The effects of lead on the growth of *Helianthus annuus*

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Abstract

Bioremediation is an increasingly viable option for the cleanup of toxic metals, such as lead, in soil. Phytoremediation is a special kind of bioremediation in which living plants are used for the removal or conversion of a compound into a benign substance. An effective phytoremediator would be able to immobilize lead and accumulate it in its tissue without negative impacts on its growth. In the present study, we tested whether lead negatively affects the growth of the common sunflower, *Helianthus annuus*, a well-known phytoremediator of lead for which information of growth under lead is missing. To do this, we grew sunflowers in different concentrations of lead and tested the growth of the shoot and biggest leaf of each plant after four weeks. We found that sunflowers grown in 100ppm and 600ppm lead had more growth than those grown in soils with no added lead nitrate. Our results suggest that *Helianthus annuus* is indeed an effective phytoremediator of lead.

Introduction

Leaching of toxic metals into soil, and contamination via strip, surface, and open-pit mining has become one of the major trade-offs of human economic development (Zhang et al. 2012). Finding effective techniques to clean up toxic metals from the environment has attracted a lot of research in the field of environmental engineering. A reclamation technique commonly used is bioremediation (Zhang et al. 2012) Bioremediation is the use of microorganisms or other living things to clean up pollutants (Dixit et al. 2015). Phytoremediation, a type of bioremediation, is typically used to clean up heavy metals from the soil through immobilization. Much research has been done on analyzing the quantities of heavy metals that plants can capture from the soil (Wuana and Okieimen 2011), but less is known about how these toxic contaminants affect plant growth. In the present study, we will assess how the growth of *Helianthus annuus*, a common phytoremediator, is affected by its capture of heavy metals from the soil.

It has been shown that *Helianthus annuus* is a hyperaccumulator at low levels of lead, meaning it has the capability of accumulating lead in its tissue but there is less research on this ability at higher concentrations and the effect of the lead on the sunflower. To be of consequence as a viable soil decontamination method, they must be hardy enough to evade the lethality of the dose of the heavy metal, while still growing fast enough for the roots to uptake a sizeable portion of the Pb from the soil. The question is whether or not lead will inhibit sunflowers from achieving their normal growth rate when grown without the presence of lead in concentrations above 100 ppm. It is for this reason our study aimed to assess whether the sunflower is capable of growing at normal rates across different levels of lead concentration in the soil.

In the present study, we assessed whether high levels of lead in soil inhibit sunflower growth. We predicted that plant growth would be unaffected by high lead concentrations. If this is shown, then sunflowers can be categorized as a viable phytoremediator. To test our hypothesis, we grew sunflowers in different lead concentrations, then measured shoot length and the length of the biggest leaf after four weeks.

Methods

The Species

Helianthus annuus is present throughout North America, but is concentrated in central and western United States (Strasburg and Rieseberg 2008). It belongs to the family *Asteraceae* (Dwivedi and Sharma 2014). In the wild, they are widely branched with many flower heads, but have only a single Fibonacci spiral head and an unbranched stem when domesticated (Reagon and Snow 2006). Its leaves are broad, coarsely toothed, rough, and mostly alternate. During the bud stage, they displays heliotropism, but once they reach anthesis, they are fixated in an eastward direction (Atamian et al. 2016). They are allogamic and pollinated by bees as their pollen is heavy and not easily transferable by wind (Chambo et al. 2011). They can be used in phytoremediation to extract toxic ingredients from soil. They can also be used for rhizofiltration, the removal of contaminants from water (Prasad 2006). It has been found that at different points in a plant's life cycle, the amount of heavy metal which it can uptake differs (Adesodun et al. 2010). For this particular species, within the first 4 weeks after germination, the sunflowers are able to accumulate the most lead in their tissue before the rate of accumulation diminishes. While plants do require certain metals, several other types are unnecessary and are detrimental to plant health (Mustafa and Komatsu 2016).

Procedure

We measured out 0.6L of soil and placed it into a 4.5 inch wide square planter, replicating this for a total of thirty two identical soil samples. We planted a single sunflower seed 2cm deep in the soil. After a week, we watered ten of the planters with 100ppm Pb(NO3)2 solution. We carried out the same procedure with our 600ppm Pb(NO3)2 solution, creating ten replicates of each concentration. For our control we used ten potting media samples left uncontaminated with a Pb(NO3)2 concentration of 0ppm. For husbandry information, see Appendix A.

Experimental design

In order to determine the effect of lead on the growth of *Helianthus annuus*, we cultivated the sunflowers over the course of four weeks in soil with lead concentrations of 600 ppm $Pb(NO3)_2$ and 100 ppm $Pb(NO3)_2$. We are using 100ppm and 600ppm solutions because the EPA deems up to 400ppm safe for soil in children's play areas. Therefore, at higher concentrations, phytoremediation or some type of cleanup should be utilized to reduce lead concentration. We also used as a control a treatment where no $Pb(NO3)_2$ was added. This control was used as opposed to a nitrate control due to the reactivity of nitrate and its transient existence as a lone compound.

Analysis

After four weeks, we measured the length of the shoots and the length of the biggest leaf of each plant to the nearest 1/16 of an inch using a ruler (see Figures 1 and 2).

The independent variable is lead concentration and the response sunflower growth. We used a one-way ANOVA to assess whether plant growth varied across lead treatments. We tested for normality using the Shapiro test and for equal variance using the Levene test. All analysis were performed using the statistical software R.

Results

Mean shoot length differed across lead concentrations (one-way ANOVA, F=8.14, DF=2 P=0.00156, see Figure 3). The assumptions of normality and equal variances were met (Shapiro test, P=0.4775; Levene test, F=1.3753, DF=2, P=0.2688). The 0ppm lead conentration had significantly less growth than the other two concentrations (Tukey-HSD, P<0.05; see Table 1).

Mean length of the biggest leaf was different across lead concentrations (one-way ANOVA, F=21.85, DF= 2, P=1.36e-06, see Figure 4). The assumptions of normality and equal variances were met (Shapiro test, P= 0.8174; Levene test, F=1.1297, DF=2, P=0.3369). The 0ppm had significantly less growth than the other two concentrations (Tukey-HSD, P<0.05; see Table 2).

Discussion

Our study found that lead does not negatively affect growth. The Tukey test showed that 0ppm had less growth than 100ppm or 600ppm. However, there was no significant difference between the 100ppm and 600ppm. This shows that up to at least this point, lead does not negatively affect sunflower growth. In addition, while the presence of lead nitrate affects sunflower growth, there is not a monotonic increase in the effect of lead in the growth of sunflowers. This suggests that sunflowers might be effective phytoremediators as lead does not impair their growth.

As our lead treatment was applied in the form of lead nitrate, and nitrate is used as a fertilizer, the nature of our impure lead compound may have acted as a confounding variable. Our results could have been affected by a lack of a manipulative control. In our experiment, we used a negative control in which no lead nitrate was added. However, we also should have had a control to test the effects of nitrate on plant growth. The beneficial effect of the nitrate as a fertilizer could have overcome the detrimental effects of the lead (Chen et al 2004). This likely contributed to the similar plant growth observed in both the low and high experimental treatments. Regardless, it is promising that the sunflower did not show signs of being negatively affected by very high levels of lead in the soil.

Studies on hyperaccumulators suggest that those plants that are classified as such overexpress genes encoding transmembrane transporters (Rascio and Navari- Izzo 2011). This allows them to have high levels of toxic metals in their tissues, which acts as a natural defense against herbivores. Glutathione and organic metabolism also contribute to the ability to hyperaccumulate (Prasad 2007). Therefore, sunflowers may also have the same overexpression of certain genes as well as high levels of glutathione production and organic metabolism.

To provide further support to the idea that lead accumulation in plant tissues does not seem to affect growth, the study should also have considered the amount of lead being accumulated by the plant. However, we did not have the resources to perform this measurement at this time.

Although some studies have found that lead nitrate leads to decreased sunflower growth, contradicting our experiment, other studies have found that sunflowers are hyperaccumulators. Paliwal et al. (2013) found that lead in the form of lead nitrate decreases sunflower growth. However, they measured growth after 120 days as opposed to our 28, and used a lead concentration made from stock solution with 1/10 the potency of ours. However, many studies have found that sunflowers can hyperaccumulate lead, suggesting that the accumulated lead has minimal effect on their growth and health (Adesoden 2010; Seth et al. 2011).

The above suggests that sunflowers are indeed effective phytoremediators of lead compounds. Perhaps our results suggest that with the addition of a soluble, benign compound such as NO3, the negative effects of lead can be neutralized, rendering the heavy metal compound even beneficial for plant growth. To further support this idea, the maximum lead tolerance and amount of lead that a sunflower can uptake should be tested. In addition, what happens to the lead after uptake by the sunflower and the effectiveness of its containment and potential recovery for smelting must be investigated to completely neutralize the lead. This will allow sunflower cultivation to be implemented into ecosystems to carry out phytoremediation.

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Tables

Table 1. ANOVA post-hoc test (Tukey test) for shoot length for different lead concentrations

	diff	Lwr	Upr	P adj
sixhundred-onehundred	-2.256790	-7.88427	3.3706898	0.5886885
zero-onehundred	-8.405812	-13.79371	-3.0179108	0.0016755
zero-sixhundred	-6.149022	-11.53692	-0.7611208	0.0227150

Table 2. ANOVA post-hoc test (Tukey test) for length of the biggest leaf for different lead concentrations

	diff	Lwr	Upr	P adj
sixhundred-onehundred	0.980	-0.2014232	2.1614232	0.1186905
zero-onehundred	-1.965	-3.0961266	-0.8338734	0.0005166
zero-sixhundred	-2.945	-4.0761266	-1.8138734	0.0000015

Figures

Figure 1. Measurement of shoot length

Figure 2. Measurement of leaf

Figure 3. Boxplot showing the shoot length of different concentrations

Figure 4. Boxplot showing the length of the biggest leaf for different concentrations

Figure 1

Figure 2

Figure 3

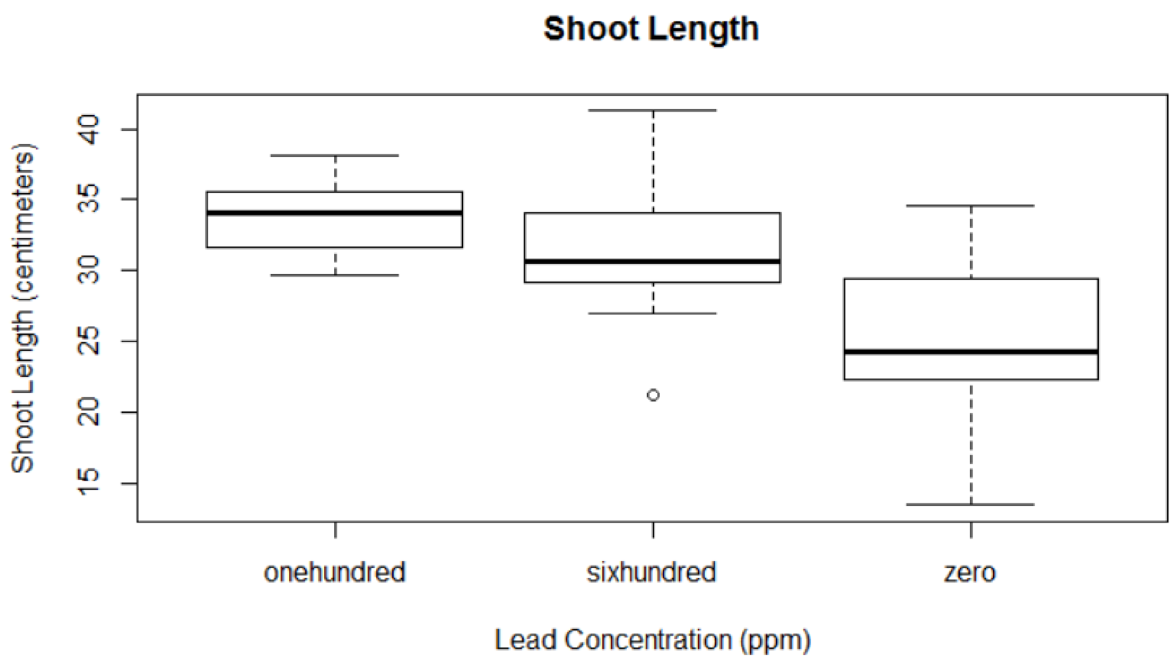
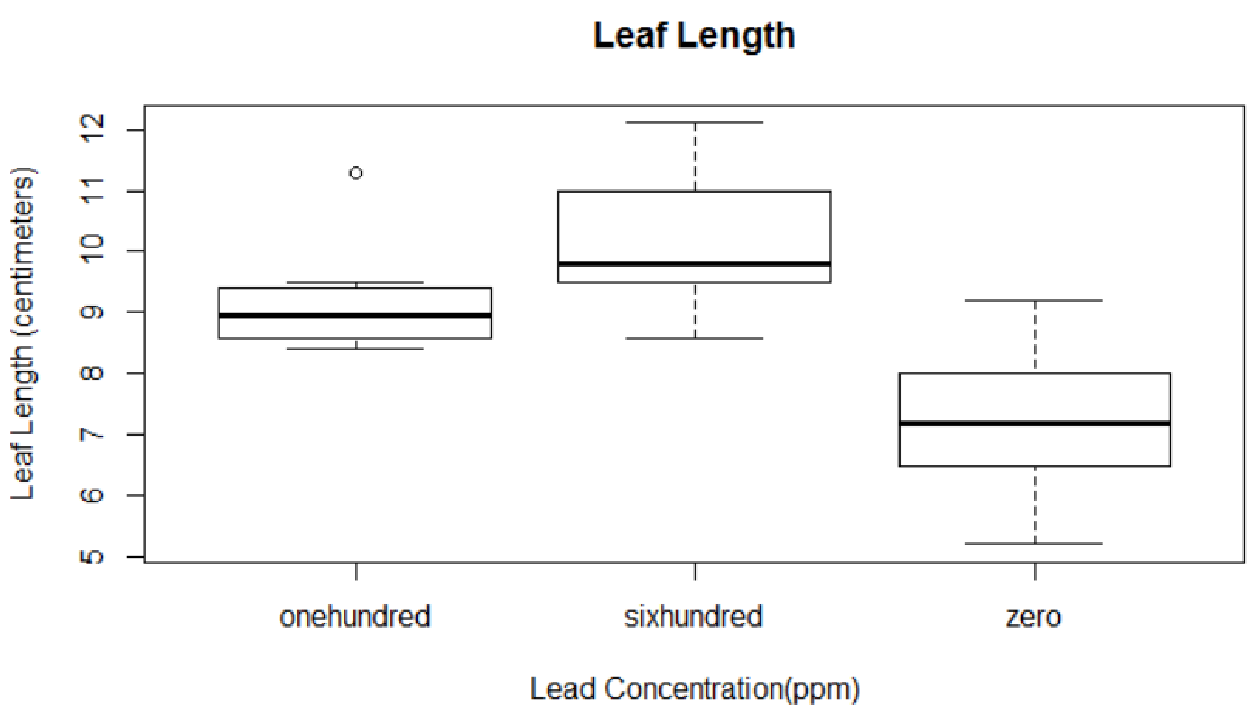


Figure 4



Appendix A

Because sunflowers are a hardy plant, it was relatively easy for them to thrive. We checked every three days for pests and disease. In addition, they were watered daily or as needed by Shane and the other greenhouse regulators. The pH was kept between 6 and 8, the optimal acidity for sunflowers (Sutradhar et al. 2014).