

# Meddling With Metals

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## 1. Background

Phytotoxicity in vascular plants can be assessed by using seeds as bioassays and looking at their germination rates. Seeds are convenient bioassay test organisms because they remain dormant and can be stored for long periods. In addition, seeds can be tested in large numbers which adds to the results' statistical accuracy. *Triticum aestivum* (wheat) seeds were specifically used because they enter the stage of primary dormancy before they begin to mature. Measuring shoot and root length of the seeds after a controlled germination allows the effect of metal concentrations to be assessed. Correlations to the seed viability can be made by statistically examining the germination rates.

## 2. Purpose/Hypothesis:

Varying the concentrations of the metals *Potassium, Magnesium, Calcium, Copper, and Lead* should have an effect on root and shoot lengths, as well as the germination rate of wheat seeds. *Potassium*, as a primary macronutrient, used in the given concentrations, should increase growth of the roots and shoots. Because *calcium* and *magnesium* are secondary macronutrients, it is expected that they will impede seed growth at a lower concentration than potassium. *Copper*, a micronutrient, should have a greater negative impact than the macronutrients. Finally, because *lead* is a heavy metal, it should decrease the root and shoot length the most.

## 3. Materials:

- Organic wheat seeds
- Pre-cut filter paper
- 90 mL sealable containers
- Petri dishes
- Vernier Caliper
- 10 groups of seeds (15seeds×3repetitions = 45seeds/group)
- Metal solutions: PbCl<sub>2</sub>, CuCl<sub>2</sub>, CaCl<sub>2</sub>, MgCl<sub>2</sub> and KCl
  - Metal concentrations (mM): 0, 0.2, 0.5, 0.7, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 7.0
- 1mL, 10mL, and 100mL pipettes
- Thermometer
- Scale

## 4. Controls:

- All seeds used are wheat and identical (no discoloured, off-sized or broken seeds used)

- De-ionized water (used when the concentration is 0mM so that the seeds are still moist)
  - Pre-cut filter paper will be wet with either 10mL of de-ionized H<sub>2</sub>O for 0mM or the given concentration of the given metal chloride
- Temperature: 25° C
- All germination tests were kept dark

## 5. Variables:

**Independent Variables:**

- Concentrations (mM): 0, 0.2, 0.5, 0.7, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 7.0
  - For Copper, Potassium, Lead, Calcium, Magnesium

**Dependent Variables:**

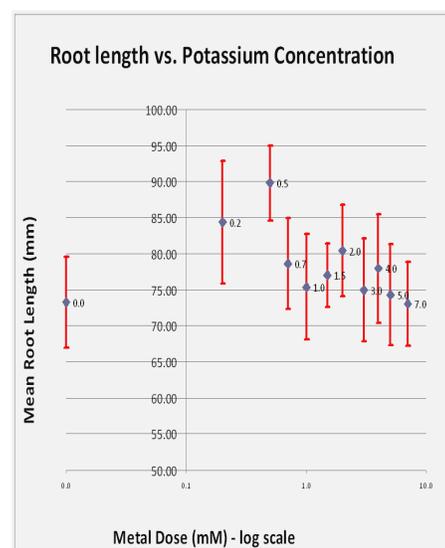
- Root and Shoot length (measurement after 96h of exposure)
- Percent Germination (Percent of germinated seeds over the total number of seeds)

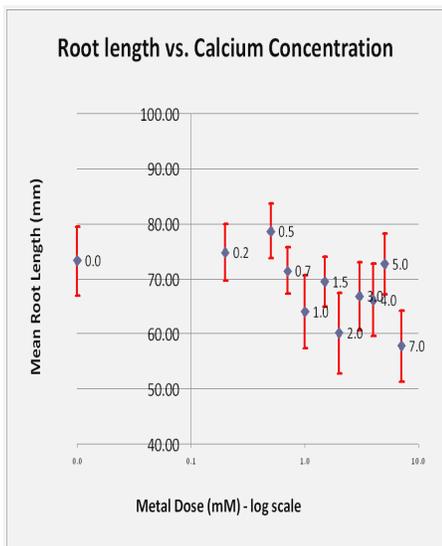
## 6. Procedure:

1. Soak 45 seeds for 10 hours in 40ml of CuCl<sub>2</sub>, MgCl<sub>2</sub>, CaCl<sub>2</sub>, KCl, and PbCl<sub>2</sub> for concentrations (mM): 0, 0.2, 0.5, 0.7, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, and 7.0. (0mM will be soaked in dH<sub>2</sub>O)
2. Add 20mL of deionized H<sub>2</sub>O to the filter papers designated to go into the 0mM concentration petridishes and put the filter papers inside.
3. Repeat step 2 for the rest of the concentrations for all the metal chloride solutions (instead chloride solutions using 20mL of the metal solution on the filter papers).
4. Place 15 soaked wheat seeds of each concentration in each corresponding petri-dish.
5. Seal the petri-dishes in plastic bags and keep them incubated for approximately 96hours.
6. Remove the seeds and place them in a freezer until the measurements are performed.
7. Measure the root length and the shoot length of the seeds.
8. Record the number that have germinated, the root length, and shoot length in a table.

## 7. Results

*Potassium* is generally used in larger quantities by plants and is found in soil, organic materials, and fertilizers. It has many functions within a plant: protein assembling, photosynthesis, fruit quality, osmoregulation, and carbon assimilation. Overall, it appears that potassium had the expected effect on the seeds: it helped the growth of the seed roots which is seen through the fact that all the values of the average root length were above that of the control. As predicted, this



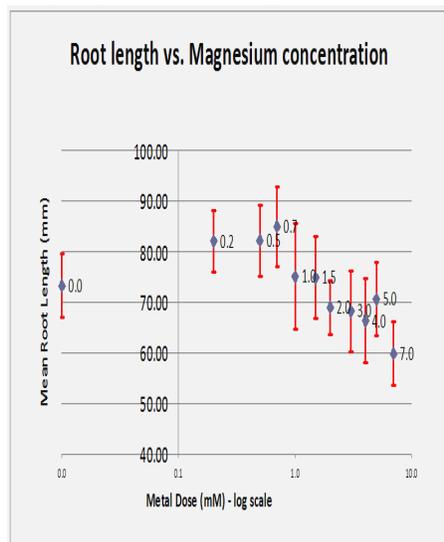
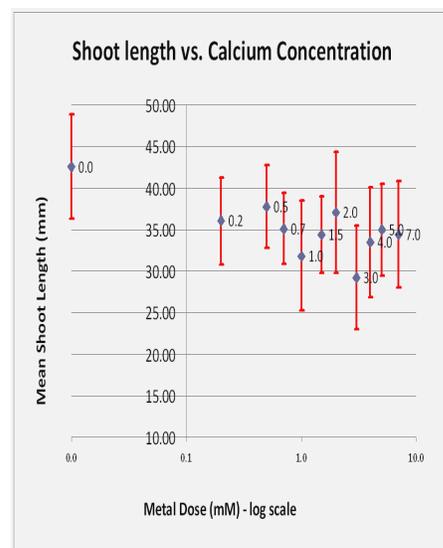


experiment indicated that potassium can be tolerated at higher concentrations in wheat seeds. From the data collected, it is expected that total inhibition would take place at a much higher level of concentration than any of the other metals because the growth does diminish at higher concentrations.

*Calcium* plays a role in transport and conservation of other elements. At the seed level, calcium is important for the growth of

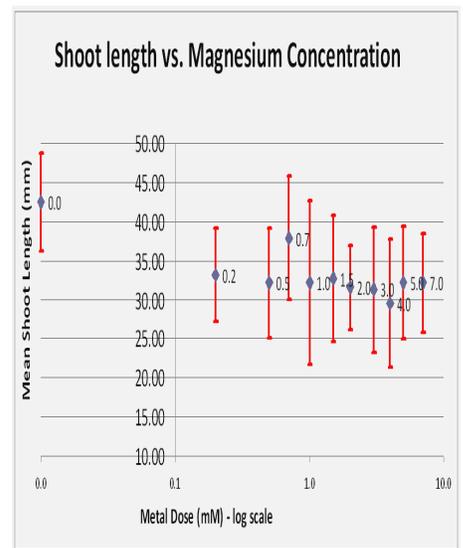
root systems, shoot tips, and storage organelles. It provides strength for a plant due to its role in the formation of pectin in the middle lamella between primary cell walls.

It was expected that the calcium as a secondary nutrient would become detrimental at a lower concentration than potassium. This is seen in the results because even though  $Ca^{2+}$  caused the mean root length to increase by 4.8% until 0.7mM, at this point the mean root length started to decrease until it was below the control group; however, potassium did not decrease root length below the control group. Furthermore, calcium also decreased the shoot length on average by 17% while potassium did not have any effect.



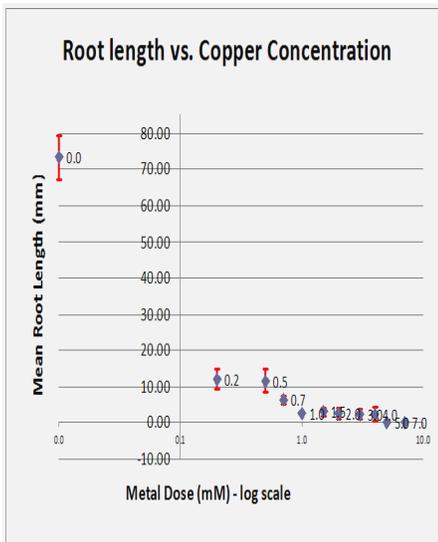
*Magnesium* is a secondary macronutrient which plays an essential role in photosynthesis because of its presence in chlorophyll. It is also said to be an activator of enzymes which plants need for growth.  $Mg^{2+}$  increased the root length until 2.0mM then it consistently decreased the shoot length as the concentrations increased. On the other hand, it increased root length at first until 2.0mM where it then decreased. Evidently,

Mg<sup>2+</sup> became detrimental to the seeds at a lower concentration than potassium but at a higher concentration than calcium. It was unexpected that even though magnesium and calcium are both secondary nutrients, the seeds would have a slightly higher tolerance for magnesium.

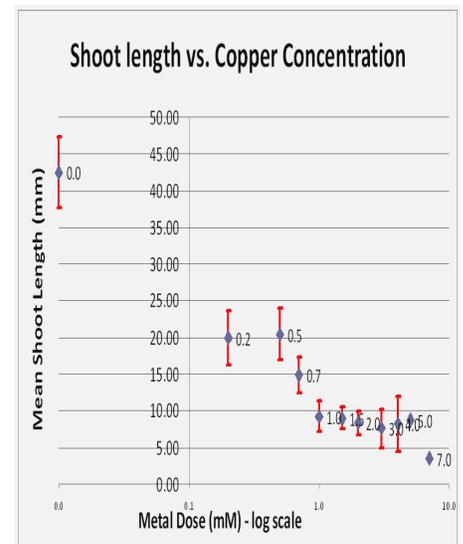


*Copper* is a micronutrient

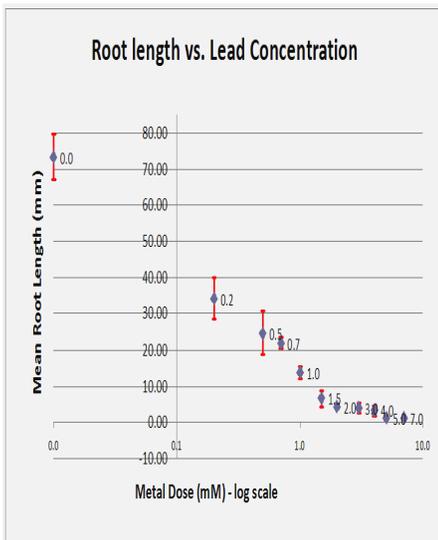
which aids in reproductive growth, root metabolism, and assembling proteins. It plays a role in photosynthesis and respiration and adds strength to plant cell walls and the plant overall. The data indicated that the mean root and shoot length decreased drastically when the concentration of copper increased. The results also showed that the length of the root was



affected more by Cu<sup>2+</sup> than the shoot growth. This might be due to a higher accumulation of the heavy metals in the roots than in the shoots or due to a faster detoxification in the shoots than the roots. Overall, *copper* was the metal which decreased germination the most

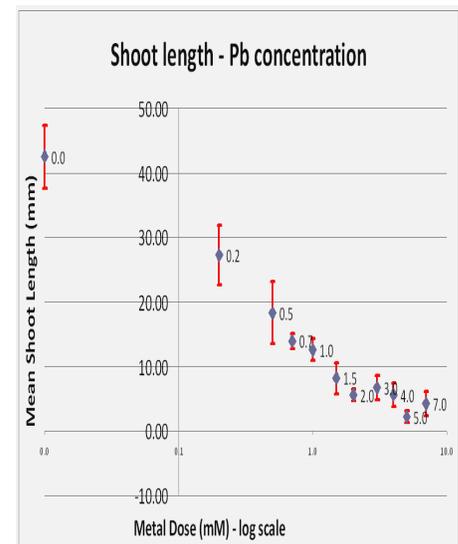


and had the greatest negative effect the length of the roots and shoots.



*Lead* is a heavy metal which has no biological function in plants and is considered to be harmful. It alters the biosynthesis of chlorophyll and forms deposits in intercellular spaces, cell walls, and vacuoles. It is known to inhibit enzyme activity, affect the regulation

of minerals and water, and upset membrane structure. The data shows that the roots were more affected than the shoots. It is thought that the heavy metal concentrations may have increased levels of phenolic compound. This may have inhibited germination because phenolic acids are thought to have a drastic impact on the permeability and electrical potentials of membranes. In general, when the concentration of lead increased, the mean root and shoot length decreased. Copper had the greatest effect – not lead. Interestingly, some fertilizers used by local farmers contain up to 3% lead. Because *Triticum aestivum* is a widely harvested in North America, it is possible that it has become more resistant to lead. However, it also is possible that while lead only forms deposits within the germinated seeds, copper was reaching more of the seeds' cells through the seeds' own internal transport mechanisms which would already be in place to transport copper to cells for photosynthesis, cell respiration, etc.



## 8. Conclusion

The order of metals which had the greatest negative effect on the seeds was: Copper, Lead, Calcium, Magnesium, and Potassium. It was expected that Potassium would have the least effect. It was anticipated that copper would have a higher affect than calcium, magnesium, and potassium; however, it was unexpected that it also would have a higher affect than lead. Essentially, what is important about these results is that metals in certain concentrations do have an effect on primary producers. Moreover, they do not decay – they accumulate in the environment. This provides a challenge for the environment to recover from a contamination. It is true that the natural environment has created its own solutions to counteract this; however, these metals will continually find ways back into the soils through the natural life cycles of plants and consumers. This study encourages society to change the environmental fate of toxic metals by demonstrating the health risks to primary producers and, indirectly, consumers. In this way, the general population may be stimulated to prevent contaminations from occurring.

- Attachment -

**9. References**

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