Lead Accumulation of Siam Weed (*Chromolaena odorata*) Grown in Hydroponics Under Drought-stressed Conditions

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**ABSTRACT**

The phytoremediation potential of Siam weed (*Chromolaena odorata*) was tested in lead (Pb) contaminated nutrient media with 5% (w/v) of polyethylene glycol (PEG) 6000 induced drought stress conditions. The plant was treated with 0, 5, 10, 20, and 50 mg/L Pb for 15 days. Different concentrations of Pb or in combination with PEG had no effect on plant growth parameters. Drought reduced water content (WC) (*p*<0.05), but did not affect the reduction of chlorophyll content and photochemical efficiency in plant tissues after 15 days of treatment. Under drought conditions, plants showed the largest Pb accumulation in roots (5,503.7 mg/kg) and exhibited the highest uptake at 50 mg/L solution (18.24 g/plant), but the translocation factor values (TFs) of Pb from root to shoot were all less than 1. Under both drought and non-drought conditions, the bioconcentration factor values (BCFs) decreased with increasing Pb concentrations. According to BCFs and TFs, *C. odorata* may be promising for phytostabilization of Pb. Based on high biomass, tolerance, and Pb uptake, the result of this hydroponic study test reveals that *C. odorata* has a good potential for developing Pb phytoremediation strategies in drought-stressed conditions.

1. INTRODUCTION

Lead (Pb) is a major toxicological concern of the present day that demands immediate attention and has been listed as a hazardous heavy metal pollutant due to its high toxicity (Qi et al., 2018). Pb 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). Excessive Pb results in reduced soil fertility and health, affecting plant growth and leading to reduced crop production (Hassan et al., 2014). Phytoremediation has generated a great deal of interest as a cost-effective plant-based technology for the removal of toxic heavy metals from contaminated soil under natural field and greenhouse conditions (Jabeen et al., 2009). Even if the phytoremediation technique seems to be one of the best alternatives, however, abiotic factors such as drought (or water deficit) may restrict the rate of plants growth that can be affected through the use of plants for the phytoremediation process (Tangahu et al., 2011).

Drought is one of the most serious constraints to agricultural crops, causing plant growth inhibition and low productivity (Seleiman et al., 2021). Meanwhile, Pb has been released into the agricultural area from metallurgical mining activities and the long-term use of agrochemicals (Alengebawy et al., 2021). Thailand has encountered drought almost every year, and extreme droughts occur frequently during the summer season. Increasing droughts have also increasingly impacted the phytoremediation efficiency of heavy metal contamination. Due to drought stress, which causes many physiological and biochemical changes in plants, making osmotic adjustment maintenance critical and leading to a reduction in the biomass of plants (Ozturk et al., 2020). Hence, drought is an important aspect to consider when selecting suitable plants for metal extraction purposes. There are some drought-tolerant plants that show potential for accumulating heavy metals with soil remediation, such as Bermuda grass (*Cynodon dactylon*) (Sekabira et al., 2011) and Shrub Violet (*Hybanthus floribundus*) (Kachenko et al., 2011). Nevertheless, several drought-tolerant plant species have rather limited uses. This is because they each show low biomass production and slow growth.
habit, and the responses of plant drought tolerance to drought stresses and impacts on heavy uptake have not yet been comprehensively studied. For this reason, the identification of novel plant species with high biomass yield, coupled with the ability to tolerate and accumulate multiple metals, has become an important aspect of phytoremediation research (Hemen, 2011).

At present, it is important to overcome the problem of metals toxicity and drought stress due to water shortage. *Chromolaena odorata*, known as Siam weed, is widely distributed throughout the country, especially in areas with a pronounced dry season. Studies show that it succeeds in the accumulation of multiple heavy metals (Cd, Zn, and Pb), with phytoremediation potential even in the presence of high heavy metal concentrations (Phaenark et al., 2009; Jampasri et al., 2021). Due to the ubiquity of this native species, it has a relatively high biomass with the ability to accumulate high concentrations of heavy metals (Tanhan et al., 2007; Phaenark et al., 2009; Khaokaew and Landrot, 2015). In addition, Naidoo and Naidoo (2018) indicated that *C. odorata* is a drought-avoider species. However, there are no reports on the combined effects of drought and Pb on the phytoremediation efficiency of *C. odorata*, including the ability to deal with drought. This study aims to investigate the drought tolerance and phytoremediation potential of *C. odorata* on Pb accumulation in a hydroponic experiment. The effects of drought on chlorophyll content, fluorescence parameters, and water content (WC) were also determined.

2. METHODOLOGY
2.1 Plant materials and hydroponic conditions

*Chromolaena odorata* used in this study was obtained from the campus of Srinakharinwirot University, Nakorn Nayok Province, Central Thailand, where there is no history of heavy metal contamination. Plants were grown from stem cuttings in a greenhouse under natural conditions for two months. The uniform plants were grown in a semi-enclosed container for one week prior to the experiment in 400 mL of 20% Hoagland solution at pH 5.5. All plants were treated with different concentrations of lead (II) nitrate [Pb(NO$_3$)$_2$], while drought stress was applied by adding 5% (w/v) of PEG-6000 to Hoagland solution for 15 days (Ranjbarfordoei et al., 2000). The experiment was arranged into five treatments: nutrient solutions with PEG only (T0), and 5, 10, 20, and 50 mg/L of Pb combinations with PEG (T1-T4) without both the combined effects of PEG and Pb (C0), and the same for all Pb concentrations without the addition of PEG served as control (C1-C4). Those metal concentrations were the initial range recommended by previous research for *C. odorata* screening tests in hydroponic experiments (Tanhan et al., 2007). All experiments were conducted with three replicates per treatment (each replicate consisted of one plantlet). *C. odorata* grown in the Hoagland solutions enriched with PEG-6000 (w/v-5%, 10%, and 20%) without contamination was conducted to find the effect of drought stress on chlorophyll contents, fluorescence parameters, and WC for 15 days. All 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. All solutions were not aerated and were topped up with the original solution daily.

2.2 Determination of plant growth

The shoot heights and the root lengths for controlled and treated plants were measured using a metric ruler. Plant samples were thoroughly washed with tap water and deionized water, separated into shoots and roots, and oven-dried (65°C for 72 h). Then, the dry weight of the shoots and roots was recorded using an analytical balance. Reduction (%) in shoot heights and the root length was calculated as a percentage of the control.

2.3 Determination of chlorophyll content, chlorophyll fluorescence parameters and water content

The leaf chlorophyll content was measured using a spectrophotometer from acetone (80% v/v) extract, and calculated using the equation of Porra et al. (1989) and Holm (1954) on the basis of mg chl/g fresh weight (FW) (Korkmaz et al., 2010):

\[
\text{Chl a (mg/g FW)} = 12.25 \times A_{663.6} - 2.55 \times A_{646.6}
\]

\[
\text{Chl b (mg/g FW)} = 20.31 \times A_{646.6} - 4.91 \times A_{663.6}
\]

\[
\text{Chl a + b (mg/g FW)} = 17.76 \times A_{646.6} - 7.34 \times A_{663.6}
\]

Where: Chl a=chlorophyll a; Chl b=chlorophyll b; $A_{663.6}$=absorbance at a wavelength of 663.6 nm; $A_{646.6}$=absorbance at a wavelength of 646.6 nm.
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 maximum quantum use efficiency (Fv/Fm) of photosystem II (PSII) for dark adapted leaves was calculated as \([-\frac{(F_{m}-F_{0})}{F_{m}}]\), according to the equations reviewed by Stirbet and Govindjee (2011).

The Performance Index (PI) was 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.

### 2.4 Determination of Pb

The dried plants were grounded into powder and sieved through a 2 mm mesh sieve. A subsample (0.5 g) of each group’s plant sample was digested in 2:1 HNO\(_3\):HCIO\(_4\) (v/v) using the open tube digestion method (Simmons et al., 2005). Aqueous extracts of the plants were analyzed by a flame atomic absorption spectrometer (FAAS) (SpectrAA 55B, Varian) for Pb determination. The FAAS with a hollow cathode lamp was used. The wavelengths were set at 283.3 nm, while hollow cathode lamps were operated at 7.5 mA. 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 Pb on the FAAS were calculated by the calibration curve.

### 2.5 Data analysis

Bioconcentration factor (BCF): the BCF was calculated as a ratio between the Pb concentration in the plant tissue and the Pb-spiked concentration in the solution (Garg and Chandra, 1994). The calculation of BCF was expressed as shown below:

$$\text{BCF} = \frac{\text{Pb concentration in whole plant (mg/kg dry weight)}}{\text{Initial Pb concentration in solution (mg/kg dry weight)}}$$

Translocation factor (TF): the Pb translocation in these plants from root to shoot was measured using TF (Cui et al., 2007), which is given below:

$$\text{TF} = \frac{\text{Concentration of Pb in shoot (mg/kg dry weight)}}{\text{Concentration of Pb in root (mg/kg dry weight)}}$$

Where: TF>1 indicates that the plant translocated Pb effectively from the roots to the shoots (Baker and Brooks, 1989).

Pb uptake: the total uptake of Pb in plants was calculated as follows (Zhang et al., 2012):

$$\text{Pb uptake} = \frac{\text{Total Pb concentration in plant (mg/kg) \times plant dry weight (g/plant)}}{1,000}$$

Relative growth rate (RGR) was calculated according to Hunt’s equation (1978):

$$\text{RGR} = \frac{\ln W_f - \ln W_i}{T_2 - T_1}$$

Where: RGR is the relative growth rate (g/g/d), and W1, T1, W2, and T2 are the initial and final dry weights and times for each treatment respectively.

### 2.6 Statistical analysis

The mean and standard errors of the three replicates were calculated and the statistical significance evaluated using the SPSS-23.0 statistical software package (SPSS, Inc.) with the one-way analysis of variance (ANOVA). A significance level of 0.05 was used in all treatment comparisons and applied using the least significant difference (LSD).

### 3. RESULTS AND DISCUSSION

#### 3.1 The combined effects of Pb and PEG on plant growth

As shown in Table 1, Pb and PEG had no significant inhibitory effects on dry plant biomass, root length, stem height, and RGR values ($p > 0.05$), although T1-T4 root lengths were reduced to 87.6-93.0% of the controls (T0; 100%). Plants grown in all single Pb treatments showed a slight but non-significant reduction in stem height (90.7-99.7%) ($p > 0.05$). This 15-day hydroponic experiment confirmed the tolerance of C. odorata to low levels of drought stress created by adding PEG-6000 (PEG) at 5% (w/v) when combined with Pb concentrations ranging from 10 to 50 mg/L. In the case of Pb, our result was similar to that reported by Swapna et al. (2014), who performed a hydroponic experiment with the concentrations of 1 and 20 mg/L Pb for 30 days, though without drought. In the present study, nevertheless, a slight decrease (≈5-15%) in the root length of plants was observed in all treatments. This may be the result of root adaptation in the growth responses of C. odorata under heavy metal influence, or it may be because roots are one of the main drivers of water in the response to drought, which regulates their growth, root length, and organizational characteristics (Salazar et al., 2015; Omoregie and Ikhajiagbe, 2021).
Table 1. Dry biomass, root length, stem height, and RGR values of *C. odorata* grown in hydroponics with different concentrations of Pb combined or uncombined with PEG

| Treatment          | Dry biomass (g/plant) | Root length (cm) | Stem height (cm) | RGR (g/g/day) |
|--------------------|-----------------------|------------------|------------------|---------------|
| T0: 0 mg/L Pb+PEG  | 2.2±0.1               | 12.9±1.5         | 56.7±1.1         | 0.03±0.02     |
| C0: 0 mg/L Pb      | 2.6±0.8               | 15.6±0.7         | 60.2±0.9         | 0.05±0.01     |
| T1: 5 mg/L Pb+PEG  | 2.8±0.3               | 11.6±1.3         | 59.1±1.4         | 0.05±0.01     |
| C1: 5 mg/L Pb      | 3.3±0.1               | 13.1±0.9         | 56.2±0.3         | 0.06±0.01     |
| T2: 10 mg/L Pb+PEG | 3.1±0.4               | 12.0±1.7         | 58.2±1.1         | 0.07±0.02     |
| C2: 10 mg/L Pb     | 2.4±0.3               | 15.2±0.9         | 60.0±2.9         | 0.04±0.01     |
| T3: 20 mg/L Pb+PEG | 2.6±0.4               | 11.7±1.9         | 57.7±1.4         | 0.06±0.03     |
| C3: 20 mg/L Pb     | 3.3±0.3               | 14.9±1.1         | 55.8±2.1         | 0.05±0.01     |
| T4: 50 mg/L Pb+PEG | 2.9±0.4               | 11.3±1.9         | 56.8±0.6         | 0.06±0.04     |
| C4: 50 mg/L Pb     | 3.4±0.3               | 13.9±2.9         | 54.6±1.7         | 0.06±0.05     |

Values are expressed as mean±SE; columns indexed by the same letter are not significantly different according to LSD (p<0.05).

3.2 Effects of drought stress on chlorophyll content, fluorescence parameters and WC

Polyethylene glycol (PEG-6000; w/v-5%, 10% and 20%) was used for drought stress induction in *C. odorata* for 15 days. The concentration of total chlorophyll content, chlorophyll a and chlorophyll b, changed slightly (decreased/increased by around 10%) in drought-stressed leaves of all PEG-treated plants compared to control, where the chlorophyll a content predominated chlorophyll b in all treatments, as shown in Figure 1. The 20% PEG treatment produced a marked decrease in total chlorophyll content (13.38 mg/g FW) compared to the other treatments. However results suggested that the chlorophyll content of the plants remained unaffected by PEG.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Effects of PEG on chlorophyll content of *C. odorata* after 15 days of treatment

Although PEG-induced drought is not severe enough to inhibit total chlorophyll content significantly after 15 days of treatment, the highest decrease was recorded with 20% PEG treatment. This was in accordance with the other species, which reported decreased or unchanged chlorophyll levels during drought stress, depending on the duration and severity of the drought (Kpyoarissis et al., 1995). On the other hand, there are many reports indicating that the application of some heavy metals substantially increases (up to 10.5% by the application of zinc (Zn)) chlorophyll content, Fv/Fm, and photosynthetic characteristics under drought conditions (Karim et al., 2012; Ma et al., 2017).

Our results indicated that the photosynthetic and fluorescence parameters of *C. odorata* were unaffected by PEG levels, but that drought stress imposed by PEG-6000 caused a decrease in the WC of the plant (Table 2). The lowest percentage of WC (71.2%) in the plant leaves was observed in the final concentration of PEG. WC was significantly (P<0.05) reduced in PEG-treated plants, with an increased
The concentration of PEG compared to the control. The results are illustrated in Table 2, which shows that the values of PI and Fv/Fm showed similar trends, with the highest values of 5.88 and 0.82 in 5% of the PEG-treated plants, respectively.

### Table 2. The values of PI, Fv/Fm, and WC (%) of C. odorata grown under different PEG stress conditions after 15 days of treatment

| PEG supply (%) | PI       | Fv/Fm   | WC (%)   |
|---------------|----------|---------|----------|
| 0             | 4.98±0.011<sup>a</sup> | 0.81±0.008<sup>a</sup> | 92.3±0.293<sup>b</sup> |
| 5             | 5.88±0.002<sup>a</sup> | 0.82±0.004<sup>a</sup> | 78.9±0.551<sup>a</sup> |
| 10            | 5.11±0.009<sup>a</sup> | 0.79±0.007<sup>a</sup> | 76.4±0.551<sup>a</sup> |
| 20            | 4.08±0.005<sup>a</sup> | 0.70±0.009<sup>a</sup> | 71.2±0.365<sup>a</sup> |

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

Healthy plants with an efficient photosynthetic apparatus have typical Fv/Fm values of 0.82-0.83 (Adams and Demmig-Adams, 2004), whilst values of Fv/Fm below 0.60 are indicative of severe drought stress (Vilagrosa et al., 2010). In the present study, results in maximum quantum yield of PSII (Fv/Fm) range from 0.70 to 0.82 to indicate that the photosystems were functioning efficiently. According to Baker and Rosenqvist (2004), water stress has no major impact on the efficiency of PSII. Although Chromolaena sp. exhibits profuse vegetative growth when water is abundant, during drought stress, stomatal closure results in decreased leaf conductance, photosynthesis, and transpiration, which may be a sensitive response of the leaf to decreasing leaf water content, resulting in higher WC (%) reduction (Mandal and Joshi, 2014; Hailemichael et al., 2016). Based on the above criteria of Fv/Fm values, however, C. odorata was not affected when PEG was supplied in the range of 5-20%.

### 3.3 Pb accumulation in plant tissues

Lead concentrations in the roots and shoots of C. odorata were significantly higher (p<0.05) for all Pb concentrations. Moreover, the roots contained much higher concentrations, with 8-10 times more than in shoots (Figure 2 and Figure 3). Under drought conditions, both the root and shoot accumulation of Pb was significantly greater (3,173.3-5,503.7 and 441.6-786.3 mg/kg) than that in the control treatments (2,714.6-4,992.8 and 302.9-607.7 mg/kg) for all Pb solutions (p<0.05) except in the roots and shoots of 5 and 5-10 mg/L Pb treatment respectively. In its roots, at the highest Pb level in solution, C. odorata exhibited the highest accumulation (T4; 5,503.7 mg/kg) under drought stress compared to the others. The effect of PEG on Pb uptake, BCF, and TF values is shown in Table 3. Plants had a strong tolerance to PEG stress with no significant difference in Pb uptake between all nutrient solutions (p>0.05). Nevertheless, the BCFs of all treatments decreased and exhibited a range of 112.0-649.6 when Pb concentrations increased. In this study, all treatments had TFs<1 in all Pb solutions with a range of 0.04-0.14, and these values did not differ significantly between the single Pb treatment and that in combination with PEG. The findings of the BCF and TF tests show the ability of the plant to bioconcentrate the Pb in the root, implying that C. odorata is capable of Pb phytostabilization. By the end of the trial, the amount of Pb accumulation in C. odorata was unaffected by drought, while the translocation of Pb from the root to the shoot was affected by either single or combined stress.

![Figure 2](image-url) Pb accumulation in the roots of C. odorata grown for 15 days in increasing Pb concentrations with (T1-T4) and without PEG (C1-C4)
Our results showed that *C. odorata* accumulates high root Pb concentrations, which is supported by other hydroponic studies under non-drought conditions. Tanhan et al. (2007) indicated *C. odorata* had the capacity for higher accumulations of Pb in roots (51,493-60,655 mg/kg) than in shoots, while a similar pattern can be also found in other species such as *Salix lucida* (11,535 mg/kg), *S. nigra* (14,091 mg/kg), *S. serissima* (7,036 mg/kg), and *Acacia mangium* and *Eucalyptus camaldulensis* (>40,000 mg/kg) (Zhivotovsky et al., 2011; Yongpisangphop et al., 2017). In most plants, 90% of the total Pb is accumulated in the roots (Kumar et al., 1995). It possible that the cell walls of root plants are the first barrier against Pb stress and can immobilize and accumulate some or even most Pb ions. Pb in roots is localized in the insoluble fraction of cell walls and nuclei, which is linked to the detoxification mechanism (Piechalak et al., 2002).

In order to indicate the efficiency of accumulation ability, Pb uptake and BCF values were used. Evidently, PEG added to a Pb contaminated solution did not generate significant reductions in Pb uptake of *C. odorata* when compared to uncombined PEG. On the other hand, the combined effect of Pb and PEG significantly decreased BCFs (from 461.7 to 125.8) with increased Pb concentrations, which indicates that *C. odorata* shows a relatively low bioaccumulation potential by adding Pb concentrations. In hydroponic tests, a BCF value ≥1,000 is used to identify the capacity of a plant to accumulate metals (Syuhaida et al., 2014). According to Tanhan et al. (2007), *C. odorata* was discovered to be a Pb hyperaccumulator based on a hydroponic test without drought. However, for this study, it is possible that *C. odorata* must deal with both Pb and PEG stress as a critical situation. Interestingly, in our comparison of combined and uncombined with PEG, the combined PEG treatment of 10-50 mg/L Pb solution displayed the higher BCFs, suggesting drought will cause a small increase in the ability of *C. odorata* to absorb Pb. In addition, the previous study found that the
accumulation of a few heavy metals, such as copper (Cu) and zinc (Zn) in soybeans, while accumulation in French marigold (Tagetes patula) under drought-stress conditions was largely increased (Aziz, 2015; Kleiber et al., 2020). Unlike Cu and Zn, which are taken up by plants as essential plant nutrient elements and play an important role in a plant’s metabolism, Pb is not an essential element for plants. Moreover, our results with BCFs<1.000 might be explained by the type of experiment conducted (i.e., hydroponics, greenhouse, field). In hydroponics, the number of consistent nutrient supplies might restrict the availability of Pb due to competition to limit non-essential element accumulation in plant tissues.

According to our results, all TF values less than 1 indicate that C. odorata is suitable for phytostabilization of Pb, as reported in other plant species grown under hydroponics such as Avicennia marina (Yan et al., 2010), Sedum alfredii (Gupta et al., 2010), Allium sativum (Jiang et al., 2019) and Phyllostachys pubescens (Liu et al., 2015). This suggests that a limited translocation of Pb occurs from the root to the other parts of the plant due to the precipitation of insoluble Pb salts in intercellular spaces, the accumulation of plasma membranes, or sequestration in the vacuoles (Yongpisaphop et al., 2017).

4. CONCLUSION
The capability of C. odorata to survive in a Pb-polluted solution is a clear indication of its tolerance, which is notable in its capacity for Pb uptake in the face of Pb- and PEG-induced stress. The application of PEG in nutrient solutions had no significant effect on plant growth, biomass, chlorophyll content, and fluorescence parameters. The reduction in WC (%), however, was caused by drought, while the translocation efficiency of Pb is limited by either Pb or drought. Based on the hydroponic BAFs and TF criteria, it indicates that C. odorata has the ability to accumulate Pb in the root and shows high Pb phytostabilization efficiency after 15 days of treatment. C. odorata may be considered, therefore, for Pb phytoremediation in contaminated soils under drought environmental conditions due to its tolerance to Pb and PEG combinations. In future studies, these hydroponic testing results will need to be confirmed by pot and field trial studies under drought conditions, which, considered together, significantly affect their capacity to absorb Pb.

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