated that under regulated deficit irrigation, such as the T2 treatment, the shoot Cd content would increase, but when drought stress was increased, the shoot Cd content would decrease.

The chard root Cd contents in T1, T2, and T3 were 1.70, 1.24, and 1.28 mg/kg, respectively (Table 3). In contrast to the Cd content in the shoots, the T1 treatment resulted in the highest Cd content in the roots, significantly higher than that in T2 and T3. This indicated that the chard plants in the T2 treatment were subjected to drought stress, the treatments settings in this trial were reasonable.

The total Cd extracted by a single chard plant (PU_{Cd}) was calculated (Eq. 1) based on the Cd content in both the roots and shoots. In T1, T2, and T3, the chard PU_{Cd} values were 47.0, 51.6, and 30.6 μg/plant, respectively (Table 3). Obviously, chard in the T2 treatment had the highest PU_{Cd} value, which was 9.8% and 68.6% higher than that in T1 and T3, respectively.

From a statistical point of view, chard plants in the T1 and T2 treatments had the same Cd uptake capacity for phytoremediation purposes. However,

Table 3. Chard shoots and roots Cd concentrations and PU_{Cd} and PE values under three regulated deficit irrigation treatments (T1: 300 L, T2: 200 L, T3: 100 L per block during each irrigation event during the organogenesis stage; T1 was the control); data shown as mean ± SD (n = 15, α = 0.05).

Treatments	Cd in shoots mg kg^{-1} DW	Cd in roots mg kg^{-1} DW	PU_{Cd} μg $plant^{-1}$	PE (shoots) g ha^{-1}
T1	1.12±0.09ab	1.70±0.20a	47.0±1.0a	3.88±0.19b
T2	1.37±0.16a	1.24±0.07b	51.6±4.3a	5.42±0.43a
T3	1.01±0.18b	1.28±0.06b	30.6±5.7b	3.35±0.74b

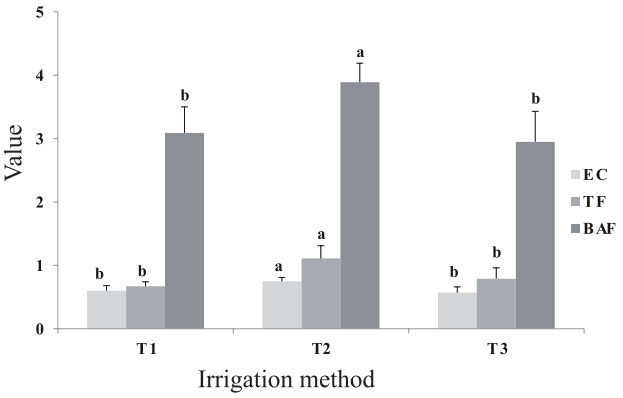

Fig. 2. Extraction coefficients (EC), translocation factor (TF), and bioaccumulation factor (BAF) in chard under different irrigation treatments (T1: 300 L, T2: 200 L, T3: 100 L per block during each irrigation event during the organogenesis stage; T1 was the control); data shown as mean ±SD (n = 3, $\alpha = 0.05$).

in practice, only the aboveground parts of chard are harvested; therefore, the PU_{Cd} does not represent the real remediation efficiency. Therefore, PE is a more suitable indicator to describe the Cd phytoremediation efficiency of chard [26]. The Cd remediation PE of chard plants in the T1, T2, and T3 treatments was 3.88, 5.42, and 3.35±0.74 g ha^{-1}, respectively (Table 3). The PE of chard plants was significantly higher in the T2 than in the T1 and T3 treatments. Although the PU_{Cd} value did not differ between chard plants treated with T1 and T2, the PE value differed significantly between these treatments because of the different effect of the irrigation treatments on the shoot and root Cd contents. The PE value of T2 was 39.7% higher than that of T1.

Effect of Irrigation Treatment on Chard Phytoremediation Capacity

To explore the reason why T2 improved Cd phytoremediation efficiency, EC, TF, and BAF values for chard under the three irrigation treatments were calculated (Fig. 2). EC, TF, and BAF are three main indicators used to assess the metal uptake capacities of plants. Generally, higher EC, BF, and TF values indicate a higher phytoremediation efficiency [12, 26, 28]. The EC, TF, and BAF values of chard plants in the T2 treatment were 0.75, 1.11, and 3.89, respectively, which were significantly higher than those in the T1 and T3 treatments. The EC, TF, and BAF values did not differ significantly between T1 and T3.

The EC, TF, and BAF values did not reach the standard of Cd-hyperaccumulators, and the Cd concentrations in chard shoots and roots were also very low in this study. The reason was that the soil Cd concentration in this study was 1.87±0.07 mg kg^{-1}, and the DTPA extractable Cd was only 0.36±0.03 mg kg^{-1}. These values are far lower than the soil Cd concentrations published by [26, 30], who also used chard in their experiments. The migration of Cd in alkaline soil is slower compared with acidic soil; under this condition, BAF is a more suitable indicator.

Putting the biomass and the leaf chlorophyll content into consideration, it can be easily seen that chard biomass accumulation was weakened, but the leaf chlorophyll content was not effected when changed from T1 to T2 treatment while both the chard biomass accumulation and leaf chlorophyll content were weakened when changed from T1 to T3 treatment. Under slight drought stress such as T2 treatment, the chard Cd uptake capacity would be increased, although the biomass accumulation rate may be restrained. For T2 treatment the reducing Cd accumulation amount caused by degradation of biomass can be denied by adding Cd concentration in shoots.

Conclusions

According to the results from this study, the regulated deficit irrigation treatment (T2) can be used to enhance phytoremediation efficiency while saving water. This treatment decreased the *Beta vulgaris* L. var. cicla shoot biomass by 15.8% but increased the Cd concentration in shoots by 23% and maintained a constant root-shoot ratio. The Cd remediation PE of T2 was 5.42 g ha^{-1}, i.e., 39.7% higher than that of T1 and 61.8% higher than that of T3.

This study indicated that regulated deficit irrigation can be used to enhance Cd phytoremediation and save water, but should be applied in a suitable way. Furthermore, this study also revealed that sufficient irrigation can reduce Cd uptake in plants, which will be useful in Cadmium pollution control. And for further research, we will test the applicability of our results on Cd phytoremediation by Cd-hyperaccumulators.

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Conflict of Interest

The authors declare no conflict of interest.

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