

Introduction

Cadmium (Cd) is a toxic heavy metal that has negative effects on plant growth and human health. Cd concentrations for soils in agricultural areas of the United States range from <0.01 to 2.0mg/Kg. These concentrations continue to rise as a result of human anthropogenic activities. Plants like spinach and wheat, which serve as dietary staples, have the ability to accumulate the heavy metal in edible tissues. In fact, dietary Cd is the leading cause of Cd exposure to non-smokers (ATSDR, 2008). Understanding the physiological mechanisms in plants has been a key concern of researchers whose goal it is to limit Cd transfer to humans. Unfortunately, these efforts may be of no use as environmental factors which may potentially govern Cd uptake, atmospheric CO₂ for instance, are drastically changed.

Carbon dioxide (CO₂) levels are rising at an alarming rate. Over the last century atmospheric Carbon dioxide levels have increased from 280ppm to greater than 400ppm (IPCC 2014) with no sign of slowing down. Research had originally shown that increase CO₂ in the atmosphere would benefit plants, allowing them to grow faster and fuller with higher grain yields (Pritchard et al. 1999). Recent findings however have indicated that while plants are producing bigger and more abundant grains, increased CO₂ also improved their ability to accumulate heavy metal contaminants. Work on *Sedum alfredii*, Indian mustard, and sunflower have all shown increased accumulation Cd or Cu as a result of being exposed to elevated CO₂ (Li et al. 2012; Tang et al. 2003).

Understanding the effects elevated carbon dioxide has had on non-crop species, it is vital that the effects of increased CO₂ on leafy vegetables are explored. In this project, we explore the hypothesis "elevated levels of atmospheric carbon dioxide will cause an increase in Cd accumulation in leafy tissue of spinach". Results will reveal the dangers of increased atmospheric gases and climate change on our food source, as well as give a key insight into the mechanisms that wheat uses to accumulate Cd in edible tissues. With this knowledge, steps can be taken to reduce our carbon footprint and produce nutritious, uncontaminated plant foods for all to enjoy.



Fig 1: The Spinach (*Spinacia Oleracea*) grown in hydroponic solution

Acknowledgements

I would like to thank the STEM Scholars Program at Lehman College and Hostos Community College for this opportunity. I would like to thank my mentor Dr. Renuka Sankaran for her guidance with the project and Michael Tavarez for his support in the lab.

Materials and Methods

Spinach (*Spinacia oleracea*) seeds were surface sterilized with 1.0% sodium hypochlorite for 10 minutes, and imbibed overnight. They were then germinated in soil under controlled conditions of the Lehman College greenhouse. After 1 week, germinated seedlings were transferred to 4L pots containing a modified Johnson's nutrient solution that was replaced weekly. The nutrient solution comprised of 2.0 mM KNO₃, 1.0 mM Ca(NO₃)₂, 1.0 mM MgSO₄, 1.0 mM KH₂PO₄, 25 μM H₃BO₃, 25 μM CaCl₂, 2.0 μM MnSO₄, 2.0 μM ZnSO₄, 0.5 μM CuSO₄, 0.5 μM H₂MoO₄, 0.1 μM NiSO₄, 20 μM FeHEDTA N(2Hydroxyethyl)ethylenediaminetriacetic acid, and the solutions were buffered with 2 mM MES[2(morpholino)ethanesulfonic acid] so that the pH level can be regulated at 5.5. Solutions were aerated continuously and replaced every 10 days.

When the plants reached the 2 leaf stage, experimental pots were treated with 0.5μM CdSO₄. Plants were tagged and separated into two growth chambers containing either increased or ambient carbon dioxide. For the ambient CO₂ condition, the plants were placed in a growth chamber where the CO₂ concentrations were 400ppm. For the elevated CO₂ conditions, the plants were placed in a CO₂ controlled chamber where the CO₂ concentrations were 550ppm. Both control and Cd treated plants were placed in ambient and elevated CO₂ conditions for the duration of the study. Growth chamber conditions were set to 16-h day and 8-h night photoperiod, 25 ± 3 C day/23 ± 3 C night. There were 10 replicates per treatment condition.

Chlorophyll measurements were recorded every other day using a SPAD chlorophyll reader. One leaf per plant was measured 3 times with averages taken as measurement. After 21 days of treatment, plants were harvested and dried in an oven at 65 °C for 24 hours so that plant dry weights may be analyzed. The dried tissues will be digested in nitric acid and Cd concentrations will be analyzed in the leaf tissues using an Atomic Absorption Spectrophotometer (AAS).



Fig 2: Relative chlorophyll content measurement using a SPAD Chlorophyll meter

Fig3: Digestion block for digesting leaf tissue samples

References

ATSDR (2008) Cadmium
 Intergovernmental Panel on Climate Change (2014) <http://www.ipcc.ch/>
 Li DD, Zhou DM. Ecotoxicol Environ Saf 2012
 Tang S et al. Bull Environ Contam Toxicol 2003
 Page, A., et al, Springer. p. 33-74, 1986
 Pritchard SG, et al. Glob Chang Biol [Internet]. 1999

Results and Conclusions

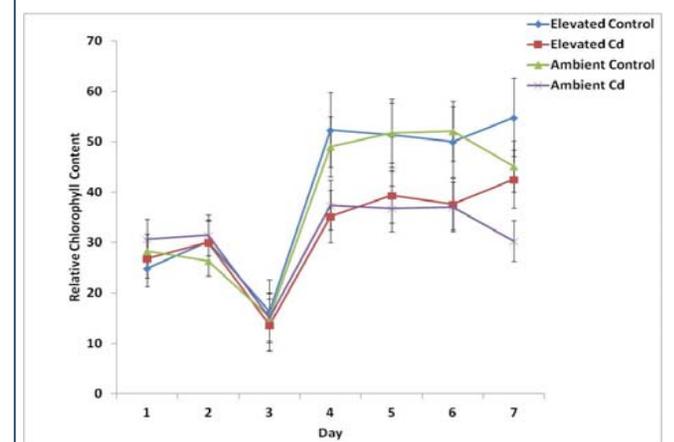


Fig 4: Relative Chlorophyll Content (RCC) of *Spinach* plants grown in different concentrations of Cd under ambient and elevated CO₂ conditions. Values represent mean ± s.e

The controls grown in both elevated and ambient CO₂ conditions in general had higher RCC compared to the Cd treatments. Plants grown in elevated CO₂ did not significantly differ from the plants grown in ambient conditions suggesting that CO₂ did not have any effect on RCC in spinach. These results suggest that Cd had an effect on the RCC in spinach while CO₂ conditions did not have any effect.

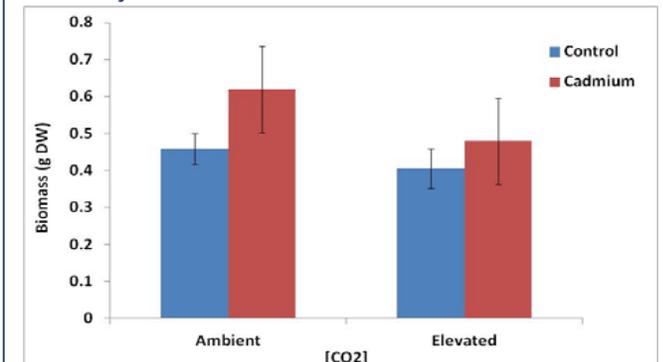


Fig 5: Tissue biomass (g DW) of spinach plants grown in different concentrations of Cd under ambient and elevated CO₂ conditions. Values represent mean ± s.e

There were no significant effects of Cd treatments as well as CO₂ conditions on biomass which indicates that the Cd treatment were not high enough to cause a significant decrease in biomass. Elevated CO₂ condition also did not seem to cause an increase in growth.

Tissue Cd concentrations will help us understand if Cd accumulation is altered because of elevated CO₂ conditions.