

Exogenous foliar thiamine application as an alternative strategy to mitigate lead stress in *Lepidium sativum*

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SUMMARY

As global populations continue to rise, there is an increasing international need to improve agricultural output to ensure food security. Lower production costs and more efficient utilization of available resources are essential to increase agricultural productivity. However, pollution (i.e., particularly heavy metal contamination from lead) reduces soil productivity and crop yield. Plant development is adversely impacted by the presence of abiotic stressors like heavy metals. Vitamins, such as thiamine, partake in various important plant physiological and developmental pathways. We investigated whether foliar application of thiamine (10 mM) on *Lepidium sativum* (garden cress) could mitigate lead-induced stress. We hypothesized that thiamine application could rescue phenotypic measurements like biomass and plant height in lead-exposed *L. sativum*. To test this, we exposed *L. sativum* to various concentrations of lead (II) nitrate, with and without thiamine treatment. Our results showed that exposure to lead reduced shoot length, root length, and overall biomass. However, thiamine-treated plants showed increased shoot length and overall plant length when compared to untreated controls under the same lead concentrations. These findings suggest that foliar application of thiamine may help rescue growth deficits caused by exposure to lead stress informing both key agricultural practices and knowledge of plant stress responses.

INTRODUCTION

With world population size on the rise, it is necessary to maintain sufficient agricultural production to match that growth (1). The United Nations Treaty, the *International Covenant on Economic, Social and Cultural Rights*, recognizes access to food as a human right (2). Currently, food insecurity is a major concern throughout the world. According to the World Health Organization, nearly 1 in 11 people worldwide (~733 million people) do not have proper access to food every year, and 2.8 billion people (~35% of the global population) cannot afford a healthy diet (3). Lower production costs and more efficient utilization of resources could make produce more available (4). In 2021, the World Bank reported that only 10.8% of the world's land is arable (5). This small percentage of arable land highlights the importance of increasing agricultural productivity either through sustainable agricultural processes or technological innovations. Heavy metal contamination, which can result from factory runoff or excessive fertilizer use, reduces soil productivity and ultimately decreases crop yield (6). Heavy metals are metallic elements with a density greater than 5 g/cm³ (7). These heavy metals include essential metals necessary for biological function such as copper, zinc, iron,

chromium, manganese, and cobalt; non-essential metals like lithium, aluminum, barium, and zirconium; and toxic non-essential metals such as tin and arsenic, cadmium, mercury, and lead (8).

Although lead is non-essential to plants, it can still be absorbed through the roots from contaminated soil. Lead pollution in the environment mainly stems from the use of explosives, factory chimneys, additives in pigments and gasoline, electric and solar cell waste, effluents from battery and mining industries, use of fertilizer and pesticides, and the smelting of ores (9). Lead is primarily trapped within the root cells of plants due to blockage by the Casparian strips within the endodermis (10). Casparian strips are an apoplastic or non-living barrier in roots with roles in controlling nutrient uptake and supports adaptations to stress (11). The accumulation of lead in plant cells can lead to inhibition of adenosine triphosphate (ATP) production, lipid peroxidation, changes in cell membrane structure, DNA damage, inhibition of germination, and decrease in chlorophyll production (10). Lead's impacts are not just limited to established plants; lead toxicity can impact germination as well. In a recent study, lead contamination was found to reduce rates of germination, seedling growth, root expansion, and biomass (12). The New York Natural Heritage Program also reported that 59% of native plants examined were considered vulnerable to the impacts of climate change (13). Lead contamination may pose an additional threat to these already vulnerable native plant species, and the loss of these species can cause long-standing harm to the other organisms within the ecosystems that depend on them. Additionally, a study on soil contamination in New York City reported average lead concentrations of 1,200 mg/kg in soil, nearly 6 times the current Environmental Protection Agency standard of 200 ppm (14). However, this lead contamination is not limited to urbanized areas. Interestingly, concentrations of lead in the northeastern United States have long been documented to exceed the natural background level of 18 mg/kg of lead in the United States (14).

There is a need to understand plant reactions to lead stress and explore treatments to protect against lead exposure. Thiamine (vitamin B1) is a water-soluble vitamin that plants can uptake from their environment. Thiamine, in the form of thiamine diphosphate (TDP), plays an important role as an enzymatic cofactor in metabolic pathways like glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle (15). High levels of TDP are associated with increased flux through the citric acid cycle and a general increase in respiration (15). Recent studies have suggested that thiamine increases plant tolerance to abiotic and biotic stress agents like salt, temperature, and osmotic stress (15). These findings

provide evidence that thiamine may act as a potential stress mediator in plants and may increase plant robustness which to better inform treatments to protect plants from heavy metal contamination.

Lepidium sativum (garden cress) is a fast-growing plant in the Brassica family with the capacity to grow in various soil and climate conditions, establishing itself as a versatile model plant (16). In addition to its adaptivity to a large range of climates and quick growth, its environmental sensitivity is well suited to environmental stress studies (16).

We aimed to further explore thiamine as a potential lead stress alleviator and to research potential techniques to increase global agricultural output. In this study, we hypothesized that exogenous application of thiamine could alleviate the physical impacts of lead-induced stress in *L. sativum* by enhancing physiological parameters such as biomass, root length, and shoot length. We found that shoot length and total length were significantly greater in plants treated with thiamine than those not treated in one of our lead conditions. We hope to further evaluate if thiamine has the same effect on *L. sativum* exposed to other heavy metals.

RESULTS

To examine the impacts of foliar thiamine treatment on plants experiencing lead stress, we counted groups of 75 *L. sativum* seeds and planted them evenly spaced apart on the surface of plastic containers filled with potting soil under artificial light in an 8/16-hour light/dark cycle (Figure 1). We treated groups with lead (II) nitrate solutions after the initial five days of growing to limit the impact of lead stress on germination rate. After five days, we measured the heights of the *L. sativum* seedlings to find starting shoot height for each group. After the initial measurements, we sprayed each group every two days with different lead solution depending on the treatment: 0 ppm (controls), 1,600 ppm (L1), 3,200 ppm (L2), or 4,800 ppm (L3). One spray of either 10mM thiamine or distilled water (non-thiamine) treatment was applied on each plant. The experimental plots consisted of eight pots, with four experimental groups and four controls (Figure 1). We sprayed the negative control with water in place of thiamine without any lead stress. The positive control (PC) received 10mM thiamine treatment without lead exposure.

After the initial measurement of plant height, for the next ten days, we recorded average plant height daily for each experimental plot (Figure 2). Ten days after the collection of the initial heights, we collected the plants to assess the results of the thiamine treatments. Before final data collection began, we rinsed plant shoots and roots in distilled water to remove any excess dirt. After we counted plants from each group, we took the total weights using a digital weighing balance. We measured roots and shoot length alongside the biomass of each sample. We decided to compare plant vitality using phenotypic analysis to provide us an insight to plant stress, nutrient deficiency, and overall health of the plant.

We observed statistical significance in some phenotypic measurements when comparing plants treated with thiamine as opposed to without as lead concentration irrigated increased from 0 ppm to 4,800 ppm. This can be seen when we compare shoot length between thiamine treated groups irrigated with 0 ppm and 4,800 ppm lead. We observed no

significant difference in shoot length in thiamine treated plants irrigated with 0 ppm of lead compared to non-thiamine treated plants (adjusted p -value [p_{adj}] = 0.6701, Figure 3). However, we observed significantly different shoot length measurements in thiamine treated plants irrigated with 4,800 ppm lead indicating a positive effect on shoot growth in thiamine treated garden cress plants irrigated with 4800 ppm lead (p_{adj} = 0.0016, Figure 3). The same trend can be seen when we measured overall length between thiamine treated groups irrigated with 0 ppm and 4,800 ppm lead, where plants that were not irrigated with lead (0 ppm condition) had no significant increase in overall length (p_{adj} = 0.7161) but thiamine treated groups irrigated with 4,800 ppm lead had significantly higher overall length when compared to 4,800 ppm lead irrigated plants not treated with thiamine (p_{adj} = 0.0265, Figure 4). The addition of thiamine may help mitigate the reductions shoot length caused by lead stress. In the 3,200 ppm lead groups, we observed that mean shoot length was 6.371 ± 1.233 cm in the thiamine treated group compared to the mean shoot length of the non-thiamine treated group, 4.121 ± 1.302 cm (p_{adj} = 0.0016, Figure 3). For 3,200 ppm lead-treated plants, overall plant length in plants treated with thiamine showed higher plant length values, 9.350 ± 2.073 cm, than those without thiamine treatment, 6.586 ± 2.673 cm (p_{adj} = 0.0265, Figure 4). Overall length for 1600 ppm was also significantly greater than that of the non-thiamine treated group with the average overall length for those treated with thiamine being higher than those not treated with thiamine (10.086 ± 2.049 and 7.546 ± 3.312 cm, respectively; p_{adj} = 0.0204; Figure 4).

However, although the differences in phenotypic measurements in most of our conditions were not significant, the magnitude of these differences appeared to increase as the lead concentration increased (Figures 3, 4). The baseline in our study was the group without any exposure to lead or thiamine.

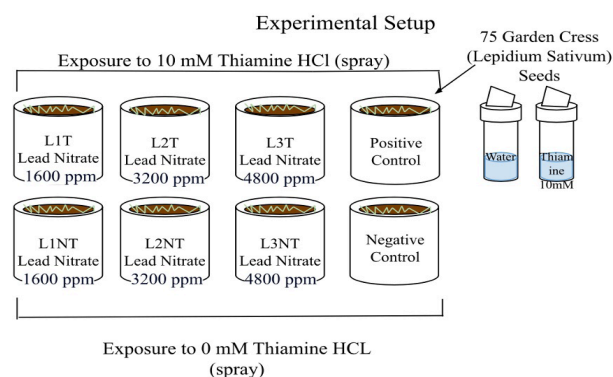


Figure 1: Experimental setup for lead irrigation and foliar thiamine treatments of *L. sativum*. Eight containers marked with their respective lead solutions and thiamine treatment conditions. On the far right, two white cylinders representing spray bottles with either 10mM thiamine or water and indicate thiamine or non-thiamine treatment (T and NT, respectively). The brown oval symbolizes the potting soil, and the green line represents the *L. sativum*. The container called positive control is a separate condition without exposure to lead but treated with thiamine. L1 represents groups irrigated with 1,600 ppm lead, L2 represented groups irrigated with 3,200 ppm, and L3 represents groups irrigated with 4,800 ppm.

Thiamine application increased root length in 1,600 ppm lead-treated plants by 138.5% (**Figure 5**). Thiamine application increased shoot length in plants exposed to 1,600 ppm, 3,200 ppm, and 4,800 ppm of lead by 138.9%, 152.8%, 145.6% respectively when compared to the no thiamine groups (**Figure 3**). Interestingly, there appears to be no statistical difference in the shoot length when comparing no lead exposure control groups ($p.adj = 0.670$, **Figure 3**). This trend can also be seen in other phenotypic measurements. Thiamine treatment alone in the positive control didn't significantly impact phenotypic measurements when compared to the negative control in total length ($p.adj = 0.7161$, **Figure 4**), or root length ($p.adj = 0.9354$, **Figure 5**).

Thiamine application increased overall plant length in plants exposed to 1,600 ppm, 3,200 ppm, and 4,800 ppm of lead by 138.7%, 130.8%, and 139.8% respectively when compared to their no thiamine counterparts (**Figure 4**). Similar to the data for shoot length, there was no significant difference in overall plant length when comparing the two control groups ($p.adj = 0.7161$, **Figure 4**). Thiamine also increased average weight per plant when applied to plants exposed to 1,600 ppm, 3,200 ppm, and 4,800 ppm of lead by 145.2%, 158.8%, and 172.2%

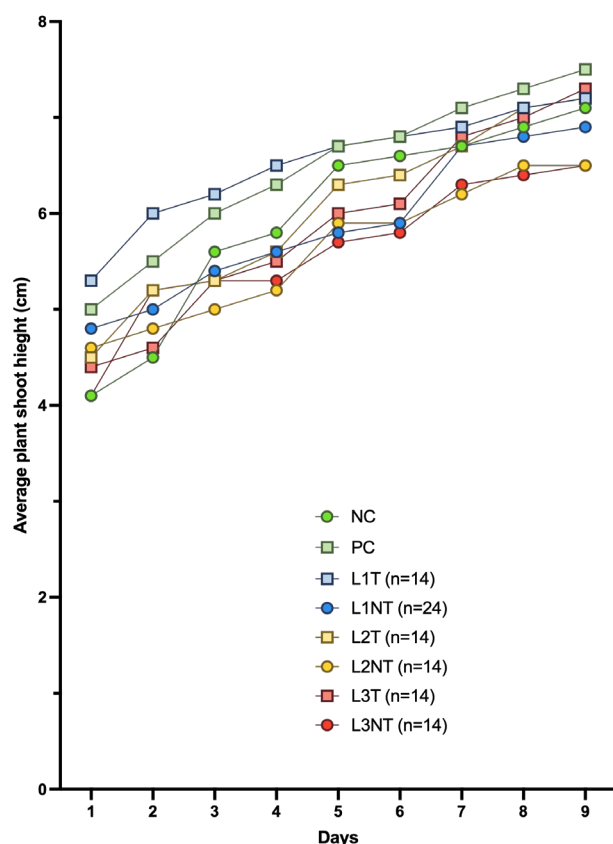


Figure 2: Average *L. sativum* heights across 9 days during irrigation of lead (II) nitrate and thiamine conditions before harvesting. Average height of each group after the initial growing stage where 10 plants heights recorded by random. NC stands for negative control, PC for positive control, and we present our no lead groups in green, our 1,600 ppm groups in blue, our 3,200 ppm groups in yellow, and 4,800 ppm group in red, with each concentration including both thiamine- and non-thiamine-treated groups (T and NT, respectively).

(**Figure 6**). Interestingly, shoot length ($p.adj = 0.8126$, 0.9180 , 0.3113 respectively), overall plant length ($p.adj = 0.1976$, 0.9770 , 0.6091 respectively), and the roots ($p.adj = 0.0583$, >0.999 , >0.999 respectively) of lead-treated plants were not statistically different when compared to the negative control (**Figures 3–5**). Our values confirm that high concentrations of lead irrigated groups (4,800 ppm) have significant differences in average shoot length and total length while thiamine treatment in moderate levels of lead exposure (1,600 ppm) experienced significant differences in average overall length when comparing both the thiamine-treated and the non-thiamine treated groups (**Figures 3, 4**). However, there did not appear to be any statistically significant differences when comparing root length between the groups ($p.adj = 0.9354$, 0.0778 , 0.8555 , 0.8356 , **Figure 5**).

DISCUSSION

Our study supported our hypothesis that foliar application of 10 mM exogenous thiamine can mitigate reductions in physiological parameters in *L. sativum* when exposed to lead concentrations of 1,600 ppm, 3,200 ppm, and 4,800 ppm. Under metal stress conditions, total plant length and shoot length decreased significantly compared to control plants. This disparity could be caused by lead's disruption of the processes of cell division and elongation, mineral absorption, and photosynthesis (17). This hindered function leads to smaller and shorter plants with decreased biomass. Accumulation of lead in the roots can inhibit root elongation and cause chlorosis, necrosis, and leaf curling, which may suggest reduced photosynthetic efficiency and reduced nutrient intake (12). However, it is important to note that there seems to be no root length differences between the controls and experimental groups, although this deviation could be attributed to the fragmentation of fragile roots during harvesting.

Nevertheless, decreased plant shoot length may provide indirect evidence of how root structures might be impacted by lead. In our study, lead exposure may have inhibited nutrient uptake and ultimately reduced the height of the plants. Increasing lead concentration increases the presence of free radicals (12). Free radicals such as hydrogen peroxide (H_2O_2) and oxygen (O_2) change the structure of certain macromolecules and consequentially impact the activity of metabolic pathways that involve said macromolecules (12). Accumulation of lead reduces water balanced nutrients, enzymatic activity, and increases the oxidative stress in plants (12).

Additionally, lead competes with macronutrients like potassium, calcium, and magnesium for plant absorption (12). This mechanism may contribute to the decreased plant height we observed (**Figure 2**). This decreased nutrient uptake decreases plant growth and lowers biomass (18). Interestingly, plants have mechanisms to reduce heavy metal accumulation; roots of plants accumulate higher amounts of lead than any other part of the plant, and the Casparian strip epidermis is a hydrophobic barrier allowing selective nutrients to travel through (12). Additionally, when plants are exposed to heavy toxic metals like lead, they sustain oxidative stress (8). Oxidative stress occurs when there are too many unstable reactive radicals and too few antioxidants

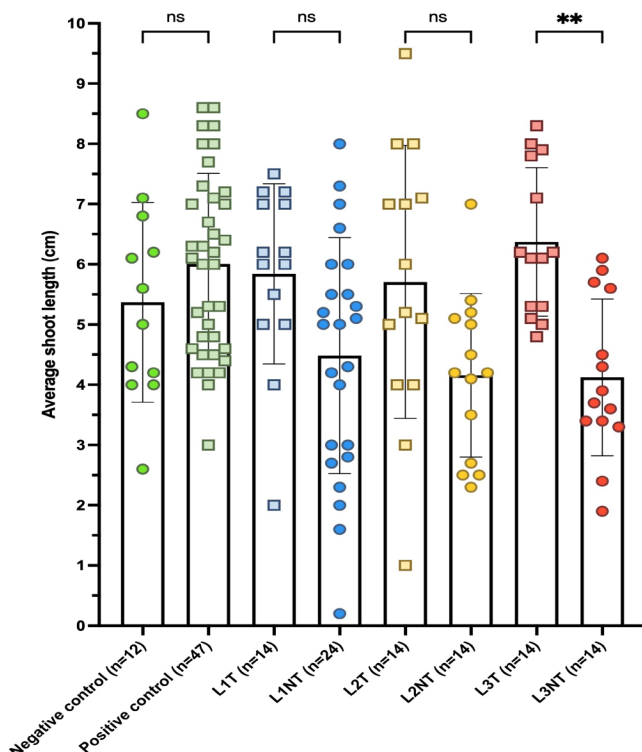


Figure 3: Thiamine exposure increased average shoot length in *L. sativum* exposed to lead stress. Mean $\pm$ standard deviation shoot length in cm for each experimental group are represented as bars and error bars. We present our no lead groups in green, our 1,600 ppm groups in blue, our 3,200 ppm groups in yellow, and 4,800 ppm group in red, with each concentration including both thiamine- and non-thiamine-treated groups (T and NT, respectively). Statistical significance was determined using ordinary one-way ANOVA where $\alpha = 0.05$ and the number of comparisons per family was 4. Using Sidák correction for multiple comparisons, ns = not- significant, ** p -adj < 0.01

to get rid of them, which ultimately leads to cellular damage to plant cells. Nevertheless, plants can adapt to heavy metal contamination in moderate levels by compartmentation in vacuoles, sequestration by phytochelatins, and an antioxidant defense system (8). However, it is important to note that low generation of reactive oxygen species occurs even in no stress conditions usually because of metabolic processes in organelles such as the chloroplast and mitochondria (19). For example, species like *Allium sativum* and *Dunaliella tertiolecta* experience lead-derived vacuolar changes in plant cells (20, 21).

Our results showed that exogenous application of thiamine improves shoot length and overall plant length in plants exposed to high levels of lead stress (Figures 3, 4). Other studies have shown that thiamine application increased measurements of biomass, plant length, and photosynthetic pigments in lentil plants (*Lens culinaris*) when exposed to 1 mM lead and in *Gossypium hirsutum* experiencing salt and drought stress (8, 22). TDP plays an important role as an enzymatic cofactor in metabolic pathways like glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle (15). Elevated levels of TDP are attributed to an increased level of respiration, and TDA has been demonstrated to alter plant

metabolite abundance, particularly photosynthetic pigments (8). This increase in glucose due to increased thiamine availability could increase plant growth and vitality. The effects of thiamine alone may have potentially offset some of the negative impacts of lead stress. These processes may help explain the effects observed following exogenous thiamine application in *L. sativum*. However, as seen in our study, the *L. sativum* group exposed to thiamine but not lead showed no significant increase in phenotypic measurements like root length, shoot length, biomass, and overall plant weight. Our results indicate that there is a significant increase in biomass and growth compared to the plants that were not treated with thiamine, suggesting that thiamine could potentially be used to improve tolerance to lead exposure and possibly other forms of heavy metal accumulation. These increases in the phenotypic measurements were only observed in the groups exposed to lead. The vitamin works as a cofactor in NADPH production and has a role in neutralizing free radicals such as reactive oxygen species (15).

Human activities such as urbanization and industrialization release heavy metal pollutants, including lead, into the environment (9). Reactive oxygen species (ROS) are molecules with an unpaired electron that can damage macromolecules such as lipids due to their instability and high level of reactivity (23). ROS in plants can be produced under

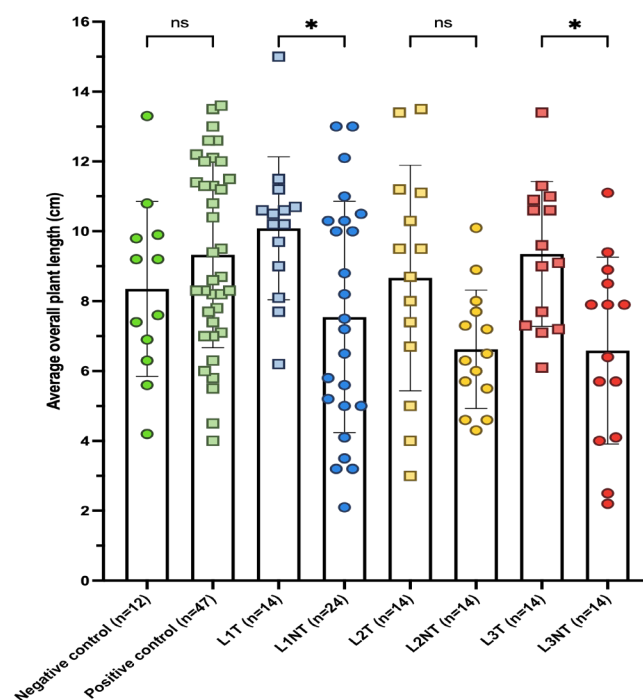


Figure 4: Thiamine exposure significantly increased overall plant length in *L. sativum* irrigated with 1600 ppm and 4800 ppm lead (II) nitrate. Mean $\pm$ standard deviation plant length in cm for each experimental group are represented as bars and error bars. We present our no lead groups in green, our 1,600 ppm groups in blue, our 3,200 ppm groups in yellow, and 4,800 ppm group in red, with each concentration including both thiamine- and non-thiamine-treated groups (T and NT, respectively). Statistical significance was determined using ordinary one-way ANOVA where $\alpha = 0.05$ and the number of comparisons per family was 4. ns = not- significant, * p -adj < 0.05.

the impact of abiotic stressors like drought, high salinity, and heavy metal content (24). Due to the antioxidant nature of thiamine, the compound can alleviate some of the radical buildup from heavy metal stress.

However, it is important to note, not all containers germinated an equal number of individuals, so there were some deviations in the space available for the growth of each plant. While this deviation would result in some potential inconsistencies within our data, we still expected that the relatively large initial sample size would provide us enough plant samples for comparison (12–38 shoots). Although the plants were all equally exposed to artificial UV light, fluctuations in natural light intensity may have impacted the daily rate of growth of the plant groups utilized in this study. However, it is noteworthy that when extracting the plant samples from the substrate, pieces of roots could have been cut short due to their delicate and intricate nature. As previously mentioned, the Casparian strip traps lead in root cells. This accumulation could be attributed to the lack of significant difference in root length for many of the experimental groups. Additionally, since we couldn't control the number of seedlings that germinated the deviations in available space for root growth and expansion could be a main factor in the lack of significant data,

We focused on lead stress; however, because contaminated environments often contain various types of heavy metal compounds, there is still much to understand on thiamine's affect across many heavy metal compounds to establish a clear overview of thiamine's potential impact on plant stress responses and plant health. Additional studies can be conducted to further investigate the impacts that thiamine has on other factors relating to growth such as pigment concentrations and other environmental stress factors such as salt stress. Thiamine is a common compound, and exogenous applications of thiamine can help vulnerable native plants survive in areas with lead contamination and increase agriculture across the globe. A recent study on lead accumulation in edible plant parts in urban gardens found that most lead accumulated in the roots, although lead dispersion in the leaves and stems was also seen (25). Thus, for the application of thiamine in agriculture, future research must emphasize thiamine's impact on the accumulation of lead in produce grown in lead-contaminated soils.

MATERIALS AND METHODS

Experimental Protocol

We counted 8 groups of 75 *L. sativum* seeds (OVM Seeds, Cress Curled Peppergrass Non-GMO Seeds – *Lepidium sativum*). These groups were kept in dry paper towel (Bounty Paper Towels) sachets secured with a paper pin. To prepare the soil groups, eight clear plastic cups with a diameter of 7.0 cm and a height of 8.5 cm were counted and labeled with their respective groups using a marker. After labeling, the plastic containers were filled with 300 g of potting soil (Miracle-Gro Potting Mix). We added 50 mL of water to each soil-filled container to saturate our soil and to ensure that there was enough moisture for the *L. sativum* to grow. We placed the 75 seeds on the surface of each container, making sure to space them evenly apart.

After placing the *L. sativum* seeds, the surface of the

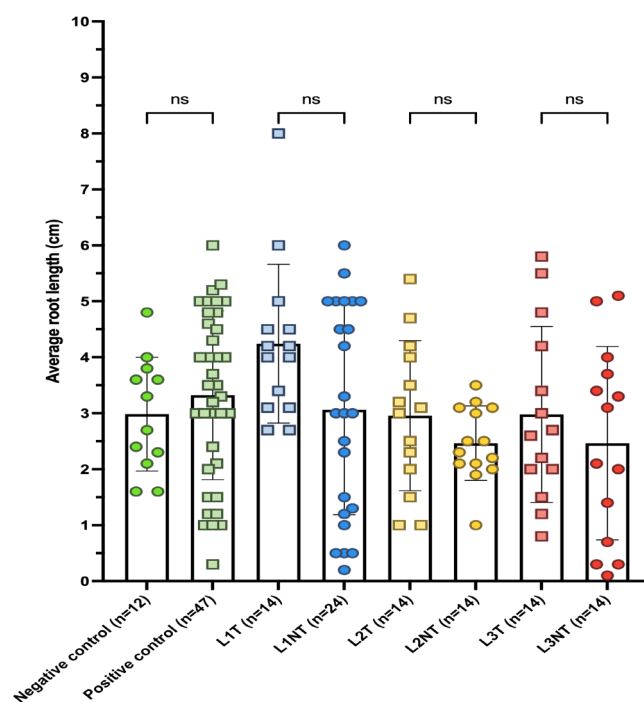


Figure 5: Thiamine exposure did not significantly change average root length in *L. sativum* exposed to lead stress. Mean $\pm$ standard deviation root length in cm for each experimental group (x-axis) are represented as bars and error bars. The average root length was found by taking the sum of all root lengths and dividing by the amount of *L. sativum* plants in each experimental group after harvesting. We present our no lead groups in green, our 1,600 ppm groups in blue, our 3,200 ppm groups in yellow, and 4,800 ppm group in red, with each concentration including both thiamine- and non-thiamine-treated groups (T and NT, respectively). Statistical significance was determined using ordinary one-way ANOVA with Šidák correction, where $\alpha = 0.05$ and the number of comparisons per family was 4. ns= not- significant.

soil was misted 10 times using a plastic spray bottle with the maximum capacity of 60 mL (Amazon, HAALII small spray bottle). We irrigated the groups with their respective lead (II) nitrate (Sigma-Aldrich) solutions after the initial five days of growing so lead stress would not impact germination rate for each container. Plants were watered every 2 days with 10 mL of distilled water. After five days, the average heights of the *L. sativum* seedlings were measured. We selected ten random plant individuals and find the mean height. These initial heights were measured with a ruler perpendicular to the plane of the soil. We made sure that the ruler did not compress down the soil as that can affect growth and skew our data.

A solution of 50 mL of 10 mM thiamine HCl (Pure Bulk) was created and stored within a small cylindrical spray bottle with capacity of roughly 60 mL (Amazon, HAALII small spray bottle). As a control, 50 mL of distilled water was placed within another spray bottle of the same size. The same type of spray bottle was used to ensure that each spray would dispense the same amount of solution. We made sure to put ample pressure on the nozzle when using the spray bottle. After the initial measurements of the *L. sativum* seedlings were collected, four 25 mL lead (II) nitrate solutions of 0.0000M (0 ppm), 0.0048M (1,600 ppm), 0.0096M (3,200 ppm), and

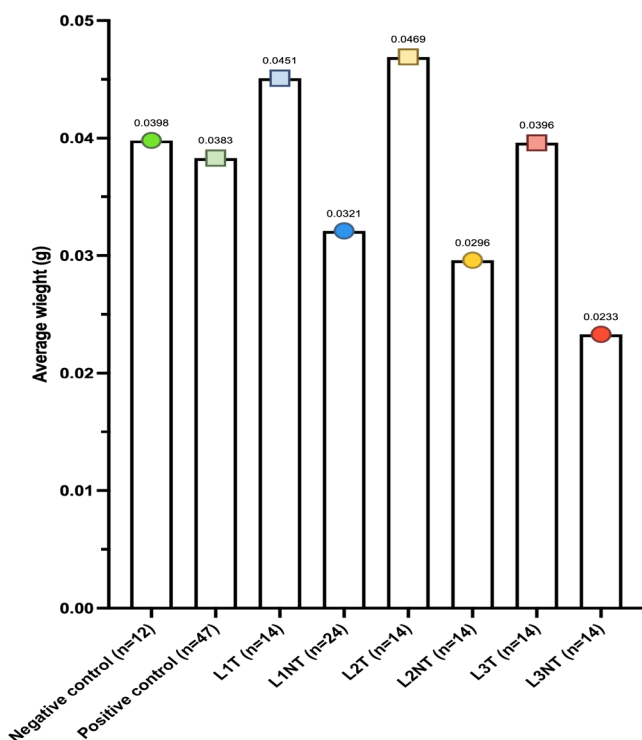


Figure 6: Thiamine exposure across 4 lead conditions (0 ppm, 1600 ppm, 3200 ppm, 48800 ppm) increased average plant mass. Mean plant mass in grams for each experimental group following harvesting. The average plant mass was found by taking the sum of all plant mass and dividing by the amount of *L. sativum* plants in each experimental group after harvesting. We present our no lead groups in green, our 1,600 ppm groups in blue, our 3,200 ppm groups in yellow, and 4,800 ppm group in red, with each concentration including both thiamine- and non-thiamine-treated groups (T and NT, respectively).

0.0145M (4,800 ppm) were irrigated every 2 days to the respective plants. One spray of either thiamine or water was applied on each plant.

After the initial measurement of plant height, for the next ten days, the average plant height out of ten random plants per group was recorded daily for each experimental plot. Ten days after the collection of the initial heights, the plants were harvested and measured to assess the results of the experimental treatments. To harvest the plants, we gently squished the sides of the plastic container. Once the soil was loosened, we poured out the contents of the container on a piece of paper towel. After gently separating each plant from the soil, we made sure to preserve the integrity of the root. After harvesting, we rinsed the shoots and roots in distilled water to remove any excess dirt. After the plants from each group were counted, we recorded the total and average weights using a digital weighing balance (Caroline Electric Balance, item #: 702010). The mean plant weight was determined by dividing the total weight of all plants by the number of plants weighed. A plastic ruler was used to measure the root and shoot length.

Statistical Analysis

Initial statistical analyses were carried out in Microsoft

Excel. We ran a pre-hoc *t*-test within each lead condition comparing thiamine plants to non-treated plants using the Analysis Toolpak extension in Excel. We had an initial alpha threshold of 0.05 but due to the large number of comparisons, we corrected our alpha threshold to 0.0125 using the Bonferroni correction. The significance of our data was determined using the Analysis Toolpak extension establishing *p*-values under a *t*-test and standard deviations. However, we used Prism 11 to generate bar graphs comparing phenotypic measurements. The bar graphs include standard deviation error bars and significance values via an ordinary one-way ANOVA assuming equal variances. Šidák correction was used in generating the graphs to adjust significance threshold.

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