



Effects of Drought Stress on the Growth and Heavy Metal Accumulation by *Chromolaena odorata* Grown in Hydroponic Media

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Abstract

This study aimed to investigate the effects of drought stress on cadmium (Cd) and zinc (Zn) accumulation in *Chromolaena odorata* grown in an artificially contaminated nutrient solution for 15 days. Polyethylene glycol (5% PEG) was used as a drought stressor. The presence of PEG did not affect the chlorophyll content and photochemical efficiency, while drought stress induced by PEG caused a decrease in water content in the plant tissues. The bioaccumulation factor (BAF) of Cd were higher than the BAF of Zn and accumulated mainly in the roots of *C. odorata*. The highest concentrations (4273.7 mg/kg Cd, 2135.4 mg/kg Zn) were found in the 20 mg/L treatment. The results suggested that Cd and Zn accumulation in *C. odorata* was not affected by PEG, while a translocation factor (TF) value < 1 was caused by either PEG or contaminants. Based on the hydroponic BAF criterion, the study confirmed that *C. odorata* was useful for phytoremediation of Cd with low drought stress.

Keywords *Chromolaena odorata* · Phytoremediation · Bioaccumulation · Drought stress tolerance · Hydroponic conditions

Drought is one of the most affecting environmental stresses to plant productivity (Hesham and Fahad 2020; Seleiman et al. 2021). In some cases, plants may die under extreme drought stress (Zargar et al. 2017). Thailand has faced drought situations almost every year, and extreme droughts often occur in the dry season. The effects of drought on agriculture are aggravated due to the depletion of water resources. Meanwhile, heavy metal contamination of agricultural soils can be as a result of long-term farming or the excessive use of agrochemicals and heightens the risk of health problems (Kumar et al. 2020). Due to the long-term use of fertilizers, cadmium (Cd) and zinc (Zn) have a higher accumulation potential in agricultural soil (Wang et al. 2020). To date, it is extremely challenging to recover the soil environment after heavy metals contaminate the soil during drought.

Evidently, phytoremediation is an eco-friendly approach that could be a successful mitigation measure to remediate heavy metal-polluted soil in a cost-effective way (Yan et al. 2020). Nevertheless, drought is an important aspect to consider when selecting plants suitable for metal remediation

purposes, because it can induce osmotic stress in plants, resulting in decreased cell expansion, a decrease in root hair, and changes in the number and area of leaves. Hence, selection of drought resistance species can be considered to be an important trait in phytoremediation of soils polluted with heavy metals (Meher et al. 2018; Yang et al. 2021).

However, the combined responses of heavy metal accumulation in plants to drought conditions are relatively scant, for example in maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) (Azizian et al. 2013; Khan et al. 2018). For this reason, testing for improved plant drought resistance with high accumulation and tolerance to heavy metals is a rather difficult and time-consuming process. Moreover, while the influence of drought stress on the Siam weed (*Chromolaena odorata*) in the accumulation of various heavy metals has been described with different parameters, the effects of drought stress on the photochemical efficiency and water content (WC) of the Siam weed leaf have not yet been comprehensively analyzed.

In this study, we used polyethylene glycol (PEG-6000) solutions to induce drought stress in higher plants. *C. odorata* was investigated for its metal tolerance and accumulation under drought conditions in an artificially contaminated nutrient solution. This is a quickly growing plant and was used as a target species with great potential to remove toxic metals from soil and water (Atagana 2011; Tanhan et al.

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2011). Meanwhile, the photosynthetic performance and WC in response to drought stress were also investigated as our understanding of the growth responses and metal accumulation of *C. odorata* to drought stress is still limited. For this reason, understanding the combined effects of these stresses is important. The purpose of this study, therefore, was to test the ability of *C. odorata* to accumulate Cd and Zn under low drought stress through a hydroponic experiment.

Materials and Methods

Plants were obtained from areas where there is no history of heavy metal contamination. They were propagated by stem cuttings and grown in a greenhouse under natural conditions for 2 months. Approximately 40 cm-long cuttings selected for uniformity based on diameter and height were transplanted to plastic containers filled with 20% Hoagland solution at pH 5.5 and acclimatized for 1 week prior to the experiment. The plants were treated with different concentrations of Cd (CdSO_4) and Zn (ZnSO_4) in 400 mL of Hoagland solution, which contained one heavy metal. A treatment group was attributed to drought stress by adding 5% of PEG-6000. The experiment was arranged into four treatments: nutrient solutions with PEG only (T0), and 5, 10, and 20 mg/L of each metal combination with PEG (T1–T3). The corresponding controls were 5, 10, and 20 mg/L of each metal without the addition of PEG (C1–C3). Each treatment was prepared in triplicate, and each replicate consisted of one plantlet. Meanwhile, the Hoagland solutions enriched with PEG at 0%, 5%, and 10% served as means of photochemical efficiency and WC measurement. All the experiments were conducted in a controlled environment chamber in a greenhouse under the long-day photoperiod with a temperature of 27–30°C during the light and dark periods. The solutions were not aerated, and the nutrient solutions were changed every 5 days. Deionized water was added to maintain a constant volume of solution every day. The pH level of the solutions was adjusted to 5.5 daily either with 1 M NaOH or 1 M HCl using a digital pH meter.

After 15 days, the plant growth parameters, WC, performance index (PI), photosynthetic pigments, and heavy metal accumulation were determined. The plant samples were collected, washed with tap water twice, and rinsed with distilled water. The samples were then oven-dried at 65 °C for 3 days and homogenized with a mill (IKA: Japan). Dried plant tissues (0.5 g) were extracted with 5 mL of 69% HNO_3 in a digestion block until the solution was clear. The samples were filtered using Whatman No. 42 filter paper, and the filtrates were adjusted to 25 mL in a volumetric flask with deionized water. For Cd and Zn determination, a flame atomic absorption spectrometer (FAAS) (SpectrAA 55B, Varian) with a hollow cathode lamp was used. The wavelengths were set at 228.8 (Cd)

and 213.9 nm (Zn), while hollow cathode lamps were operated at 4 and 5 mA for Cd and Zn, respectively. Quantification was carried out with a calibration curve obtained from a series of diluted standard solutions with a coefficient of determination (r^2) higher than 0.995. The levels of detection for Cd and Zn on the FAAS were calculated from the calibration curve. The stem height and root length were measured, while the toxicity symptoms were visually assessed throughout the experiment. The phytoremediation efficiency of the plants was calculated according to the following equations (Hunt 1978; Cui et al. 2007; Wilson and Pyatt 2007):

$$\text{BAF} = \frac{\text{Metal concentration in shoot (mg/kg DW)}}{\text{Metal concentration in medium (mg/kg DW)}}$$

$$\text{TF} = \frac{\text{Metal concentration in shoot (mg/kg DW)}}{\text{Metal concentration in root (mg/kg DW)}}$$

$$\text{RGR} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$

where RGR is the relative growth rate (g/g/d) and W_1 and T_1 as well as W_2 and T_2 are the initial and final dry weights and times for each treatment, respectively.

Chlorophyll fluorescence was assessed using a portable photosynthesis meter. The photochemical efficiency of PSII (F_v/F_m) for dark adapted leaves was calculated from the measured parameters (Maxwell and Johnson 2000). The PI was also determined using a chlorophyll fluorometer (Pocket PEA, Hansatech Instruments Ltd, King's Lynn, Norfolk, UK). At least 30 readings from a leaf were used to get one final average reading.

The chlorophyll content was calculated according to the equations of Korkmaz et al. (2010) based on mg chl/g fresh weight (FW):

$$\text{Chl a} = 12.25 \times A_{663.6} - 2.55 \times A_{646.6}$$

$$\text{Chl b} = 20.31 \times A_{646.6} - 4.91 \times A_{663.6}$$

$$\text{Chl a} + \text{b} = 17.76 \times A_{646.6} + 7.34 \times A_{663.6}$$

where Chl a and b are chlorophyll a and chlorophyll b, respectively.

The WC was measured and expressed as a percentage according to the following formula (Xu et al. 2006):

$$\text{WC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

The mean and standard errors of the three replicates were calculated, and the statistical significance was evaluated using the SPSS-23.0 statistical software package (SPSS Inc.) with ANOVA at $p < 0.05$ according to SPSS.

Results and Discussion

A slight decrease in root length under the PEG treatment was observed with the increase of heavy metal concentration and showed a significant difference in comparison to the controls (C1–C3) (Table 1). Meanwhile, a slight but non-significant reduction in stem height was observed in plants grown in Cd with the PEG treatments ($p > 0.05$). The dry plant biomass and RGR values in each treatment also showed no significant mean difference ($p > 0.05$). However, 15 days after the initiation of the treatments, only root length reduction was significantly influenced by drought stress in all the treatments ($p < 0.05$). This effect might be due to the reduction in relative turgidity and protoplasm dehydration caused by turgor loss that results in reduced cell expansion and cell division, which ultimately reduces the root length. According to drought tolerance mechanisms in plants, root length, density, and size are key responses of plants to drought (Salehi-Lisar and Bakhshayeshan-Agdam 2016). Obviously, root length was not influenced by the increasing concentration of heavy metals, which was consistent with the results of several other. They emphasized that the toxicity of heavy metal stress in the medium depends on the available concentration added (Zeng et al. 2011; Li et al. 2012). Additionally, the heavy metal alone or combined with drought had no significant effect on the dry biomass of plants at the highest concentration of 20 mg/L compared to unstressed plants.

This phenomenon might be due to the increased synthesis of cell wall polysaccharides in the nutrient solution (Liu et al. 2015). Furthermore, large biomass may induce the dilution effect on the heavy metal, thereby leading to less toxicity (Wu et al. 2016).

The chlorophyll content, WC, and fluorescence parameters of the plants under various PEG treatments are depicted in Table 2. The results clearly show that the chlorophyll a content surpassed that of chlorophyll b. Under all three levels of the PEG treatment used, however, the observed differences in the total chlorophyll content of chlorophyll a and chlorophyll b were not significant ($p > 0.05$) after a 15-day trial. With an increase in PEG concentrations, there was no decrease in leaf chlorophyll content of *C. odorata*. It is indicated that *C. odorata* shows low sensitivity to drought stress. In other species, it has been reported that the chlorophyll level decreases or is unchanged depending on the duration and severity of the drought (Salehi-Lisar and Bakhshayeshan-Agdam 2016). Hence, chlorophyll content seems to be a useless tool to study the photosynthetic performance under low levels of drought-stressed conditions for a short period of 15 days. No significant differences were observed in the values of PI and Fv/Fm between planted PEG and unstressed plants ($p > 0.05$). The unaffected Fv/Fm values confirm the high stability of the quantum yield of primary photochemistry under drought stress, as reported by Tezara et al. (2003).

However, PEG-induced drought stress markedly decreased WC in plants ($p < 0.05$). A significant decline in

Table 1 Dry biomass, root length, stem height, and RGR of *C. odorata* were measured in hydroponics with various concentrations of Zn and Cd with and without 5% PEG

Treatment	Dry biomass (g/plant)		Root length (cm)	Stem height (cm)	RGR (g/g/day)
	Initial	Final			
Zn					
T0	1.3 ± 0.05 ^{a,A}	2.6 ± 0.16 ^{a,A}	11.8 ± 1.26 ^{a,A}	47.8 ± 1.35 ^{a,B}	0.087 ± 0.02
T1	1.2 ± 0.12 ^{a,A}	2.3 ± 0.09 ^{a,A}	10.8 ± 0.33 ^{a,A}	46.2 ± 0.88 ^{a,A}	0.042 ± 0.01
C1	1.0 ± 0.02 ^{a,A}	2.1 ± 0.24 ^{a,A}	13.1 ± 1.37 ^{b,A}	46.7 ± 1.09 ^{a,A}	0.044 ± 0.01
T2	1.3 ± 0.19 ^{a,A}	2.4 ± 0.43 ^{a,A}	10.0 ± 0.50 ^{a,A}	44.2 ± 0.88 ^{a,A}	0.038 ± 0.02
C2	1.4 ± 0.11 ^{a,A}	2.3 ± 0.32 ^{a,A}	13.8 ± 0.33 ^{b,A}	48.8 ± 0.88 ^{a,A}	0.033 ± 0.01
T3	1.2 ± 0.19 ^{a,A}	2.2 ± 0.13 ^{a,A}	11.1 ± 1.37 ^{a,A}	47.2 ± 0.88 ^{a,B}	0.041 ± 0.02
C3	1.4 ± 0.09 ^{a,A}	2.1 ± 0.27 ^{a,A}	15.6 ± 1.37 ^{c,A}	45.5 ± 1.15 ^{a,A}	0.024 ± 0.02
Cd					
T0	1.3 ± 0.24 ^{a,A}	2.1 ± 0.95 ^{a,A}	13.1 ± 1.23 ^{a,A}	43.7 ± 0.92 ^{a,A}	0.053 ± 0.02
T1	1.4 ± 0.11 ^{a,A}	1.9 ± 0.22 ^{a,A}	12.3 ± 0.44 ^{a,A}	44.6 ± 0.31 ^{a,A}	0.020 ± 0.01
C1	1.2 ± 0.12 ^{a,A}	1.5 ± 0.18 ^{a,A}	15.8 ± 1.45 ^{c,B}	45.4 ± 2.33 ^{a,A}	0.013 ± 0.01
T2	1.2 ± 0.12 ^{a,A}	2.4 ± 0.30 ^{a,A}	10.8 ± 0.33 ^{a,A}	45.2 ± 3.94 ^{a,A}	0.043 ± 0.01
C2	1.0 ± 0.02 ^{a,A}	2.4 ± 0.21 ^{a,A}	14.2 ± 0.50 ^{b,A}	47.2 ± 5.18 ^{a,B}	0.056 ± 0.01
T3	1.3 ± 0.17 ^{a,A}	2.1 ± 0.24 ^{a,A}	11.3 ± 0.60 ^{a,A}	43.7 ± 9.63 ^{a,A}	0.031 ± 0.02
C3	1.4 ± 0.11 ^{a,A}	2.5 ± 0.43 ^{a,A}	14.8 ± 0.33 ^{b,A}	46.7 ± 1.20 ^{a,A}	0.036 ± 0.02

Value expressed as mean ± SE; lower case letters represent differences between the same treatments within each heavy metal concentration; different uppercase letters represent significant differences between the Zn and Cd treatments. Columns indexed by the same letter are not significantly different according to the Duncan test ($p < 0.05$)

Table 2 Chlorophyll content, fluorescence parameters, and WC of *C. odorata* grown under different PEG stress conditions after 15 days of treatment

PEG supply	Chlorophyll content (mg/g FW)			PI	Fv/Fm	WC (%)
	Total	a	b			
0%	19.65 ± 1.51 ^a	11.69 ± 1.12 ^a	7.96 ± 1.04 ^a	4.76 ± 0.003 ^a	0.82 ± 0.006 ^a	91.3 ± 0.393 ^b
5%	21.87 ± 0.54 ^a	13.65 ± 1.08	8.22 ± 0.96	6.29 ± 0.006 ^a	0.81 ± 0.003 ^a	77.8 ± 0.751 ^a
10%	20.91 ± 0.68 ^a	12.19 ± 0.66	8.12 ± 0.94 ^a	5.38 ± 0.004 ^a	0.79 ± 0.003 ^a	76.8 ± 0.265 ^a

Values are mean ± SE; columns indexed by the same letter are not significantly different according to the Duncan test ($p < 0.05$)

WC was clearly observed in the plant tissues, which suggests that drought stress imposes a greater negative impact on water balance in *C. odorata* than photochemical efficiency and chlorophyll content. Evidently, the exposure of drought resulted in decreasing plant water potentials, indicating that osmotic stress was involved (Mohammadkhani and Heidari 2007; Meher et al. 2018).

The heavy metal accumulation levels by the studied plants are summarized in Table 3. Zn and Cd accumulated by the roots and shoots of *C. odorata* increased significantly with an increase in applied metal solution concentration. Zn accumulation increased significantly in combined stress compared to the controls (metal alone) but to a much smaller degree than Cd ($p < 0.05$). The highest Cd and Zn contents were found in roots with concentrations of 4273.7 mg/kg Cd and 2135.4 mg/kg Zn at the 20 mg/L treatment. In the case of Cd, the plant exhibited higher BAF values than that of Zn, with the highest BAF value of 24.3 at 5 mg/L Cd under drought stress, indicating *C. odorata* has a stronger ability to accumulate Cd. This is consistent with findings from previous studies indicating *C. odorata* had a higher potential for Cd accumulation in both hydroponics and pot trials by the

absence of drought (Jampasri et al. 2021). According to our results, all TF values less than 1 indicate that *C. odorata* is suitable for phytostabilization. In hydroponic systems, the consistent nutrient supplies might restrict the translocation of heavy metals due to the competition of carrier channels to limit non-essential elements or due to the precipitation of metals in plant tissues. Furthermore, metal ions must first pass through a Casparian strip in the roots, which serves as a limiting step in heavy metal translocation to the shoots. This characteristic could help the plant avoid the toxic effects of the heavy metal and cellular damage (Hamim et al. 2018). However, a BAF value > 1 is used in hydroponic tests to determine a plant's ability to accumulate metals (Sabeen et al. 2013). The results revealed that the decrease in BAF was caused by an increase in heavy metal concentration, but was not by drought. It is possible that PEG-induced drought is not severe enough to reduce the ability of *C. odorata* to accumulate heavy metals.

In case of drought stress, our data shows that overall, the BAF of Cd were higher than the BAF of Zn, which indicates the strong ability of *C. odorata* to accumulate and transfer the Cd-contaminated nutrient solution.

Table 3 Zn and Cd accumulation in the roots and shoots, TF, and BAF of *C. odorata*

Metal	Concentration (mg/L)	Metal accumulation (mg/kg)		TF	BAF
		Root	Shoot		
Zn	5 + PEG	752.8 ± 1.49 ^{a,A}	72.8 ± 0.14 ^{b,A}	0.10	14.6
	5	1039.0 ± 0.52 ^{b,B}	53.3 ± 0.60 ^{a,A}	0.05	10.7
	10 + PEG	1597.5 ± 0.40 ^{b,D}	99.8 ± 0.28 ^{a,B}	0.06	10.0
	10	1298.5 ± 0.60 ^{a,C}	98.6 ± 0.22 ^{a,B}	0.08	9.9
	20 + PEG	2135.4 ± 0.38 ^{a,D}	130.9 ± 1.15 ^{b,C}	0.06	6.5
	20	1902.0 ± 0.55 ^{b,D}	112.2 ± 0.41 ^{a,C}	0.06	5.6
Cd	5 + PEG	2059.3 ± 0.63 ^{a,B}	121.9 ± 1.15 ^{b,B}	0.06	24.3
	5	2302.6 ± 0.60 ^{b,B}	91.8 ± 0.32 ^{a,A}	0.04	18.3
	10 + PEG	3039.5 ± 0.25 ^{a,C}	182.6 ± 0.32 ^{a,C}	0.06	18.3
	10	2936.4 ± 0.44 ^{a,C}	176.9 ± 0.40 ^{a,C}	0.06	17.7
	20 + PEG	4273.7 ± 0.84 ^{b,D}	253.3 ± 0.92 ^{b,D}	0.06	12.7
	20	3973.8 ± 0.63 ^{a,D}	216.7 ± 0.69 ^{a,D}	0.05	10.8

Each value is the mean ± SE of three replicates. Lower case letters represent differences between the same treatments within each heavy metal concentration; different uppercase letters represent significant differences between the Zn and Cd treatments. The columns indexed by the same letter are not significantly different according to the Duncan test ($p < 0.05$)

Similar results reported elsewhere show that *C. odorata* has a higher accumulating ability for Cd than Zn in the hydroponic experiment without drought (Swapna et al. 2014). On the other hand, Eissa and Negim (2018) found that the accumulation of Zn was higher than that of Cd in the case of spinach (*Spinacia olivera* L.) and lettuce (*Lactuca sativa* L.) irrigated with sewage wastewater, which was not the case for *C. odorata*. This different accumulation could result from a specific mechanism that confers a broad resistance to several different metals or may involve a series of metal-specific mechanisms in different plants (Weerakoon 2019). Consequently, the results displayed in Table 3 reveal that the levels of Zn and Cd accumulation in *C. odorata* were unaffected by PEG. This finding was supported by Naidoo and Naidoo (2018), who discovered that *Chromolaena* is a drought-avoidant species. Similar to other plant species, Zn accumulation in maize and Cd uptake in sunflower were unaffected by drought stress in pot experiments (Castillo-Michel et al. 2009; Khan et al. 2018).

By the end of the trial, the results revealed that the accumulation of heavy metals in *C. odorata* was unaffected by drought. However, *C. odorata* was not efficient in translocating Zn and Cd from root to shoot under either heavy metals or drought stress. This 15-day hydroponic study test confirmed the tolerance of *C. odorata* to low levels of drought created by adding PEG. Based on the BAF criterion, *C. odorata* makes a noteworthy choice in Cd phytoremediation under drought-stressed conditions. Additional studies are required to be confirmed by pot or field trials which, considered together, significantly affect the capacity of heavy metal absorption in drought environmental conditions.

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