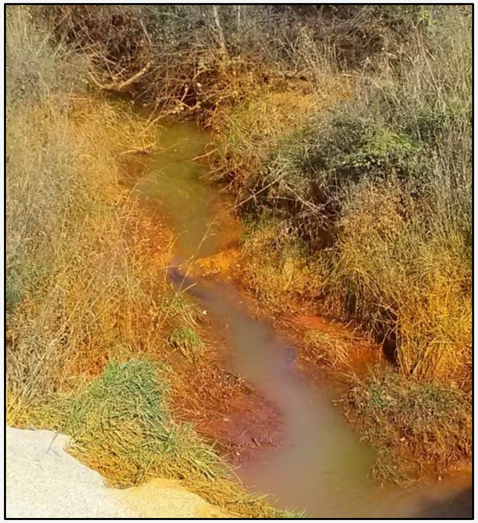
The Heavy Metal Movement: Phase II

A field and laboratory study of *Panicum virgatum L.* and its ability to phytoremediate soil from the Tar Creek Superfund Site



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Purpose

The purpose of this project is to compare *Panicum virgatum L.* (Kanlow Switchgrass) seeds collected from plants that have been growing in toxic soil versus seeds from *Panicum virgatum L.* that has not been growing in toxic soil for their abilities to phytoremediate heavy metals from soil.

Additionally to collect *Panicum virgatum L.* plants from Tar Creek at various stages of growth and dormancy, in order to analyze the tissues for bioaccumulation of heavy metals.

Hypotheses

I. It is hypothesized that *Panicum virgatum L.* grown from seeds collected from plants at the Tar Creek Superfund Site will be more effective at phytoremediation of heavy metals than *Panicum virgatum L.* grown from seeds collected from plants not exposed to toxic soil.

Null Hypothesis I: States that *Panicum virgatum L.* grown from seeds collected from plants at the Tar Creek Superfund Site will not be more effective at phytoremediation of heavy metals than *Panicum virgatum L.* grown from seeds collected from plants not exposed to toxic soil.

II. It is hypothesized that the level of heavy metals in *Panicum virgatum L.* collected at the Tar Creek Superfund Site will be higher during active growth, and lower during dormancy.

Null Hypothesis II: The null hypothesis states that there will be no differences in the level of heavy metals in the *Panicum virgatum L.* collected at the Tar Creek Superfund Site during active growth and dormancy.

Background Information

- **Previous Study**
  - In a previous study *Schizachyrium scoparium* (Little bluestem) and *Panicum virgatum* (Switchgrass) were tested for their ability to remove heavy metals from soil through phytoextraction.
  - Contaminated soil was obtained from Tar Creek Superfund Site for this test.
  - The plant tissues were also examined to see whether heavy metals were bioaccumulated in the roots or the shoots of the grasses.
    - **Both grasses were successful in the phytoextraction of heavy metals, and there were higher levels of metals in the roots of the plants.**
  - While collecting soil, it was observed that various plants were growing along the banks of Tar Creek, raising new questions. One particular plant that stuck out in the group was a grass that was growing abundantly. The next step was to identify this grass.
- **Switchgrass Identification and Information**
  - After thorough research, the grass was identified to be *Panicum virgatum L.* (Kanlow switchgrass).
  - Kanlow is a lowland type of switchgrass, warm-season, native, and perennial. It is six to eight feet tall in maturity and can have a root system ten feet deep or more.
  - Switchgrass is a popular choice for phytoremediation due to its fibrous root system.
  - Switchgrass and its seed are readily available in the United States; from Canada, the Atlantic coast, and all the way to the Rocky Mountains.

Background Information

- **Heavy Metal Contamination and Tar Creek**
  - Soil can become contaminated through mining, manufacturing, and the use of synthetic products.
  - While some heavy metals can occur naturally in soil, it is rarely at toxic levels.
  - A few metals of concern are cadmium, zinc, lead, and iron. Some of the maximum concentrations for these heavy metals in soil are:
    - 0.44 ppm for cadmium
    - 200 ppm for lead
    - 1100 ppm for zinc
    - 185 ppm for iron.
  - One area that is greatly affected by heavy metal contamination is Tar Creek in northeastern Oklahoma.
  - Tar Creek was extensively mined for lead and zinc ore in the early and mid-1900's.
  - Due to the extensive contamination, Tar Creek was listed on the National Priorities List in 1983, making it a Superfund site.
  - The site encompasses Picher, Cardin, Quapaw, North Miami, and Commerce, effecting nearly 30,000 people.

Tar Creek

All photographs were taken by student research or student's sponsor

Background Information

Phytoremediation

- It is imperative that a solution is found for this contamination.
- One possible solution could be phytoremediation, which uses various types of plants to remove, transfer, stabilize, and/or destroy contaminants in soil and groundwater.
- The focus of this study was phytoextraction.
  - In phytoextraction, plant roots absorb the contaminants, which can be stored in the vacuole of the cells or translocate through the cell membranes into the xylem and up into the stems and leaves of the plant.
  - Hyperaccumulators, plants that have an ability to absorb high levels of contaminates, are the ideal plants for phytoremediation.
- Technologies that are typically used to remediate contaminated soil resort to excavation and landfilling.
- Phytoremediation allows the soil to stay in place, and is also much more cost effective.
  - Traditional methods cost between $10 and $300 per cubic meter while phytoremediation could cost approximately $0.05 per cubic meter.

Method 1. Identification/ Collections at Tar Creek

Multiple samples and pictures were taken to identify the switchgrass. Books, researchers, and various grass experts were utilized.

The grass was finally identified to be Panicum virgatum L. (Kanlow switchgrass).

The following collection materials were obtained: gloves, shovel, paper bags, and bucket.

Seed collections took place in mid-September, due to the timing of natural seed viability.

Seeds were removed from the plant by running hands along the seed head.

Once the seeds were removed they were placed in a paper bag.

Specimens were collected using a shovel to remove entire plant, along with the root system.

One plant sample was taken at the end of every month, beginning in July.

This is repeated for five months.

Method 2. Preparation and Stratification of Seeds

Seeds were sent to Oklahoma State University to be cleaned. The seeds were still in their spikelets and needed to be removed with means not available in our school lab.

Regular Panicum virgatum L. seeds were obtained from a professor at Oklahoma State University.

Once seeds were cleaned and returned, both seed types were stratified by soaking and refrigerating for two weeks.

Method 3. Soil Preparation

Soil collected from Tar Creek first was sifted, to remove plant material, rocks, etc., under a vent hood.

Tar Creek soil was mixed with potting soil and seed starter.

This was to give the plants their best chance to grow, as the soil is very toxic.

The ratio of potting soil and seed starter to Tar Creek soil was 2:1.

A soil mixture of potting soil and seed starter was also prepared at a 1:1 ratio for the control group

Method 4. Making Growing Chambers

12 containers, PVC pipe, and braided cotton rope were obtained.

PVC pipe was cut and glued to the bottom of the container, for support.

The edges of the container and the pipe were roughed with sand paper to ensure they would stay intact, and then glued into place.
Two holes were drilled in the bottom of the container.

The wick was cut into 90 centimeter segments, and pulled through holes and looped up into the container.
Seeds were dispersed evenly across the surface of the container, and misted

Plants were watered in trays with approximately 2,000 mL of distilled water as needed.

500 mL of pea gravel was measured and poured into the bottom of the container.

3,000 mL of soil mixture was measured and added to growing chamber.

4.5g of stratified seeds were added to each container.

Method 5. Taking and Preparing Samples

Plants were removed from growing chambers.

Roots were rinsed with tap water to remove initial dirt.

Using stainless steel scissors, the roots were separated from the shoots.

Roots and shoots were rinsed with deionized water until thoroughly clean.

Once samples were cleaned, they were placed glassware to dry in a ventilated oven for three days.

Once samples were dry, a biomass was taken using a digital scale

Then, the samples were ground up using a coffee grinder.

This process was repeated three times for each of the four testing groups (twelve in all). 5

Method 6. Soil Analysis

1 gram of soil was measured and placed into a test tube.

10 mL of 1:1 nitric acid and deionized water was added.

The solution was allowed to heat for 10 – 15 minutes.

5 mL of nitric acid was added.

This was allowed to evaporate to 5 mL, which took two hours.

2 mL of water and 4 mL of 30% hydrogen peroxide was added.

30% hydrogen peroxide was added 1 mL at a time due to possibility of reaction in the samples.

The solution was allowed to evaporate to 5 mL before 5 mL of water and 1.25 mL of hydrochloric acid were added.

Water was added to the 25 mL line for a 25 times dilution.

Method 7. Plant Tissue Analysis

.5 grams of plant sample was measured out.
5 mL of nitric acid was added.

The solution was allowed to predigest for 30 – 45, minutes.

Temperature was raised to 60 degrees Celsius.

3 mL of 30% hydrogen peroxide was added.

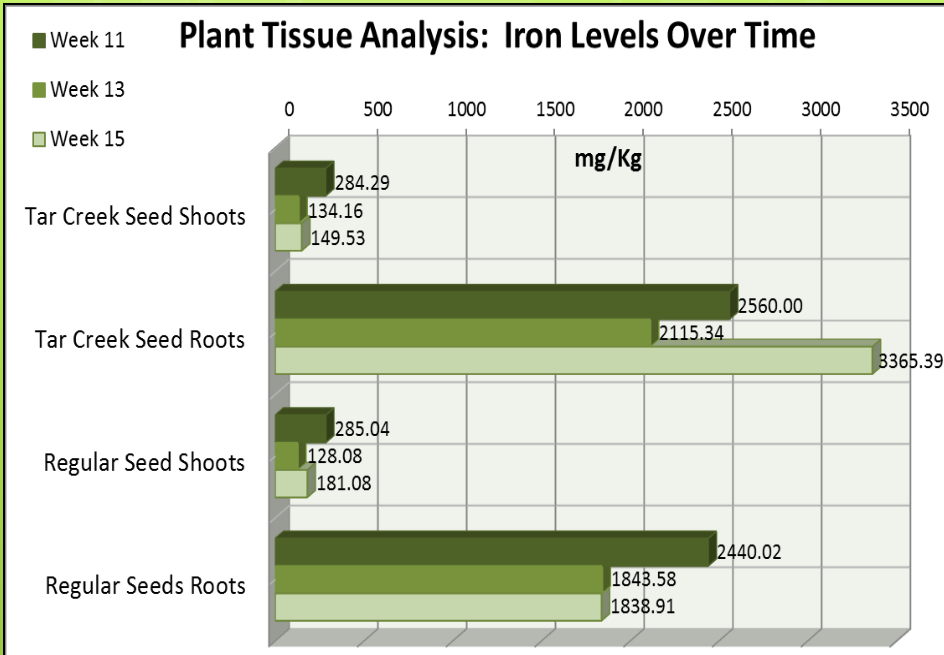
30% hydrogen peroxide was added 1 mL at a time due to possibility of reaction in the samples.

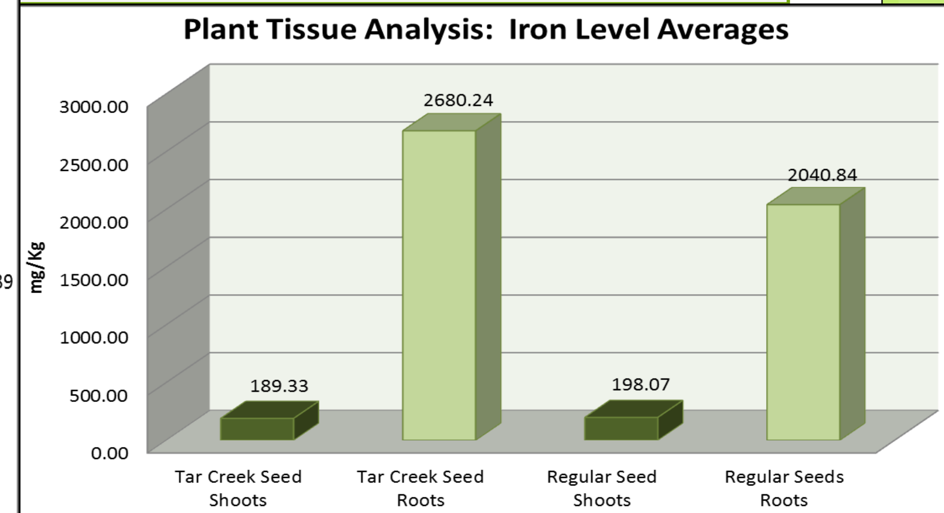
Solution digested at 110 degrees Celsius for approximately 25 minutes or until it reached the volume of 5 mL.

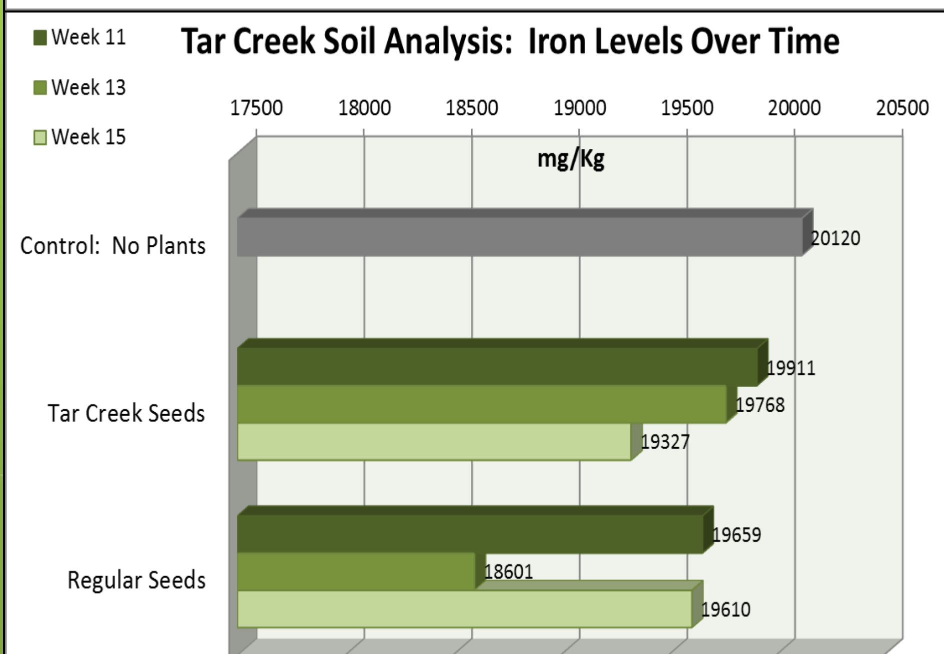
Water was added to the 20 mL line for a 40 times dilution.

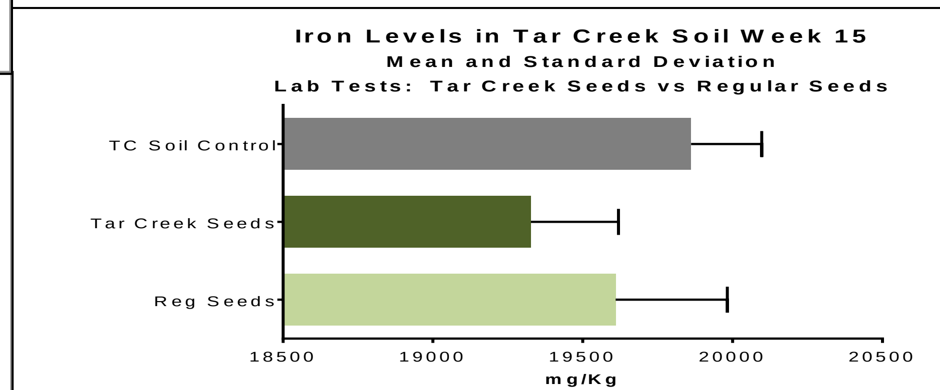
Both soil and plant tissues were analyzed using an ICP-OES Spectro Arcos machine.

Iron





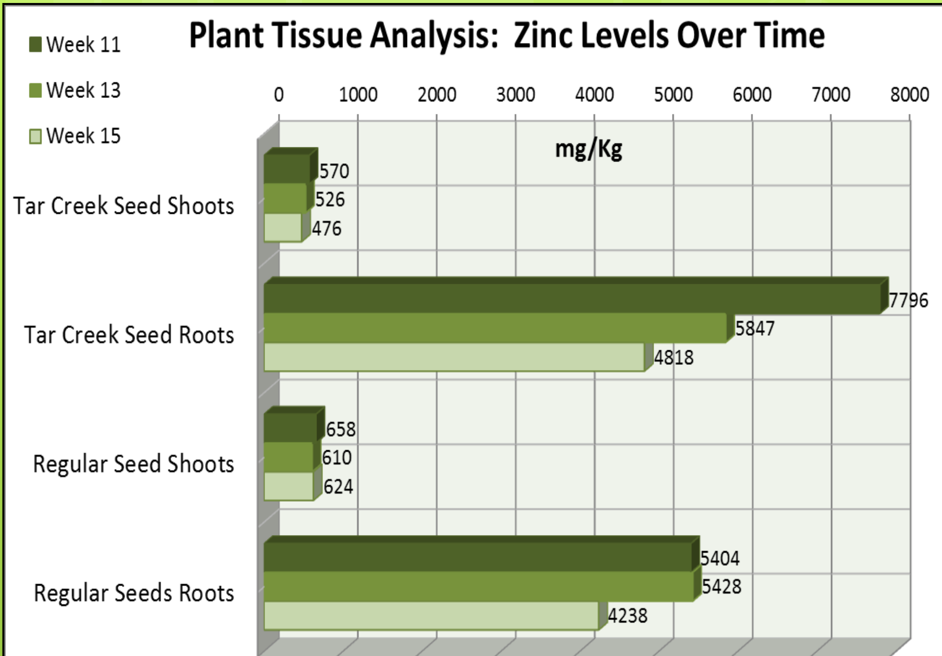


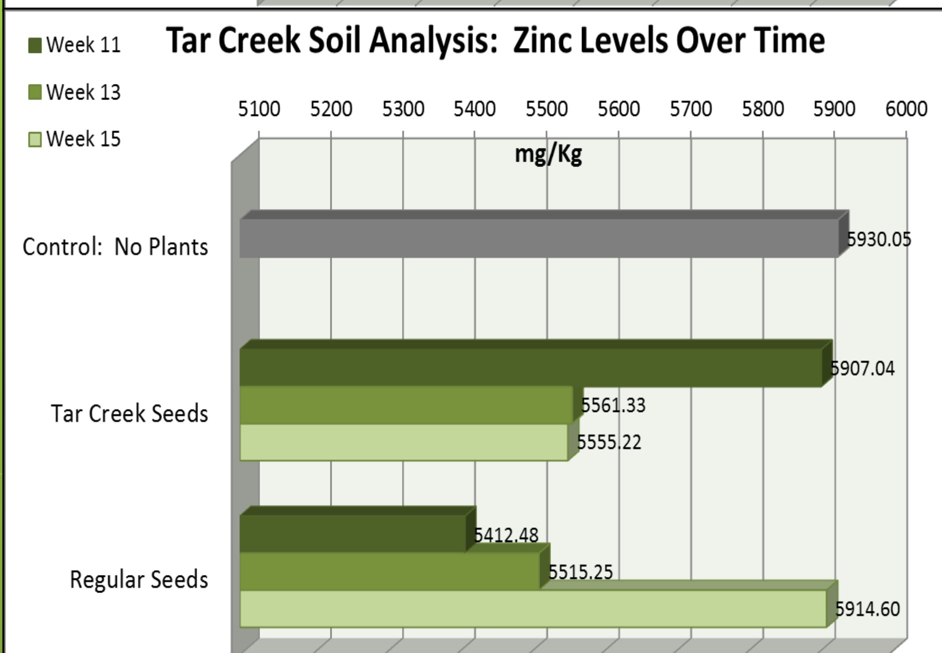


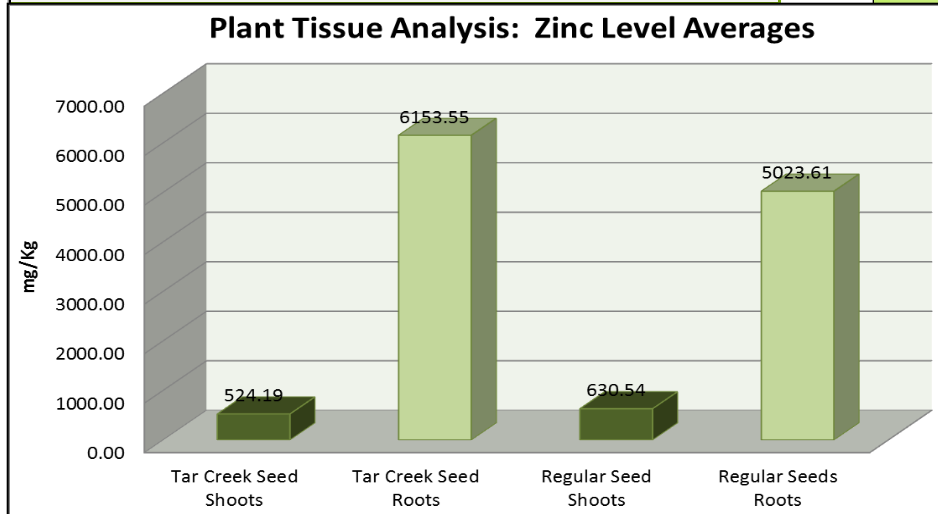
Plant tissue: The Tar Creek seeds accumulated more iron in their roots, as compared to the regular seeds. Also, the Tar Creek seeds translocated less iron to their shoots, compared to the regular seeds.

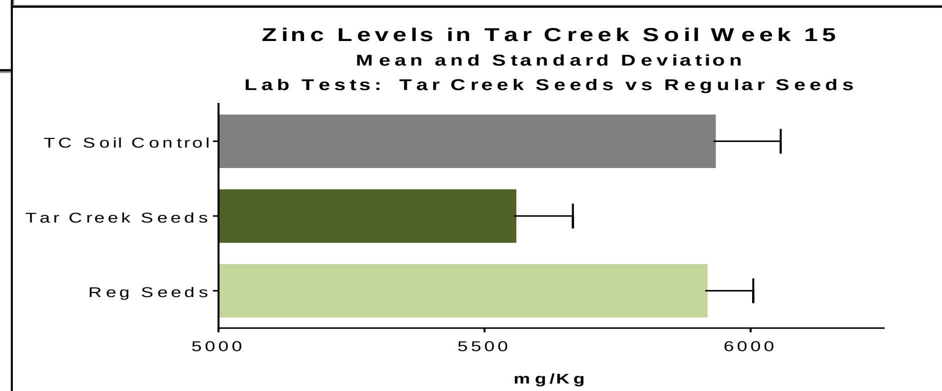
Soil: The soil that had Tar Creek seeds grown in it had continual removal of heavy metals, while the soil with regular seeds was not as effective by week fifteen. Statistically, neither seed was significantly different.

Zinc





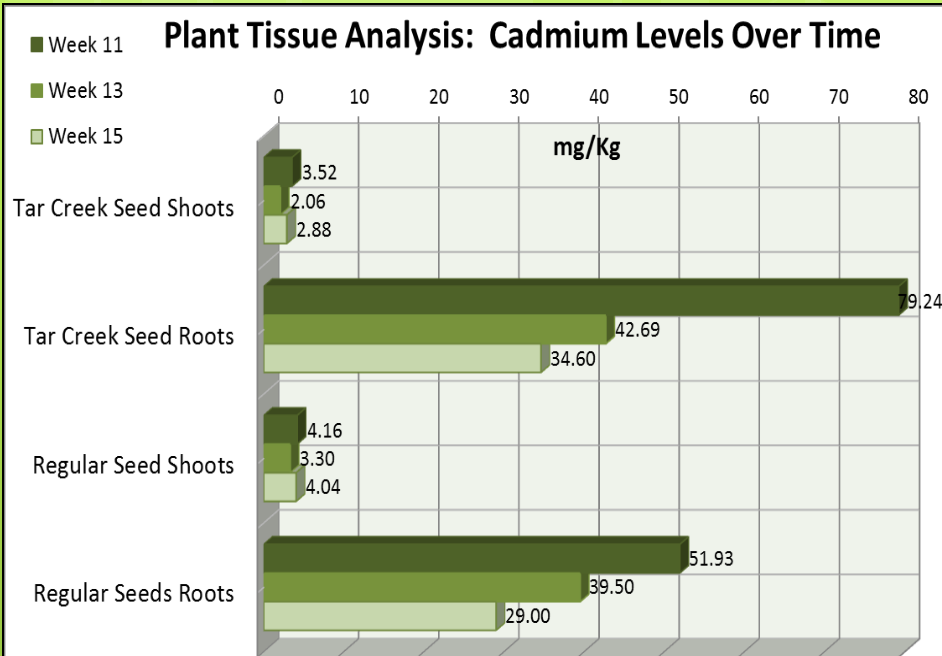


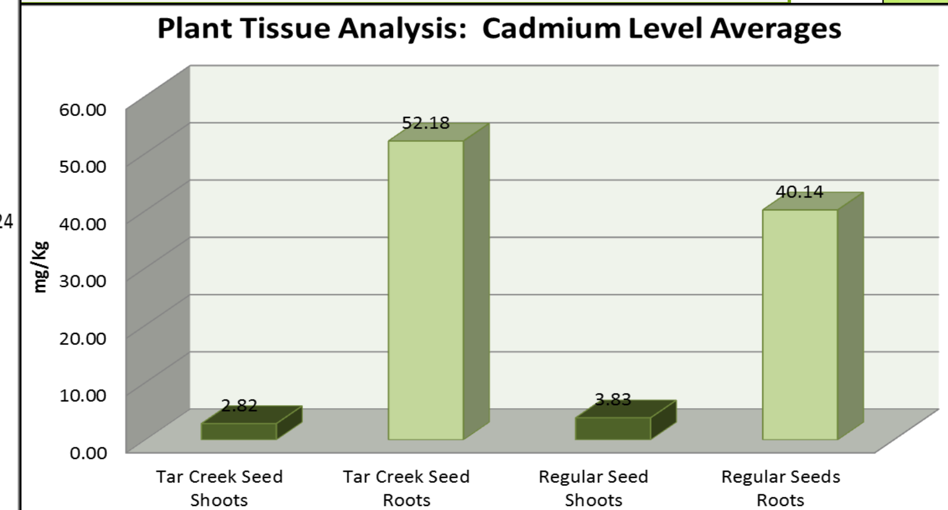


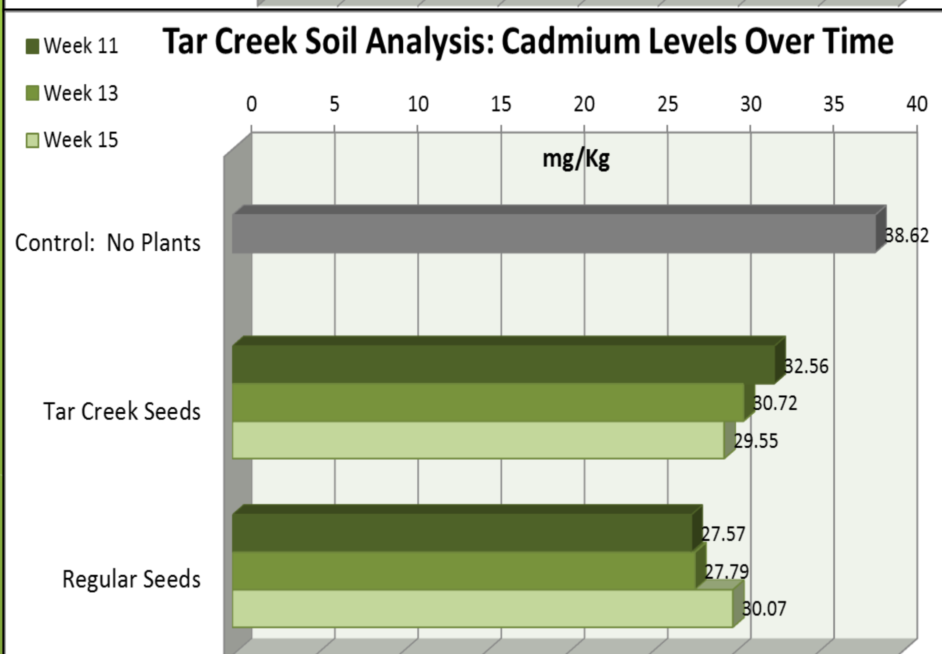
Plant tissue: The Tar Creek seeds accumulated more zinc in their roots, as compared to the regular seeds. Also, the Tar Creek seeds translocated less zinc to their shoots than the regular seeds.

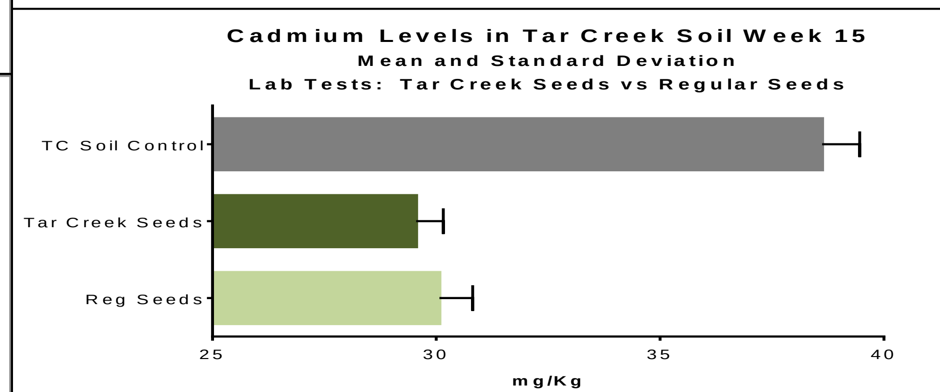
Soil: The soil with Tar Creek seeds grown in it had continual removal of heavy metals. The soil with regular seeds was effective at first, but not as much by week fifteen. Statistically, only the Tar Creek seeds were very significantly different.

Cadmium





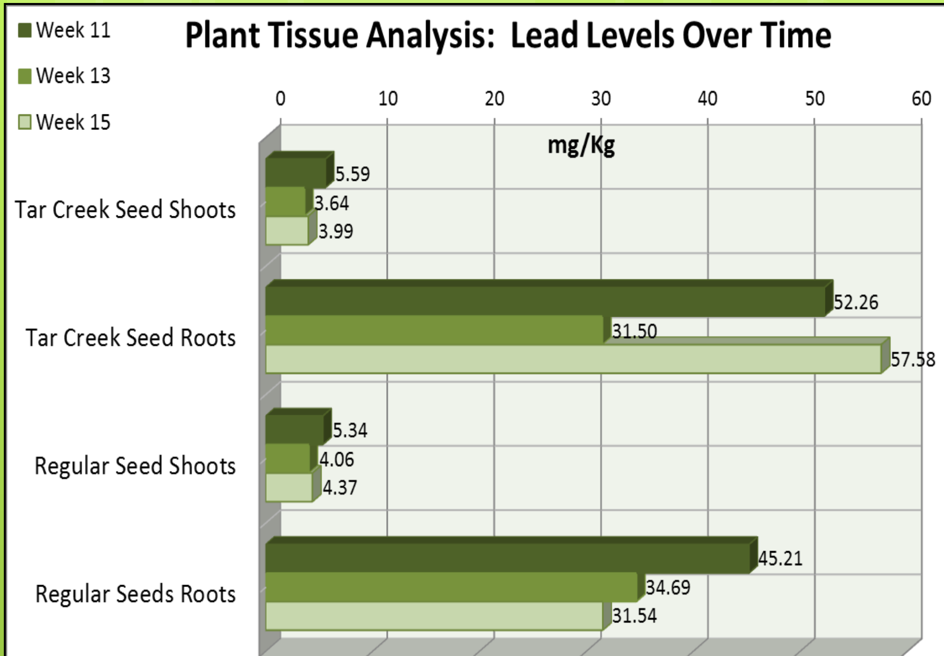


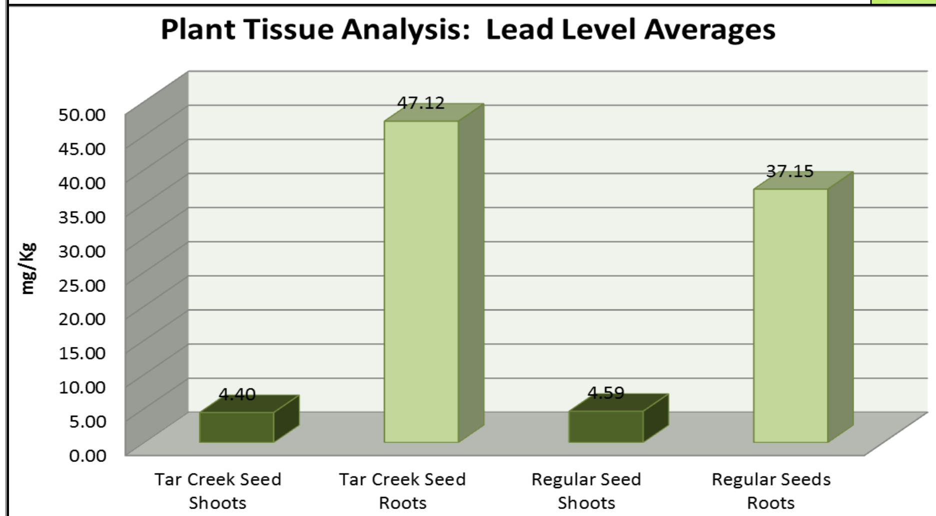


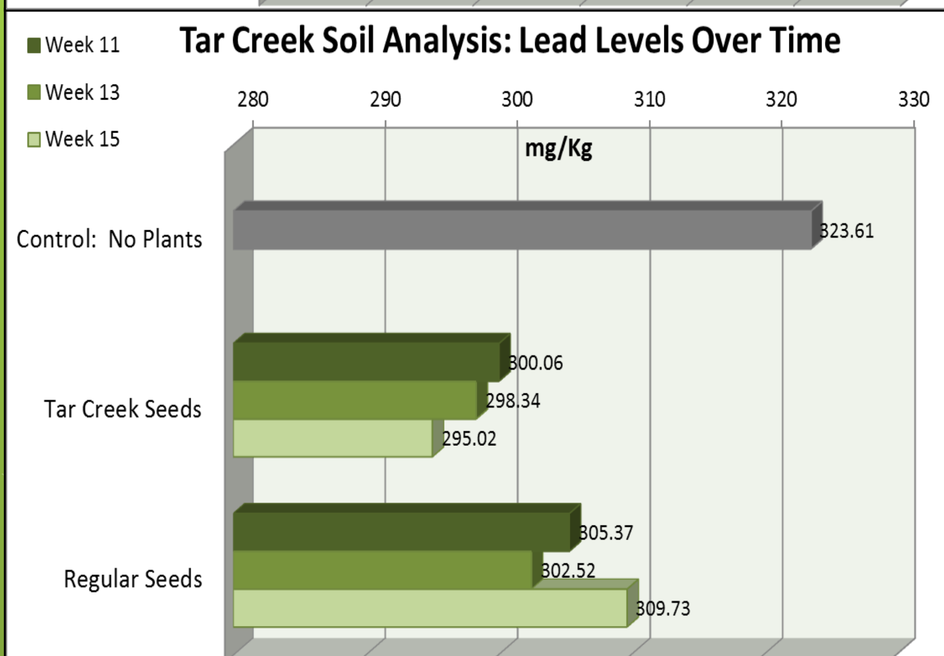
Plant tissue: The Tar Creek seeds accumulated more cadmium in their roots, as compared to the regular seeds. Also, the Tar Creek seeds translocated less cadmium to their shoots than the regular seeds.

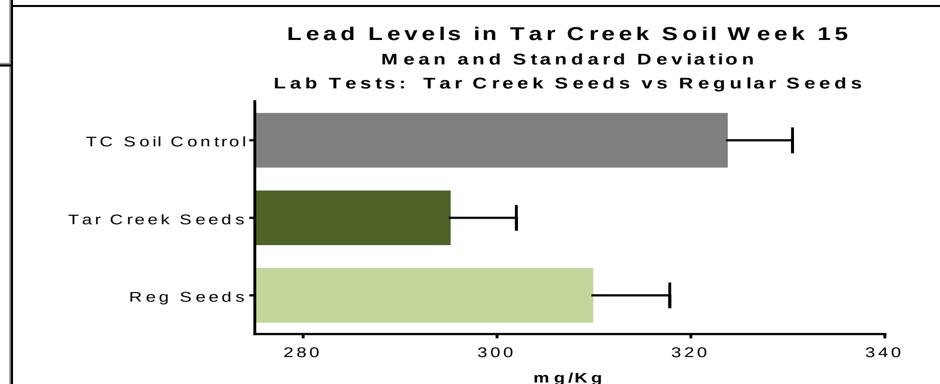
Soil: The soil with Tar Creek seeds grown in it had continual removal of heavy metals. The soil with regular seeds was effective at first, but not as much by week fifteen. Statistically, however, both seed types were extremely significantly different.

Lead





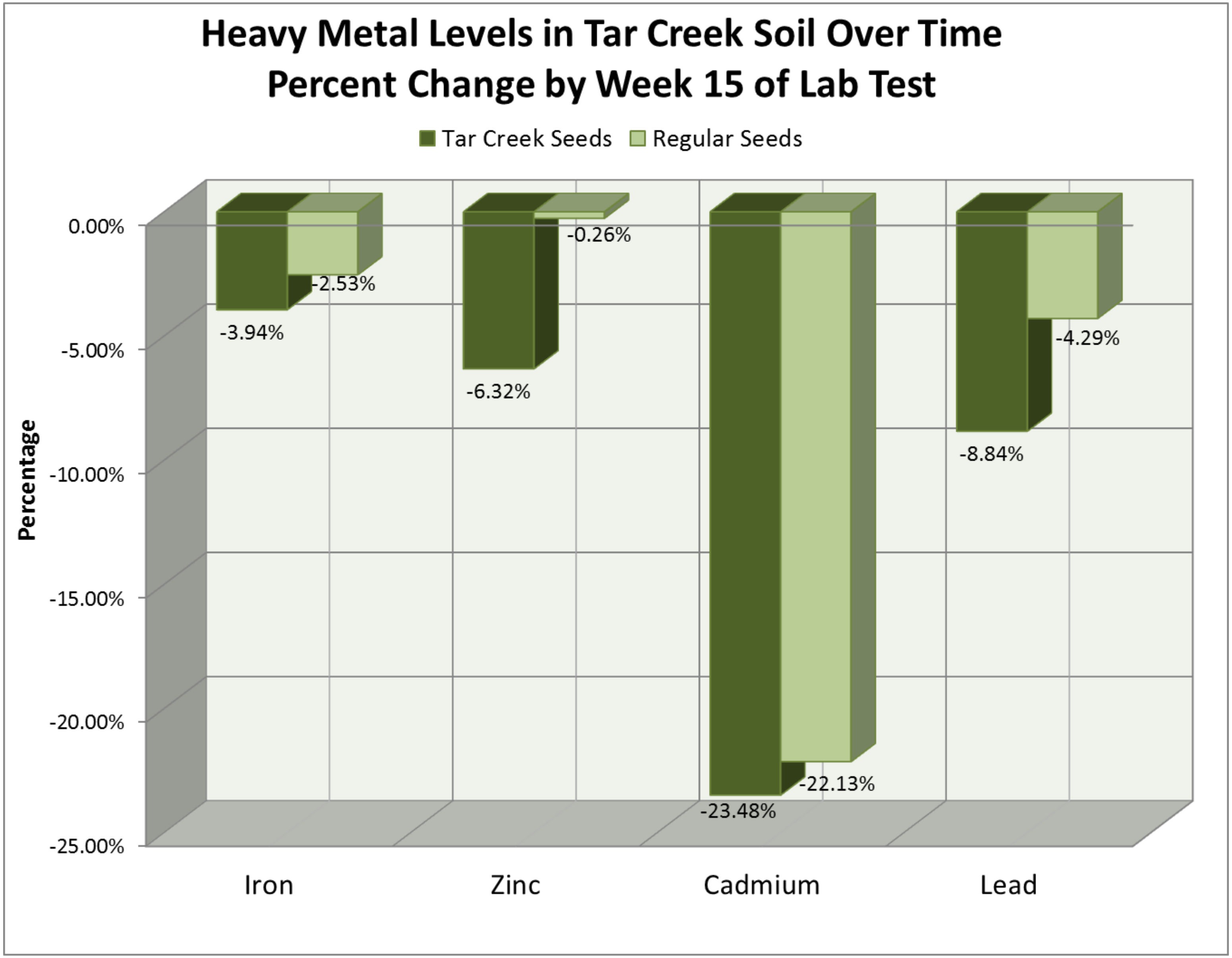




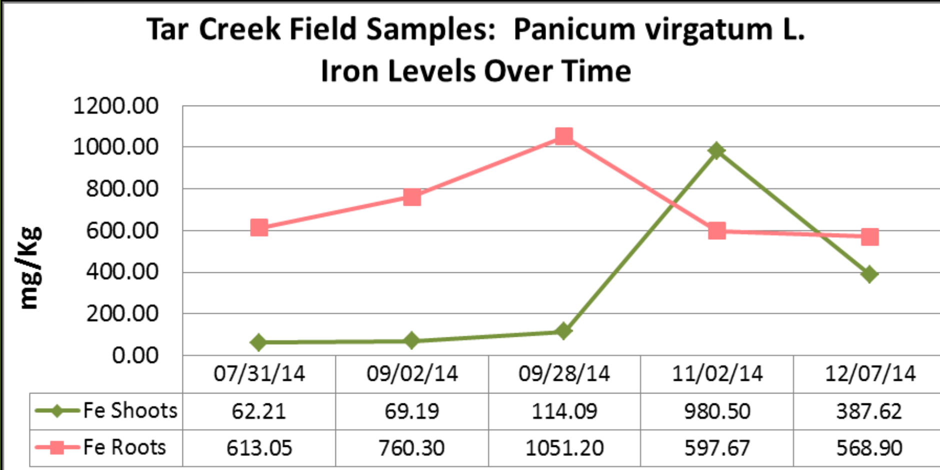
Plant tissue: The Tar Creek seeds accumulated more lead in their roots, as compared to the regular seeds. Also, the Tar Creek seeds translocated less lead to their shoots than the regular seeds.

Soil: The soil with Tar Creek seeds grown in it had continual removal of heavy metals. The soil with regular seeds was effective at first, but not as much by week fifteen. Statistically, only the Tar Creek seeds were very significantly different.

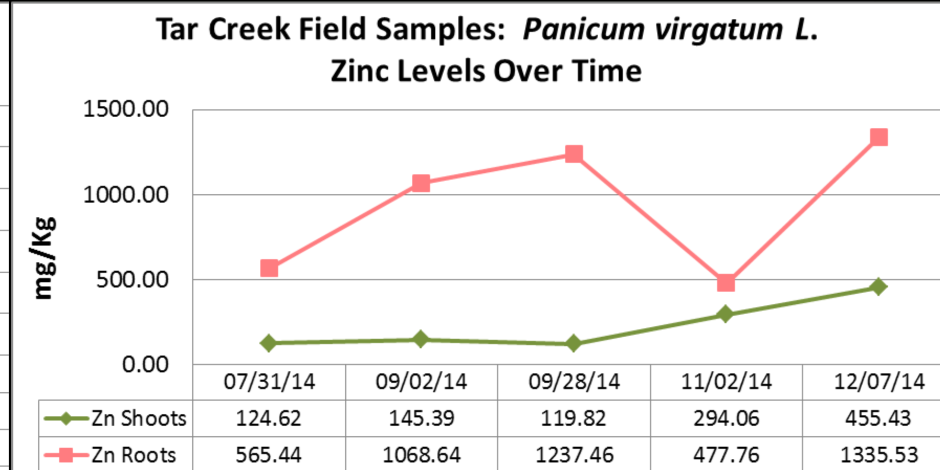
Percent Change in Soil



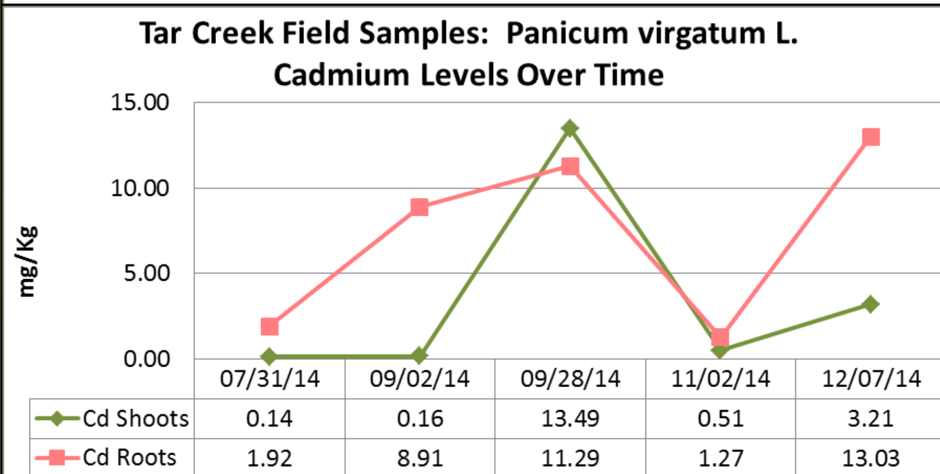
Field Study



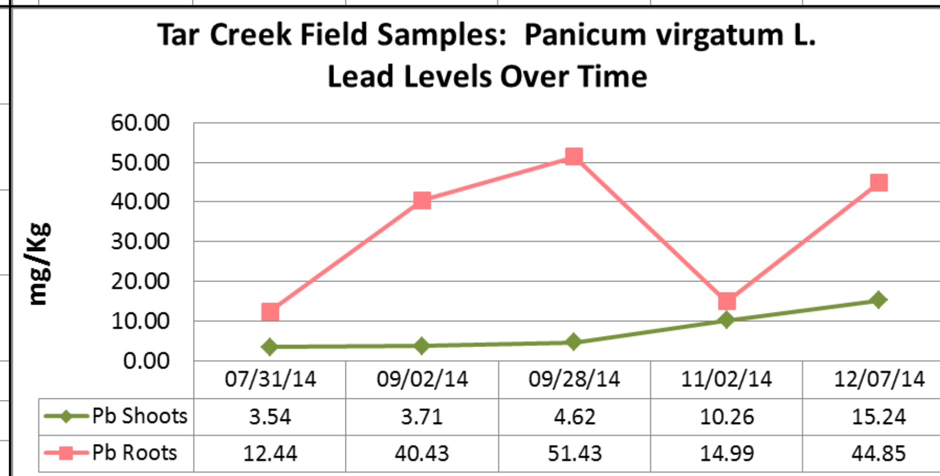
	07/31/14	09/02/14	09/28/14	11/02/14	12/07/14
Fe Shoots	62.21	69.19	114.09	980.50	387.62
Fe Roots	613.05	760.30	1051.20	597.67	568.90



	07/31/14	09/02/14	09/28/14	11/02/14	12/07/14
Zn Shoots	124.62	145.39	119.82	294.06	455.43
Zn Roots	565.44	1068.64	1237.46	477.76	1335.53



	07/31/14	09/02/14	09/28/14	11/02/14	12/07/14
Cd Shoots	0.14	0.16	13.49	0.51	3.21
Cd Roots	1.92	8.91	11.29	1.27	13.03



	07/31/14	09/02/14	09/28/14	11/02/14	12/07/14
Pb Shoots	3.54	3.71	4.62	10.26	15.24
Pb Roots	12.44	40.43	51.43	14.99	44.85

The plants collected at Tar Creek had higher levels of all the heavy metals in the roots of the plants as compared to the shoots, except for cadmium on the 9/28/14 collection and iron on the 11/02/14 collection. The levels of heavy metals in the roots decreased on the 11/02/14 collection for all of the metals, while the level of metals increased in the roots on the 12/07/14 collection. These trends could have been caused by the weather; a freeze happened the day before the 11/02/14 collection, and warmer temperatures occurred the week before the 12/07/14 collection.

Statistical Data

Statistical Analysis using Unpaired T-Test
Lab Study: Tar Creek Soil Levels at Week 15
Comparison of Tar Creek Seeds vs Regular Seeds

Comparison	P Value	Significance
Iron: Control Soil vs Soil with Tar Creek Seeds	0.0617	Not Significant
Iron: Control Soil vs Soil with Regular Seeds	0.3679	Not Significant
Zinc: Control Soil vs Soil with **Tar Creek Seeds**	0.0072	Very Significant
Zinc: Control Soil vs Soil with Regular Seeds	0.8407	Not Significant
Cadmium: Control Soil vs Soil with **Tar Creek Seeds**	<0.0001	Extremely Significant
Cadmium: Control Soil vs Soil with **Regular Seeds**	<0.0001	Extremely Significant
Lead: Control Soil vs Soil with **Tar Creek Seeds**	0.0042	Very Significant
Lead: Control Soil vs Soil with Regular Seeds	0.0718	Not Significant

Applications/ Impact

- The results of this project could be applicable in many ways.
- Large scale:
  - Grass could be used to remediate extremely polluted sites such as a Superfund site like Tar Creek.
  - From the field study results, it is understood that the switchgrass growing at Tar Creek is removing heavy metals from the soil, and storing them in the plant tissue.
  - The plants could help remediate the area in a nondestructive way.
- Small scale:
  - Grass could be beneficial when used in buffer or riparian zones to limit nonpoint source pollution.
  - Also, switchgrass could be used in rain gardens to remediate pollutants coming off of a parking lot.
  - Since the grass was able to grow and remove heavy metals in such high levels, they would more than likely be very effective in these nonpoint source pollution areas.
- Since the seeds collected from Tar Creek showed the trend of being more effective than regular seeds, this project could shed light on a genetic evolution of the plant. More research would need to be conducted to determine exactly what is allowing the plants to be successful at phytoremediation.
- Using phytoremediation would be a much more cost effective to traditional clean-up methods, while preserving a habitat and allowing an area to heal naturally.

Analyses

Lab Study:

- **The hypothesis that that *Panicum virgatum L.* grown from seeds collected from plants at the Tar Creek Superfund Site will be more effective at phytoremediation of heavy metals was accepted.**
  - While the regular seeds showed signs of removal in the first sample, this trend did not continue.
  - By week fifteen of growth the Tar Creek seeds had removed more heavy metals from the soil as compared to the regular seeds, with a down ward trend in all of the metals.
  - Looking at the plant tissue, the Tar Creek seeds accumulated more of the metals in their roots, while the regular seeds allowed the metals to translocate to the shoots.

Field Study:

- **The hypothesis that the level of heavy metals in *Panicum virgatum L.* collected at the Tar Creek Superfund Site will be higher during active growth, and lower during dormancy was accepted.**
  - The plants exhibited a general pattern of having higher levels of heavy metals during active growth.
  - The level of metals in the plant tissue decreased on November 2, 2014, possibly due to a freeze that occurred the day before.
  - The level of metals increased in the plant tissue of the last collection in December, possibly due to the fact that even though the plants were dormant, the warmer temperatures that week allowed the metals to increase in the plants

Conclusions

- **Challenges:**
  - Firstly, the challenge of determining exactly which plants were growing at Tar Creek was a key component for this study.
  - Secondly, the collection of seeds also proved challenging. It was imperative that the seeds were collected at the height of viability.
    - Since the seeds had to be stratified and then planted there was only a small window for taking samples.
- **Future Studies:**
  - It would be interesting to watch the plants in the lab over a longer period of time, to see if the trends found in this study would continue.
  - Another field study would be interesting.
    - The plants could be examined over a year, or longer, along with soil and water samples.
  - Since the Tar Creek seeds showed a trend of removing more heavy metals than the regular seeds, it might be beneficial to do some genetic testing on the plants.
  - It would also be interesting to find a way to remove the metals from the plants so that they could be recycled and used in industry.

Conclusions

- **The pros to using switchgrass:**
  - It could remediate an area naturally and cost effectively.
  - Since most of the metals stayed in the roots of the plants there would be less of a chance of bioaccumulation through the food chain.
  - The results from this study also suggest that switchgrass may be able to develop a mechanism to deal with high levels of heavy metals, making it an ideal plant for phytoremediation.
- **The con to using switchgrass**:
  - Since the metals stay in the roots, it may be harder to remove the grasses from the environment if needed.

Acknowledgements

- Dr. Yanqi Wu, switchgrass geneticist from Oklahoma State University for helping me identify the switchgrass variety at Tar Creek, for lending me some seed of the same variety and for helping me clean and prepare my collected seeds.
- Nancy Wolf, lab manager at University of Arkansas's Soil and Plant Laboratory for allowing me to work in her lab and for all the information about procedures and for teaching me to use the ICP-OES Spectro Arcos machine.
- My mother and teacher Keli Steen for her continued support and guidance throughout this project.

Bibliography

Albright, Vurtice. "Investigations into the Capabilities of Switchgrass to Phytoremediate Atrazine Contamination in Surface Water Runoff." University of Iowa, 2011. Web. Oct. 2014. <http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=1474&context=etd>.

"Cadmium." *Safety and Health Topics*. United States Department of Labor, n.d. Web. Nov. 2014. <https://www.osha.gov/SLTC/cadmium/>.

Beavington, F. "Heavy Metal Soil Contamination." *Environmental Pollution* 9.3 (n.d.): 1-7. USDA/ NRCS, Sept. 2000. Web. 30 Oct. 2014. <http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_053279.pdf>.

Grubinger, Vern, and Don Ross. *Interpreting the Results of Soil Tests for Heavy Metals*. University of Vermont, n.d. Web. 4 Oct. 2013.

Hottle, Troy. *Grounds For Change Activating Vacant Land*. N.p., 2015. Web. Jan. 2015. <http://www.gfcactivatingland.org/explore/precedents/almono-brownfield/>.

Jeliazkova, Ekaterina A., and Lyle E. Craker. "HEAVY METALS AND SEED GERMINATION IN SOME MEDICINAL AND AROMATIC PLANTS." University of Massachusetts, n.d. Web. Oct. 2014. <http://www.cprm.gov.br/pgagem/Manuscripts/jeliazkovaea.htm>.

"'Kanlow' Switchgrass Panicum Virgatum L." *Plant Materials Program* (n.d.): n. pag. May 2011. Web. 5 Nov. 2014. <http://www.nrcs.usda.gov/Internet/FSE_PLANTMATERIALS/publications/kspmcrb10373.pdf>.

Lasat, Mitch M. "The Use of Plants for the Removal of Toxic Metals from Contaminated Soil." (n.d.): n. pag. Web. 2 Dec. 2014. <http://clu-in.org/download/remed/lasat.pdf>.

"Phytoremediation." *Teach Tree*. N.p., n.d. Web. 28 Oct. 2014. <http://www.cpeo.org/techtree/ttdescript/phytrem.htm>.

"Using Phytoremediation to Clean Up Sites." *EPA*. Environmental Protection Agency, n.d. Web. 6 Oct. 2014. <http://www.epa.gov/superfund/accomp/news/phyto.htm>.

United States. DEQ. *Oklahoma Plan for Tar Creek*. N.p.: n.p., n.d. Print.

West, Chuck. "Establishing and Maintaining Perennial Grass Crops for Energy: Emphasis on Switchgrass." (n.d.): 1-2. University of Arkansas. Web. 23 Oct. 2014. <http://www.farmfoundation.org/news/articlefiles/1712-West%20hndout.pdf>.