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Honors Thesis

SUNFLOWER SEEDLINGS FAIL TO REMOVE URANIUM POLLUTION IN THE  
NAVAJO NATION: PARTICIPATORY SCIENCE AS A PATH TO BUILD  
COMMUNITY AND ADDRESS ENVIRONMENTAL INJUSTICE

by  
Zak Webber

Submitted to Brigham Young University in partial fulfillment of graduation requirement  
for University Honors

Molecular Biology  
Brigham Young University  
April 2020

Advisor: Benjamin Abbott  
Honors Coordinator: Paul Evans



## ABSTRACT

### SUNFLOWER SEEDLINGS FAIL TO REMOVE URANIUM POLLUTION IN THE NAVAJO NATION: PARTICIPATORY SCIENCE AS A PATH TO BUILD COMMUNITY AND ADDRESS ENVIRONMENTAL INJUSTICE

Zak Webber  
Molecular Biology  
Bachelor of Science

Mid-20<sup>th</sup> century mining on Naabeehó Bináhásdzo (Navajo Nation) polluted groundwater with high concentrations of uranium and arsenic. The Navajo Nation and other rural residents of this region use groundwater for drinking, livestock, and irrigation. However, many individuals and communities must purchase and transport treated water from locations that are often hours away. Sunflowers have been shown to preferentially take up heavy metals, including uranium and arsenic, potentially representing a tool to improve water quality through on-site, low-cost phytoremediation. We carried out a collaborative research project with a high school class on the Navajo Nation in 2018 and 2019. The students collected surface water from wells and streams near where they lived, which we analyzed for general chemistry parameters. We then performed a laboratory experiment with sunflower seedlings grown in local soil to assess whether phytoremediation could be effective at removing arsenic and uranium. We found that arsenic concentration did not change over the course of the experiment and uranium concentration increased, potentially associated with weathering of the geologically young

soil in this area. Though it appears phytoremediation is not a feasible solution for heavy-metal contamination in the Navajo Nation, the participatory science approach created meaningful relationships and an important collaboration. Additionally, this project provided experiential learning opportunities for Navajo high school students and the BYU undergraduate students who worked with them.



## ACKNOWLEDGEMENTS

This project is the result of many individual's passion for science within their community. Thanks to the community at Brigham Young University, I have had the wonderful opportunity to pursue a question that was important to me. I am grateful for the insight given from many professors and the enthusiasm of the honors advisors. The funding from the honors project allowed me to pursue my thesis question and pay for much of the analysis.

I was extremely fortunate to be connected to Tommy Rock who was instrumental in giving me the initial direction for this project. His research and connection to Navajo Nation greatly impacted the success of our project. I thank him for his patience with me as I tried to approach a project that was in many ways outside of my field.

Sarah Groenwald went above and beyond the expectations of our collaboration. Her students and classes performed the experiments and willingly served as our laboratory technicians. Without her collaboration I could not have been successful in this endeavor. I am grateful to her for her hospitality and dedication to the project.

I am grateful to Isaac St. Clair, Rebekah Frei, and other members of the lab who worked to teach me analysis, learn how to acid rinse all sorts of objects. I was amazed by the kindness that both demonstrated to me throughout this process. They were helpful connecting me to lab resources and tools.

Above all I express my gratitude to Ben Abbott who was willing to take on a unique project as I pursued a "what if?". In many moments of discouragement, I felt buoyed up by his interest in the research and his concern for me. He has mentored me in

experimental design, writing, and data analysis. Without him this project would not have been possible. I am deeply touched by the energy and investment Dr. Abbott has put into this project.





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## **Important terms and definitions**

*Phytoremediation*- Restoring an environment using plant life.

*Rhizofiltration*- Removing a solute or particulate constituent through root processes.

*Endemic*- Plant or soil that originally comes from the referenced area.

*Regolith*- Unweathered or poorly weathered rocky material present above bedrock.

*Soil matrix*- The living and nonliving constituents (soil particles, roots, animals) that make up the solid portion of the soil.

*Soil solution*- The water and dissolved constituents that exist within and around the soil matrix.

*Abiotic interactions*- Non-biological relationships such as physical or chemical reactions involving water, regolith, light, etc.

*Helianthus annuus*- A “mammoth” sunflower species cultivated commonly in many areas of the world.

*Participatory science*- An approach that involves non-professionals (e.g. community members, students, etc.) in the research process. Participating individuals may contribute to identifying the research question, setting priorities, collecting data, and interpreting and communicating results.

## Background

Uranium mining sponsored by the U.S. government in the late 20<sup>th</sup> century degraded public health, agriculture, and the biosphere of the Four-Corners region<sup>1</sup> of the United States<sup>2</sup>. These effects have directly affected the lifestyle and livelihood of the Diné people, commonly referred to as the Navajo<sup>3</sup>. The hundreds of abandoned mine structures in the Navajo Nation have been linked with the presence of trace heavy metals, such as uranium and arsenic in soils and water<sup>4</sup>. Exposure to uranium causes many adverse health effects including hypertension, renal



**Figure 1.** Photo of a contaminated well on the Navajo Nation. Groundwater and surface water were polluted with uranium, arsenic, and other heavy metals from widespread mining during the 20<sup>th</sup> century.

<sup>1</sup>Dashner-Titus, E. J., Hoover, J., Li, L., Lee, J.-H., Du, R., Liu, K. J., Traber, M. G., Ho, E., Lewis, J., & Hudson, L. G. (2018). Metal exposure and oxidative stress markers in pregnant Navajo Birth Cohort Study participants. *Free Radical Biology and Medicine*, 124, 484–492. <https://doi.org/10.1016/j.freeradbiomed.2018.04.579>

<sup>2</sup> Navajo Nation: Cleaning Up Abandoned Uranium Mines. (2020, January 14). Retrieved from <https://www.epa.gov/navajo-nation-uranium-cleanup>

<sup>3</sup> *Uranium Contamination in the Navajo Nation: An Environmental Justice Impact Analysis | Global Ecological Humanities*. (2017). Duke.Edu. [https://sites.duke.edu/lit290s-1\\_02\\_s2017/2017/03/03/uranium-contamination-in-the-navajo-nation-an-environmental-justice-impact-analysis/](https://sites.duke.edu/lit290s-1_02_s2017/2017/03/03/uranium-contamination-in-the-navajo-nation-an-environmental-justice-impact-analysis/)

<sup>4</sup> Brugge, Doug, and Rob Goble. “The History of Uranium Mining and the Navajo People.” *American Journal of Public Health* 92, no. 9 (September 2002): 1410–19.

failure, and autoimmune disease <sup>5</sup> and is considered unsafe for human consumption in concentrations of 30 µg/L<sup>6</sup>. Arsenic is also commonly found in water sources and even small amounts of arsenic can be toxic and cause vomiting and even death. Many wells have been shut down due to uranium and arsenic concentrations in exceedance of the Environmental Protection Agency (EPA) standards. Despite these measures many Navajo were in the 95<sup>th</sup> percentile of urine concentrations of U indicating ongoing contamination<sup>7</sup>. The EPA has acknowledged the seriousness of uranium mining contamination, but despite increased scientific investigation<sup>8</sup>, a cost-effective solution to resolve this issue has yet to be found.

Many members of the Navajo Nation have limited access to clean water because mine tailings have contaminated their groundwater<sup>2</sup>. Consequently, families must travel to wells that are often hours away to collect water that is safe for consumption, leading to health and resource disparities<sup>9</sup>. This is a classic example of environmental injustice, when the individuals who benefited from environmental degradation are not those who

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<sup>5</sup> US EPA,REG 09. (2016, June 15). *Providing Safe Drinking Water in Areas with Abandoned Uranium Mines* | US EPA. US EPA. <https://www.epa.gov/navajo-nation-uranium-cleanup/providing-safe-drinking-water-areas-abandoned-uranium-mines>. See also: “Health Effects of Uranium” on website.

<sup>6</sup> Table 2. Navajo Nation Contaminated Unregulated Water Sources. (2012). Epa.gov. Retrieved 30 October 2018, from <https://www.epa.gov/sites/production/files/2016-06/documents/watersourcestable-with-mcls.pdf>

<sup>7</sup> *Evaluation of Water Cistern Contamination and Health Risks in the Navajo Nation*. (2020). [https://www.cdc.gov/nceh/hsb/cwh/navajo\\_nation.htm](https://www.cdc.gov/nceh/hsb/cwh/navajo_nation.htm)

<sup>8</sup> US EPA,OA. (2018, September 10). *U.S. EPA awards \$429,000 to Diné College for abandoned uranium mine studies*. US EPA. US EPA. <https://www.epa.gov/newsreleases/us-epa-awards-429000-dine-college-abandoned-uranium-mine-studies>

<sup>9</sup> Fact Sheets, Disparities. (2013). *Disparities. Fact Sheets*. Newsroom. <https://www.ihs.gov/newsroom/factsheets/disparities/>

bear its cost<sup>10</sup>. In this case, the general U.S. population benefitted from the uranium mining, which enhanced national security and provided energy, but the residents of the sovereign Navajo Nation alone are suffering the environmental consequences of this mining.

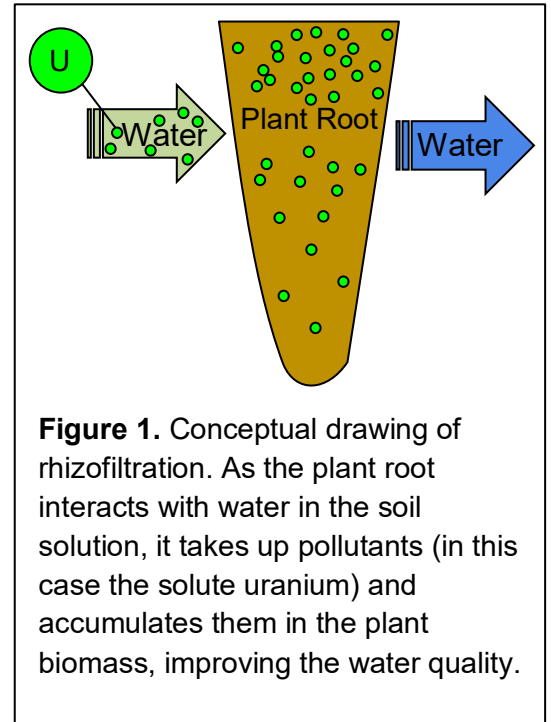
I have personally seen the individual and family difficulties caused by groundwater contamination in the Navajo Nation. In 2018, I had the opportunity to teach Navajo students in 7<sup>th</sup> to 10<sup>th</sup> grade. When I learned that a third of Navajo still do not have access to potable water at home<sup>2</sup>, I felt the urgency of addressing this injustice and decided to focus on this subject for my thesis. Long trips to collect clean water were a part of their life, and I saw how this burden affected students and families. Additionally, while families will travel great distance for personal drinking water, many unregulated wells are used by livestock, despite concentrations of uranium that are considered unsafe for human consumption<sup>3</sup>. It is unknown whether uranium is bioaccumulating in the meat or milk of the livestock drinking contaminated water or in the tissues of crops watered with contaminated water in the Navajo Nation, though this is a potential vector for human harm<sup>11</sup>. To my knowledge, there is no testing of livestock or crops produced in the Navajo Nation, though these are widespread food sources<sup>7</sup>. The local community has gone to great lengths to solve water contamination. They have dug new wells, trucked water to aquifers, and investigated wells which are contaminated. However, many of these solutions are costly and require specialized equipment.

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<sup>10</sup> Daniels, Brigham, Michalyn Steele, and Lisa Grow Sun. "Just Environmentalism." SSRN Scholarly Paper. Rochester, NY: Social Science Research Network, May 1, 2018. <https://papers.ssrn.com/abstract=3178353>.

<sup>11</sup> Corlin, L., Rock, T., Cordova, J. et al. Health Effects and Environmental Justice Concerns of Exposure to Uranium in Drinking Water. *Curr Envir Health Rpt* 3, 434–442 (2016). <https://doi.org/10.1007/s40572-016-0114-z>

In the past few decades, phytoremediation (using plant growth to reduce soil pollutants) has been applied in other areas with heavy-metal contamination. This approach has substantial benefits because of its low cost and relatively simple infrastructure requirements<sup>12</sup>. Several different processes are involved in phytoremediation, including rhizofiltration, where plant roots accumulate contaminants through passive or active uptake (Fig. 1). When active uptake occurs, the plant preferentially removes the contaminant from the soil solution faster than it takes up water for transpiration. This has been shown to work for many heavy metals, including



**Figure 1.** Conceptual drawing of rhizofiltration. As the plant root interacts with water in the soil solution, it takes up pollutants (in this case the solute uranium) and accumulates them in the plant biomass, improving the water quality.

arsenic<sup>13</sup>. Until recently, few plants had been tested for their ability to remediate uranium-contaminated water<sup>14</sup>. However, Lee and Yang recently discovered that, in a hydroponic environment, *Helianthus annuus* (the common sunflower) successfully removes uranium with a peak removal of 90% in less than 10 hours<sup>15</sup>. Their research

<sup>12</sup> Lone, M. I., He, Z. L., Stoffella, P. J., & Yang, X. E. (2008). Phytoremediation of heavy metal polluted soils and water: progresses and perspectives. *Journal of Zhejiang University. Science. B*, 9(3), 210-20.

<sup>13</sup> Abbas, Ghulam et al. "Arsenic Uptake, Toxicity, Detoxification, and Speciation in Plants: Physiological, Biochemical, and Molecular Aspects." *International Journal of Environmental Research and Public Health* 15.1 (2018): 59. PMC. Web. 10 Oct. 2018.

<sup>14</sup> "Memorandum: May 16, 2005. Phytoremediation Pilot Study at the Monument Valley UMTRA site". Madeline Roanhorse. Navajo AML/UMTRA Department. Accessed Oct. 24 2018. file:///home/chronos/u-6be40415b559bbcf34ed78cf14fa8eac8d27c89/Downloads/MON000033.pdf

<sup>15</sup> Lee M, Yang M. "Rhizofiltration using sunflower (*Helianthus annuus* L.) and bean (*Phaseolus vulgaris* L. var. *vulgaris*) to remediate uranium contaminated groundwater". *J Hazard Mater.* 2010 Jan 15;173(1-3):589-96. Doi:10.1016/j.jhazmat.2009.08.127. Epub 2009 Sep 1. PubMed PMID: 19783370.



suggests that sunflowers and related species could be effective in the removal of uranium from contaminated water.

The discovery of sunflower rhizofiltration of uranium raises several questions with scientific and societal implications. Would this method be effective at removing uranium from contaminated water in a soil matrix, rather than only in a hydroponic environment? Specifically, does the filtration work in the arid soil of Navajo Nation as effectively as in a hydroponics system without mineral interactions? How might differences in chemistry and soil organisms increase or decrease the filtration rate? Is this method scalable to a rural area with many end consumers and no centralized water distribution system? To address these questions, I initiated a project with my faculty adviser to collaborate with high school students in the Navajo Nation and undergraduate students from BYU. Using this participatory approach, we evaluated the efficacy of phytoremediation in a laboratory incubation using soil from the Navajo Nation.

## Methods

### *Initiating the collaboration*

We undertook this project from a framework of environmental justice and community collaboration. This approach, which is commonly referred to as citizen science or participatory science, can increase the relevance and quality of scientific research, particularly when the area of scientific inquiry has implications for society<sup>16</sup>. As opposed to a “scientist-only” approach, which may or may not respond to external input and priorities, a community centered approach seeks input from community collaborators and other stakeholders. A community centered approach seeks to determine the relevance of the research by integrating the input of non-professionals in the scoping, implementation, and interpretation of the work. While there has been some resistance to and criticism of participatory approaches for potential issues in data quality and control, these methods have effectively been implemented in many fields of research including environmental hydrology<sup>17</sup>. Because they involve the community in the whole scientific process, the benefit of science is more apparent and can deliver valuable experiences to local participants before the sometimes-long time lags between data collection and peer-reviewed publication of results<sup>16</sup>.

We began our project by identifying collaborators within the Navajo Nation. We

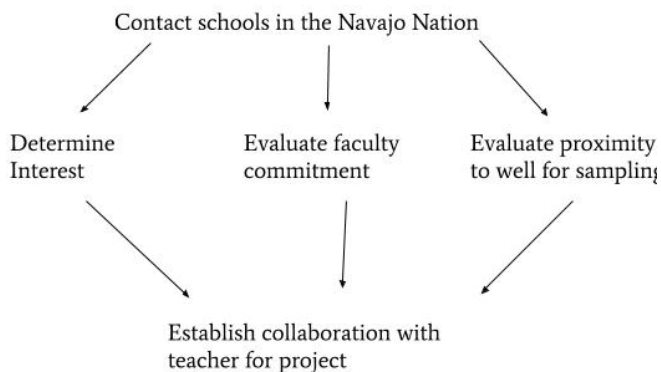
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<sup>16</sup> Buytaert, W., Zulkafli, Z., Grainger, S., Acosta, L., Alemie, T. C., Bastiaensen, J., De Bièvre, B., Bhusal, J., Clark, J., Dewulf, A., Foggin, M., Hannah, D. M., Hergarten, C., Isaeva, A., Karpouzoglou, T., Pandeya, B., Paudel, D., Sharma, K., Steenhuis, T., ... Zhumanova, M. (2014). Citizen science in hydrology and water resources: opportunities for knowledge generation, ecosystem service management, and sustainable development. *Frontiers in Earth Science*, 2. <https://doi.org/10.3389/feart.2014.00026>

<sup>17</sup> Abbott, Benjamin W., Florentina Moatar, Olivier Gauthier, Ophélie Fovet, Virginie Antoine, and Olivier Ragueneau. “Trends and Seasonality of River Nutrients in Agricultural Catchments: 18 Years of Weekly Citizen Science in France.” *Science of The Total Environment* 624 (May 15, 2018): 845–58. <https://doi.org/10.1016/j.scitotenv.2017.12.176>.

contacted high schools to gauge interest and considered the research objectives in discussion with potential collaborators within the Navajo Nation prior to formulating the full scope of our project. We identified a list of schools and contacted administrators via phone and email. We made additional contacts through social media platforms such as LinkedIn ©. Schools were selected based on interest and proximity to well data provided by our collaborator Tommy Rock (Ph.D.) who sampled well data for the Navajo Nation Environmental Protection Agency. Of eight schools contacted, two expressed interest and one had an environmental science teacher (Ms. Groenwald) who readily committed to the project. For these reasons, we worked with Chinle high school as our collaborating school (Fig. 2).

**Figure 2. Method of establishing high school collaboration:**



Once we had established a collaboration with Ms. Groenwald, we arranged a visit on April 5, 2019 to meet the students at Chinle High School and review the experimental design. Dr. Rock presented us to the class who would be participating in the research project.

In addition to the engagement at the high-school level, we contacted the Navajo Nation Environmental Protection Agency and inquired whether they were interested in collaborating. Members of the Navajo Nation Protection Agency felt the project was

important and should be presented to the tribal chapters for approval. They expressed interest in the project and provided information about the history of uranium problems in the Navajo Nation water supply. An appointment was made with the Many Farms Tribal Chapter. We prepared an informational packet and proposal that requested approval from the council of elders. We provided these materials to Ms. Groenwald who presented them to the chapter on April, 8 2019 explaining the goals of the project. The elders were very accommodating and provided the location of wells for sampling and offered additional assistance if necessary.

### **Experimental design**

The collaboration consisted of two major components: 1. collection of surface-water by students and 2. a student-led phytoremediation experiment. I describe both of these below.

During our first visit to Chinle High School, we initiated a sample collection project to catalyze reflection in students of the causes of water quality. We invited each student to select a location from surface-water sources and wells near where they lived (Table 1). Prior to departing, we taught students the procedure for collecting a water sample and distributed 125 mL and 60mL high-density polyethylene (HDPE) bottles, syringes, and filters. We left a detailed protocol with images and illustrations of the process for each student.

**Table 1.** Outline of procedure for sample collection used by citizen scientists.

<b>Step</b>	<b>Process</b>
1. Locate site	Find site, note weather conditions, note temperature, record bottle ID and any other observations.
2. Fill the 250 mL bottle	Fill bottle with unfiltered water. Avoid solid things, leaves, floating objects, etc. If possible, find an area where the water is flowing.

3. Fill the 30 mL bottle with filtered water	Using syringe, suck up water using the same guidelines as step 2. Next, place the filter on the syringe end and gently expel the water through into the bottle. Be careful not to push too hard or the filter could break.
4. Preserve the sample	When returning to class have the teacher place 1 drop of HCl in the filtered bottle. Keep samples cool.

We analyzed the student-collected samples for several parameters to help students think about the water quality in the area they live. From the unfiltered bottle, we used a field-deployable spectrophotometer (s::can Messtechnik GmbH, Vienna) to quantify nitrate, total organic carbon, dissolved organic carbon, and turbidity, common water quality parameters. We also used an ion chromatography system (Thermo Scientific Dionex, Sunnyvale) to quantify major anions and cations (dissolved constituents in the water that vary with water source and other conditions). We shared these data with students (Table 2) and invited them to compare the concentrations with the notes they took about the conditions of the sites from which they sampled.

For the phytoremediation experiment, we needed endemic (site-specific) soil and water to test if rhizofiltration could be effective in the Navajo Nation. During our first visit to Chinle, we collected 40 L of soil from the school yard to be used as endemic (site-specific) soil in the phytoremediation experiment. Soil was gathered using trowels and was stored in buckets until the experiment (details below). With input from the tribal chapters, we selected livestock well 10T-254 (36.4409, -109.6385) for collection of water. This well is an artesian well that flows from a spring that has been contained in a spring box. This well had lower levels of uranium than expected previously. During our second visit to Chinle High School on May 20, 2019, we collected the water and soil for the experiment. At the time of water collection from the livestock well, there were many

cattle and a decomposing cow carcass near the site. We collected the water on May 20, 2019 with a temperature of about 20°C. We collected 8L of sample in acid-rinsed, 2-L HDPE bottles. The water was stored in the bottles until use in the experiment two hours later with young student collaborators.

#### *Phytoremediation experimental conditions*

We purchased seeds for mammoth sunflowers (*Helianthus annuus*) from a local greenhouse. Students planted the seeds in 13 plastic cups (500 mL) during our initial visit to Chinle. Sprouting the plants with the students accomplished two important goals. First, it verified that they would grow in the endemic soil and conditions. Second, it increased the involvement and engagement of the students by extending the contact time and providing an opportunity to develop relationships during the cultivation. For example, students chose the plant ID names, assigning humorous and personal names, that we used throughout the experiment.

In case the local seedlings failed to sprout, we grew additional plants for 16 days in the Brigham Young University (BYU) greenhouse using soil collected from the first visit to Chinle. For both sets of seedlings (Chinle and BYU), we followed the same procedure. Two seeds were buried ~1 cm deep in plastic cups that were filled two-thirds with noncompacted soil using a small trowel. We prepared 7 control cups without seedlings to account for the effects of soil alone on the uranium concentrations. The plant containers were perforated to allow water to seep through the bottom for collection and analysis. To allow the seedlings to develop, plants and controls were kept at ~24°C and watered daily for 16 days. Plants used for the experiment were from the greenhouse at BYU. Chinle plants were donated to the high-school community garden.

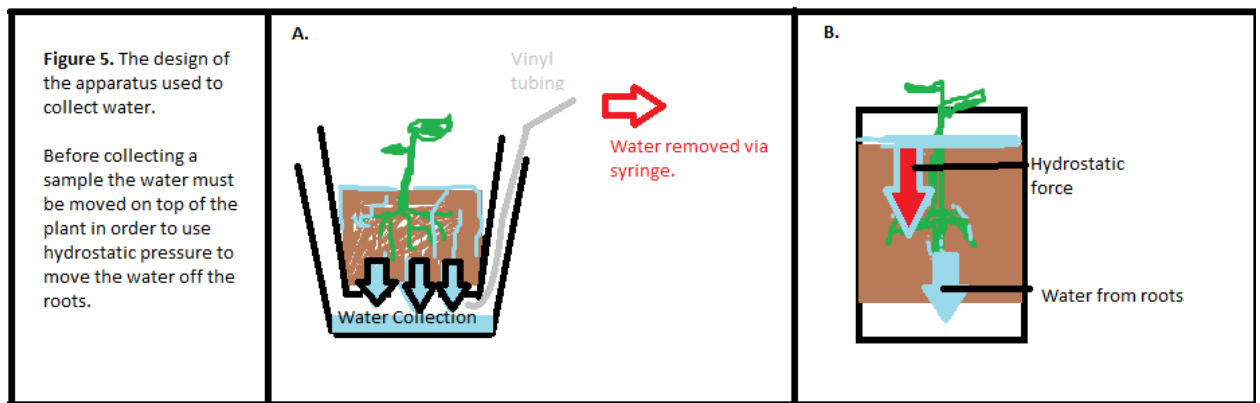


**Figure 4.** Photo of Chinle, the town where the high school we collaborated with was located.

During the experiment itself, we placed each perforated cup into an overlapping cup that had a section of 9-mm inner diameter vinyl tubing laying in it. This design allowed the collection of the water which had infiltrated through the soil matrix, including plant roots for the cups with seedlings (Fig. 5). The other end of the tubing fit on a syringe to collect water at the bottom of the container. A collection container ensured that the plant roots were fully submerged in water and allowed for water collection.

We conducted the experiment at the high school working directly with the teacher and students. First, we added 150 mL of water to each sample at the beginning of the experiment (0 hours). We removed water samples 0, 2, 4, and 12 hours after beginning.

To ensure the water was representative of what was in contact with the soil, the water from the bottom of the collecting cup via syringe and placed on the top of the plant to force all the water below to descend via hydrostatic pressure. Hydrostatic pressure is created by the force of gravity on the water layer above, which forces the water below to exit into the collection duct (Fig. 5). At this point we vacuumed the water and filtered through a glass-fiber filter paper with an effective pore size of 0.7  $\mu\text{m}$ . The filter removes solids, allowing analysis of the solute fraction. We placed filtered samples in acid-rinsed 5 ml polystyrene containers labeled with the plant ID and corresponding time interval. Students performed this portion of the experiment under supervision of the instructor and investigators.



For the 13 plants and 7 controls, we collected water at the different time steps to calculate uptake rate and test if there were nonlinearities due to saturation kinetics. Many plants in excess water show reduced transport efficiency as the plant absorbs more water and becomes more saturated relative to the solution outside the root<sup>18</sup>. For this reason, the

<sup>18</sup> Welch, R. M. (1973). Vanadium Uptake by Plants. *Plant Physiology*, 51(5), 828–832.  
<https://doi.org/10.1104/pp.51.5.828>



active uptake of water and solutes by the plant roots may decrease through time (Fig. 6). One plant and two controls were excluded because they were labeled improperly or not sampled at the correct time.

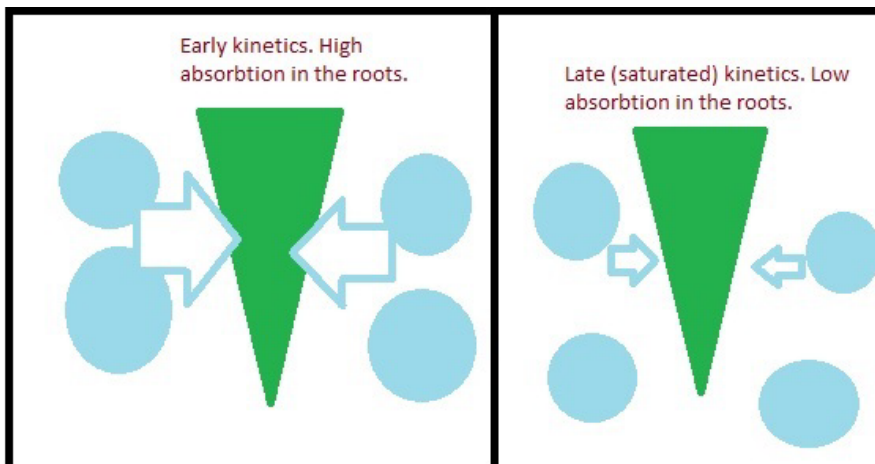


Figure 6. Saturation kinetics in the roots.

Working with the students, we hypothesized that the plants would have lower concentrations of Uranium (U) and arsenic (As) at the end of the experiment compared to the controls due to the active uptake of the roots, as suggested by the experiments used by Lee et al<sup>11</sup>. Antimony (Sb) was also included as a control as it is not an element we expected to be present in the water. Furthermore, we hypothesized that due to the saturation kinetics the roots would demonstrate less active uptake over time as the experiment proceeded.

All collected samples were analyzed for U, Sb, and As concentrations using inductively coupled plasma mass spectrometry (ICP-MS) at the University of Utah. This technique dissociates individual elements dissolved in a water sample. The element concentration is quantified by comparing the mass of each element in the sample to a known standard.

### *Statistical analysis*

We used the open-source statistical software package R to analyze the data and create figures<sup>19</sup>. In order to test for changes in concentration through time, we calculated linear regressions. Given the moderate number of comparisons relative to our sample size, we used a decision criterion of  $\alpha = 0.05$ , which reduces the probability of a false positive to 5% for each statistical test. To compare concentrations in plant and control treatments, we calculated the smoothed conditional means over the course of the experiment as well as the 95% confidence intervals around those means. When the confidence intervals overlapped between the two treatments, we concluded there was not a significant difference (i.e. we rejected the hypothesis that presence of sunflower seedlings altered concentration of the various heavy metals).

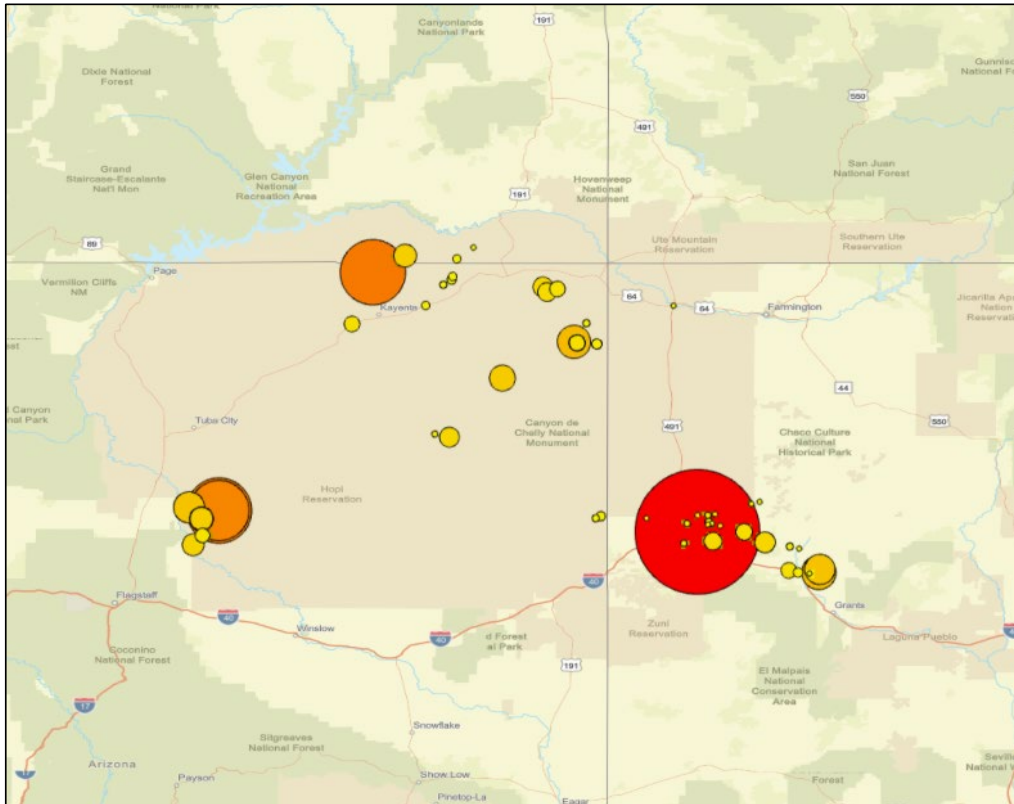
## **Results**

### *Mapping of uranium concentration in groundwater*

Based on the Navajo EPA data provided by Dr. Rock, we plotted concentration of U in wells across the Navajo Nation (Fig. 7). Approximately 47 % of tested wells were above the recommended level of 30  $\mu\text{g}$  per liter. The highest levels of contamination appeared to be near abandoned mines, though we did not have enough data to quantitatively test for a spatial correlation.

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<sup>19</sup> R Core Team. "R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing," 2016. <http://www.R-project.org/>.



**Red:**  
 $>200\mu\text{g/L}$

**Yellow:**  
 $>10\mu\text{g/L}$

EPA requires  
 $<30\mu\text{g/L}$  in  
 useable wells.

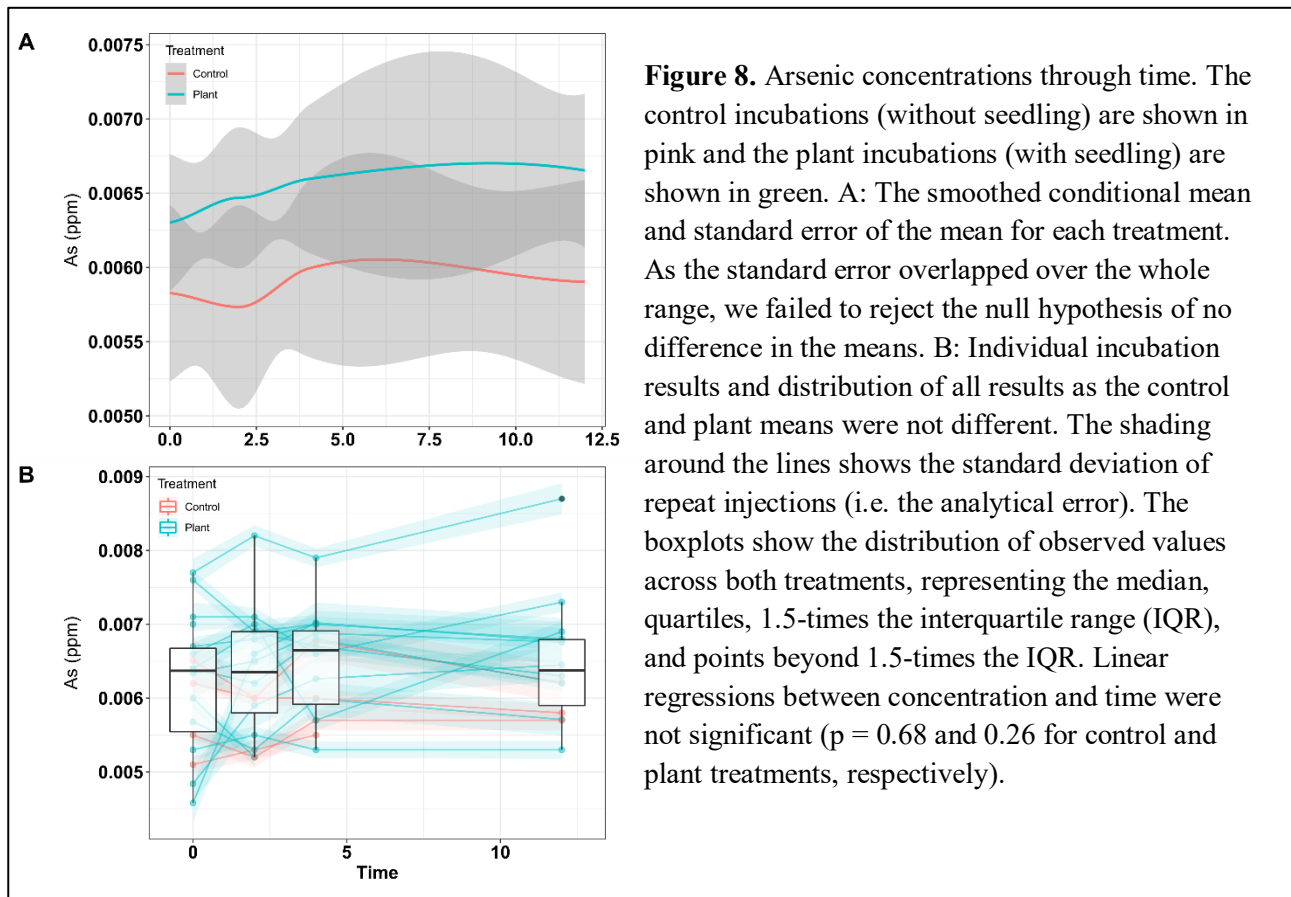
**Figure 7.** Uranium concentration in wells on the Navajo Nation. Color and size of circle denote concentration, with light yellow representing  $10\mu\text{g/L}$  and red representing  $200\mu\text{g/L}$ . Data provided by the Navajo Environmental Protection Agency.

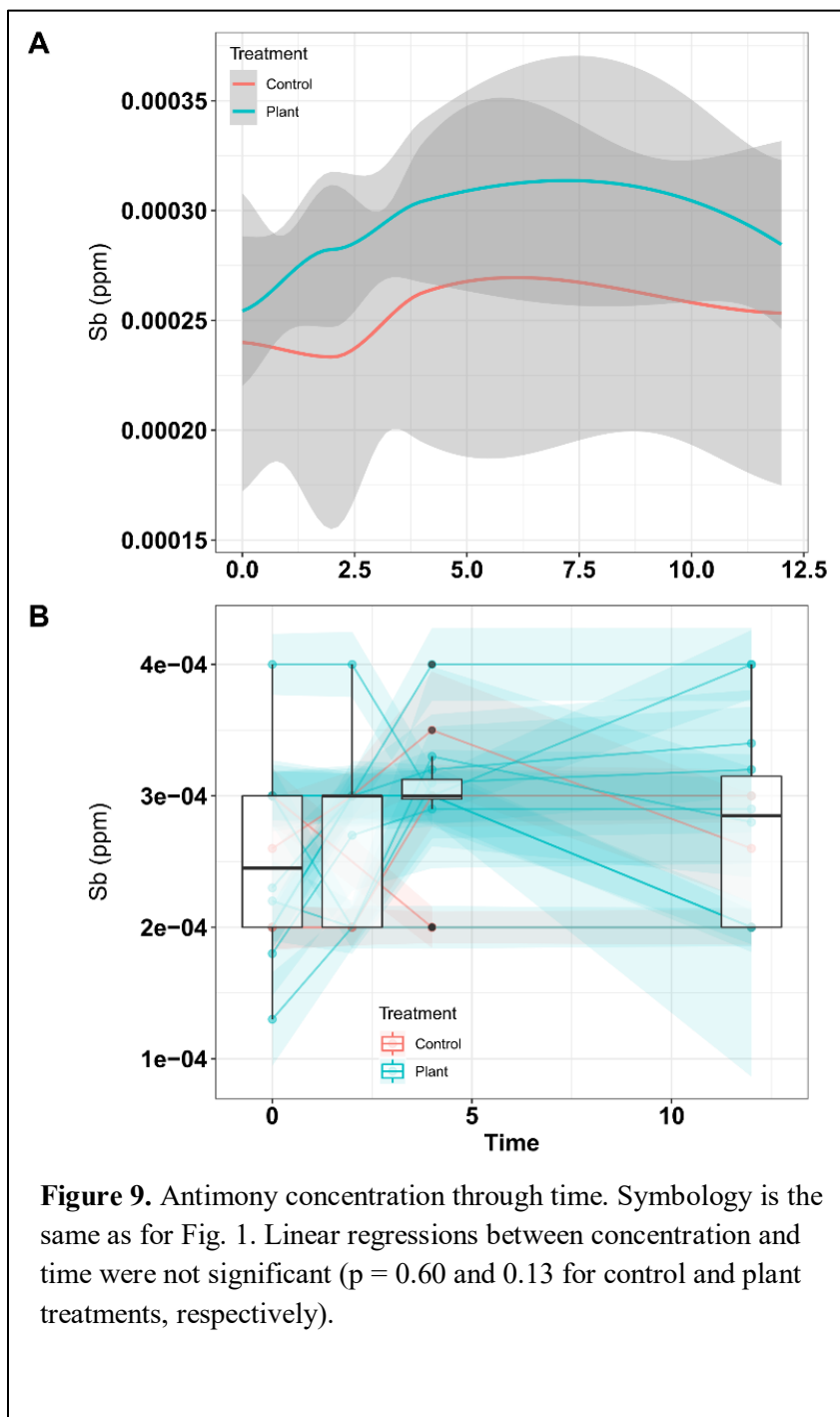
### *Results from student sampling of surface water*

Surface-water chemistry sampled by the students was highly variable across parameters (Appendix, Table 2). Some concentrations of nitrate, nitrate, and phosphate far exceeded water quality standards. However, this could be highly local contamination rather than a systemic problem because the students collected samples primarily from isolated pools that could be experiencing evaporation or direct contamination from livestock or people.

### *Sunflower uptake of uranium and arsenic*

During the 12-hour rhizofiltration experiments outlined above, we observed no change in arsenic (As) concentration (Fig. 8). This suggests there was no preferential uptake in As, or that the release rate of As from the soil exceeded the uptake rate. The control and plant treatments were statistically identical throughout the experiment, based on the overlap in the 95% confidence intervals (Fig. 8A). This further suggests that abiotic processes in the soil and soil solution overwhelmed any potential activity in or around the roots.



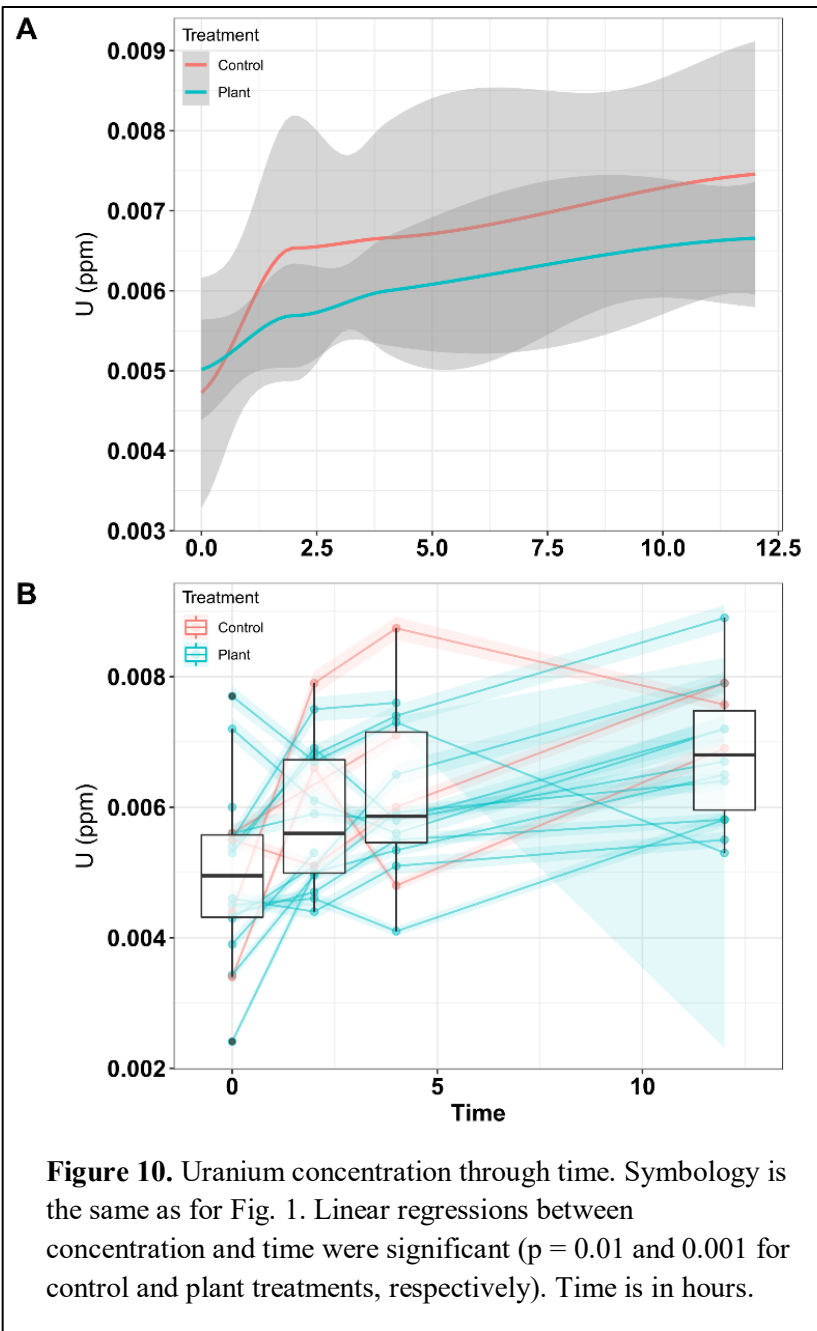


## Antimony

(Sb) showed the same pattern as As: no change in concentration through the experiment and no significant difference between control and plant treatments (Fig. 9). This further suggests that active uptake may not have been appreciable when compared to the effects on the roots exerted by the heterogenous solution from the soil.

However, uranium (U) showed

significant increases over the course of the experiment (Fig. 10). For both plant and control treatments, U concentration increased by approximately 20% over the first two



hours of the experiment, and then increased more slowly through 12 hours (Fig. 10). The plant treatment was statistically indistinguishable from the control treatment, based on the 95% confidence intervals of the smoothed means.

## Discussion

The nonlinear increase in U concentration fits observations of rapid abiotic dissolution (i.e. weathering) of soil and regolith soon after wetting<sup>20 21</sup>. The relatively dilute water stimulates release of soluble elements from the soil matrix, rapidly moving towards equilibrium. The continued increase of U concentration through the 12-hour experiment (Fig. 10) demonstrates that the soil is geologically young and unmodified, which is what we expected for such an arid and geologically active area as the Navajo Nation.

There are several potential explanations for our findings. First, the lack of a difference between plant and control treatments across the three heavy metals could be due to the seedlings having too low of root density in the soil matrix. Second, previous rhizofiltration experiments have been carried out in hydroponic environments with uranium dissolved in solution (i.e. in a nutrient solution devoid of the soil) to allow assessment of the root-solution interactions. However, controlled, hydroponic environments are poor analogues for actual soils, where multiple processes can enhance or impede root activity.

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<sup>20</sup> Jean Marçais, Alexandre Gauvain, Thierry Labasque, Benjamin Abbott, Gilles Pinay, et al.. Dating groundwater with dissolved silica and CFC concentrations in crystalline aquifers. *Science of the Total Environment*, Elsevier, 2018, 636, pp.260-272. [ff10.1016/j.scitotenv.2018.04.196](https://doi.org/10.1016/j.scitotenv.2018.04.196)ff. [ffinsu-01779877f](https://doi.org/10.1016/j.scitotenv.2018.04.196)

<sup>21</sup> Alam, M. S., & Cheng, T. (2014). Uranium release from sediment to groundwater: Influence of water chemistry and insights into release mechanisms. *Journal of Contaminant Hydrology*, 164, 72–87. <https://doi.org/10.1016/j.jconhyd.2014.06.001>



We chose to conduct our experiment to test specifically the potential efficacy of rhizofiltration in an actual soil from a contaminated area. The age of the seedlings and the volume of the soil solution were comparable to other laboratory tests<sup>22</sup>, so we reject the conclusion of a failed experiment. Instead, we hypothesize that because soils on the Navajo Nation are arid and relatively undeveloped, when they are exposed to water flow, they rapidly weather, releasing trace elements, including heavy metals, which are present in low quantities in these parent materials. When surveyed in many sites the soil profile was loose, gravelly, and had large amounts of carbonate<sup>23</sup> and were generally alkaline. Studies with rice plants have correlated increased pH with reduced metal uptake<sup>24</sup>. We suspect that the increased alkali properties of the soils near Chinle could predict the ineffectiveness observed in this study. Consequently, our data suggests that rhizofiltration is unlikely to be an effective remediation technique on the Navajo Nation, at least not if the sunflowers are planted in soils which are alkaline. A hydroponic setup could be effective at reducing U concentration in small-scale wells<sup>25</sup> but given the material and maintenance costs of such systems, it may not be a feasible solution. A more effective method would be a hydroponic filtration system to reduce adverse effects from the soil.

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<sup>22</sup> Jain, R., Peräniemi, S., Jordan, N., Vogel, M., Weiss, S., Foerstendorf, H., & Lakaniemi, A.-M. (2018). Removal and recovery of uranium(VI) by waste digested activated sludge in fed-batch stirred tank reactor. *Water Research*, 142, 167–175. <https://doi.org/10.1016/j.watres.2018.05.042>

<sup>23</sup> *United States Department of Agriculture Natural Resources Conservation Service*. (n.d.). Retrieve March 3, 2020, from [https://www.nrcs.usda.gov/Internet/FSE\\_MANUSCRIPTS/arizona/chinleAZ\\_NM2011/CHINLE%20AREA%20SOIL%20SURVEY.pdf](https://www.nrcs.usda.gov/Internet/FSE_MANUSCRIPTS/arizona/chinleAZ_NM2011/CHINLE%20AREA%20SOIL%20SURVEY.pdf)

<sup>24</sup> Zeng, F., Ali, S., Zhang, H., Ouyang, Y., Qiu, B., Wu, F., & Zhang, G. (2011). The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. *Environmental Pollution*, 159(1), 84–91. <https://doi.org/10.1016/j.envpol.2010.09.019>

<sup>25</sup> Magwaza, S. T., Magwaza, L. S., Odindo, A. O., & Mditshwa, A. (2020). Hydroponic technology as decentralised system for domestic wastewater treatment and vegetable production in urban agriculture: A review. *Science of The Total Environment*, 698, 134154. <https://doi.org/10.1016/j.scitotenv.2019.134154>

Further studies should be done to investigate the mechanism by which plants actively uptake heavy metals. To understand the nature of the regolith present in many regions of the Navajo Nation, these studies should be compared to geospatial mapping of wells to observe the trends of well data in the solution. In future experiments a soil matrix plant analysis of uranium may have many confounding factors and make it difficult to determine the efficacy of rhizofiltration of U. The initial pH of the soil and root diameter can greatly affect the absorption of uranium by the roots. The presence of other ions in the soil and water can also affect the uptake by the plant<sup>26</sup>.

Although the results may have rejected our hypothesis, this project led to important collaboration with members of the Navajo Nation through citizen science. Using a community centered approach to research we succeeded in raising awareness of scientific issues and allowing young collaborators to participate in scientific research<sup>27</sup>. Many of our collaborators actively took notes in their sampling of wells and documented the growth of the seedlings in endemic soil internalizing scientific skills. This suggests that with further mentoring high school aged students could participate in additional research projects. Furthermore, the project instilled an excitement of scientific inquiry in the community. Students, tribal leaders, Navajo Nation EPA members, and teachers became interested in a research question that was relevant to their community. Consequently, this project could lead to more research questions based on the findings and data gathered in this endeavor.

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<sup>26</sup> *A Model of Uranium Uptake by Plant Roots Allowing for Root-Induced Changes in the soil.* (2018). Environmental Science & Technology. <https://pubs.acs.org/doi/10.1021/acs.est.7b06136>

<sup>27</sup> Yurth, Cindy. *The Sunflower Project - Navajo Times.* Navajo Times. (2019, May 9). <https://navajotimes.com/ae/community/the-sunflower-project/>

An obstacle in our research process was the geographic distance between us and our collaborators in Chinle, Arizona. While we began the process with the vision of providing closely supervised hands-on experimentation the 400-mile distance to our collaborators made it difficult to make frequent trips and develop relationships with our collaborators. Future recommendations would be a temporary residence in the community where collaborators are found in order to have frequent connections with them. Furthermore, we would have liked students to take part in the dissemination of the project; however, since many graduated before the completion of the project it was difficult to remain in contact. We recommend that parties interested in high-school collaborative projects begin their research early in the school year in order to have more time to gather data and conduct experiments.

Regarding experimentation with rhizofiltration, further studies should include multiple plants at different stages of growth to control growth mediated variables in active uptake. Hydroponic solutions should not be associated with a plant's ability as a phytoremediator in soil conditions. Since soil matrices may exhibit complex dynamics due to the dissolved heterogenous mixture of components found within<sup>26</sup>. Given this information ongoing studies would be prudent to include soil analysis with filtration rate data. Recent studies have also investigated age specific root dynamics. There is also evidence to support that age of the root is negatively correlated with root uptake<sup>28</sup>. This supports the rejection of our hypothesis since our plants were relatively young. Future research would provide greater insight to the soil-specific effects of soils on plant root

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<sup>28</sup> COMAS, L. H., BAUERLE, T. L., & EISSENSTAT, D. M. (2010). Biological and environmental factors controlling root dynamics and function: effects of root ageing and soil moisture. *Australian Journal of Grape and Wine Research*, 16, 131–137. <https://doi.org/10.1111/j.1755-0238.2009.00078.x>

dynamics. Although many factors could be considered when analyzing root dynamics our data clearly demonstrates that the compilation of these factors make sunflowers an ineffective rhizofilter of U and As in soil conditions of the Chinle region.

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### **Advisors Credentials:**

Ben Abbott- Ecohydrologist. Experienced in biogeochemistry and hydrology. Led and organized multiple citizen science projects in France and Utah.

Abbott, Benjamin W., Florentina Moatar, Olivier Gauthier, Ophélie Fovet, Virginie Antoine, and Olivier Ragueneau. “Trends and Seasonality of River Nutrients in Agricultural Catchments: 18 Years of Weekly Citizen Science in France.” *Science of The Total Environment* 624 (May 15, 2018): 845–58.  
<https://doi.org/10.1016/j.scitotenv.2017.12.176>.

## **Additional materials:**

*Bulletin sent to potential collaborators:*

### **Mentored High School Student Research Opportunity**

#### **Aims**

- Help High school students participate in academic research.
- Engage in environmental science project within the local community.
- Help students communicate in Navajo.

#### **Summary**

Mining in the Navajo nation has caused heavy metal contamination of water sources. New evidence suggests that plants effectively remove contaminants from water. We will try to look into new ways of removing heavy metal contaminants from water using sunflower plants.

Student from the community will learn to collect water samples, cultivate sunflowers, and analyze data. Students can use this experience in their college applications and will have the opportunity to help share the findings of this project at conferences in their communities.

This will be a competitive opportunity for students as they prepare for college. Students will be mentored and present the data to their communities. Students will also have the opportunity to advance their Navajo language skills by creating posters and presentations that connect with a traditional audience.

#### **Collaborators:**

Students will work with undergraduate college students, local community leaders, and professional researchers.

#### **Locations:**

This project will take place in schools across the Navajo Nation.

#### **Timeline:**

The project will begin in April 2019 and data will be presented in Fall of 2019. Some time may be required during summer months for students to meet and work on projects.

#### **Costs:**

No cost will be required of students. Students will be provided transportation from their school to the research locations. Students will also be covered for the costs of conferences and posters.

#### **Contact:**

Zak Webber



## Time-Dependent Filtration Experiment

### Overview

1. Label tubes, name each plant.
2. Pour well-water into each of the samples.
3. Collect samples after 15 minutes and 4 hours.
5. Analysis of filtration.

### Labeling

We are going to label each tube before starting. We also will name each cup of dirt. This allows us to organize and keep track of the samples.

1. Write a fun name on tape for each plant and put it on the plant.
2. Using tape label 4 bottles for each plant with the same plant name in quotation marks.
3. Label each bottle the date and T-1, T-2, T-3, T-4

### Setting up the experiment

We need to measure the water that is in contact with the roots the most. For this reason we will need to transplant the sunflowers in buckets to smaller cups so they can be used next week for the same experiment.

1. Carefully dig a plant out from the ground. Keep all the roots intact and use gloves. If the roots have grown together keep the plants together. Place the plant in a cup and bury with dirt until it is mostly upright. Disturbing the roots can cause the plant to change its behavior.
2. Lightly water the soil using school tap water.

To test the filtration rate on 3-week old plants we will use plants in the red cups. Each cup has had holes poked in it so that water can drain into the bottom and be collected through the tube and syringe.

1. For each plant (or cup of dirt) measure 150mL of well-water into a graduated cylinder. It is important that this is as precise as possible.
2. Pour the water gently onto the plant. Take care to avoid pouring it to quickly and causing an indent in the dirt.

### Taking water samples

Water samples will be taken four times after 0 hours, 4 hours, 20 hours, and 40 hours. Twice during school. The next sample will be taken by Mr. Webber after 20 hours and again after 40 hours.

1. Gently vacuum all the water by pulling the syringe back slowly. When it's full, gently twist it off the tube (without removing the tube) and expel the water into the cup with the soil. This will force out water that is in contact with the roots.
2. Set a timer for 5 minutes. Relax, listen to some good music, talk about how you think "End Game" should have ended.
3. Vacuum 10mL water using the syringe and tube. Detach it gently from the tube and using gloved hands screw on the filter. Gently push the water into the bottle labeled T-#
4. Push gently, hard force can break the filter and ruin the sample. If the filter gets clogged get Mr. Webber's attention.
5. Once the sample is collected take the sample to the teacher with HCl. Have them add 1-2 drops. Keep gloves on until done with step 6.
6. Seal the bottle tightly and label the time it was sampled on the data sheet.

### Analysis

Data can take some time to analyze. We always need more scientists and often scientists are balancing many projects at once. It will take 3 weeks or more before the data samples can be analyzed using a machine that will measure concentrations of Uranium, Arsenic, Copper and other water contaminants.

Once the data has been run it takes additional time to write the entire project in a way that is professional and able to be interpreted by all. If the sunflowers are effective there are many future research questions that could be asked. How could sunflowers benefit the Navajo Nation? What effect would this have on drinking water? While science often does not give us quick answers I hope you know you have participated in research and a project that helps benefit the community you live in. It may not have seemed that you did much but in science every drop of counts.

Email me if you have questions, comments, or ideas: Zak Webber (fransisco.webber@gmail.com) ■ ■ ■

## Project: Water Filtration via Sunflowers

### Overview

We're going to see if Sunflowers are effective in removing Uranium from the water we give them. We will do this by testing the content of the water before and after we use it to water the plants.

If this works then we will know whether sunflowers would make a useful crop for areas affected by high amounts of uranium in the water or soil. This project will allow us to not only investigate the quality of the water, but do something about it.

### Experimental Procedure

#### a. Growing Sunflowers

Sunflowers will need to be sprouted to seedling size before planting in the affected areas. We will need to record any factors that may contribute to their growth. Only the healthy seedlings should be planted.

To keep good track of the plants we will plant sunflowers in per each bucket so that the roots are sufficiently dense. These plants will be watered with water collected from designated sources. In addition, 4 buckets will be left empty in random locations. No other treatments should be made to the plants. This will ensure that the experiment is not affected by unaccounted variables.

#### b. Measuring Water Quality

Using water from the source we will measure 5 samples of each source. One source will be industrial water from the school, and the other source will be one known to contain heavy metals.

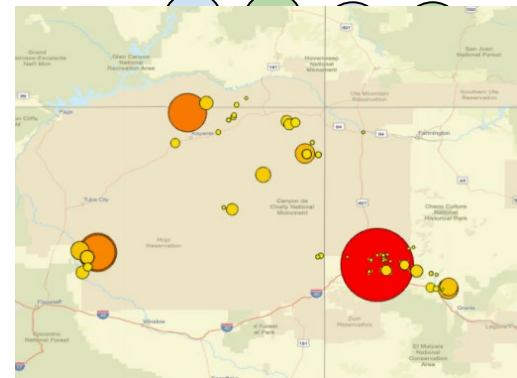
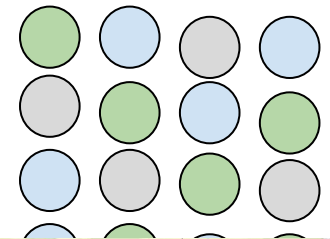
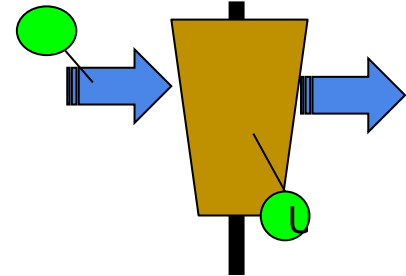
We will also map the areas the water is sampled from. If it is a well, this will be labeled with the well identification. The mapping can easily be done using excel and arcGIS.

#### e. Drying the Sunflowers

At the conclusion of the experiment sunflowers must gently be removed from the buckets to measure the content in the plants. They can be placed in ziplock bags after removing the dirt. Isolating them for assessment will help us know where the sunflowers retain different materials, such as Uranium.

#### f. Student Presentations

Students will create posters describing what they learn and the experimental design. With each action careful recording should be taken so we know exactly what happens during the experiments. Student groups should also research academic articles and cite their sources in their presentation. The final presentation should be prepared in Navajo.





## The Sunflower project: Using sunflowers to provide clean water to those living in areas polluted by uranium mining via partnership with high-school aged students

Zak Webber, Ben Abbott, Isaac St. Clair

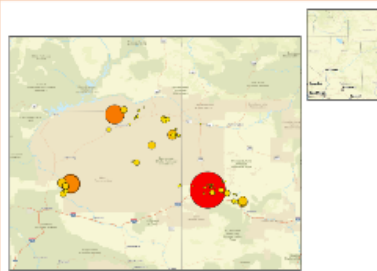
### Mining in the Navajo Nation

Uranium mining in the early 20 century has caused adverse effects to public health, agriculture, and biosphere in the four-corners region of the United States. These effects have directly affected the lifestyle and livelihood of the Diné people, commonly referred to as the Navajo. The over 1000 abandoned mine structures in this region have been linked with the presence of trace heavy metals, such as uranium (U), in soils and water. Uranium contamination has caused many adverse health effects(4) and is considered unsafe for human consumption in concentrations of 30 µg/L by the Safe Drinking Water Act.

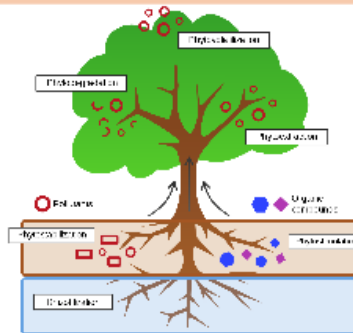
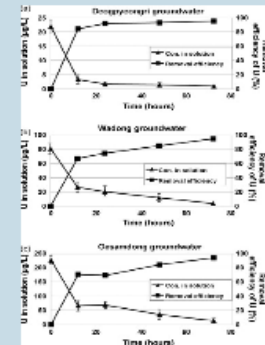


Year	Uranium (µg/L)
1980	100
1985	150
1990	200
1995	250
2000	300
2005	350
2010	400
2015	450
2020	500

### Well Data



### Using Sunflowers to improve Water Quality



### Citizen Science

An important aspect of this project is to involve local high school students (Chinle, Monument Valley high school) in the sampling, analyzing, and presenting of this research. This will allow them to have exposure to inquisition and prepare them for careers in science.



